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Metconazole

Document M-CA, Section 1

IDENTITY OF THE ACTIVE SUBSTANCE

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Date	Data points containing amendments or additions and brief description	Document identifier and version number

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CA 1 IDENTITY OF THE ACTIVE SUBSTANCE

CA 1.1 Applicant

BASF Agro BV
Arnhem (NL) Zürich Branch
8036 Zürich - Wiedikon
Switzerland

(a) Contact: [redacted]
Telephone No: [redacted]
E-mail: [redacted]

(b) Alternative: [redacted]
Telephone No: [redacted]
E-mail: [redacted]

CA 1.2 Producer

Manufacturer of Metconazole (legal entity)

Metconazole is manufactured for:

BASF Agro BV
Arnhem (NL) Zürich Branch
8036 Zürich - Wiedikon
Switzerland

Affiliates or representatives:

BASF SE
Crop Protection Division:
P.O. Box 120
67114 Limburgerhof
Germany

Contact person: [redacted]
Telephone No: [redacted]
Telefax: [redacted]
E-mail: [redacted]

Further information is regarded CONFIDENTIAL and provided in Document J.

Location of the manufacturing site of Metconazole

CONFIDENTIAL information - data provided separately (Document J)

CA 1.3 Common Name Proposed or ISO-accepted and synonyms

ISO common name: metconazole

CA 1.4 Chemical Name (IUPAC and CA nomenclature)

IUPAC name: (1RS,5RS;1RS,5SR)-5-(4-chlorobenzyl)-2,2-dimethyl-1-(1H-1,2,4-triazol-1-ylmethyl) cyclopentanol

CA nomenclature: 5-[(4-chlorophenyl)methyl]-2,2-dimethyl-1-(1H-1,2,4-triazol-1-ylmethyl) cyclopentanol

CA 1.5 Producer's Development Code Numbers

BASF Number: BAS 555 F
BASF Registry Number: Reg.No. 4056343 (mixture of cis and trans diastereomers)

Reg. No. 4079468 (cis-isomers; racemic mixture of two enantiomers),
Reg. No. 4079654 (trans-isomers; racemic mixture of two enantiomers)
AC 900768
CL 900,768
WL 148271
KNF-S-474M

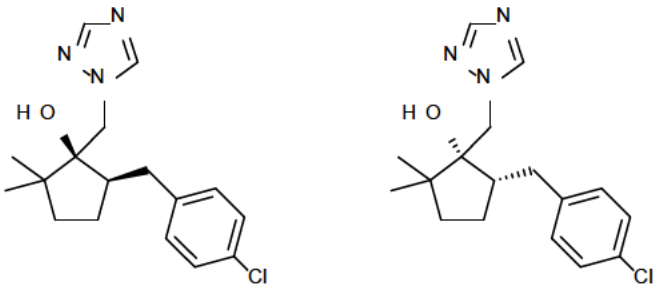
CA 1.6 CAS, EC and CIPAC Numbers

CAS No.: 125116-23-6 (unstated stereochemistry)
CIPAC No.: 706
EC No.: Not assigned

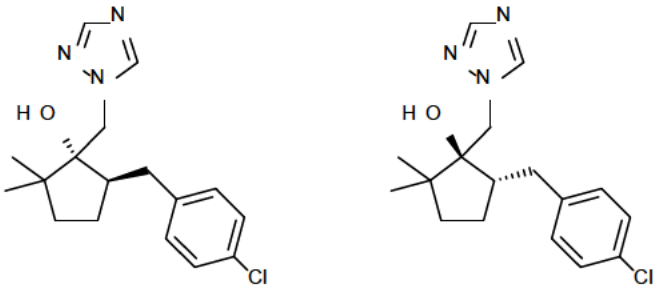
CA 1.7 Molecular and Structural Formula, Molar Mass

Molecular formula: $C_{17}H_{22}ClN_3O$
Molar mass: 319.8 g/mol

Structural formulae:



cis-isomers
Reg.no. 4079468



trans-isomers
Reg.no. 4079654

CA 1.8 Method of Manufacture (synthesis pathway) of the active substance

CONFIDENTIAL information - data provided separately (Document J)

CA 1.9 Specification of Purity of the Active Substance in g/kg

- sum of cis- and trans-metconazole (RegNo. 4079468 and RegNo. 4079654): min. 940 g/kg
  and
- cis-metconazole (RegNo. 4079468): min. 800 g/kg and max. 950 g/kg

CA 1.10 Identity and Content of Additives (such as stabilisers) and impurities

CA 1.10.1 Additives

CONFIDENTIAL information - data provided separately (Document J)

CA 1.10.2 Significant impurities

CONFIDENTIAL information - data provided separately (Document J)

CA 1.10.3 Relevant impurities

Technical metconazole does not contain impurities that are considered to be of toxicological, ecotoxicological or environmental concern.

CA 1.11 Analytical Profile of Batches

CONFIDENTIAL information - data provided separately (Document J)

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Document M-CA, Section 2

PHYSICAL AND CHEMICAL PROPERTIES OF THE ACTIVE SUBSTANCE

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CA 2 PHYSICAL AND CHEMICAL PROPERTIES OF THE ACTIVE SUBSTANCE

Test or Study & Data Point	Guideline and method	Test material purity and specification	Findings	GLP Y/N	Reference
CA 2.1 Melting point and boiling point	EEC A1	98.6 %	Information previously reported and peer-reviewed: Melting point range 100.0 – 108.4 °C Melting point was determined without decomposition taking place		EFSA Scientific Report (2006) 64, 1-71, Conclusion on the peer review of Metconazole
	OECD 103 ≈ EEC A2	98.1%	Information previously reported and peer-reviewed: Boiling point 315 °C		
CA 2.2 Vapour pressure, volatility	EEC A4	98.6 %	Information previously reported and peer-reviewed: Vapour pressure 2.1 x 10^{-8} Pa at 20 °C		EFSA Scientific Report (2006) 64, 1-71, Conclusion on the peer review of Metconazole
	calculation	98.6%	Information previously reported and peer-reviewed: Henry's law constant 2.21 x 10^{-7} Pa·m^3·mol^{-1} at 20 °C		
CA 2.3 Appearance (Physical state, colour)		98.6%	Information previously reported and peer-reviewed: White powdered solid, odourless		EFSA Scientific Report (2006) 64, 1-71, Conclusion on the peer review of Metconazole

Test or Study & Data Point	Guideline and method	Test material purity and specification	Findings	GLP Y/N	Reference
CA 2.4 Spectra (UV/VIS, IR, NMR, MS), molar extinction at relevant wavelengths, optical purity	Standard IR, NMR and MS methodology	Pure Isomers: cis-isomer (98.8% purity) trans-isomer (98.6% purity)	Information previously reported and peer-reviewed: The different spectra (IR, ^{1}H-NMR, ^{13}C-NMR, MS-CI, MS-EI) were found to be in agreement with the proposed chemical structures.		Draft Assessment Report, Vol. 3, Annex B, B.2, Physical and chemical properties, January 2004.
	OECD 101	AC12140-17: 98.1 %	UV/Vis solution in watery medium (methanol/water (1:9)): λ_{max} (nm) ε (L·mol^{-1}·cm^{-1}) 268 364.73 276 285.25 290 3.08 295 2.82	Y	[see KCA 2.4/1 2014/1158207]
CA 2.5 Solubility in water	EEC A6	98.6%	Information previously reported and peer-reviewed: 30.4 mg/L at 20 °C in distilled Milli-Q water (pH ca. 7.5) no effect of pH		EFSA Scientific Report (2006) 64, 1-71, Conclusion on the peer review of Metconazole
CA 2.6 Solubility in organic solvents	EEC A6	98.6%	Information previously reported and peer-reviewed: Solubility at 20 °C in hexane : 1.40 g/L toluene : 103 g/L dichloromethane : 481 g/L methanol : 403 g/L 2-propanol : 132 g/L acetone : 363 g/L ethyl acetate : 260 g/L		EFSA Scientific Report (2006) 64, 1-71, Conclusion on the peer review of Metconazole

Test or Study & Data Point	Guideline and method	Test material purity and specification	Findings	GLP Y/N	Reference
CA 2.7 Partition co-efficient n-octanol/water	EEC A8	98.6%	Information previously reported and peer-reviewed: 3.85 at 20 °C (pH 7.2 - 8) Effect of pH was not investigated since there is no dissociation in water in the environmentally relevant pH-range		EFSA Scientific Report (2006) 64, 1-71, Conclusion on the peer review of Metconazole
CA 2.8 Dissociation in water - dissociation constant(s) (pKa values) - identity of dissociated species - dissociation constant(s) (pKa values) of the active principle	OECD 112	98.6%	Information previously reported and peer-reviewed: <u>Dissociation constant</u> pKa_1 = 11.38 pKa_2 = 1.08		EFSA Scientific Report (2006) 64, 1-71, Conclusion on the peer review of Metconazole
CA 2.9 Flammability and self-heating	EEC A10	96 %	Information previously reported and peer-reviewed: Not highly flammable		EFSA Scientific Report (2006) 64, 1-71, Conclusion on the peer review of Metconazole
CA 2.10 Flash point			Not applicable, due to melting point > 40°C		
CA 2.11 Explosive properties	EEC A14	96 %	Information previously reported and peer-reviewed: Not explosive		EFSA Scientific Report (2006) 64, 1-71, Conclusion on the peer review of Metconazole

Test or Study & Data Point	Guideline and method	Test material purity and specification	Findings	GLP Y/N	Reference
CA 2.12 Surface Tension	EEC A5	COD-001163 98.7 %	48.6 mN/m at 20 °C (90% saturated solution in pure water) Remark: This study is required as the study in the Annex I Registration process had been carried out with TGAI of a purity of 97.4 %. However, regulation EU 283/2013 requires PAI or material of a purity of ≥ 98 %.	Y	[see KCA 2.12/1 2015/1193306]
CA 2.13 Oxidising properties	EEC A17	96 %	Information previously reported and peer-reviewed: not oxidizing		EFSA Scientific Report (2006) 64, 1-71, Conclusion on the peer review of Metconazole
CA 2.14 Other studies			Not required		

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Document M-CA, Section 3

FURTHER INFORMATION ON THE ACTIVE SUBSTANCE

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Date	Data points containing amendments or additions and brief description	Document identifier and version number

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CA 3 FURTHER INFORMATION ON THE ACTIVE SUBSTANCE

CA 3.1 Use of the Active Substance

Metconazole is a triazole fungicide which is used worldwide in several crops for the control of a broad range of important pathogens. Metconazole is active against different fungal stages both on the plant surface and in the plant tissue. After application to the plant, the active ingredient is taken up via the leaf and then translocated via the transpiration flow. Due its mobility, it shows systemic and translaminar activity. By that, it can control fungal stages which have already become established in deeper tissue layers. Metconazole is thus suitable for preventative and curative treatments.
Since the vapour pressure of metconazole is very low, a marked gas phase activity was not observed.

CA 3.2 Function

Metconazole is used as a fungicide to control harmful diseases in a broad range of crops. Furthermore, metconazole is used as plant growth regulator in oilseed rape.

CA 3.3 Effects on Harmful Organisms

Metconazole is active against different fungal stages on and in the plant. When applied protectively, metconazole inhibits further development of germinated fungal spores. Due to its ability to enter into the leaf and its further translocation as well as its high intrinsic activity, it can also control fungal stages that have already become established in deeper tissue layers. Metconazole is thus suitable for preventative and curative treatments.

CA 3.4 Field of Use Envisaged

Agriculture

CA 3.5 Harmful Organisms Controlled and Crops or Products Protected or Treated

Metconazole is used to control a broad range of important fungal diseases in cereals and oilseed rape such as

- *Alternaria* spp.
- *Blumeriella* spp.
- *Erysiphe* spp.
- *Drechslera* spp.
- *Fusarium* spp.
- *Leptosphaeria* spp.
- *Puccinia* spp.
- *Pyrenopeziza* spp.
- *Pyrenophora* spp.
- *Rhynchosporium* spp.
- *Sclerotinia* spp.
- *Septoria tritici / Zymoseptoria tritici*

Furthermore, metconazole is used as plant growth regulator in oilseed rape.

CA 3.6 Mode of Action

Metconazole belongs to the triazole group of fungicides and the primary mode of action is the blocking of ergosterol biosynthesis through inhibition of cytochrome P450 sterol 14α-demethylase (CYP51). The depletion of ergosterol and accumulation of non-functional 14 α - methyl sterols results in inhibition of growth and cell membrane disruption. Because of the mode of action triazoles belong to the demethylation inhibitors (DMI). DMIs and morpholines together are named sterolbiosynthesis inhibitors (SBI).

CA 3.7 Information on Occurrence or Possible Occurrence of the Development of Resistance and Appropriate Management Strategies

Four major mechanisms are associated with changes in DMI-sensitivity: Mutations in the target gene (cyp51), elevation of intracellular CYP51 levels, reduced intracellular accumulation of triazoles by overexpression of efflux-pumps and altered sterol biosynthesis. It is assumed that these resistance mechanisms may be combined in an additive manner. Mutations of a single gene result in a low degree of resistance and resistance levels may increase by additional mutations of other genes. This results in a quantitative (directional) type of resistance and changes in the sensitivity of a population are gradual.
A current summary of the situation for some of the main indications where DMIs are used can be found in the FRAC SBI Working Group report (FRAC 2015). In summary, it can be said that some pathogens have shown a shift towards lower sensitivity in the period since their introduction, but that in most cases the situation has now stabilised (FRAC 2015). For the intensive cereal growing regions of Northern Europe, the sensitivity situation of Zymoseptoria tritici towards DMIs was widely discussed in recent years. Since the early 2000s, a shift in Zymoseptoria tritici to a reduced sensitivity towards different DMIs has been determined in microtitre assays with isolates taken from the most important cereal-growing regions in Europe (FRAC 2015). Molecular biological analyses have shown that mutations and mutation combinations in CYP51, and also other factors, such as the activity of efflux transporters or CYP51 overexpression, can be linked to the sensitivity changes observed (Cools and Fraaije 2006, Leroux et al. 2007, Brunner et al. 2008, Stammler et al. 2008, Cools et al. 2010, Walker et al. 2010, Stammler et al. 2010, Stammler and Semar 2011, Cools et al. 2012). Isolates belonging to different CYP51-haplotypes showed variation in their sensitivity response to different DMIs (Glättli et al. 2009, Fraaije et al. 2007), that means, correlation of sensitivity between various DMIs can be low or even negative. This is confirmed by frequency analyses of CYP51-haplotypes in field trials after the application of DMI treatments, which showed that DMIs select CYP51-haplotypes differently (Fraaije et al. 2007, Stammler et al. 2008). Sensitivity changes observed in microtitre plates do not always correlate with DMI efficacy observed in the field (Mehl et al. 2010, Stammler et al. 2008, Strobel et al. 2010), since other factors such as application timing, weather conditions and disease pressure may influence fungicide efficacy. Despite sensitivity changes measured in microtiter plates, some DMIs (including metconazole) at registered dose rates has shown reliable field performance against Zymoseptoria tritici throughout the past decade, whereas the efficacy of some other DMIs has significantly decreased (Stammler et al. 2006, Defra 2007, Strobel et al. 2010, Clark et al. 2010).

Cross resistance studies over many years, with different pathogens and different DMI-fungicides indicated that a clear statement is not possible. There are DMIs which show a good correlation of the sensitivities in Zymoseptoria tritici, but correlations for others are low to moderate, especially when sensitivities of imidazoles and triazoles are correlated (Figures 1, 2). While R-types containing I381V have higher EC50 values to metconazole, EC50 values for prochloraz are on the wild type level or even lower (at least in case I381V is not combined with V136A and S524T).

There exist also several reports in the past on other fungal pathogens that complete cross resistance within DMI fungicides is not always be present (Leroux et al. 2000, 2006, Kendall et al. 1986, Steva et al. 1990). The present recommendation of the FRAC SBI Working Group, however, is to consider all DMIs as one product group in which in general cross resistance exists.

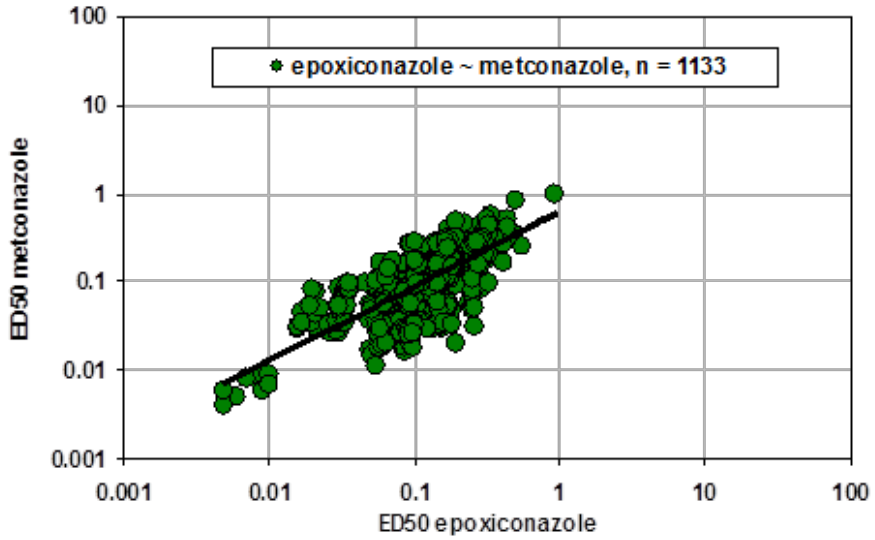

Figure 3.7-1: Correlation of the sensitivity of *Zymoseptoria tritici* to metconazole and epoxiconazole, determined by microtiter assays (BASF internal data)

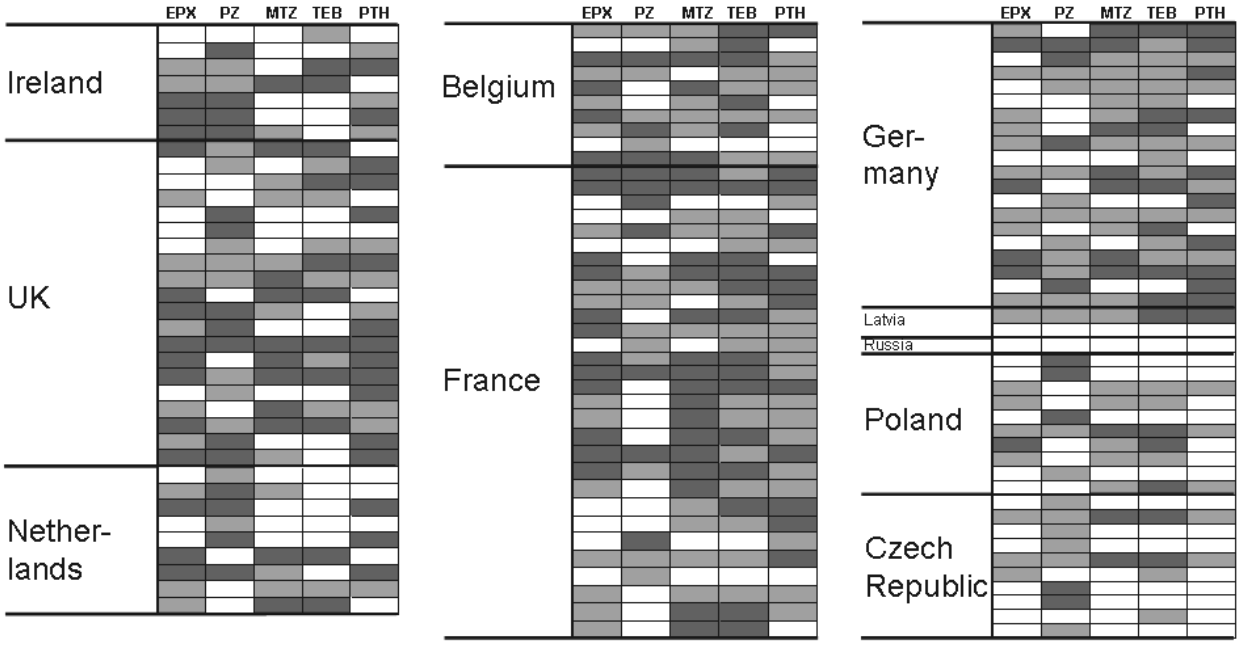

Figure 3.7-2: **119 isolates *of Zymoseptoria tritici* from different European countries (isolated in 2009) were analyzed for their sensitivity to 5 DMIs (EPX = epoxiconazole, PZ = prochloraz, MTZ = metconazole, TEB = tebuconazole and PTH = prothioconazole). ED_{50} values for each individual DMI were assigned to one of three classes, defined as 1/3 of isolates with low ED_{50}, 1/3 of isolates with moderate ED_{50} and 1/3 with high ED_{50}. White squares are for low, gray for moderate and black for high ED_{50} classification. The figure indicates that isolates have different sensitivity patterns and that sensitivity of one isolate to the 5 DMIs is in most cases heterogeneous.**

Within the SBI-group, there is no cross resistance between morpholines (e.g. fenpropimorph) and DMI fungicides. There is no cross resistance or a correlation of the sensitivity to DMI fungicides and other modes of action.

Sensitivities of *Zymoseptoria tritici* and *Leptosphaeria* spp. to metconazole are monitored on a regular basis.

In case of field failure which cannot be explained by other agronomic parameters, the sensitivity of the target pathogens of this Resistance Risk Analysis to metconazole will be analysed.
Regulatory authorities will be informed at an early stage about all cases of field failure known to be due to resistance. Changes in sensitivity will be communicated in the FRAC working groups and may result in modifications to the recommended resistant management strategies.

CA 3.8 Methods and Precautions Concerning Handling, Storage, Transport or Fire

Report: CA 3.8/1
Anonymous, 2015b
Safety data sheet - Metconazole tech.
2015/1189719
Guidelines: EEC 1907/2006
GLP: no

Exposure Controls / Personal Protection

Control parameters

Components with workplace control parameters

No occupational exposure limits known.

Exposure controls

Personal protective equipment

Respiratory protection:
Suitable respiratory protection for higher concentrations or long-term effect: Particle filter with medium efficiency for solid and liquid particles (e.g. EN 143 or 149, Type P2 or FFP2)

Hand protection:
Suitable chemical resistant safety gloves (EN 374) also with prolonged, direct contact (Recommended: Protective index 6, corresponding > 480 minutes of permeation time according to EN 374): E.g. nitrile rubber (0.4 mm), chloroprene rubber (0.5 mm), butyl rubber (0.7 mm) and other.

Eye protection:
Safety glasses with side-shields (frame goggles) (e.g. EN 166)

Body protection:
Body protection must be chosen depending on activity and possible exposure, e.g. apron, protecting boots, chemical-protection suit (according to EN 14605 in case of splashes or EN ISO 13982 in case of dust).

General safety and hygiene measures

Handle in accordance with good industrial hygiene and safety practice. Wearing of closed work clothing is recommended. Store work clothing separately. Keep away from food, drink and animal feeding stuffs.

Handling and Storage

Precautions for safe handling
No special measures necessary if stored and handled correctly. Ensure thorough ventilation of stores and work areas. When using do not eat, drink or smoke. Hands and/or face should be washed before breaks and at the end of the shift.

Protection against fire and explosion:
Avoid dust formation. Dust can form an explosive mixture with air. Prevent electrostatic charge - sources of ignition should be kept well clear - fire extinguishers should be kept handy.

Conditions for safe storage, including any incompatibilities
Segregate from foods and animal feeds.
Further information on storage conditions: Protect against moisture. Keep away from heat. Protect from direct sunlight.

Storage stability:
Storage duration: 60 Months

First-Aid Measures

Description of first aid measures
Remove contaminated clothing.

If inhaled:
Keep patient calm, remove to fresh air, seek medical attention.

On skin contact:
Wash thoroughly with soap and water.

On contact with eyes:
Wash affected eyes for at least 15 minutes under running water with eyelids held open.

On ingestion:
Immediately rinse mouth and then drink 200-300 ml of water, seek medical attention.

Most important symptoms and effects, both acute and delayed
Symptoms: The most important known symptoms and effects are described in the labelling (see section 2) and/or in section MCA 11, further important symptoms and effects are so far not known.

Indication of any immediate medical attention and special treatment needed
Treatment: Treat according to symptoms (decontamination, vital functions), no known specific antidote.

Transport Information
Land transport

ADR

UN number	UN3077
UN proper shipping name:	ENVIRONMENTALLY HAZARDOUS SUBSTANCE, SOLID, N.O.S. (contains METCONAZOLE)
Transport hazard class(es):	9, EHSM
Packing group:	III
Environmental hazards:	yes
Special precautions for user:	Tunnel code: E

RID

UN number	UN3077
UN proper shipping name:	ENVIRONMENTALLY HAZARDOUS SUBSTANCE, SOLID, N.O.S. (contains METCONAZOLE)
Transport hazard class(es):	9, EHSM
Packing group:	III
Environmental hazards:	yes
Special precautions for user:	None known

Inland waterway transport

ADN

UN number	UN3077
UN proper shipping name:	ENVIRONMENTALLY HAZARDOUS SUBSTANCE, SOLID, N.O.S. (contains METCONAZOLE)
Transport hazard class(es):	9, EHSM
Packing group:	III
Environmental hazards:	yes
Special precautions for user:	None known
Transport in inland waterway vessel:	Not evaluated

Sea transport

IMDG

UN number:	UN 3077
UN proper shipping name:	ENVIRONMENTALLY HAZARDOUS SUBSTANCE, SOLID, N.O.S. (contains METCONAZOLE)
Transport hazard class(es):	9, EHSM
Packing group:	III
Environmental hazards:	yes
Marine pollutant:	yes
Special precautions for user:	None known

Air transport

IATA/ICAO

UN number:	UN 3077
UN proper shipping name:	ENVIRONMENTALLY HAZARDOUS SUBSTANCE, SOLID, N.O.S. (contains METCONAZOLE)
Transport hazard class(es):	9, EHSM
Packing group:	III
Environmental hazards:	yes
Special precautions for user:	None known

Fire-Fighting Measures

Extinguishing media

Suitable extinguishing media:
dry powder, foam, water spray

Unsuitable extinguishing media for safety reasons:
carbon dioxide

Special hazards arising from the substance or mixture

hydrogen chloride, carbon monoxide, carbon dioxide, nitrogen oxides, organochloric compounds
The substances/groups of substances mentioned can be released in case of fire.

Advice for fire-fighters

Special protective equipment:
Wear self-contained breathing apparatus and chemical-protective clothing.

Further information
Collect contaminated extinguishing water separately, do not allow to reach sewage or effluent systems. Dispose of fire debris and contaminated extinguishing water in accordance with official regulations. In case of fire and/or explosion do not breathe fumes. Keep containers cool by spraying with water if exposed to fire.

CA 3.9 Procedures for Destruction or Decontamination

Report: CA 3.9/1
Anonymous, 2015b
Safety data sheet - Metconazole tech.
2015/1189719
Guidelines: EEC 1907/2006
GLP: no

For purposes of disposal, combustion of Metconazole or its pesticide products in a licensed incinerator is recommended. This method of disposal applies also to contaminated packages, which cannot be cleaned or reused.

Although it is possible to incinerate the product at lower temperatures, combustion at approximately 1100°C with a residence time of about 2 seconds is advised.
By doing so, i.e., operating the incinerator according to the conditions laid down in council directive 94/67/EEC resp. directive 2000/76/EC of the European Parliament, one will achieve complete combustion and minimize the formation of undesired by-products in the off-gases.

Contaminated packaging:
Contaminated packaging should be emptied as far as possible and disposed of in the same manner as the substance/product.

CA 3.10 Emergency Measures in Case of an Accident

Report: CA 3.10/1
Anonymous, 2015b
Safety data sheet - Metconazole tech.
2015/1189719
Guidelines: EEC 1907/2006
GLP: no

Personal precautions, protective equipment and emergency procedures
Use personal protective clothing. Avoid contact with the skin, eyes and clothing. Avoid dust formation.

Environmental precautions
Do not discharge into the subsoil/soil. Do not discharge into drains/surface waters/groundwater.

Methods and material for containment and cleaning up
For small amounts: Contain with dust binding material and dispose of.
For large amounts: Sweep/shovel up.
Avoid raising dust. Dispose of absorbed material in accordance with regulations. Clean contaminated floors and objects thoroughly with water and detergents, observing environmental regulations.
Collect waste in suitable containers, which can be labeled and sealed.

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Document M-CA, Section4

ANALYTICAL METHODS

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Version history[1]

Date	Data points containing amendments or additions and brief description	Document identifier and version number
07/Jan/2016	CA 4.1.2 Methods for risk assessment - Further analytical methods, used in support of environmental fate studies, amended. - Further analytical methods, used in support of toxicological studies, amended. - Further analytical methods, used in support of residue or feeding studies, amended. - Correction of typing error, CA 4.1.2/8, Linearity. - Numbering of studies amended CA 4.2 Methods for post-approval control and monitoring purposes - Table for already evaluated multi residue method plants amended.	Document MCA Section 4 Version 2 (BASF DocID 2016/1030843)
	Numbering of studies amended	

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CA 4 ANALYTICAL METHODS

CA 4.1 Methods used for the generation of pre-approval data

CA 4.1.1 Methods for the analysis of the active substance as manufactured

(a) Determination of the pure active substance in the active substance as manufactured and specified in the dossier submitted in support of approval under Regulation (EC) No 1107/2009

Determination by method M-2417.01 and SOP-PA.2080

The analytical method M-2417.01 (present number: APL0437/01) was submitted, peer-reviewed and accepted in Annex-I-inclusion process. This method has slightly been stated in a more precise way and thus a new version (APL0437/02, also described as SOP-PA.2080) has been generated, whose respective validation is submitted.

Report:	CA 4.1.1/1 Luz L.A. da, 2014h SOP-PA.2080_E Rev.00 - Determination of isomers in Technical Grade Active Ingredient (Tgai) Metconazole (BAS 555 F) by gas chromatography (GC) 2014/3003802
Guidelines:	none
GLP:	yes (certified by Instituto Nacional de Metrologia, Normalizacao e Qualidade Industrial - INMETRO, Rio de Janeiro, Brazil)

Report:	CA 4.1.1/2 Luz L.A. da, 2014g Validation of SOP-PA.2080 - Determination of isomers in Technical Grade Active Ingredient (Tgai) Metconazole (BAS 555 F) by gas chromatography (GC) 2014/3000002
Guidelines:	SOP-PA.2020, SOP-PA.2080
GLP:	yes (certified by Instituto Nacional de Metrologia, Normalizacao e Qualidade Industrial - INMETRO, Rio de Janeiro, Brazil)

Principle of the method

The analytical method SOP-PA.2080 (= APL0437/02) is used for the determination of the active ingredient content in technical metconazole (BAS 555 F) employing gas chromatography (GC) with flame ionization detector (FID).

The following table compares both method versions:

Table 4.1.1-1: Comparison between APL0437/02 (=SOP-PA.2080) and APL0437/01 (=M-2417.01)

Method	**SOP-PA.2080 (= APL0437/02)**			**M-2417.01 (= APL0437/01**
Column	DB-1301, 30 m x 0.32 mm ID, 0.25 µm film, or equivalent			DB-1301, 30 m x 0.32 mm ID, 0.25 µm film
Injector temperature	300°C			300°C
Detector temperature	320°C			320°C
Oven temperature	Time [min]	temperature	temperature increase	Temperature program
	Initial	220°C	---	Initial: 220°C
	1	220°C	isocratic	220°C for 1 min.
	5	260°C	10°C/min	program to 260°C at 10°C/min.
	9	260°C	isocratic	hold (260°C) for 4 min.
	10	280°C	20°C/min	then to 280°C at 20°C/min.
	22	280°C	isocratic	hold (280°C) for 12 min.
Carrier gas	Helium			Helium
Detector gas	H_2/synthetic air optimized mixture			Hydrogen at 30 mL/min, Air at 250 mL/min
Split ratio	1:40			1:40
Column flow	1.8 mL/min (constant pressure)			33 cm/s (column head pressure: 15 psi, measured at 220°C)
Injection volume	2 µL			2 µL
Analysis time	22 min			22 min
Internal standard	Dioctyl adipate			Dioctyl adipate
RT	Internal standard: 5.7 min cis-metconazole: 8.0 min trans-metconazole: 8.7 min			Internal standard: 5.1 – 6.1 min cis-metconazole: 7.4 – 8.4 min trans-metconazole: 8.2 – 9.2 min

Identity
The identity of the internal standard and of each isomer of metconazole (Reg.No. 4079468, Reg.No.4079654), was confirmed by gas chromatography–mass spectrometry (GC-MS).

Specificity
The selectivity of the method was proven by comparing the chromatograms of the solvent (acetonitrile), the internal standard (dioctyl adipate), each isomer reference item (Reg.No.4079468 and Reg.No.4079654), the reference item mixture, the reference item of metconazole PAI (Reg.No.4056343) and metconazole TGAI (Reg.No.4056343).
No co-elution was detected.

Linearity
Linearity was evaluated based on detector response ratio of seven different concentrations of reference items cis-metconazole (Reg.No. 4079468) and trans-metconazole (Reg.No. 4079654) in the range between 160 mg/L and 1.650 mg/L – which corresponds to 160 g/kg (16%) to 1,650 g/kg (160%) with regard to the active ingredient – and the internal standard (ISTD) dioctyl adipate (DOA), which can be expressed by an equation in the format $y = ax \pm b$. Each solution had triple injection. The correlation coefficients were found above 0.999 and the results prove the linearity of the detector responses of reference items in all investigated ranges.

The following table and figures summarizes the results.

Table 4.1.1-2: Linearity data obtained by GC-FID

Component Reg.no	Concentration range (mg/L)	Concentration range (%)	y-axis intercept	slope	correlation coefficient
Reg.No. 4079468 (cis-metconazole)	160 - 1650 mg/L	16 - 160%	0.00323	0.00167	0.99956
Reg.No. 4079654 (trans-metconazole)	160 - 1650 mg/L	16 - 160%	-0.00487	0.00168	0.99960

In addition, a calibration curve of three different concentrations of the reference item Reg.No.4056343 PAI – (metconazole PAI) – was done based on the statement of the Certificate of Analysis in the range of 340 mg/L to 1.349 mg/L – which corresponds to 340 g/kg (34%) to 1,349 g/kg (135%) with regard to the active ingredient – related to Reg.No.4079468 and 60 mg to 238 mg/L – which corresponds to 60 g/kg (6%) to 238 g/kg (23.8%) with regard to the active ingredient – related to Reg.No.4079654. The obtained linearity parameters agree within the error of measurement.

Table 4.1.1-3: Linearity – Metconazole PAI, Reg.No. 4056343 (related to cis-metconazole, Reg.No. 4079468)

Component Reg.no	Concentration range (mg/L)	Concentration range (%)	y-axis intercept	slope	correlation coefficient
Reg.No.4056343 (Metconazole PAI)	340 - 1349 mg/L	34 - 135%	-0.01291	0.00168	0.99988

Table 4.1.1-4: Linearity – Metconazole PAI, Reg.No. 4056343 (related to trans-metconazole, Reg.No. 4079654)

Component Reg.no	Concentration range (mg/L)	Concentration range (%)	y-axis intercept	slope	correlation coefficient
Reg.No.4056343 (Metconazole PAI)	60 - 238 mg/L	6 - 23.8%	-0.00305	0.00166	0.99983

Precision/Repeatability

The evaluation of repeatability was performed analyzing five preparations of the test item at one concentration level for both isomers. The results were calculated using the calibration curves for linearity (see above). The repeatability was determined as the relative standard deviation in% of the mean concentration determined in the commercial batch.

The acceptability of the%RSD (coefficient of variation, CV) for repeatability was proved by the Horwitz equation, an exponential relationship between the inter laboratory relative standard deviation (RSD_R) and the concentration C (expressed as decimal fraction):

$$\% RSD_R = 2^{(1-0.5 \log C)}$$

which is modified for estimation of repeatability (RSD, intra laboratory) to:

$$\% RSD_r = \% RSD_R \times 0.67$$

The acceptability of the%RSD (coefficient of variation, CV) for intermediate precision was proved by the modified Horwitz equation. The$\%RSD_r$ values for the quantified components (Reg.No.4079468 and Reg.No.4079654) were between 0.708% and 1.465% (vide infra) in metconazole TGAI which is within the calculated Horwitz limit value for these concentration levels.

Table 4.1.1-5: Precision data obtained by GC-FID detection

Reg.No.	average conc. (%)	%RSDr Limit	%RSD found	%RSD accepted
Reg.No. 4079468 (cis-metconazole)	80.82	1.384	0.708	YES
Reg.no. 4079654 (trans-metconazole)	16.24	1.762	1.465	YES

Conclusion
The results showed that the analytical conditions used in the analytical method (SOP-PA.2080 = APL0437/02) are suitable for the quantification of the respective isomers in technical metconazole (BAS 555 F).

(b) Determination of significant and relevant impurities and additives (such as stabilisers) in the active substance as manufactured

Metconazole does not contain relevant impurities.

For information on significant impurities and stabilizers please refer to document JCA, as this is CONFIDENTIAL information.

CA 4.1.2 Methods for risk assessment

Note:
The order of the study summaries is different compared to the information given in the application submitted for renewal of approval. In case references are summarized that were not contained in the application or in case references listed in the application are not contained in this chapter, comments are made where appropriate.

For the reviewers convenience a concordance list of naming and designations of reference compounds mentioned in M-CA 4.1.2 and 4.2 as well as the respective sections of Doc N is given below.

Table 4.1.2-1: List of Analytes

Common/Descriptive Name	Actual Metabolite Code	Reg.No.	CL Number
Metconazole	M555F000	4056343	189635
Metconazole CIS	M555F000 cis	4079468	354801
Metconazole TRANS	M555F000 trans	4079654	354802
Metconazole (-)trans	M555F000-SS	5836047	--
Metconazole (+)cis	M555F000-RS	5836046	--
Metconazole (+)trans	M555F000-RR	5836048	--
Metconazole (-)cis	M555F000-SR	4677200	--
M11	M555F011	4111112	382390
M21	M555F021	4558878	382391
M30	M555F030	4110625	382389
1,2,4-Triazole	M555F020	87084	196719
Triazolyl alanine	--	270412	147267
triazole acetic acid	--	137281	--
triazole lactic acid	--	--	--

(a) Methods in soil, water, sediment, air and any additional matrices used in support of environmental fate studies

The following methods cover the compounds necessary for the discussion on the residue definition for risk assessment for the environmental compartments as summarized in Document N and discussed in M-CA 7.5. These methods were validated within stand-alone validation studies.
If further methods have been used and validated within environmental fate studies, these are addressed within the respective study reports, and therefore presented in the corresponding dossier chapters.

Soil

Analytical methods for the determination of Metconazole residues in soil were reviewed in the context of the inclusion in Annex I under Directive 91/414/EEC. These methods evaluated previously are summarized in Table 4.1.2-2 for the reviewer's convenience.

Table 4.1.2-2: Summary of peer-reviewed analytical methods for determination of Metconazole residues in soil

Method No.	Matrix	Method principle	Target analytes	LOQ mg/kg	Year	DocID	EU reviewed
FAMS 055-02 and DFG S19	soil	GC-NPD	Metconazole, as sum of *cis*- and *trans*-isomer	0.01	1995	1995/7000218	yes

To cover actual guidelines and current state of the art techniques used in analytical laboratories, new analytical methods for the determination of the relevant analytes in soil were developed and validated.

Validation of method L0206/01

Report:	CA 4.1.2/1 Obermann M.,Sopena-Vazquez F., 2015a Validation of analytical method L0206/01 for the determination of the Metconazole BAS 555 F enantiomers Reg.No.5836046, Reg.No.4677200, Reg.No.5836047 and Reg.No.5836048 in soil by LC-MS/MS 2013/1376999
Guidelines:	SANCO/825/00 rev. 8.1 (16 November 2010), SANCO/3029/99 rev. 4 (11 July 2000), EEC 91/414 Annex II (Part A Section 4), EEC 91/414 Annex III (Part A Section 5), EPA 850.7100
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Principle of the method

BASF analytical method L0206/01 was developed for the determination of metconazole (BAS 555 F) enantiomers M555F000-RS (Reg. No. 5836046), M555F000-SS (Reg. No. 5836047), M555F000-RR (Reg. No. 5836048) and M555F000-SR (Reg. No. 4677200) in soil. The method was developed and validated at BASF, Limburgerhof, Germany.

A 5 g soil sample is extracted twice with a mixture of acetonitrile/water (70/30, v/v). After centrifugation, the extracts are combined. An aliquot (1 mL) of the combined extract is concentrated by evaporation to dryness under a stream of nitrogen, reconstituted with methanol and subsequently analysed by LC-MS/MS monitoring two mass transitions of metconazole.

Recovery findings

The method is suitable to determine residues of the enantiomeric forms of metconazole in soil. Samples are fortified with the analytes at the limit of quantification of 0.002 mg kg^{-1} and 10 times higher (0.02 mg kg^{-1}).

Mean recovery values (mean of five replicates per fortification level and analyte) are between 96% and 114% for all enantiomers of metconazole and both mass transitions monitored (see table below).

Table 4.1.2-3: Recoveries for the enantiomers of metconazole (M555F000-RS, M555F000-SS, M555F000-RR and M555F000-SR) in soil

Matrix	Analyte	Mass transition	Fortification Level [mg/kg]	Number of replicates	Mean Recovery [%]	RSD [%]	Overall Recovery [%]	RSD [%]
Soil LUFA 2.2	Metconazole (+)cis M555F000-RS (Reg. No. 5836046)	320→70	0.002	5	110	2.1	106	3.9
			0.020	5	103	1.4		
		320→125	0.002	5	102	1.4	103	2.8
			0.020	5	104	3.7		
	Metconazole (-)trans M555F000-SS (Reg. No. 5836047)	320→70	0.002	5	112	1.4	108	4.0
			0.020	5	105	2.1		
		320→125	0.002	5	110	1.1	109	1.6
			0.020	5	109	1.9		
	Metconazole (+)trans M555F000-RR (Reg. No. 5836048)	320→70	0.002	5	114	1.1	109	4.7
			0.020	5	105	2.2		
		320→125	0.002	5	109	0.3	108	1.9
			0.020	5	108	2.8		
	Metconazole (-)cis M555F000-SR (Reg. No. 4677200)	320→70	0.002	5	111	2.8	107	3.9
			0.020	5	104	1.6		
		320→125	0.002	5	111	1.5	110	2.1
			0.020	5	109	2.1		
Soil LUFA 5M	Metconazole (+)cis M555F000-RS (Reg. No. 5836046)	320→70	0.002	5	104	2.7	104	3.7
			0.020	5	104	4.8		
		320→125	0.002	5	103	1.1	106	3.2
			0.020	5	109	1.9		
	Metconazole (-)trans M555F000-SS (Reg. No. 5836047)	320→70	0.002	5	101	2.4	101	3.3
			0.020	5	101	4.3		
		320→125	0.002	5	101	0.9	104	3.6
			0.020	5	107	2.9		
	Metconazole (+)trans M555F000-RR (Reg. No. 5836048)	320→70	0.002	5	101	2.6	101	3.0
			0.020	5	102	3.7		
		320→125	0.002	5	102	1.3	104	2.8
			0.020	5	106	2.8		
	Metconazole (-)cis M555F000-SR (Reg. No. 4677200)	320→70	0.002	5	96	2.6	97	4.3
			0.020	5	97	5.7		
		320→125	0.002	5	98	2.0	101	4.0
			0.020	5	104	3.2		

Linearity
Good linearity (r > 0.995) is observed in the range of 0.025 ng mL^{-1} to 1.0 ng mL^{-1} for the two mass transitions of each test item using solvent-based standards (diluted in methanol).

Specificity
Significant interferences (> 30% of LOQ) are not observed at the retention times and mass transitions considered for each analyte.
LC-MS/MS is a highly specific self-confirmatory technique. Due to the high selectivity and specificity of LC-MS/MS, monitoring two mass transitions, an additional confirmatory technique is not necessary.

Matrix Effects
It could be demonstrated that the matrix load in the soil samples has no significant influence on the analyte analysis (matrix effect < 20%). Only for analyte M555F000-RS a matrix effect is observed on the lowest concentration level (response factor: 124-125%). However, taking this into consideration, the calibration curve is still in an acceptable range.

Limit of Quantification
The limit of quantification (LOQ) is defined by the lowest fortification level successfully tested. It is 0.002 mg kg^{-1} for all four individual enantiomers of metconazole.

Limit of Detection
The limit of detection (LOD) is defined as 20% of the LOQ; equivalent to 0.0004 mg/kg for all four enantiomers of metconazole, corresponding to the lowest calibration level used.

Repeatability
The relative standard deviations (RSD, %) for all fortification levels are below 20%.

Standard Stability
Calibration solutions (dissolved in methanol) of each analyte are stable (less than 5% decline) for at least 4 weeks when stored refrigerated at 4 ± 2°C.

Extract Stability
Mean recovery values of the soil extracts and of the methanol-reconstituted samples at 4 ± 2°C after a storage period of 7 days are within a range of 70-110% recovery.

Reproducibility
Reproducibility of the method was not determined within this validation study.

Conclusion

The analytical method L0206/01 for analysis of metconazole (BAS 555 F) enantiomers M555F000-RS (Reg. No. 5836046), M555F000-SS (Reg. No. 5836047), M555F000-RR (Reg. No. 5836048) and M555F000-SR (Reg. No. 4677200) in soil uses LC-MS/MS for final determination, with a limit of quantification of 0.002 mg kg^{-1}.

It could be demonstrated that the method fulfils the requirements with regard to specificity, repeatability, limit of quantification and recoveries and is therefore applicable to correctly and accurately determine residues of the enantiomers of metconazole in soil.

Validation of method L0203/01

Report:	CA 4.1.2/2 Geschke S., 2014a Validation of an analytical method for determination of BAS 555 F (Metconazole) and its metabolite 1,2,4-(1H)-Triazole in soil 2013/1377001
Guidelines:	SANCO/825/00 rev. 8.1 (16 November 2010)
GLP:	yes (certified by Landesanstalt fuer Umwelt, Messungen und Naturschutz Baden-Wuerttemberg, Karlsruhe, Germany)

Principle of the method

The analytical method L0203/01 for the determination of metconazole (BAS 555 F) *cis*-isomer (Reg. No. 4079468) and *trans*-isomer (Reg. No. 4079654) and its metabolite 1,2,4-(1H)-triazole (Reg. No. 87084) in soil, was validated at Eurofins Agroscience Services EcoChem GmbH, Niefern-Öschelbronn, Germany.

A 5 g soil sample is extracted twice with a mixture of acetonitrile/water (70/30, v/v). After centrifugation, the extracts are combined and the residues of metconazole (*cis*- and *trans*-isomer) and 1,2,4-(1H) triazole are analyzed by LC-MS/MS monitoring two mass transitions of metconazole and three mass transitions of 1,2,4-(1H)-triazole.

Recovery findings

The method proved to be suitable to determine metconazole (*cis*- and *trans*-isomer) and its metabolite 1,2,4-(1H)-triazole in soil. Samples were fortified with the analytes at the limit of quantification of 0.002 mg kg^{-1} and 10 times higher (0.02 mg kg^{-1}). No residues of metconazole (*cis*- and *trans*-isomer) were detectable in blank specimens. For metabolite 1,2,4-(1H)-triazole residues were detectable in blank specimens, so blank correction in the recovery data, for one of three mass transitions, was needed.

Mean recovery values (mean of five replicates per fortification level and analyte) were between 79% and 105% for all analytes (see table below), which fulfils the legal requirements.

Table 4.1.2-4: Summary of the recovery data in soil matrices

Soil	Analyte	m/z	Forti-fication Level [mg kg^{-1}]	Number of replicates	Mean Recovery [%]	RSD [%]	Overall Recovery [%]	RSD [%]
Lufa 2.2	*cis*-metconazole Reg.No. 4079468	320 → 125	0.002	5	94	3	97	4
			0.02	5	100	3		
		320 → 70	0.002	5	96	1	99	3
			0.02	5	101	3		
Lufa 5M		320 → 125	0.002	5	97	5	97	5
			0.02	5	97	5		
		320 → 70	0.002	5	99	6	100	5
			0.02	5	100	6		
Lufa 2.2	*trans*-metconazole Reg.No. 4079654	320 → 125	0.002	5	97	6	98	4
			0.02	5	98	2		
		320 → 70	0.002	5	96	6	97	5
			0.02	5	99	3		
Lufa 5M		320 → 125	0.002	5	104	2	103	5
			0.02	5	103	7		
		320 → 70	0.002	5	104	3	104	5
			0.02	5	103	7		
Soil (L120313)*	1,2,4-(1H)-triazole Reg.No. 87084	70 → 28	0.002	5	79	18	88	16
			0.02	5	97	6		
		70 → 43	0.002	5	87	19	89	14
			0.02	5	92	6		
		70 → 70	0.002	5	92	15	95	12
			0.02	5	98	7		
Soil (L120317)*		70 → 28	0.002	5	95	8	91	9
			0.02	5	87	8		
		70 → 43	0.002	5	105	16	100	13
			0.02	5	94	3		
		70 → 70	0.002	5	84	19	90	14
			0.02	5	95	5		

RSD = Relative standard deviations
* soil originate from a field dissipation study (DocID 2015/1000221)

Linearity
Good linearity ($r \geq 0.999$) was observed in the range of 0.03 ng mL^{-1} to 10 ng mL^{-1} for the quantifier mass transitions of metconazole (*cis*- and *trans*-isomer) (320→125) and for the quantifier and qualifier mass transitions of metabolite 1,2,4-(1H)-triazole ($r \geq 0.997$). For metconazole (*cis*- and *trans*-isomer) qualifier mass transition (320→70) the detector response was second order within the range of 0.03 ng mL^{-1} to 10 ng mL^{-1}.
Standards used for calibration curves were prepared in acetonitrile/water (70/30, v/v).

Specificity
No significant interferences (> 30% of LOQ) were observed at the retention times and mass transitions of metconazole (*cis*- and *trans*-isomer). Significant interferences (> 30% of LOQ) were observed at the retention time and for one mass transition considered for 1,2,4-(1H)-triazole. Therefore, interferences in the control samples were determined and blank correction in the recovery data was needed for this mass transition.
Due to the high selectivity and specificity of LC-MS/MS, monitoring at least two mass transitions, an additional confirmatory technique was not necessary.

Matrix Effects
To check possible ion enhancement or suppression effects in HPLC/MS-MS analysis, final extracts from soil blank samples were spiked with defined concentrations of *cis*- and *trans*-metconazole and 1,2,4-(1H)-triazole. Results demonstrated that the matrix load in the tested soil samples had no significant influence on the analyte analysis. Therefore, calibration was performed with standards in acetonitrile/water (70:30, v/v).

Limit of Quantification
The limit of quantification (LOQ) is defined by the lowest fortification level successfully tested. It is 0.002 mg kg^{-1} for metconazole (*cis*- and *trans*-isomer) and 1,2,4-(1H)-triazole.

Limit of Detection
The limit of detection (LOD) was estimated at 30% of the LOQ; equivalent to 0.0006 mg kg^{-1} for metconazole (*cis*- and *trans*-isomer) and 1,2,4-(1H)-triazole, corresponding to the lowest calibration level used.

Repeatability
The relative standard deviations (RSD, %) for all fortification levels were below 20%.

Stability of Working Solutions
Stock solutions of each analyte were stable (less than 10% decline) for at least 4.5 months refrigerated. The stock solutions were prepared within an ongoing parallel field study.

Extract Stability
Mean recovery values of the soil extracts after a storage period of 7 days at 1-10°C (nominally) in the dark are within a range of 70-120% recovery, hence soil extracts were considered stable for this period of time.

Reproducibility
Reproducibility of the method was not determined within this validation study.

Conclusion

The analytical method L0203/01 for analysis of metconazole (*cis*- and *trans*-isomer) and its metabolite 1,2,4-(1H)-triazole in soil uses LC-MS/MS for final determination, with a limit of quantification of 0.002 mg kg^{-1}.

It could be demonstrated that the method fulfils the requirements with regard to specificity, repeatability, limit of quantification and recoveries and is therefore applicable to correctly determine metconazole (*cis*- and *trans*-isomer) and its metabolite 1,2,4-(1H)-triazole in soil.

Extraction efficiency of the soil methods

In general the extraction efficiency has been proven within the respective soil metabolism studies. The extraction procedure of the metabolism trials, provided in MCA section 7.1.1 demonstrates good extractability using acetonitrile and acetonitrile/water (50/50, v/v) solvents mixtures. This has been adopted in the residue analytical methods L0203/01 and L0206/01, wherein acetonitrile/water (70/30, v/v) was used for extraction. In addition the volume of solvent used per soil mass was increased at least 8-fold in the residue analytical method with the result of increased extractability. Therefore, the extraction procedure used in residue analysis can be regarded as adequate.

Table 4.1.2-5: Reference list of soil metabolism studies

Number	DocID	Study	Details provided in section
1	2014/1000901	Soil metabolism of Metconazole (BAS 555 F) under aerobic conditions	CA 7.1.1
2	2014/1000922	Metconazole: Route and rate of degradation in anaerobic soil	CA 7.1.1

Water

Analytical methods for the determination of Metconazole residues in water were reviewed in the context of the inclusion in Annex I under Directive 91/414/EEC. These methods evaluated previously are summarized in Table 4.1.2-6 for the reviewer´s convenience.

Table 4.1.2-6: Summary of peer-reviewed analytical methods for determination of Metconazole residues in soil

Method No.	Matrix	Method principle	Target analytes	LOQ µg/L	Year	DocID	EU reviewed
FAMS 058-01	drinking water, surface water	GC-NPD	Metconazole, as sum of *cis*- and *trans*-isomer	0.05	1996	1996/7000341	yes
FAMS 058-01 (ILV)	drinking water	GC-NPD	Metconazole, as sum of *cis*- and *trans*-isomer	0.05	1998	1998/7000224	yes

To cover actual guidelines and current state of the art techniques used in analytical laboratories, new analytical methods for the determination of the relevant analytes in water were developed and validated.

Validation of method D1501/01 (L0273/01)

Report:	CA 4.1.2/3 Andrews R.S., 2015a Validation of method D1501/01 (L0273/01): Method for the determination of enantiomers of BAS 555 F (Reg.No. 5836046, Reg.No. 4677200, Reg.No. 5836047 and Reg.No. 5836048) in surface and drinking water by LC-MS/MS 2015/7000428[*)]
Guidelines:	EPA 850.6100, SANCO/3029/99 rev. 4 (11 July 2000), SANCO/825/00 rev. 8.1 (16 November 2010)
GLP:	yes (certified by United States Environmental Protection Agency)

Principle of the method

BASF analytical method D1501/01 (L0273/01) was developed for the determination of metconazole (BAS 555 F) enantiomers M555F000-RS (Reg. No. 5836046), M555F000-SS (Reg. No. 5836047), M555F000-RR (Reg. No. 5836048) and M555F000-SR (Reg. No. 4677200) in drinking (well) water and surface (lake) water. The method was developed and validated at BASF, Research Triangle Park, USA.

Residues of parent metconazole in water samples are directly injected, and the residues of metconazole are determined by high performance liquid chromatography (HPLC) column with detection by electrospray ionization tandem mass spectrometry (MS/MS-ESI), monitoring two ion transitions (for quantitation and confirmatory purpose).

The limit of quantification of the method is 25 ng L^{-1} and the limit of detection is 5 ng L^{-1} for all four enantiomers of metconazole.

Recovery findings

The method is suitable to determine residues of the enantiomeric forms of metconazole in drinking and surface water. Water samples were fortified with analytes at the limit of quantification of 25 ng L^{-1} and ten times higher (250 ng L^{-1}).

Mean recovery values (mean of five replicates per fortification level and analyte) are between 92% and 109% for all enantiomers of metconazole (see table below), which fulfils the legal requirements.

Table 4.1.2-7: Recoveries of Metconazole from Drinking Water and Surface Water

Analyte	Matrix	Forti-fication level (ng L^{-1})	Number of replicates	Recovery (%)	Mean Recovery (%)	Standard Deviation	% RSD[a]	Overall Recovery (%)
Metconazole (+)trans M555F000-RR (Reg. No. 5836048)	Drinking water (well water)	Primary Quantitation (m/z 320→70)						
		25	5	111, 89, 110, 107, 109	105	9	9	103
		250	5	99, 102, 103, 100, 101	101	2	2	
		Confirmatory Quantitation (m/z 322→70)						
		25	5	110, 107, 110, 109, 111	109	2	2	105
		250	5	99, 101, 102, 105, 101	101	2	2	
	Surface water (pond water)	Primary Quantitation (m/z 320→70)						
		25	5	94, 108, 89, 108, 109	102	10	9	100
		250	5	95, 97, 98, 100, 100	98	2	2	
		Confirmatory Quantitation (m/z 322→70)						
		25	5	105, 109, 104, 107, 114	108	4	3	103
		250	5	96, 97, 101, 98, 93	97	3	3	
Metconazole (+)cis M555F000-RS (Reg. No. 5836046)	Drinking water (well water)	Primary Quantitation (m/z 320→70)						
		25	5	107, 103, 105, 101, 108	105	3	3	103
		250	5	97, 99, 102, 97, 103	100	3	3	
		Confirmatory Quantitation (m/z 322→70)						
		25	5	108, 105, 103, 103, 102	104	2	2	101
		250	5	97, 101, 97, 96, 101	98	2	2	
	Surface water (pond water)	Primary Quantitation (m/z 320→70)						
		25	5	108, 107, 105, 105, 116	108	4	4	102
		250	5	93, 96, 97, 96, 99	96	2	2	
		Confirmatory Quantitation (m/z 322→70)						
		25	5	108, 109, 104, 104, 113	108	4	3	102
		250	5	95, 95, 95, 96, 98	96	1	2	

Analyte	Matrix	Forti-fication level (ng L^{-1})	Number of replicates	Recovery (%)	Mean Recovery (%)	Standard Deviation	% RSD[a]	Overall Recovery (%)
Metconazole (-)cis M555F000-SR (Reg. No. 4677200)	Drinking water (well water)	Primary Quantitation (m/z 320→70)						
		25	5	106, 103, 103, 101, 106	104	2	2	102
		250	5	95, 101, 101, 98, 99	99	2	2	
		Confirmatory Quantitation (m/z 322→70)						
		25	5	108, 105, 100, 98, 102	103	4	4	101
		250	5	94, 101, 98, 97, 102	98	3	3	
	Surface water (pond water)	Primary Quantitation (m/z 320→70)						
		25	5	106, 104, 100, 102, 112	105	5	4	100
		250	5	92, 97, 93, 94, 100	95	3	3	
		Confirmatory Quantitation (m/z 322→70)						
		25	5	101, 100, 100, 101, 109	102	4	4	97
		250	5	91, 91, 89, 95, 93	92	2	3	
Metconazole (-)trans M555F000-SS (Reg. No. 5836047)	Drinking water (well water)	Primary Quantitation (m/z 320→70)						
		25	5	111, 106, 107, 106, 110	108	2	2	104
		250	5	98, 103, 105, 97, 97	100	4	4	
		Confirmatory Quantitation (m/z 322→70)						
		25	5	112, 106, 105, 106, 110	107	3	3	105
		250	5	96, 108, 111, 98, 101	103	6	6	
	Surface water (pond water)	Primary Quantitation (m/z 320→70)						
		25	5	105, 108, 106, 102 ,112	107	4	4	102
		250	5	94, 96, 96, 96, 101	96	3	3	
		Confirmatory Quantitation (m/z 322→70)						
		25	5	110, 98 ,87, 94, 118	101	13	12	98
		250	5	90, 95, 97, 90, 99	94	4	4	

[a] Relative Standard Deviation = (Standard Deviation ÷ Average Recovery) × 100

Linearity
Acceptable linearity is observed for the standard range and the two mass transitions tested for each analyte: The method-detector response is linear over the 5 - 100 ng L^{-1} range ($r = \geq 0.998$) for the analysis of metconazole enantiomers using solvent-based standards (diluted in water).

Specificity
Significant interferences (> 30% of LOQ) were not observed at the retention time and mass transitions of the analytes.
LC-MS/MS is a highly specific self-confirmatory technique. Due to the high selectivity and specificity of LC-MS/MS an additional confirmatory technique is not necessary.

Matrix Effects
Since the method requires no sample extraction or clean-up, the method validation analyses serves to evaluate the effect of matrix load on the analysis. The sample recoveries show that the matrix load in the samples has no influence on analysis; therefore, the validation samples are analyzed using solvent-based calibration standard solutions.

Limit of Quantification
The limit of quantification (LOQ) is defined by the lowest fortification level successfully tested. The validated LOQ for all four enantiomers of metconazole in water is 25 ng L^{-1}.

Limit of Detection
The limit of detection (LOD) for all four enantiomers of metconazole in water is set at 20% of the LOQ, equivalent to 5 ng L^{-1}, corresponding to the lowest calibration level used.

Repeatability
The relative standard deviations (RSD, %) for all fortification levels are below 20%.

Stability of Working Solutions
The stability of each analyte in standard solutions has been determined. In previous studies, metconazole was shown to be stable in stock solutions prepared in methanol for at least 3 months and fortification solutions prepared in methanol for 1 month when held under refrigeration. In this study, metconazole was shown to be stable in calibration solutions prepared in water and refrigerated for at least 1 month (31 days), the longest interval tested. During the course of this study, all solutions are stored in a refrigerator and all solutions are used within the demonstrated time period of stability.

Extract stability
As the methodology is based on direct injection, instead of the extract stability the stability of the analytes in sample matrix under refrigerated conditions was tested to establish final volume stability. The recoveries from stored solutions generated during final volume stability experiments indicate that metconazole is stable in the two water matrices tested for at least 7 days, which is sufficient to support the storage intervals and conditions incurred by the samples in the subject study.

Reproducibility
Reproducibility of the method was successfully tested within an independent laboratory validation study, presented in section M-CA 4.2/10, BASF DocID 2015/7000429).

Conclusion
The method D1501/01 (L0273/01) for analysis of the metconazole (BAS 555 F) enantiomers M555F000-RS (Reg. No. 5836046), M555F000-SS (Reg. No. 5836047), M555F000-RR (Reg. No. 5836048) and M555F000-SR (Reg. No. 4677200) in drinking (well) water and surface (lake) water uses LC-MS/MS for final determination, with a limit of quantification of 25 ng L^{-1}.
It could be demonstrated that the method fulfils the requirements with regard to specificity, repeatability, limit of quantification, and recoveries and is, therefore, applicable to correctly determine residues metconazole enantiomers in drinking and surface water.

Air

Analytical methods for the determination of Metconazole residues in air were reviewed in the context of the inclusion in Annex I under Directive 91/414/EEC. These methods evaluated previously are summarized in Table 4.1.2-8 for the reviewer's convenience.

Table 4.1.2-8: Summary of peer-reviewed analytical methods for determination of Metconazole residues in air

Method No.	Matrix	Method principle	Target analytes	LOQ µg/m^3	Year	DocID	EU reviewed
FAMS 067-01	air	GC-NPD	Metconazole, *cis*- and *trans*-isomer	0.28	1997	1997/7000323	yes

To cover actual guidelines and current state of the art techniques used in analytical laboratories, new analytical methods for the determination of the relevant analytes in air were developed and validated.

Report:	CA 4.1.2/4 Siekmann D., 2014a Validation of an analytical method for the determination of BAS 555 F in air 2014/1045705[*)]
Guidelines:	SANCO/825/00 rev. 8.1 (16 November 2010), SANCO/3029/99 rev. 4 (11 July 2000)
GLP:	yes (certified by Landesamt fuer Umwelt, Messungen und Naturschutz Baden Wuerttemberg, Karlsruhe, Germany)

Principle of the method

The analytical method for the determination of metconazole (BAS 555 F) in air, was validated at Eurofins Agroscience Services EcoChem GmbH, Niefern-Öschelbronn, Germany.

The analyte metconazole is spiked onto the front filter of an adsorbent tube. The tubes are then placed in a climatized chamber without light and air is sucked with a flow rate of 1 L min^{-1} from the climatized chamber which is held at a temperature of 35 ± 2°C and a relative humidity ≥ 80% for 8 hours. After sucking air through the tube, the test item is extracted from the adsorbent material with acetone. The residue is determined by using LC-MS/MS monitoring two mass transitions of metconazole.

Recovery findings

The method is suitable to determine metconazole in air. Samples are fortified with the analyte at the limit of quantification of 0.04 µg m^{-3} and ten times higher (0.4 µg m^{-3}).

Mean recovery values (mean of five replicates per fortification level and analyte) are between 84% and 99% (see table below), which fulfils the legal requirements.

Table 4.1.2-9: Results of method validation of metconazole in air

Analyte	Mass transition	Fortification Level [µg m^{-3}]	Number of replicates	Mean Recovery [%]	RSD [%]	Overall Recovery [%]	RSD [%]
Metconazole Reg.No. 4056343	320 → 125	0.0429	5	84	2	92	9
		0.351	5	99	5		
	320 → 70	0.0429	5	86	2	93	9
		0.351	5	99	6		

RSD Relative standard deviation

Linearity

For analysis of metconazole by HPLC-MS/MS, the detector response for the quantifier mass transition (320→125) is linear (r = ≥0.999) within the range of 0.1 ng mL^{-1} to 5 ng mL^{-1}. The best fit of detector response for the qualifier mass transition (320→70) is second order (r = ≥1.00) within the range from 0.1 ng mL^{-1} to 5 ng mL^{-1}. Second order calibration results in much lower deviations from all nominal concentrations than linear calibration and is therefore more accurate. Standards used for calibration curves are prepared in acetonitrile/water (50/50, v/v).

Specificity

Under the described conditions, the method is specific for the determination of metconazole in air. No significant interferences (> 30% of LOQ) are observed at the retention times and mass transitions of metconazole. Due to the high selectivity and specificity of LC-MS/MS an additional confirmatory technique is not necessary.

Matrix Effects

No matrix effects were identified within the control or blank measurements.

Limit of Quantification

According to SANCO/825/00 rev. 8.1, the limit of quantification should comply with the concentration C (highest tolerable limit of quantification) calculated from the AOEL (Acceptable Operator Exposure Level). The study used an $AOEL_{systemic}$ value of 0.01 mg kg^{-1} body weight day^{-1}. Considering a body weight of 60 kg, a safety factor of 0.1 and an average respiratory volume of 20 m^3 per day, the limit of quantification (C) for the air method should be 3 µg m^{-3}.

The analytical limit of quantification used in this method is 0.0429 µg m^{-3} in air of metconazole, resulting from the lowest concentration level successfully tested within recovery experiments.

Limit of Detection

The limit of detection is estimated at 30% of the LOQ, equivalent to 0.013 µg m^{-3} air of metconazole, corresponding to the lowest calibration level used.

Repeatability

The relative standard deviations (RSD, %) for all fortification levels are below 20%.

Stability of Working Solutions

The stability of metconazole in tubes and extracts is checked over a period of 7 days. Storage of tubes (at room temperature, in a refrigerator or in a freezer) and extracts (in a refrigerator or in a freezer) is possible for up to 7 days without any significant loss. The stability in acetonitrile/water has already been tested for method L0203/01, DocID 2013/1377001.

Reproducibility

Reproducibility of the method was not determined within this validation study.

Conclusion
The analytical method for analysis of metconazole in air uses LC-MS/MS for final determination, with a limit of quantification of 0.0429 µg m^{-3} air.
It could be demonstrated that the method fulfils the requirements with regard to specificity, repeatability, limit of quantification and recoveries and is therefore applicable to correctly determine metconazole in air.

Further analytical methods used in support of environmental fate studies

The following method(s) were used, validated and therefore presented within studies, listed in section CA 7 "Fate and Behaviour in the Environment". To provide a complete overview of all analytical methods, these are additionally described in the following executive summaries.

Report:	CA 4.1.2/5 [see KCA 7.1.4/1 2014/1306965] Sandt H.J. van de, 2014 a Determination of dislodgeable foliar residues of Metconazole (BAS 555 F) and determination of foliar DT50 after application of BAS 555 01 F to wheat, 2013-2014
Guidelines:	EPA 875.2100, EEC 1607/VI/97 rev. 2 10.06.1999, SANCO/3029/99 rev. 4 (11 July 2000)
GLP:	yes (certified by Ministry of Health, Welfare and Sport, The Hague, The Netherlands)

Executive Summary

Principle of the method
The method for the determination of metconazole (BAS 555 F) in dislodgeable foliar residues, resulting from one application of BAS 555 01 F to wheat, was validated at PTRL Europe GmbH, Ulm, Germany. The limit of quantification (LOQ) is defined by the lowest fortification level successfully tested. It is 5 µg L^{-1} for metconazole.
A 20 mL dislodging solution (0.01% Aerosol OT-B in water) is diluted with a mixture of methanol/water (1/1, v/v) prior to LC-MS/MS analysis, monitoring two mass transitions of metconazole (m/z 320→70 for quantification and m/z 320→125 for confirmation).

Recovery findings
The method proved to be suitable to determine metconazole in dislodgeable foliar residues, resulting from one application of BAS 555 01 F to wheat. Fresh dislodging solutions (lab fortification) and field quality control samples (field fortification) were fortified with the analyte at the limit of quantification of 5 µg L-^{1}, 50 µg L^{-1} (10 x LOQ) and 5000 µg L^{-1} (1000 x LOQ). None of the analyzed control specimens had any residues exceeding the LOQ of 5 µg L^{-1}. Mean recovery values (mean of three/five replicates per fortification level and analyte) were between 70% and 110% (Table 7.1.4-11), which fulfils the legal requirements.

Table 4.1.2-10: Summary of the residue data in dislodgeable solutions of field and lab fortification samples

Matrix	Analyte	m/z	Forti-fication level [µg L^{-1}]	Number of replicates	Mean recovery [%]	RSD [%]	Overall recovery [%]	RSD [%]
DFR solution – field fortification	Metconazole	320 → 70	50	3	106	2	105	3
			5000	3	104	3		
		320 → 125	50	3	104	1	104	3
			5000	3	105	4		
DFR solution – lab fortification		320 → 70	5	5	97	2	102	6
			5000	5	108	1		
		320 → 125	5	5	94	5	102	9
			5000	5	109	1		

DFR = Dislodgeable foliar residues
RSD = Relative standard deviations

Linearity
Good linearity ($r \geq 0.999$) was observed in the range of 0.1 ng mL^{-1} to 10 ng mL^{-1} for both mass transitions of metconazole. At least five standards, prepared in methanol/water (1/1, v/v), were used for calibration.

Specificity
No significant interferences (> 20% of LOQ) were observed at the retention times and mass transitions of metconazole.
Due to the high selectivity and specificity of LC-MS/MS, monitoring two mass transitions, an additional confirmatory technique was not necessary.

Matrix Effects
The influence of the matrix load on the analysis of metconazole was tested by preparation of matrix-matched standards. Final extracts of control samples were fortified with metconazole. No significant effects of matrix (enhancement or suppression) on the LC-MS/MS responses were observed.

Limit of Quantification
The limit of quantification (LOQ) is defined by the lowest fortification level successfully tested. It is 5 µg L^{-1} for metconazole.

Limit of Detection
The limit of detection (LOD) was set to 20% of LOQ or 1.0 µg L^{-1}.

Repeatability
The relative standard deviations (RSD, %) for all fortification levels were below 20%.

Stability of Working Solutions

The recoveries in the fortified samples (matrix-matched standards) prepared in methanol/water (1/1, v/v) were within an acceptable range of 70% and 110%, thus stability was sufficiently proven.

Reproducibility

The method was validated concurrently by processing laboratory fortifications and field fortifications, thus demonstrating transport and storage stability.

Conclusion

The method for analysis of metconazole in dislodgeable foliar residues, resulting from one application of BAS 555 01 F to wheat, uses LC-MS/MS for final determination, with a limit of quantification of 5 µg L^{-1}.

It could be demonstrated that the method fulfils the requirements with regard to specificity, repeatability, limit of quantification and recoveries and is therefore applicable to correctly determine metconazole in dislodgeable foliar residues, resulting from one application of BAS 555 01 F to wheat.

Report:	CA 4.1.2/6 [see KCA 7.1.4/2 2013/1386123] Roussel C.H., 2015 a Determination of dislodgeable foliar residues of Metconazole (BAS 555 F) and determination of foliar DT50 after application of BAS 555 01 F to oil seed rape, 2013
Guidelines:	EC 1107/2009 of the European Parliament and of the Council of 21 Oct 2009, European Commission Regulation No 283/2013, European Commission Regulation No 284/2013, SANCO/3029/99 rev. 4 (11 July 2000), OECD (2011) Guidance Document on Crop Field Trials (Series on Testing and Assessment No. 164 and Series On Pesticides No. 66)
GLP:	yes (certified by Groupe Interministeriel des Produits Chimiques, France)

Executive Summary

Principle of the method

The method for the determination of metconazole (BAS 555 F) in dislodgeable foliar residues, resulting from one application of BAS 555 01 F to oilseed rape, was validated at PTRL Europe GmbH, Ulm, Germany. The limit of quantification (LOQ) is defined by the lowest fortification level successfully tested. It is 5 µg L^{-1} for metconazole.

A 20 mL dislodging solution (0.01% Aerosol OT in water) is diluted with a mixture of methanol/water (1/1, v/v) prior to LC-MS/MS analysis, monitoring two mass transitions of metconazole (m/z 320→70 for quantification and m/z 320→125 for confirmation).

Recovery findings

The method proved to be suitable to determine metconazole in dislodgeable foliar residues, resulting from one application of BAS 555 01 F to oilseed rape. Fresh dislodging solutions (lab fortification) and field quality control samples (field fortification) were fortified with the analyte at the LOQ of 5 µg L^{-1}, 50 µg L^{-1} (10 x LOQ) and 5000 µg L^{-1} (1000 x LOQ). None of the analyzed control specimens had any residues exceeding the LOQ of 5 µg L^{-1}. Mean recovery values (mean of three/five replicates per fortification level and analyte) were between 70% and 110% (Table 7.1.4-12), which fulfils the legal requirements.

Table 4.1.2-11: Summary of the residue data in dislodgeable solutions of field and lab fortification samples

Matrix	Analyte	m/z	Forti-fication level [µg L^{-1}]	Number of replicates	Mean recovery [%]	RSD [%]	Overall recovery [%]	RSD [%]
DFR solution – field fortification	Metconazole	320 → 70	50	3	100	0.3	96	5
			5000	3	92	3		
		320 → 125	50	3	99	0.6	95	5
			5000	3	91	3		
DFR Solution – lab fortification		320 → 70	5	5	96	1	93	4
			5000	5	91	5		
		320 → 125	5	5	95	3	92	5
			5000	5	90	6		

DFR = dislodgeable foliar residues
RSD = relative standard deviations

Linearity
Good linearity ($r \geq 0.99$) was observed in the range of 0.1 ng mL^{-1} to 10 ng mL^{-1} for both mass transitions of metconazole. At least five standards, prepared in methanol/water (1/1, v/v), were used for calibration.

Specificity
No significant interferences (> 20% of LOQ) were observed at the retention times and mass transitions of metconazole.
Due to the high selectivity and specificity of LC-MS/MS, monitoring two mass transitions, an additional confirmatory technique was not necessary.

Matrix Effects
The influence of the matrix load on the analysis of metconazole was tested by preparation of matrix-matched standards. Final extracts of control samples (diluted with methanol/water, 1/1, v/v) were fortified with metconazole. No significant effects of matrix (enhancement or suppression) on the LC-MS/MS responses were observed at the tested concentration levels 1.0 and 10 ng mL^{-1}.

Limit of Quantification
The limit of quantification (LOQ) is defined by the lowest fortification level successfully tested. It is 5 µg L^{-1} for metconazole.

Limit of Detection
The limit of detection (LOD) of the method was set to 20% of LOQ or 1.0 µg L^{-1}.

Repeatability
The relative standard deviations (RSD, %) for all fortification levels were below 20%.

Stability of Working Solutions
The recoveries in the fortified samples (matrix-matched standards) prepared in methanol/water (1/1, v/v) were within an acceptable range of 70% and 110%, thus stability was sufficiently proven.

Reproducibility
The method was validated concurrently by processing laboratory fortifications and field fortifications, thus demonstrating transport and storage stability.

Conclusion
The method for analysis of metconazole in dislodgeable foliar residues, resulting from one application of BAS 555 01 F to oilseed rape, uses LC-MS/MS for final determination, with a limit of quantification of 5 µg L^{-1}.
It could be demonstrated that the method fulfils the requirements with regard to specificity, repeatability, limit of quantification and recoveries and is therefore applicable to correctly determine metconazole in dislodgeable foliar residues, resulting from one application BAS 555 01 F to oilseed rape.

(b) Methods in soil, water and any additional matrices used in support of efficacy studies

No stand-alone validation of analytical methods were required in support of efficacy studies.

(c) Methods in feed, body fluids and tissues, air and any additional matrices used in support of toxicological studies

No stand-alone validated methods for toxicological studies were required; hence, there are no separate methods to be listed in chapter CA 4.1.2. The analytical methodology, used in toxicological studies, is in general described and validated with the corresponding toxicological study, and therefore presented in the corresponding dossier ~~chapter~~ section CA 5 “Toxicological and Metabolism Studies in the Active Substance”. To provide a complete overview of all analytical methods, these are additionally described in the following executive summaries.

Report: CA 4.1.2/7
[see KCA 5.5 MK-427-003] (previously peer-reviewed)

Metconazole cis/trans / WL148271: A two year chronic toxicity feeding study in rats ([redacted], 1992a)

Guidelines: Protocol in compliance with test methods B.32 of directive 92/69/EEC
GLP: Yes
Acceptance: The study was accepted.

Executive summary of the analytical method used within the analytical phase (MK-427-003)

Principle of the method
The analytical method used in study MK-427-003 was validated for the determination of *cis*-metconazole (BAS 555 F) in animal test diet. The study was performed by [redacted]
Residues were extracted from test diet samples by Soxhlet extraction with 30% acetone in hexane. The extracts were diluted to a known volume with hexane. For control samples as well as samples fortified at a low concentration, an acetonitrile/hexane partition step was performed prior to analysis. Measurements are based on the peak due to the *cis*-isomer alone, and results are expressed in terms of the test item as received, no purity correction being applied. Residues were analyzed by gas-liquid chromatography (GLC) coupled to a nitrogen thermionic detector (TID). Analysis was performed on an Ultrabond II column.

Recovery findings
The mean recovery values were between 70% and 110% for metconazole. The detailed results are given in the table below.

Table 4.1.2-12: Validation results of method applied in study MK-427-003 using GLC-TID: *cis*-metconazole (BAS 555 F) in animal test diet

Matrix	Analyte	Detector settings	Fortification level (mg/kg)	No of replicates	Mean recovery (%)	RSD (%)
Animal test diet	*Cis*-metconazole	Nitrogen thermionic detector, 300°C	10	60	102	6.1
			100	10	104	5.8
			300	6	101	4.9
			1000	12	107	16.6
			1000 [a]	11 [a]	103 [a]	7.0 [a]
			Overall [a]	87 [a]	102 [a]	6.0 [a]

[a] Excluding one recovery value of 155%

Linearity
The linearity was tested using standards at concentrations between of 1.0 to 4.0 µg/mL. Calibration solutions were prepared in hexane and contained about 2 to 4% by volume of the extract from control diet to improve the performance of the method.

Specificity
Final detection has been applied by nitrogen thermionic detection (TID). As only known analytes of known concentration were analyzed for dose verification purposes, no additional confirmatory technique is necessary.

Interference
No relevant interferences from the reagent blank at the relevant retention times have been reported.

Limit of Quantitation
The limit of quantitation (LOQ) is defined as the lowest fortification level successfully tested. During this study, a limit of quantitation of 10 mg/kg for *cis*-BAS 555 F was obtained in animal test diet.

Repeatability
The relative standard deviations (RSD, %) for all fortification levels were below 20%.

Stability of solutions
The stability of the analytes in working solutions was tested within this study. Test substance solutions were stable for up to one month when stored in the dark at ambient temperature.

Conclusion
The analytical method used in study MK-427-003 for the analysis of *cis*-metconazole in animal test diet uses GLC-TID for final determination, with an LOQ of 10 mg/kg.
It could be demonstrated that the method generally fulfils the requirements with regard to repeatability, specificity, LOQ and recoveries and is therefore considered suitable for the analysis of *cis*-metconazole in animal test diet.

Report: CA 4.1.2/8
[see KCA 5.5 MK-428-001] (previously peer-reviewed)

Metconazole cis/trans / WL148271: A two year oncogenicity feeding study in rats [redacted], 1992b)

Guidelines: Protocol in compliance with test methods B.32 of directive 92/69/EEC
GLP: Yes
Acceptance: The study was accepted.

Executive summary of the analytical method used within the analytical phase (MK-428-001)

Principle of the method
The analytical method used in study MK-428-001 was validated for the determination of *cis*-metconazole (BAS 555 F) in animal test diet. The study was performed by [redacted]
Residues were extracted from test diet samples by Soxhlet extraction with 30% acetone in hexane. The extracts were diluted to a known volume with hexane. For control samples as well as samples fortified at a low concentration, an acetonitrile/hexane partition step was performed prior to analysis. Measurements are based on the peak due to the *cis*-isomer alone, and results are expressed in terms of the test item as received, no purity correction being applied. Residues were analyzed by gas-liquid chromatography (GLC) coupled to a nitrogen thermionic detector (TID). Analysis was performed on an Ultrabond II column.

Recovery findings
The mean recovery values were between 70% and 110% for *cis*-metconazole. The detailed results are given in the table below.

Table 4.1.2-13: Validation results of method applied in study MK-428-001 using GLC-TID: *cis*-metconazole (BAS 555 F) in animal test diet

Matrix	Analyte	Detector settings	Fortification level (mg/kg)	No of replicates	Mean recovery (%)	RSD (%)
Animal test diet	*Cis*-metconazole	Nitrogen thermionic detector, 300°C	100	10	104	5.8
			300	6	101	4.9
			1000	12	107	16.6
			1000 [a]	11 [a]	103 [a]	7.0 [a]
			Overall [a]	87 [a]	103 [a]	5.9 [a]

[a] Excluding one recovery value of 155%

Linearity
The linearity was tested using standard solutions at concentrations of 1.0 to 5.0 µg/mL. For *cis*-BAS 555 F, no information regarding linearity of calibration standard measurements was stated. Calibration solutions were prepared in hexane and contained about 2 to 4% by volume of the extract from control diet to improve the performance of the method.

Specificity
Final detection has been applied by nitrogen thermionic detection (TID). As only known analytes of known concentration were analyzed for dose verification purposes, no additional confirmatory technique is necessary.

Interference
No relevant interferences from the reagent blank at the relevant retention times have been reported.

Limit of Quantitation
The limit of quantitation (LOQ) is defined as the lowest fortification level successfully tested. During this study, a limit of quantitation of 100 mg/kg for *cis*-BAS 555 F was obtained in animal test diet.

Repeatability
The relative standard deviations (RSD, %) for all fortification levels were below 20%.

Stability of solutions
The stability of the analytes in working solutions was tested within this study. Test substance solutions were stable for up to one month when stored in the dark at ambient temperature.

Conclusion
The analytical method used in study MK-428-001 for the analysis of *cis*-metconazole in animal test diet uses GLC-TID for final determination, with an LOQ of 100 mg/kg.
It could be demonstrated that the method generally fulfils the requirements with regard to repeatability, specificity, LOQ and recoveries and is therefore considered suitable for the analysis of *cis*-metconazole in animal test diet.

Report: CA 4.1.2/9
[see KCA 5.5 MK-428-002] (previously peer-reviewed)

WL 148271: 91 Week oral (dietary administration) carcinogenicity study in the mouse ([redacted], 1992b)

Guidelines: Protocol in compliance with test methods B.32 of directive 92/69/EEC
GLP: Yes
Acceptance: The study was accepted.

Executive summary of the analytical method used within the analytical phase (MK-428-002)

Principle of the method
The analytical method HUK 579/25-02F was validated for the determination of metconazole (BAS 555 F) *cis*- and *trans*-isomers in animal test diet. The study was performed by [redacted]
Residues were extracted from test diet samples by Soxhlet extraction with acetone. Afterwards, the sample was made to a final volume of 100 mL. Control samples as well as samples fortified at a low concentration, concentrated and cleaned-up. Therefore, samples were evaporated to dryness under nitrogen. Acetonitrile was added and the sample mixed to ensure dissolution of the test item. Hexane added, the sample is shaken, and the hexane layer is transferred to another test tube and the process was repeated a second time. Afterwards, the hexane layers were combined. Acetonitrile is then added to the tube containing the combined hexane washes. The acetonitrile combined extract was evaporated to dryness under nitrogen. Acetone is then added and the tube was mixed to ensure dissolution of the test item. Residues were analyzed by gas liquid chromatography (GLC) coupled to a nitrogen-phosphorus detector (NPD). Analysis was performed on a CP Sil 8 CB column.

Recovery findings
The mean recovery values were between 70% and 110% for *cis*- and *trans*-metconazole. The detailed results are given in the table below.

Table 4.1.2-14: Validation results of method HUK 579/25-02F using GLC-NPD: metconazole (BAS 555 F) *cis*- and *trans*-isomers in animal test diet

Matrix	Analyte	Detector settings	Fortification level (mg/kg)	No of replicates	Mean recovery (%)	RSD (%)
Animal test diet	*Cis*-metconazole	Nitrogen-phosphorus detector 300°C	30	3	93	0.6
			1000	3	97	1.7
			Overall	6	95	2.6
	Trans-metconazole		30	4	86	2.1
			1000	4	97	1.2
			Overall	6	92	6.2

Linearity
The linearity was tested using three matrix standards at concentrations between of 30 to 3000 µg/g. Matrix calibration standards were prepared by fortifying control test diet with a respective volume of fortification solutions. Fortified control test diet was extracted by Soxhlet extraction as described above. For BAS 555 F (*cis* and *trans*), no information regarding linearity of calibration standard measurements was stated. Matrix calibration solutions were prepared in acetone.

Specificity
Final detection has been applied by nitrogen-phosphorus detection (NPD). As only known analytes of known concentration were analyzed for dose verification purposes, no additional confirmatory technique is necessary.

Interference
No interferences from the reagent blank at the relevant retention times have been reported.

Limit of Quantitation
The limit of quantitation (LOQ) is defined as the lowest fortification level successfully tested. During this study, a limit of quantitation of 30 mg/kg for BAS 555 F (each isomer, *cis* and *trans*) was obtained in animal test diet.

Limit of Detection
For BAS 555 F (*cis* and *trans*), the limit of detection (LOD) was 0.2 mg/kg in animal test diet.

Repeatability
The relative standard deviations (RSD, %) for all fortification levels were below 20%.

Conclusion
The analytical method HUK 579/25-02F for the analysis of metconazole *cis*- and *trans*-isomers in animal test diet uses GLC-NPD for final determination, with an LOQ of 30 mg/kg (per isomers).
It could be demonstrated that the method generally fulfils the requirements with regard to repeatability, specificity, LOQ and recoveries and is therefore considered suitable for the analysis of metconazole (*cis*- and *trans*-isomer) in animal test diet.

Report: CA 4.1.2/10
[see KCA 5.6.1 MK-430-003] (previously peer-reviewed)

Metconazole WL136184 cis, 2-generation main study, 0, 2, 8, 32 and 48 mg/kg bw/d in diet ([redacted], 1992a)

Guidelines: Protocol in compliance with test methods B.35 of directive 88/302/EEC
GLP: Yes
Acceptance: The study was accepted.

Executive summary of the analytical method used within the analytical phase (MK-430-003)

Principle of the method
The analytical method used in study MK-430-003 was validated for the determination of *cis*-metconazole (BAS 555 F) in animal test diet mixes. The study was performed by [redacted]
Residues were extracted from diet mixes by shaking a representative sub-sample with acetone. The extract was filtered and the filtrate was concentrated or diluted with hexane. Residues were analyzed by gas chromatography (GC) with a nitrogen thermionic detector (TID). Analysis was performed on an Ultrabond 20M column.

Recovery findings
The mean recovery values were between 70% and 110% for *cis*-metconazole. The detailed results are given in the table below.

Table 4.1.2-15: Validation results of the analytical method applied in study MK-430-003 using GC-TID: *cis*-metconazole (BAS 555 F) in animal test diet mixes

Matrix	Analyte	Detector settings	Fortification level (µg/kg)	No of replicates	Mean recovery (%)	RSD (%)
LAD II diet	*Cis*-metconazole	Nitrogen thermionic detector, 300°C	13.3	6	103	6
			341	6	101	10
			Overall	12	102.1	7.9

Linearity
The linearity was tested using five standards at concentrations up to 30 µg/mL. For *cis*-BAS 555 F, linear correlations were obtained. Calibration solutions were prepared in hexane.

Specificity
Final detection has been applied by nitrogen thermionic detection (TID). As only known analytes of known concentration were analyzed for dose verification purposes, no additional confirmatory technique is necessary.

Interference
No relevant interferences from the reagent blank at the relevant retention times have been reported.

Limit of Quantitation
The limit of quantitation (LOQ) is defined as the lowest fortification level successfully tested. During this study, a limit of quantitation of 0.01 mg/kg for *cis*-BAS 555 F was obtained.

Repeatability
The relative standard deviations (RSD, %) for all fortification levels were below 20%.

Conclusion
The analytical method used in study MK-430-003 for the analysis of *cis*-metconazole in animal test diet mixes uses GLC-TID for final determination, with an LOQ of 0.01 mg/kg. It could be demonstrated that the method generally fulfils the requirements with regard to repeatability, specificity, LOQ and recoveries and is therefore considered suitable for the analysis of *cis*-metconazole in animal test diet mixes.

Report: CA 4.1.2/11
[see KCA 5.6.2 MK-432-005] (previously peer-reviewed)

Study on metconazole cis/trans (WL148271, 85:15 cis/trans)
Teratology study in the Rat, oral (gavage) following exposure to metconazole WL148271 (cis/trans) from gestations days 6-15 ([redacted], 1991b)

Guidelines: Protocol in compliance with test method B.31 of directive 87/302/EEC
GLP: Yes
Acceptance: The study was accepted.

Executive summary of the analytical method used within the analytical phase (MK-432-003 and MK-432-005)

Principle of the method
The analytical method used in studies MK-432-003 and MK-432-005 was validated for the determination of *cis*-metconazole (BAS 555 F) in 1% methylcellulose. The study was performed by [redacted]
Residues were extracted from 1% aqueous methylcellulose by dissolving a representative sub-sample in acetonitrile. The extract was filtered and further diluted, as necessary, using acetonitrile. Residues were analyzed by high-performance liquid chromatography with ultra-violet (UV) detection. Analysis was performed on a BDH Lichrosorb Diol column equipped with guard column (BDH Lichrospher 100 RP-18e), using an acetonitrile/water (95/5, v/v) as mobile phase.

Recovery findings
The mean recovery values were between 70% and 110% for *cis*-metconazole. The detailed results are given in the table below.

Table 4.1.2-16: Validation results of the analytical method applied in studies MK-432-003 and MK-432-005 using HPLC-UV: *cis*-metconazole (BAS 555 F) in 1% aqueous methylcellulose

Matrix	Analyte	Detector wavelength (nm)	Fortification level (mg/mL)	No of replicates	Mean recovery (%)	RSD (%)
Aqueous methylcellulose	*Cis*-metconazole	221	1	9	100	1.0
			60	10	99	1.2
			Overall	19	99	1.3

Linearity
The linearity was tested using standards at concentrations between of 1 to 5 µg/mL. Calibration solutions were prepared in acetonitrile.

Specificity
Final detection has been applied by UV detection. As only known analytes of known concentration were analyzed for dose verification purposes, no additional confirmatory technique is necessary.

Interference
No interferences from the reagent blank at the relevant retention times have been reported.

Limit of Quantitation
The limit of quantitation (LOQ) is defined as the lowest fortification level successfully tested. During this study, a limit of quantitation of 1 mg/mL for *cis*-BAS 555 F was obtained.

Limit of Detection
For BAS 555 F, the limit of detection (LOD) was 0.08 mg/mL.

Repeatability
The relative standard deviations (RSD, %) for all fortification levels were below 20%.

Stability of solutions
The chemical and physical stability of the analyte in freshly prepared specimen solutions was tested within this study. Results indicated that the analyte was chemically stable in solution for at least 24 hours when stored at ambient temperature during the day and + 4°C overnight. Furthermore, results revealed that metconazole produces a homogeneous suspension in 1% aqueous methylcellulose, which can be maintained for up to 1 hour while magnetically stirred and successfully resuspended following storage in the dark (ambient temperature during the day, + 4°C overnight) for 4 and 24 hours.

Conclusion
The analytical method used in studies MK-432-003 and MK-432-005 for the analysis of *cis*-metconazole in 1% aqueous methylcellulose uses HPLC-UV for final determination, with an LOQ of 1 mg/mL.
It could be demonstrated that the method generally fulfils the requirements with regard to repeatability, specificity, LOQ and recoveries and is therefore considered suitable for the analysis of *cis*-metconazole in 1% aqueous methylcellulose.

Report: CA 4.1.2/12
[see KCA 5.6.2 MK-432-009] (previously peer-reviewed)

Teratology study in the Rat, oral (gavage) after exposure to metconazole WL136184 (95% cis) from gestations days 6-15 ([redacted], 1992b)

Guidelines: Protocol in compliance with test method B.31 of directive 87/302/EEC
GLP: Yes
Acceptance: The study was accepted.

Executive summary of the analytical method used within the analytical phase (MK-432-009)

Principle of the method
The analytical method used in study MK-432-009 was validated for the determination of metconazole (BAS 555 F) in 1% methylcellulose. The study was performed by [redacted].
Residues were extracted from 1% aqueous methylcellulose by dissolving the total sample in acetone. After further dilution with acetone, residues were analyzed by gas chromatography (GC) with nitrogen thermionic detection. Analysis was performed on an Ultrabond 20 M column.

Recovery findings
The mean recovery values were between 70% and 110% for metconazole. The detailed results are given in the table below.

Table 4.1.2-17: Validation results of the analytical method applied in study MK-432-003 using GC-TID: metconazole (BAS 555 F) isomers in 1% aqueous methylcellulose

Matrix	Analyte	Detector settings	Fortification level (mg/mL)	No of replicates	Mean recovery (%)	RSD (%)
Aqueous methylcellulose	Metconazole	Nitrogen thermionic detector, 300°C	0.6	6	94	2.8
			8.0	6	95	2.7
			Overall	12	94	2.5

Linearity
The linearity was tested using five standards at concentrations between of 6 to 30 µg/mL. For BAS 555 F, linear correlations were obtained. No coefficients of determination (R^2) were stated. Calibration solutions were prepared in acetone.

Specificity
Final detection has been applied by nitrogen thermionic detection (TID). As only known analytes of known concentration were analyzed for dose verification purposes, no additional confirmatory technique is necessary.

Interference
No interferences from the reagent blank at the relevant retention times have been reported.

Limit of Quantitation
The limit of quantitation (LOQ) is defined as the lowest fortification level successfully tested. During this study, a limit of quantitation of 0.6 mg/mL for BAS 555 F was obtained.

Repeatability
The relative standard deviations (RSD, %) for all fortification levels were below 20%.

Stability of solutions
The chemical stability of the analyte in 1% aqueous methylcellulose was tested within this study. Results indicated that the analyte was chemically stable in 1% aqueous methylcellulose for at least 6 hours when stored at 21°C.

Conclusion
The analytical method used in study MK-432-009 for the analysis of metconazole in 1% aqueous methylcellulose uses HPLC-TID for final determination, with an LOQ of 0.6 mg/mL.
It could be demonstrated that the method generally fulfils the requirements with regard to repeatability, specificity, LOQ and recoveries and is therefore considered suitable for the analysis of metconazole in 1% aqueous methylcellulose.

Report: CA 4.1.2/13
[see KCA 5.6.2 MK-432-015] (previously peer-reviewed)

Teratology study in the Rabbit, oral (gavage) following exposure to metconazole WL148271 (cis/trans) from gestations days 6-28 ([redacted], 1997a)

Guidelines: Protocol in compliance with TG 414 from OECD (2001)
GLP: Yes
Acceptance: The study was accepted.

Executive summary of the analytical method used within the analytical phase (MK-432-015)

Principle of the method
The analytical method used in study MK-432-015 was validated for the determination of metconazole (BAS 555 F) in 0.5% aqueous carboxymethylcellulose. The study was performed by [redacted].
Residues were extracted from 0.5% aqueous carboxymethylcellulose by dissolving the suspension in acetonitrile, diluting aliquots to the appropriate concentrations in the solutions, and analyzing by high-performance liquid chromatography (HPLC) using ultra-violet (UV) detection. Analysis was performed on an INERTSIL 5U ODS column using acetonitrile/water (50/50, v/v) with 0.1% phosphoric acid as mobile phase.

Recovery findings
The mean recovery values were between 70% and 110% for metconazole. The detailed results are given in the table below.

Table 4.1.2-18: Validation results of the analytical method used in study MK-432-015 using HPLC-UV: metconazole (BAS 555 F) in 0.5% aqueous carboxymethylcellulose

Matrix	Analyte	Detector wavelength (nm)	Fortification level (mg/mL)	No of replicates	Mean recovery (%)	RSD (%)
Aqueous carboxymethyl-cellulose	Metconazole	220	0.1	5	104	0.4
			4.0	5	104	1.5
			Overall	10	104	1.1

Linearity
The linearity was tested using six standards at concentrations between of 0.0017 to 0.04 µg/mL. For BAS 555 F, linear correlations with coefficients of determination (R^2) greater than 0.99 were obtained. Calibration solutions were prepared in acetonitrile.

Specificity
Final detection has been applied by ultra-violet (UV) detection. As only known analytes of known concentration were analyzed for dose verification purposes, no additional confirmatory technique is necessary.

Interference
No interferences from the reagent blank at the relevant retention times have been reported.

Limit of Quantitation
The limit of quantitation (LOQ) is defined as the lowest fortification level successfully tested. During this study, a limit of quantitation of 0.1 mg/mL for BAS 555 F was obtained.

Limit of Detection
For BAS 555 F, the limit of detection (LOD) was 0.01 mg/mL.

Repeatability
The relative standard deviations (RSD, %) for all fortification levels were below 20%.

Stability of solutions
The stability of the analytes in standard solutions was investigated within this study. Test item solutions were stored at ambient temperatures for 12 days and 11 hours and analyzed against freshly prepared standard solutions, revealing stability of the test item at least within this time period.

Conclusion
The analytical method used in study MK-432-015 for the analysis of metconazole in 0.5% aqueous carboxymethylcellulose uses HPLC-UV for final determination, with an LOQ of 0.1 mg/mL.
It could be demonstrated that the method generally fulfils the requirements with regard to repeatability, specificity, LOQ and recoveries and is therefore considered suitable for the analysis of metconazole in 0.5% aqueous carboxymethylcellulose.

Report: CA 4.1.2/14
[see KCA 5.6.2 MK-432-007] (previously peer-reviewed)

Teratology study in the Rabbit, oral (gavage) following exposure to metconazole cis (WL136148) from gestations days 7-19 ([redacted], 1992a)

Guidelines: --
GLP: Yes
Acceptance: The study was accepted.

Executive summary of the analytical method used within the analytical phase (MK-432-007)

Principle of the method
The analytical method used in study MK-432-007 was validated for the determination of metconazole (BAS 555 F) in 1% methylcellulose. The study was performed by [redacted]
Residues were extracted from 1% aqueous methylcellulose by dissolving a representative sub-sample in acetone. The extract was further diluted using acetone and a suitable volume was evaporated (T = 40°C) to dryness. The residue was redissolved in toluene. Residues were analyzed by gas-liquid chromatography (GLC) with alkali-flame ionization (FID). Analysis was performed on a DB-1701 GC column.

Recovery findings
The mean recovery values were between 70% and 110% for metconazole. The detailed results are given in the table below.

Table 4.1.2-19: Validation results of the analytical method applied in study MK-432-007 using GLC-FID: metconazole (BAS 555 F) in 1% aqueous methylcellulose

Matrix	Analyte	Detector settings	Fortification level (mg/mL)	No of replicates	Mean recovery (%)	RSD (%)
Aqueous methylcellulose	Metconazole	Alkali-flame ionization, 300°C	1.0	10	101	1.6
			20.0	10	100	3.5
			Overall	20	101	2.7

Linearity
The linearity was tested using four standards at concentrations between of 4 to 20 µg/mL. For BAS 555 F, linear correlations were obtained. Calibration solutions were prepared in toluene.

Specificity
Final detection has been applied by alkali-flame ionization detection (FID). As only known analytes of known concentration were analyzed for dose verification purposes, no additional confirmatory technique is necessary.

Interference
No relevant interferences from the reagent blank at the relevant retention times have been reported.

Limit of Quantitation
The limit of quantitation (LOQ) is defined as the lowest fortification level successfully tested. During this study, a limit of quantitation of 1.0 mg/mL for BAS 555 F was obtained.

Limit of Detection
For BAS 555 F, the limit of detection (LOD) was 0.04 mg/mL.

Repeatability
The relative standard deviations (RSD, %) for all fortification levels were below 20%.

Stability of solutions
The chemical and physical stability of the analyte in freshly prepared specimen solutions was tested within this study. Results indicated that the analyte was chemically stable in solution for at least 24 hours when stored at ambient temperature during the day and + 4°C overnight. Furthermore, results revealed that metconazole produces a homogeneous suspension in 1% aqueous methylcellulose, which can be maintained for up to 1 hour while magnetically stirred and successfully resuspended following storage in the dark at ambient temperature for 4 hours.

Conclusion:
The analytical method used in study MK-432-007 for the analysis of metconazole in 1% aqueous methylcellulose uses GLC-FID for final determination, with an LOQ of 1.0 mg/mL.
It could be demonstrated that the method generally fulfils the requirements with regard to repeatability, specificity, LOQ and recoveries and is therefore considered suitable for the analysis of metconazole in 1% aqueous methylcellulose.

Report: CA 4.1.2/15
[see KCA 5.6.2 MK-432-010] (previously peer-reviewed)

Second Teratology study in the Rabbit, oral (gavage) following exposure to metconazole cis (WL136148) from gestations days 7-19 (1992b)

Guidelines: --
GLP: Yes
Acceptance: The study was accepted.

Executive summary of the analytical method used within the analytical phase (MK-432-010)

Principle of the method
The analytical method used in study MK-432-010 was validated for the determination of metconazole (BAS 555 F) in 1% methylcellulose. The study was performed by
Residues were extracted from 1% aqueous methylcellulose by dissolving a representative sub-sample in acetone. The extract was further diluted using acetone and a suitable volume was evaporated (T = 40°C) to dryness. The residue was redissolved in toluene. Residues were analyzed by gas-liquid chromatography (GLC) with alkali-flame ionization (FID). Analysis was performed on a DB-1 GC column.

Recovery findings
The mean recovery values were between 70% and 110% for metconazole. The detailed results are given in the table below.

Table 4.1.2-20: Validation results of the analytical method applied in study MK-432-010 using GLC-FID: metconazole (BAS 555 F) isomers in 1% aqueous methylcellulose

Matrix	Analyte	Detector settings	Fortification level (mg/mL)	No of replicates	Mean recovery (%)	RSD (%)
Aqueous methylcellulose	Metconazole	Alkali-flame ionization detector, 300°C	0.125	10	98	4.2
			10	6	99	4.7
			Overall	16	98	4.3

Linearity
The linearity was tested using four standards at concentrations between of 4 to 20 µg/mL. For BAS 555 F, linear correlations were obtained. Calibration solutions were prepared in toluene.

Specificity
Final detection has been applied by alkali-flame ionization detection (FID). As only known analytes of known concentration were analyzed for dose verification purposes, no additional confirmatory technique is necessary.

Interference
No interferences from the reagent blank at the relevant retention times have been reported.

Limit of Quantitation
The limit of quantitation (LOQ) is defined as the lowest fortification level successfully tested. During this study, a limit of quantitation of 0.125 mg/mL for BAS 555 F was obtained.

Limit of Detection
For BAS 555 F, the limit of detection (LOD) was 0.005 mg/mL.

Repeatability
The relative standard deviations (RSD, %) for all fortification levels were below 20%.

Stability of solutions
The chemical and physical stability of the analyte in freshly prepared specimen solutions was tested within this study. Results indicated that the analyte was chemically stable in solution for at least 24 hours when stored at ambient temperature during the day and + 4°C overnight. Furthermore, results revealed that metconazole produces a homogeneous suspension in 1% aqueous methylcellulose, which can be maintained for up to 1 hour while magnetically stirred and successfully resuspended following storage in the dark (ambient temperature during the day, + 4°C overnight) for 6 and 24 hours.

Conclusion
The analytical method used in study MK-432-010 for the analysis of metconazole in 1% aqueous methylcellulose uses GLC-FID for final determination, with an LOQ of 0.125 mg/mL.
It could be demonstrated that the method generally fulfils the requirements with regard to repeatability, specificity, LOQ and recoveries and is therefore considered suitable for the analysis of metconazole in 1% aqueous methylcellulose.

(d) Methods in body fluids, air and any additional matrices used in support of operator, worker, resident and bystander exposure studies

No stand-alone validated data generation methods for the determination Metconazole were required for exposure studies. If needed, the analytical methodology is addressed within the respective study, and therefore presented in the corresponding dossier chapter.

(e) Methods in or on plants, plant products, processed food commodities, food of plant and animal origin, feed and any additional matrices used in support of residues studies

The following methods cover the compounds necessary for the discussion on the residue definition for risk assessment for products of plant and animal origin as summarized in Document N and discussed in CA 6.7: Metconazole (sum of *cis*- and *trans*-isomers). These methods were validated within stand-alone validation studies.
If further methods have been used and validated within residue studies, these are addressed within the respective study reports, and therefore presented in the corresponding dossier chapters, but for completion also listed at the end of this chapter.

The following recovery and repeatability criteria are required according to the OECD Guidance document on analytical methods (ENV/JM/MONO(2007)17), depending on the fortification levels:

≤1 µg/kg	50 - 120 ± 35%
>1 µg/kg ≤0.01 mg/kg	60 - 120 ± 30%
>0.01 mg/kg ≤0.1 mg/kg	70 - 120 ± 20%
>0.1 mg/kg ≤1.0 mg/kg	70 - 110 ± 15%
>1 mg/kg	70 - 110 ± 10%

Plant

Analytical methods for the determination of metconazole residues in plant matrices were evaluated in the context of the inclusion in Annex I of Directive 91/414/EEC. Methods evaluated previously are summarized in Table 4.1.2-21 for the reviewer´s convenience. The respective study is listed as fully peer-reviewed if it is part of the DAR.

Table 4.1.2-21: Summary of analytical methods for determination of metconazole residues in plant matrices

Method No	DocID	Matrix	Method principle	Target analytes	LOQ	Year	Comments	EU reviewed
FAMS 050-01	MK-244-010	Plant	GC-NPD & GC-MSD	*Cis*-metconazole *Trans*-metconazole	0.01 mg/kg (grain) 0.03 mg/kg (straw)	1996	Cereal grain & straw	Yes
FAMS 050-01	MK-244-016	Plant	GC-NPD	*Cis*-metconazole *Trans*-metconazole	0.01 mg/kg (grain) 0.03 mg/kg (straw)	1996	Cereal grain & straw	Yes
FAMS 050-01	MK-244-026	Plant	GC-NPD	*Cis*-metconazole *Trans*-metconazole	0.01 mg/kg (grain) 0.03 mg/kg (straw)	1999	Cereal whole plant, ears, rest plant, grain & straw	Yes
FAMS 050-01	MK-730-045	Plant	GC-NPD (*cis*- and *trans*-isomers) GC-MSD (triazolyl-alanine)	*Cis*-metconazole *Trans*-metconazole Triazolyl-alanine	0.01 mg/kg (*cis*- and *trans*-isomers) 0.1 mg/kg (triazolyl-alanine)	1999	Wheat grain	Yes
FAMS 059-01	MK-244-014	Plant	GC-NPD & GC-MSD	*Cis*-metconazole *Trans*-metconazole	0.01 mg/kg	1996	Oilseed rape & oil	Yes
FAMS 059-02	MK-244-019	Plant	GC-NPD & GC-MSD	*Cis*-metconazole *Trans*-metconazole	0.01 mg/kg	1997	Oilseed rape	Yes
FAMS 059-02	MK-244-025	Plant	GC-NPD	*Cis*-metconazole *Trans*-metconazole	0.01 mg/kg	1999	Oilseed rape	Yes
FAMS 059-02	MK-244-018	Plant	GC-NPD & GC-MSD	*Cis*-metconazole *Trans*-metconazole	0.01 mg/kg	1997	Oilseed rape	Yes
FAMS 057-01	MK-244-011	Plant	GC-NPD & GC-MSD	*Cis*-metconazole *Trans*-metconazole	0.01 mg/kg	1996	Sugar beet roots & leaves	Yes
DFG S19	MK-244-017	Plant	GC-NPD	*Cis*-metconazole *Trans*-metconazole	0.01 mg/kg (grain) 0.03 mg/kg (straw)	1996	Wheat grain & straw	Yes
DFG S19	MK-240-002 2003/1016623	Plant	GC-NPD & GC-MSD	*Cis*-metconazole *Trans*-metconazole	0.01 mg/kg	1999 2003	Wheat grain, grape, pea, oilseed rape	Yes
DFG S19	MK-240-003	Plant	GC-NPD & GC-MSD	*Cis*-metconazole *Trans*-metconazole	0.01 mg/kg	1999	Wheat grain, grape, pea, oilseed rape	Yes

Validation of method 550/0

Report:	CA 4.1.2/16 Lehmann A.,Mackenroth C., 2004a Validation of the analytical method 550/0 in various plant matrices 2004/1010555
Guidelines:	EPA 860.1340, SANCO/825/00 rev. 6 (20 June 2000), SANCO/3029/99 rev. 4 (11 July 2000), EEC 96/46
GLP:	yes (certified by Landesamt fuer Umweltschutz und Gewerbeaufsicht, Mainz, Germany)

Principle of the method

The analytical method No 550/0 was validated for the determination of metconazole (BAS 555 F) *cis*- and *trans*-isomers in plant matrices. The study was performed by BASF, Limburgerhof, Germany.

Metconazole (BAS 555 F, AC900768) is extracted from the sample using a mixture of methanol, water and hydrochloric acid. An aliquot of the extract is centrifuged and partitioned with dichloromethane, and the dichlormethane partition taken to dryness. After dissolving in methanol/water (80:20), the sample is quantitated by HPLC-MS/MS using the transition m/z 320 → 70, for monitoring m/z 320 → 89 or m/z 320 → 125 may be used. Analysis was accomplished using a Luna C18 column and an acetonitrile-pure water gradient with formic acid as modifier. The total amount of metconazole is calculated as the sum of the *cis*- and *trans*-isomers.

Recovery findings

The method was validated in cereal forage, grain and straw, oilseed rape seed, lemon fruit, pea seed and tomato fruit. The recoveries were determined for each isomer in five samples fortified at 0.005 mg/kg, the limit of quantitation, and at 0.05 mg/kg. The recoveries and coefficient of variation were well within the required parameters, see table below.

Table 4.1.2-22: Validation data for analytical method 550/1 for the determination of cis- and trans-metconazole residues in plant matrices

Matrix	Analyte	Fortification level (mg/kg)	No of replicates	Mean recovery (%)	RSD (%)
Cereal forage	*Cis*-metconazole	0.005	5	97	2.4
		0.05	5	95	1.2
		Overall	10	96	2.3
	Trans-metconazole	0.005	5	103	2.6
		0.05	5	97	0.8
		Overall	10	100	3.8
Cereal grain	*Cis*-metconazole	0.005	5	106	1.8
		0.05	5	103	1.8
		Overall	10	105	2.2
	Trans-metconazole	0.005	5	105	1.4
		0.05	5	102	1.2
		Overall	10	103	1.9
Cereal straw	*Cis*-metconazole	0.005	5	103	4.9
		0.05	5	101	3.0
		Overall	10	102	4.0
	Trans-metconazole	0.005	5	106	1.2
		0.05	5	101	1.4
		Overall	10	103	3.2
Oilseed rape seed	*Cis*-metconazole	0.005	5	99	4.7
		0.05	5	98	1.7
		Overall	10	98	3.4
	Trans-metconazole	0.005	5	107	1.5
		0.05	5	101	1.7
		Overall	10	104	3.3
Lemon fruit	*Cis*-metconazole	0.005	5	88	2.5
		0.05	5	97	2.3
		Overall	10	93	5.7
	Trans-metconazole	0.005	5	101	2.3
		0.05	5	98	3.7
		Overall	10	100	3.4
Pea seed	*Cis*-metconazole	0.005	5	87	1.7
		0.05	5	97	0.7
		Overall	10	92	6.2
	Trans-metconazole	0.005	5	102	1.9
		0.05	5	98	1.2
		Overall	10	100	2.7
Tomato fruit	*Cis*-metconazole	0.005	5	89	6.3
		0.05	5	99	2.2
		Overall	10	94	7.0
	Trans-metconazole	0.005	5	102	4.4
		0.05	5	101	1.7
		Overall	10	101	3.3

Linearity
The calibration curve was linear for the standard solution concentration range of 0.025-2.5 ng/mL. At least seven calibration points were used. Correlation coefficients were greater than 0.99. Calibration solutions were prepared in methanol/water (4/1, v/v).

Specificity
LC-MS/MS is a highly specific self-confirmatory technique. Under the described conditions the method is specific for the determination of *cis*- and the *trans*-isomer of metconazole in plant matrices. Due to the high selectivity and specificity of LC-MS/MS an additional confirmatory technique is not necessary.

Interference
The recovery data in the tables above are corrected for interference from matrix compounds of the appropriate unfortified sample, even the measured signal levels in extracts of controls were < 30% LOQ.
In general, no significant interferences (> 30% of LOQ) were observed.

Limit of Quantitation
The limit of quantitation (LOQ) is 0.01 mg/kg, 0.005 mg/kg each of *cis* and *trans*-isomers which are determined separately, resulting from the lowest concentration level successfully tested within recovery experiments.

Limit of Detection
The method has a limit of determination (LOD) of 0.0025 mg kg 1, corresponding to the lowest calibration level used.

Repeatability
The relative standard deviations (RSD, %) for all fortification levels were < 20%.

Reproducibility
Reproducibility of the method was not determined within the validation study.

Stability of solutions
Metconazole is stable in the HPLC-solvent at 4°C for at least 7 days; standard solutions used for fortification have been proved to be stable for at least six weeks.

Conclusion
The analytical method No 550/0 for the analysis of metconazole *cis*- and *trans*-isomers in plant matrices uses LC-MS/MS for final determination, with an LOQ of 0.01 mg/kg (as sum of isomers).
It could be demonstrated that the method fulfils the requirements with regard to repeatability, LOQ and recoveries and is therefore considered suitable for the analysis of metconazole (*cis*- and *trans*-isomer) in plant matrices.

Validation of method L0019/01 (former method number 550/0)

To meet actual requirements a new validation study, to update method 550/0 was conducted; new method number L0019/01

Report:	CA 4.1.2/17 Richter S., 2015a Metconazole: Validation of BASF method L0019/01 for the determination of cis- and trans-Metconazole in various crop types, using LC/MS/MS 2014/7000241
Guidelines:	SANCO/825/00 rev. 8.1 (16 November 2010), SANCO/3029/99 rev. 4 (11 July 2000), EPA 860.1340 (1996), OECD-ENV/JM/MONO/(2007)17
GLP:	yes (certified by Landesanstalt fuer Umwelt, Messungen und Naturschutz Baden-Wuerttemberg, Karlsruhe, Germany)

Principle of the method

The analytical method No L0019/01 was validated for the determination of metconazole (BAS 555 F) *cis*- and *trans*-isomers in plant matrices. The study was performed by PTRL Europe, Ulm, Germany.

After extraction of the plant material with a methanol/water/2 N HCl mixture and clean-up by partitioning into dichloromethane, final determination was performed by LC-MS/MS. Analysis was accomplished using a Phenomenex Luna C18 column and an acetonitrile/water gradient with formic acid as modifier. The following mass transitions were used for the analysis: m/z 320 → 70 for quantitation and m/z 320 → 125 for confirmation.

Recovery findings

For all matrices tested (lettuce, orange, wheat grain, dry bean and oil seed rape), the mean recovery values were between 70% and 110%. The detailed results are given in the table below.

Table 4.1.2-23: Validation data for analytical method L0019/01 for the determination of *cis*- and *trans*-metconazole residues in plant matrices

Matrix	Analyte	Mass transition	Fortification level (mg/kg)	No of replicates	Mean recovery (%)	RSD (%)
	Cis-metconazole	320 → 70	0.005	5	88	2.2
			0.05	5	91	3.3
			Overall	10	90	3.3
		320 → 125	0.005	5	85	3.4
			0.05	5	91	2.8
			Overall	10	88	4.8
	Trans-metconazole	320 → 70	0.005	5	94	6.6
			0.05	5	96	3.7
			Overall	10	95	5.1
		320 → 125	0.005	5	91	3.7
			0.05	5	93	3.9
			Overall	10	92	3.9
Orange	*Cis*-metconazole	320 → 70	0.005	5	97	3.9
			0.05	5	93	2.9
			Overall	10	95	4.2
		320 → 125	0.005	5	96	1.4
			0.05	5	91	3.3
			Overall	10	94	3.5
	Trans-metconazole	320 → 70	0.005	5	98	3.3
			0.05	5	97	4.0
			Overall	10	98	3.5
		320 → 125	0.005	5	100	3.1
			0.05	5	97	2.4
			Overall	10	99	3.2
Wheat grain	*Cis*-metconazole	320 → 70	0.005	5	93	1.5
			0.05	5	95	1.5
			Overall	10	94	1.9
		320 → 125	0.005	5	88	4.1
			0.05	5	89	3.2
			Overall	10	88	3.5
	Trans-metconazole	320 → 70	0.005	5	99	3.2
			0.05	5	104	1.3
			Overall	10	102	3.4
		320 → 125	0.005	5	103	5.7
			0.05	5	105	2.9
			Overall	10	104	4.3

Table 4.1.2-23: Validation data for analytical method L0019/01 for the determination of *cis*- and *trans*-metconazole residues in plant matrices

Matrix	Analyte	Mass transition	Fortification level (mg/kg)	No of replicates	Mean recovery (%)	RSD (%)
Beanoil seed rape, dry	*Cis*-metconazole	320 → 70	0.005	5	90	5.1
			0.05	5	96	1.9
			Overall	10	93	4.8
		320 → 125	0.005	5	85	1.1
			0.05	5	89	4.0
			Overall	10	87	3.7
	Trans-metconazole	320 → 70	0.005	5	95	3.3
			0.05	5	100	2.6
			Overall	10	98	3.6
		320 → 125	0.005	5	96	5.1
			0.05	5	103	2.7
			Overall	10	99	5.3
Oilseed rape seed	*Cis*-metconazole	320 → 70	0.005	5	92	2.9
			0.05	5	94	3.4
			Overall	10	93	3.1
		320 → 125	0.005	5	91	3.3
			0.05	5	93	3.6
			Overall	10	92	3.4
	Trans-metconazole	320 → 70	0.005	5	97	3.1
			0.05	5	99	3.2
			Overall	10	98	3.2
		320 → 125	0.005	5	93	2.7
			0.05	5	97	3.1
			Overall	10	95	3.5

Linearity
Good linearity was observed in the range tested. Linear correlations with coefficients >0.999 were obtained for *cis*- and *trans*-metconazole. At least 5 calibration points distributed over a range of 0.020 to 5.0 ng/mL were used. Calibration solutions were prepared in methanol.

Specificity
LC-MS/MS is a highly specific self-confirmatory technique and therefore a confirmatory technique is not required. Analysis is possible at two ion transitions.

Interference
The interferences/residues of the analyte measured in the control samples were below 30% of the limit of quantitation (LOQ) for each matrix and each mass transition.

Matrix effects
The matrix effect was tested for each matrix. No significant matrix effects (>20% suppression or enhancement) on LC-MS/MS response were observed. Thus calibration solutions in solvent were used for evaluation of the results.

Limit of Quantitation
The limit of quantitation was 0.005 mg/kg per isomer, resulting from the lowest concentration level successfully tested within recovery experiments.

Limit of Detection
The LOD was 0.001 mg/kg (20% of the LOQ) per isomer, corresponding to the lowest calibration level used.

Repeatability
The relative standard deviations (RSD, %) for all commodities were <10% at all fortification levels.

Stability of Working Solutions
Both isomers of metconazole indicated sufficient stability in stock / spike (methanol) as well as in calibration solutions for at least 2 weeks when stored frozen in the dark.
The final sample extracts in methanol/water (2/8, v/v) were re-injected after at least 5 days of storage under frozen conditions. No decrease in stability in the stored extracts could be observed when the results were evaluated with freshly prepared calibration solutions in solvent. Re-injection of final extracts resulted in recoveries within the acceptable range of 70-110%.

Conclusion
The analytical method No L0019/01 for the analysis of metconazole *cis*- and *trans*-isomers in plant matrices uses LC-MS/MS for final determination, with an LOQ of 0.01 mg/kg (sum of isomers).
It could be demonstrated that the method fulfils the requirements with regard to specificity, repeatability, LOQ and recoveries and is therefore considered suitable for the analysis of metconazole (sum of *cis*- and *trans*-isomers) in plant matrices.

Validation of method 535/1

Report:	CA 4.1.2/18 Mackenroth C.,Lehmann A., 2007a Validation of BASF method No. 535/1 in plant matrices 2006/1039427
Guidelines:	EPA 860.1340, SANCO/825/00 rev. 6 (20 June 2000), SANCO/3029/99 rev. 4 (11 July 2000), EEC 6/46, EEC 91/414 Annex III (Part A Section 5)
GLP:	yes (certified by Landesamt fuer Umweltschutz und Gewerbeaufsicht, Mainz, Germany Fed.Rep.)

Principle of the method

The analytical method No 535/1 (actual method number L0076/01) was validated for the determination of metconazole (BAS 555 F, sum of *cis*- and *trans*-isomers) in plant matrices. The study was performed by BASF, Limburgerhof, Germany.

After extraction of the plant material with a methanol/water/hydrochloric acid mixture and subsequent centrifugation of an aliquot, transfer an aliquot of the supernatant into a culture tube containing sodium hydroxide solution. For purification, perform liquid/liquid partitioning with cyclohexane. Evaporate the cyclohexane to dryness and dissolve the residue in methanol/water for HPLC-MS/MS quantitation. Analysis was accomplished using a Betasil C18 column and a methanol/water gradient with formic acid as modifier. For metconazole, the transition ions m/z 320 → 70 and 320 → 125 can be used for quantitation. The *cis*- and *trans*-isomers of metconazole elute together and are quantitated as total metconazole.

Recovery findings

Analytical method 535/1 offers the possibility to determine numerous fungicides or metabolites thereof, such as BAS 421 F, BAS 480 F, BAS 500 F, BF 500-3, BAS 510 F and metconazole (BAS 555 F) in plant matrices by means of HPLC-MS/MS. Here, only the metconazole results are reported. The method proved to be suitable for analysis of *cis*- and *trans*-metconazole in wheat, lemon, lettuce, oilseed rape seed, tomato and onion to a limit of quantitation of 0.01 mg/kg. In all matrices tested, the mean recovery values were between 70 and 110%. The detailed results are given in the table below.

Table 4.1.2-24: Validation data for analytical method 535/1 for the determination of metconazole (total of *cis*- and *trans*-) residues in plant matrices

Matrix	Analyte	Mass transition	Fortification level (mg/kg)	No of replicates	Mean recovery (%)	RSD (%)
Wheat plant without root	Metconazole	320 → 70	0.01	5	94	2.6
			0.1	5	92	6.1
			Overall	10	93	4.5
		320 → 125	0.01	5	88	12
			0.1	5	88	7.0
			Overall	10	88	9.0
Wheat grain	Metconazole	320 → 70	0.01	5	96	2.5
			0.1	5	92	4.3
			Overall	10	94	4.0
		320 → 125	0.01	5	94	4.2
			0.1	5	94	10
			Overall	10	94	7.3
Wheat straw	Metconazole	320 → 70	0.01	5	91	3.9
			0.1	5	89	5.7
			Overall	10	90	4.9
		320 → 125	0.01	5	93	11
			0.1	5	87	5.9
			Overall	10	90	9.1
Lemon fruit	Metconazole	320 → 70	0.01	5	89	3.1
			0.1	5	83	4.9
			Overall	10	86	5.2
		320 → 125	0.01	5	93	7.5
			0.1	5	83	6.7
			Overall	10	88	9.1
Lettuce	Metconazole	320 → 70	0.01	5	92	2.5
			0.1	5	88	5.6
			Overall	10	90	4.8
		320 → 125	0.01	5	91	8.7
			0.1	5	88	13
			Overall	10	90	11
Oilseed rape seed	Metconazole	320 → 70	0.01	5	97	1.8
			0.1	5	94	4.3
			Overall	10	96	3.4
		320 → 125	0.01	5	99	3.8
			0.1	5	98	13
			Overall	10	99	9.1
Tomato fruit	Metconazole	320 → 70	0.01	5	94	1.2
			0.1	5	91	2.3
			Overall	10	93	2.8
		320 → 125	0.01	5	97	4.9
			0.1	5	89	4.5
			Overall	10	93	6.4
Onion bulb	Metconazole	320 → 70	0.01	5	94	3.3
			0.1	5	89	4.7
			Overall	10	92	4.9
		320 → 125	0.01	5	98	3.2
			0.1	5	93	3.6
			Overall	10	96	4.1

Linearity
Good linearity was observed in the range of 0.05 to 0.5 ng/mL for metconazole (external reference standard). At least four calibration points, double injected, were used. Correlation coefficients were greater than 0.99. Calibration solutions were prepared in methanol/water (1:1, v/v).

Specificity
Due to the high specificity of HPLC-MS/MS and the different transitions proposed above, no other confirmatory technique is necessary.

Interference
The recovery data in the tables above are corrected for interference from matrix compounds of the appropriate unfortified sample, even the measured signal levels in extracts of controls were < 30% LOQ.
In general, no significant interferences (> 30% of LOQ) were observed.

Limit of Quantitation
The limit of quantitation was defined by the lowest fortification level successfully tested, which was 0.01 mg/kg for all metconazole in all sample materials.

Limit of Detection
The method has a limit of determination (LOD) of 0.0025 mg kg, corresponding to the lowest calibration level used.

Repeatability
The relative standard deviations (RSD, %) for all commodities and all fortification levels were well below 20%.

Stability of solutions
Stability of BAS 555 F in methanol is proven up to six weeks. Reference is given to report 2004/1010555 summarized above. Samples are stable in methanol/water if stored refrigerated for at least seven days.

Conclusion
The analytical method No 535/1 (actual method number L0076/01) for the analysis of metconazole (sum of *cis*- and *trans*-isomers) in plant matrices uses LC-MS/MS for final determination, with an LOQ of 0.01 mg/kg.
It could be demonstrated that the method fulfils the requirements with regard to specificity, repeatability, LOQ and recoveries and is therefore considered suitable for the analysis of metconazole (sum of *cis*- and *trans*-isomers) in plant matrices.

Validation of method D0508

Report:	CA 4.1.2/19 Saha M.,Gooding R., 2014b Method validation of BASF analytical method D0508 entitled: The determination of residues of Metconazole (BAS 555 F) and its metabolites in plant matrices using LC/MS/MS 2014/7002655*)
Guidelines:	EPA 860.1340
GLP:	yes (certified by United States Environmental Protection Agency)

Principle of the method

The analytical method D0508 was validated for the determination of metconazole (BAS 555 F) cis- and trans-isomers and metabolites M555F011 (Reg.No.4111112), M555F021 (Reg.No.4558878) and M555F030 (Reg.No.4110625) in plant matrices. The study was performed by BASF Crop Protection, NC, USA.

A plant sample (5 g) was extracted by homogenization with acetonitrile-water using polytron. An aliquot of the extract was diluted with methanol-water for the LC-MS/MS determination of metconazole (*cis* and *trans* isomer), and M555F021, M555F011 and M555F030. A sample clean-up procedure using liquid-liquid partition with a mixture of hexane/ethyl acetate was developed for soybean hay and forage for the analysis of metconazole (*cis* and *trans* isomer), M555F021, M555F011 and M555F030 (optional for other matrices).

The analyses are conducted with LC-MS/MS in positive ion mode.

Recovery findings

In all matrices tested, the mean recovery values were between 70% and 110% with the exception of the metabolite M555F030 in molasses with recovery values of 63% and 66%. The detailed results are given in the table below.

Table 4.1.2-25: Validation results of method D0508: metconazole (BAS 555 F) isomers and its metabolites in plant matrices

Matrix	Analyte	Mass transition	Fortification level (mg/kg)	No of replicates	Mean recovery (%)	RSD (%)
Wheat grain	*Cis*-metconazole	320 → 70	0.005	5	97	5.2
			0.05	5	97	3.5
			Overall	10	93	8.2
	Trans-metconazole	320 → 70	0.005	5	89	4.2
			0.05	5	97	9.0
			Overall	10	97	4.2
	M555F021	336 → 125	0.01	5	92	4.6
			0.1	5	101	5.2
			Overall	10	97	6.6
	M555F011	336 → 125	0.01	5	92	1.7
			0.1	5	101	1.5
			Overall	10	96	5.4
	M555F030	334 → 111	0.01	5	90	3.3
			0.1	5	102	5.4
			Overall	10	96	7.7
Wheat straw	*Cis*-metconazole	320 → 70	0.005	5	82	7.4
			0.05	5	97	6.4
			Overall	10	89	11
	Trans-metconazole	320 → 70	0.005	5	76	8.8
			0.05	5	95	3.5
			Overall	10	86	13
	M555F021	336 → 125	0.01	5	79	7.2
			0.1	5	86	4.6
			Overall	10	83	6.9
	M555F011	336 → 125	0.01	5	78	8.9
			0.1	5	86	10
			Overall	10	82	11
	M555F030	334 → 111	0.01	5	72	5.6
			0.1	5	92	8.9
			Overall	10	82	15
Soya bean seed	*Cis*-metconazole	320 → 70	0.005	5	85	8.4
			0.05	5	95	8.1
			Overall	10	94	9.4
	Trans-metconazole	320 → 70	0.005	5	87	5.1
			0.05	5	101	4.9
			Overall	10	90	9.9
	M555F021	336 → 125	0.01	5	86	11
			0.1	5	98	8.7
			Overall	10	92	12
	M555F011	336 → 125	0.01	5	74	6.6
			0.1	5	96	11
			Overall	10	85	16
	M555F030	334 → 111	0.01	5	87	9.9
			0.1	5	91	4.7
			Overall	10	89	7.6

Table 4.1.2-25: Validation results of method D0508: metconazole (BAS 555 F) isomers and its metabolites in plant matrices

Matrix	Analyte	Mass transition	Fortification level (mg/kg)	No of replicates	Mean recovery (%)	RSD (%)
Soya bean hay	*Cis*-metconazole	320 → 70	0.005	5	108	4.9
			0.05	5	110	5.2
			Overall	10	109	4.8
	Trans-metconazole	320 → 70	0.005	5	102	6.7
			0.05	5	105	6.9
			Overall	10	103	6.6
	M555F021	336 → 125	0.01	5	96	11
			0.1	5	102	12
			Overall	10	99	11
	M555F011	336 → 125	0.01	5	94	7.6
			0.1	5	93	5.6
			Overall	10	93	6.3
	M555F030	334 → 111	0.01	5	100	4.6
			0.1	5	104	3.6
			Overall	10	102	4.6
Sugar beet root	*Cis*-metconazole	320 → 70	0.005	5	102	6.7
			0.05	5	105	6.9
			Overall	10	98	9.0
	Trans-metconazole	320 → 70	0.005	5	94	12
			0.05	5	101	3.7
			Overall	10	103	6.6
	M555F021	336 → 125	0.01	5	89	12
			0.1	5	87	13
			Overall	10	88	12
	M555F011	336 → 125	0.01	5	96	6.5
			0.1	5	103	4.2
			Overall	10	100	6.3
	M555F030	334 → 111	0.01	5	92	6.4
			0.1	5	100	7.7
			Overall	10	96	7.8
Sugar beet top	*Cis*-metconazole	320 → 70	0.005	5	89	4.5
			0.05	5	95	5.7
			Overall	10	92	5.9
	Trans-metconazole	320 → 70	0.005	5	85	4.7
			0.05	5	87	8.4
			Overall	10	86	6.6
	M555F021	336 → 125	0.01	5	86	5.1
			0.1	5	95	10
			Overall	10	91	9.3
	M555F011	336 → 125	0.01	5	82	6.9
			0.1	5	88	7.6
			Overall	10	85	8.0
	M555F030	334 → 111	0.01	5	85	10
			0.1	5	97	11
			Overall	10	91	12

Table 4.1.2-25: Validation results of method D0508: metconazole (BAS 555 F) isomers and its metabolites in plant matrices

Matrix	Analyte	Mass transition	Fortification level (mg/kg)	No of replicates	Mean recovery (%)	RSD (%)
Soya bean oil	*Cis*-metconazole	320 → 70	0.005	5	84	4.6
			0.05	5	79	3.0
			Overall	10	82	5.1
	Trans-metconazole	320 → 70	0.005	5	94	11
			0.05	5	87	5.5
			Overall	10	91	9.4
	M555F021	336 → 125	0.01	5	85	13
			0.1	5	85	14
			Overall	10	85	13
	M555F011	336 → 125	0.01	5	97	11
			0.1	5	95	3.1
			Overall	10	96	8.0
	M555F030	334 → 111	0.01	5	90	8.4
			0.1	5	93	8.4
			Overall	10	91	8.0
Sugar beet juice	*Cis*-metconazole	320 → 70	0.005	5	87	6.8
			0.05	5	101	5.2
			Overall	10	94	9.8
	Trans-metconazole	320 → 70	0.005	5	95	8.0
			0.05	5	103	5.6
			Overall	10	99	7.6
	M555F021	336 → 125	0.01	5	97	6.8
			0.1	5	96	5.4
			Overall	10	96	5.7
	M555F011	336 → 125	0.01	5	92	5.9
			0.1	5	99	5.2
			Overall	10	96	6.3
	M555F030	334 → 111	0.01	5	96	5.7
			0.1	5	96	3.4
			Overall	10	96	4.5
Molasses	*Cis*-metconazole	320 → 70	0.005	5	84	7.7
			0.05	5	95	5.5
			Overall	10	90	9.1
	Trans-metconazole	320 → 70	0.005	5	100	14
			0.05	5	98	3.8
			Overall	10	98	9.8
	M555F021	336 → 125	0.01	5	83	8.3
			0.1	5	108	6.4
			Overall	10	95	16
	M555F011	336 → 125	0.01	5	76	11
			0.1	5	91	3.3
			Overall	10	84	12
	M555F030	334 → 111	0.01	5	63	3.5
			0.1	5	66	5.5
			Overall	10	64	5.3

Table 4.1.2-25: Validation results of method D0508: metconazole (BAS 555 F) isomers and its metabolites in plant matrices

Matrix	Analyte	Mass transition	Fortification level (mg/kg)	No of replicates	Mean recovery (%)	RSD (%)
Wheat flour	*Cis*-metconazole	320 → 70	0.005	5	73	6.3
			0.05	5	99	5.1
			Overall	10	86	16
	Trans-metconazole	320 → 70	0.005	5	87	7.4
			0.05	5	96	6.3
			Overall	10	91	8.3
	M555F021	336 → 125	0.01	5	86	10
			0.1	5	95	6.3
			Overall	10	91	9.3
	M555F011	336 → 125	0.01	5	96	7.0
			0.1	5	96	7.6
			Overall	10	96	6.9
	M555F030	334 → 111	0.01	5	92	0.9
			0.1	5	95	3.4
			Overall	10	94	3.0

Linearity
For metconazole (*cis* and *trans*), M555F011, M555F021 and M555F030 good linearity was observed in the range of 0.125 to ~~1.0~~2.5 ng/mL. Four calibration points distributed over this range were used. Coefficients of determination (R^2) were greater than 0.99. Calibration solutions were prepared in methanol/water (4/1, v/v).

Specificity
Significant interferences (> 30% of LOQ) were not observed at the retention time and mass transitions of the analytes.
LC-MS/MS is a highly specific self-confirmatory technique. Due to the high selectivity and specificity of LC MS/MS an additional confirmatory technique is not necessary.

Interference
The reagent blank has shown no interference peaks at the retention time of all analytes.

Limit of Quantitation
The limit of quantitation (LOQ) is defined as the lowest fortification level successfully tested. During this study, a limit of quantitation of 0.005 mg/kg for BAS 555 F (each isomer, *cis* and *trans*) and a limit of quantitation of 0.01 mg/kg for M555F021, M555F011 and M555F030 was confirmed in different plant matrices.

Limit of Detection
The limit of detection (LOD) was set at 20% of the limit of quantitation, corresponding to the lowest calibration level used.

Repeatability
The relative standard deviations (RSD, %) for all commodities and all fortification levels were below 20%.

Stability of solutions

Standard substances were stored in a freezer (<-50°C) until use. During the course of a residue study (BASD DocID 2006/7006723, see chapter 6.10), the stability of fortification and LC-MS/MS calibration standard solutions was examined. Test and reference substance solutions were stored in a refrigerator at 4°C. Stock solutions (1 mg/mL) were made fresh every three months and further diluted to proper concentration. Dilutions of stock standards for fortifications were made fresh every month. Stability of the analytes in various solvent systems used within the method (stock and fortification solutions in methanol or water, standard solutions in methanol/water or 0.1% formic acid) was proven for 30-90 days.

Table 4.1.2-26: Stability of standard, fortification and stock solutions

Solution	Stability (days)
Stock solutions of BAS 555 F (Cis and trans isomers) and its metabolites (M555F021, M555F011 and M555F030) in methanol	90
Fortification solutions of BAS 555 F (Cis and Trans isomers) and its metabolites (M555F021, M555F011 and M555F030) in methanol	41
LC-MS/MS calibration standards of BAS 555 F (Cis and Trans isomers) and its metabolites (M555F021, M555F011 and M555F030) in methanol-water, 80:20, v/v	30

Conclusion

The analytical method D0508 for the analysis of metconazole *cis*- and *trans*-isomers and metabolites M555F021, M555F011 and M555F030 in plant matrices uses LC-MS/MS for final determination, with an LOQ of 0.01 mg/kg (as sum of isomers and for individual metabolites).

It could be demonstrated that the method fulfils the requirements with regard to repeatability, LOQ and recoveries and is therefore considered suitable for the analysis of metconazole (*cis*- and *trans*-isomer) in plant matrices.

Validation of method D0604

Report:	CA 4.1.2/20 Saha M.G., 2007a Method validation of BASF analytical method D0604 Entitled: The determination of residues of BAS 555 F and its metabolites in corn and cotton matrices using LC/MS/MS 2006/7011129
Guidelines:	EPA 860.1340
GLP:	yes (certified by United States Environmental Protection Agency)

Principle of the method

The analytical method D0604 was validated for the determination of metconazole (BAS 555 F) cis- and trans-isomers and metabolites M555F011 (M11; Reg.No.4111112), M555F021 (M21; Reg.No.4558878) and M555F030 (M30; Reg.No.4110625) in plant matrices. The study was performed by BASF Agro Research, NC, USA. It is adapted from method D0508.

A plant sample (5 g) was extracted by homogenization with acetonitrile-water using polytron. An aliquot of the extract was concentrated to an aqueous phase and cleaned-up using liquid-liquid partition with a mixture of hexane/ethyl acetate (9:1, v/v). An aliquot of the organic phase was evaporated to dryness, and the residues were re-dissolved in methanol/water (8:2, v/v) for the LC-MS/MS determination of metconazole (*cis* and *trans* isomer), and M555F021, M555F011 and M555F030.

The analyses are conducted with LC-MS/MS in positive ion mode.

Recovery findings

In all matrices tested, the mean recovery values were between 70% and 110% with the exception of the metabolite M555F011 in maize stover with mean recovery values of 65% and 67% at 1.0 mg/kg fortification and overall, respectively, and in cotton gin by-products with a mean recovery value of 68% at 1.0 mg/kg fortification. The detailed results are given in the table below.

Table 4.1.2-27: Validation results of method D0604: metconazole (BAS 555 F) isomers and its metabolites in plant matrices

Matrix	Analyte	Mass transition	Fortification level (mg/kg)	No of replicates	Mean recovery (%)	RSD (%)
Maize grain	*Cis*-metconazole	320 → 70	0.005	4	99	4.3
			0.05	5	100	2.8
			Overall	9	99	3.5
	Trans-metconazole	320 → 70	0.005	5	97	4.1
			0.05	5	96	4.5
			Overall	10	96	4.1
	M555F021	336 → 125	0.01	5	100	7.9
			0.1	5	94	10
			Overall	10	97	9.1
	M555F011	336 → 125	0.01	5	82	4.1
			0.1	5	76	6.4
			Overall	10	79	6.5
	M555F030	334 → 111	0.01	4	100	4.9
			0.1	5	91	3.0
			Overall	9	95	6.1
Maize stover	*Cis*-metconazole	320 → 70	0.005	5	91	3.4
			2.5	5	88	2.8
			Overall	10	89	3.3
	Trans-metconazole	320 → 70	0.005	5	87	4.7
			2.5	5	86	4.2
			Overall	10	86	4.2
	M555F021	336 → 125	0.01	5	91	12
			1.0	5	75	12
			Overall	10	83	15
	M555F011	336 → 125	0.01	5	70	8.1
			1.0	5	65	5.1
			Overall	10	67	7.6
	M555F030	334 → 111	0.01	5	87	3.5
			1.0	5	88	7.9
			Overall	10	87	5.8
Cotton seed	*Cis*-metconazole	320 → 70	0.005	5	94	11
			0.05	5	101	8.0
			Overall	10	97	10
	Trans-metconazole	320 → 70	0.005	5	96	12
			0.05	5	100	5.8
			Overall	10	98	9.1
	M555F021	336 → 125	0.01	5	86	11
			0.1	5	94	12
			Overall	10	90	12
	M555F011	336 → 125	0.01	5	75	9.0
			0.1	5	75	11
			Overall	10	75	9.3
	M555F030	334 → 111	0.01	5	93	8.6
			0.1	5	96	13
			Overall	10	95	11

Table 4.1.2-27: Validation results of method D0604: metconazole (BAS 555 F) isomers and its metabolites in plant matrices

Matrix	Analyte	Mass transition	Fortification level (mg/kg)	No of replicates	Mean recovery (%)	RSD (%)
Cotton gin by-products	*Cis*-metconazole	320 → 70	0.005	5	88	7.3
			2.5	5	73	15
			Overall	10	80	14
	Trans-metconazole	320 → 70	0.005	5	95	5.2
			2.5	5	71	13
			Overall	10	83	17
	M555F021	336 → 125	0.01	5	85	6.8
			1.0	5	77	6.8
			Overall	10	81	8.2
	M555F011	336 → 125	0.01	5	74	3.9
			1.0	5	68	9.0
			Overall	10	71	8.0
	M555F030	334 → 111	0.01	5	77	5.2
			1.0	5	77	8.0
			Overall	10	77	6.3

Linearity
For metconazole (*cis* and *trans*), M555F011, M555F021 and M555F030 good linearity was observed in the range of 0.125 to 10.0 ng/mL. Five calibration points distributed over this range were used. Coefficients of determination (R^2) were greater than 0.99. Calibration solutions were prepared in methanol/water (4/1, v/v).

Specificity
LC-MS/MS is a highly specific self-confirmatory technique. Due to the high selectivity and specificity of LC MS/MS an additional confirmatory technique is not necessary.

Interference
The reagent blank has shown no interference peaks at the retention time of all analytes.

Limit of Quantitation
The limit of quantitation (LOQ) is defined as the lowest fortification level successfully tested. During this study, a limit of quantitation of 0.005 mg/kg for BAS 555 F (each isomer, *cis* and *trans*) and a limit of quantitation of 0.01 mg/kg for M555F021, M555F011 and M555F030 was confirmed in different plant matrices.

Limit of Detection
The limit of detection (LOD) was set at 20% of the limit of quantitation. In addition, a minimum signal to noise ratio of 3:1 was used for the lowest standard in the calibration curves.

Repeatability
The relative standard deviations (RSD, %) for all commodities and all fortification levels were within the respective repeatability criteria (<10%, 15%, 20% or 30%, depending on the fortification level) except for *cis* and *trans* metaconazole in cotton gin by-products at the 2.5 mg/kg fortification level and overall where the RSD was 13-17%.

Stability of solutions
(See method D0508, DocID 2014/7002655).
Test and reference substance solutions were stored in a refrigerator at 4°C and were refrigerated during their use in this study. Stock solutions (1 mg/mL) were made fresh every three months and further diluted to proper concentration. Dilutions of stock standards for fortifications were made fresh every month. The stability of each analyte in the various solvent systems used within the method has been previously determined elsewhere. In the current study, the stability of each analyte in calibration solutions was extended by testing, from the 30 days reported in the referenced validation study, to at least 37 days, as shown below.

Table 4.1.2-28: Stability of standard solutions

Solution	Stability (days)
LC-MS/MS calibration standards of BAS 555 F (*cis* and *trans* isomers) and its metabolites (M21, M11 and M30) in methanol/water, 4:1, v/v	37

Conclusion
The analytical method D0604 for the analysis of metconazole *cis*- and *trans*-isomers and metabolites M555F021, M555F011 and M555F030 in plant matrices uses LC-MS/MS for final determination, with an LOQ of 0.01 mg/kg (as sum of isomers and for individual metabolites).
It could be demonstrated that the method generally fulfils the requirements with regard to repeatability, specificity, LOQ and recoveries and is therefore considered suitable for the analysis of metconazole (*cis*- and *trans*-isomer) in plant matrices.

Extraction efficiency of the plant methods

Methods D0508, D0604, 550/0 and 535/1 were used in analysis of crop commodities in plant residue studies submitted in this dossier (Section M-CA 6.3). The following points are used to compare the extraction procedure used in these methods to extractions used in the plant metabolism studies.
Residue analytical methods D0503 and D0604 use acetonitrile/water for extraction. The extraction procedure of the metabolism trials show in general good extractability using acetonitrile/water solvents mixtures. In wheat grain, the sequential extraction with acetonitrile (ACN), ACN/H2O and H2O lead to extractable residues of 82 to 92% TRR (References 1+2) Taking into account, that in analytical methods a more effective homogenization technology (Polytron), and a more favorable volume of solvent to sample mass ratio are used, the extraction procedure using the acetonitrile/water approach can be regarded as acceptable.
The extraction procedure of the residue methods L0019/01 (550/0) and L0076/01 (535/1) are based on extraction with methanol/water/hydrochloric acid. These are multi residue methods developed by BASF to analyze a range of compounds for data generation. A harmonized extraction procedure is used to successfully analyze the range of compounds. The extraction procedure in these methods is based on methanol in combination with water, and this is consistent with the extraction procedure used within the metconazole metabolism studies in canola and banana. In the canola metabolism studies, high levels of residue in seed (78% to 98% TRR) were released after sequential extraction with methanol (MeOH), water and MeOH/HCl (References 3+4). In the banana metabolism study, 86 to 94% of TRR of banana fruit were extracted with MeOH with a further release of 3-12% TRR after extraction with MeOH/HCl (Reference 5).
Addition of HCl to the extraction solvent was performed as a common approach to increase extractability in the multi-residue methods. This is based on the general experience, that extractability of plant material can be increased at lower pH-values. In consequence, also the approach using methanol/ water/hydrochloric acid can be regarded as adequate.

Table 4.1.2-29: Reference list of plant metabolism studies

Number	BASF DocID	Study	EU reviewed
1	MK-640-001	[Triazole-14C] WL136184 (KNF-S-474c): Metabolism in wheat	yes
2	MK-640-002	[Cyclopentyl-14C] WL148271(KNF-S-474m): Metabolism in wheat	yes
3	MK-640-006	CL 900768 (Metconazole): Metabolism of [triazole-3,5-14C] CL 900768 in canola under field conditions	yes
4	MK-640-007	CL 900768 (Metconazole): Metabolism of [p-chlorophenyl-U-14C] CL 900768 in canola under field conditions	yes
5	MK-640-008	CL 900768 (Metconazole): Metabolism of CL 900768 in banana under greenhouse conditions	No section M-CA 6.2.1

Validation of method modification M004 of BCS method 01062

Report:	CA 4.1.2/21 Class T., 2011a Modification M004 of BCS residue analytical method 01062 for the determination of 1,2,4-Triazole, Triazolylalanine, Triazole acetic acid and Triazole lactic acid by LC/DMS/MS/MS in plant materials 2012/1294644
Guidelines:	Guidance Document on Residue Analytical Methods (SANCO/3029/99 rev.4), OECD-ENV/JM/MONO/(2007)17 (OECD No. 39)
GLP:	yes (certified by Landesanstalt fuer Umwelt, Messungen und Naturschutz Baden-Wuerttemberg, Karlsruhe, Germany)

Principle of the method

Principle of method 01062 (BASF Method Number L0170/02):
The analytes are extracted with methanol/water (4/1, v/v), an aliquot is filtered, concentrated, and cleaned-up by a simple dispersive C18-SPE step. The analytes are determined by LC-DMS/MS/MS, using two different HPLC columns/stationary phases (Thermo Aquasil C18 and Thermo Hypercarb) and an AB SCIEX QTRAP® 5500 LC-MS/MS instrument equipped with SelexIONTM ion mobility technology which is based on planar differential mobility spectrometry (DMS). Residues are quantified using isotopically labelled internal standards.

Recovery findings

The method proved to be suitable for analysis of 1,2,4-triazole (T), triazolylalanine (TA), triazole acetic acid (TAA) and triazole lactic acid (TLA) in various plant material in/on five different crop types (full validation sets performed) and in many additional plants matrices (reduced validation sets performed
In all matrices successfully tested, the mean recovery values were between 70 and 110%. Few average recoveries were above 110% but < 120%, obviously caused by analyte(s) present in control samples requiring background correction and supported by acceptable relative standard deviations (RSDs) and thus considered acceptable. The detailed results are given below.

Table 4.1.2-30 Recovery results from triazole derivative metabolites plant matrices

Crop	Fortification level (mg/kg)	Recovery (%)	Analyte[1]			
			T	TA	TAA	TLA
Tomato	0.01	Average	105	111	90	92
		RSD	10	14	5	13
		n	5	5	5	5
	1.0	Average	98	110	101	114
		RSD	5	12	5	5
		n	5	5	5	5
Cucumber	0.01	Average	90	111	100	100
		RSD	14	13	10	6
		n	3	3	3	3
	1.0	Average	100	109	105	108
		RSD	3	7	4	2
		n	3	3	3	3
Lettuce	0.01	Average	88	116	105	108
		RSD	8	6	5	6
		n	3	3	3	3
	1.0	Average	102	106	104	104
		RSD	1	9	5	7
		n	3	3	3	3
Cereal grain	0.01	Average	115	91	87	80
		RSD	4	12	9	3
		n	5	5	5	5
	1.0	Average	118	84	80	79
		RSD	5	6	5	5
		n	5	5	5	5
Cereal straw	0.01	Average	109	79	109	100
		RSD	17	9	17	6
		n	3	3	3	3
	1.0	Average	102	76	90	85
		RSD	19	1	9	12
		n	3	3	3	3

Table 4.1.2-30 Recovery results from triazole derivative metabolites plant matrices

Crop	Fortification level (mg/kg)	Recovery (%)	Analyte[1]			
			T	TA	TAA	TLA
Cereal green plant	0.01	Average	109	108	103	89
		RSD	7	8	7	7
		n	3	3	3	3
	1.0	Average	116	100	102	98
		RSD	4	4	5	5
		n	3	3	3	3
Whole orange	0.01	Average	100	90	92	95
		RSD	10	6	3	6
		n	5	5	5	5
	1.0	Average	100	100	92	92
		RSD	2	3	3	7
		n	5	5	5	5
Oilseed rape seed and sunflower seed (TA only)	0.01	Average	102	101	99	82
		RSD	7	25	13	10
		n	5	5	5	5
	1.0	Average	93	92	95	98
		RSD	6	5	4	3
		n	5	5	5	5
Melon peel	0.01	Average	94	97	92	105
		RSD	12	27	7	4
		n	3	3	3	3
	1.0	Average	108	96	96	93
		RSD	7	7	2	4
		n	3	3	3	3
Melon fruit	0.01	Average	98	101	97	106
		RSD	2	9	5	10
		n	3	3	3	3
	1.0	Average	100	107	110	109
		RSD	9	6	2	2
		n	3	3	3	3

Table 4.1.2-30 Recovery results from triazole derivative metabolites plant matrices

Crop	Fortification level (mg/kg)	Recovery (%)	Analyte[1]			
			T	TA	TAA	TLA
Melon pulp	0.01	Average	97	77	99	103
		RSD	5	29	3	10
		n	3	3	3	3
	1.0	Average	110	110	105	108
		RSD	2	7	7	4
		n	3	3	3	3
Sweet pepper	0.01	Average	87	104	106	107
		RSD	11	21	1	9
		n	3	3	3	3
	1.0	Average	107	104	110	110
		RSD	9	9	1	1
		n	3	3	3	3
Carrot leaf	0.01	Average	112	118	106	118
		RSD	6	10	11	6
		n	3	3	3	3
	1.0	Average	97	110	108	102
		RSD	6	14	3	4
		n	3	3	3	3
Carrot root	0.01	Average	90	98	104	105
		RSD	5	9	9	5
		n	3	3	3	3
	1.0	Average	98	105	105	106
		RSD	6	1	4	4
		n	3	3	3	3
Dry bean seed	0.01	Average	104	88	103	91
		RSD	8	12	11	6
		n	5	5	5	5
	1.0	Average	96	81	72	94
		RSD	8	9	7	5
		n	5	5	5	5

1 T=1,2,4-triazole, TA=triazolylalanine, TAA=triazole acetic acid, TLA=triazole lactic acid
RSD Relative standradr deviation
n Number of tests

Linearity
The internal standard procedure using stable isotopically labelled internal standards was used for calibration.. Good linearity was observed in the range of 1 to 600 ng/mL for 1,2,4-triazole (T), triazolylalanine (TA), triazole acetic acid (TAA) and triazole lactic acid (TLA). The correlation coefficients of the 1/x weighted linear regression were always < 0.99.

Specificity
Residues of some of the analytes in untreated blank control samples were frequently present and used to correct residues of samples fortified at the LOQ to obtain background corrected recoveries. Only one LC-MS/MS MRM transition per analyte was monitored. Nevertheless, the LC-DMS/MS/MS method is considered highly specific for its use for data-generation. The method determines 1,2,4-triazole (T), triazolylalanine (TA), triazole acetic acid (TAA) and triazole lactic acid (TLA) in plant matrices. There were no known interferences from plant components or from reagents, solvents and glassware used in case of tomato, cucumber, lettuce, cereal (grain, straw, green plant), orange, oilseed rape (seed), melon (peel, pulp, fruit), sweet pepper, carrot (leaf and root) and dry bean seeds.

Limit of Quantification
The limit of quantitation was defined by the lowest fortification level successfully tested and was 0.01 mg/kg for each analyte in all tested matrices.

Limit of Detection
The limit of detection are estimated to be about 0.002 mg/kg.

Stability of working solutions
The stability of the analytes and their internal standard solutions and extracts was not tested specifically. Acceptable recoveries obtained with calibration solutions sufficiently demonstrate stability.

Matrix effects
The internal standard procedure, using stable isotopically labelled internal standards, compensates for matrix effects.

Repeatability
Relative standard deviations were below 20% for all analytes and sample materials fortified at 0.01 mg/kg (LOQ), except for triazolylalanine (TA) in sunflower seed, melon peel and melon pulp (RSDs < 30%, caused by endogenous TA present in the untreated sample requiring background subtraction). Nevertheless, these results are considered acceptable.

Conclusion
The method meets in general all guideline criteria to determine at an LOQ of 0.01 mg/kg residues of 1,2,4-triazole (T), triazolylalanine (TA), triazole acetic acid (TAA) and triazole lactic acid (TLA) in/on five different crop types (full validation sets performed) and in many additional plants matrices (reduced validation sets performed).
It could be demonstrated that for the above mentioned matrices the method fulfils the requirements with regard to specificity, repeatability, limit of quantitation and recoveries.

Animal

As no new feeding studies were conducted, no new stand-alone validated analytical methods were developed for animal matrices. The validated analytical method DFG S19 is submitted as monitoring method together with its ILV in chapter M-CA 4.2.

A summary table of methods already evaluated is also presented in CA 4.2 for the reviewer's convenience.

Further analytical methods used in support of residue or feeding studies

The following method(s) were used, validated and therefore presented within studies, listed in section CA 6 "Residues in or on Treated Products, Food and Feed and Plant Metabolism". To provide a complete overview of all analytical methods, these are additionally described in the following executive summaries.

Report:	CA 4.1.2/22 [see KCA 6.4.1/1 2008/8000061] [redacted] 2008 a Magnitude of the residues of Metconazole in chicken eggs and tissues
Guidelines:	EPA 860.1480
GLP:	yes (certified by United States Environmental Protection Agency)

Executive summary - Validation of method RM-41M-2

Principle of the method

The analytical method RM-41M-2 was validated for the determination of metconazole (BAS 555 F) *cis*- and *trans*-isomers in liver. The study was performed by [redacted]

Residues were extracted from a liver sample by blending three times with acetonitrile, and combining the extracts. Residues were partitioned using hexane to remove oil and fat and the acetonitrile layer is evaporated. The residues were dissolved in hexane/ethyl acetate (90:10, v/v), partitioned with aqueous sodium chloride solution and filtered through sodium sulfate. Using acetonitrile, the residues were partitioned again, evaporated, dissolved and washed in methanol/water (5:1, v/v), passed through a C18 cartridge and evaporated. Residues were dissolved in methanol/water (2:1, v/v) and analyzed by triple-quadrupole LC-MS/MS. Analysis was performed on an Agilent Zorbax SB-C8 column using a water-methanol gradient with formic acid as modifier.

Recovery findings

The mean recovery values were between 70% and 110% for *cis*- and *trans*-metconazole. The detailed results are given in the table below.

Table 4.1.2-31: Validation results of method RM-41M-2 using LC-MS/MS: metconazole (BAS 555 F) isomers in liver

Matrix	Analyte	Mass transition	Fortification level (mg/kg)	No of replicates	Mean recovery (%)	RSD (%)
Liver	*Cis*-metconazole	320→125	0.02	5	86	17
			0.1	6	80	3.7
			Overall	11	83	12
	Trans-metconazole	320→125	0.02	5	87	17
			0.1	6	81	3.6
			Overall	11	84	12

Linearity

The linearity was tested using four standards at concentrations between of 0.01 to 0.1 µg/mL. For BAS 555 F (*cis* and *trans*), linear correlations with coefficients of determination (R^2) greater than 0.99 were obtained. Calibration solutions were prepared in methanol/water.

Specificity

LC-MS/MS is a highly specific self-confirmatory technique. Due to the high selectivity and specificity of LC-MS/MS an additional confirmatory technique is not necessary.

Interference

No interferences from the reagent blank at the relevant retention times have been reported. No residues were detected in control samples.

Limit of Quantitation

The limit of quantitation (LOQ) is defined as the lowest fortification level successfully tested. During this study, a limit of quantitation of 0.02 mg/kg for BAS 555 F (each isomer, *cis* and *trans*) was obtained in liver.

Limit of Detection

For BAS 555 F (*cis* and *trans*), the limit of detection (LOD) was 0.01 mg/kg in liver.

Repeatability

The relative standard deviations (RSD, %) for all fortification levels were below 20%.

Stability of solutions

The stability of the analytes in working solutions was no subject of this study. Test and reference substance solutions were stored in a refrigerator and were refrigerated during their use in this study. Standards were generally not used for more than one week.

Conclusion

The analytical method RM-41M-2 for the analysis of metconazole *cis*- and *trans*-isomers in liver uses LC-MS/MS for final determination, with an LOQ of 0.04 mg/kg (as sum of isomers).

It could be demonstrated that the method generally fulfils the requirements with regard to repeatability, specificity, LOQ and recoveries and is therefore considered suitable for the analysis of metconazole (*cis*- and *trans*-isomer) liver.

Executive summary - Validation of method RM-41M-3

Principle of the method

The analytical method RM-41M-3 was validated for the determination of metconazole metabolites M1 (M555F001) and M12 (M555F012) in kidney. The study was performed by [redacted]

Residues were extracted from the tissues using methanol and methanol/water (9:1, v/v). The Extract was evaporated to obtain an aqueous residue, and residues were partitioned with acetonitrile/methanol (2:1, v/v) and hexane. The acetonitrile/methanol layer was split to Fraction A (for C18 cleanup and LC-MS/MS analysis for M12) and Fraction B (for hydrolysis, C18 cleanup, and LC-MS/MS analysis for M1).

For Fraction A (M12), an aliquot of the extract was evaporated, the residues were dissolved in methanol/water (5:1, v/v), passed through a Cl8 cartridge and evaporated. Residues were dissolved in methanol/water (2:1, v/v). Analysis was performed by LC-MS/MS.

For Fraction B (M1), an aliquot of the extract was evaporated, the residues were transferred into a screw-top test tube, capped, and the residues hydrolyzed in 3 N HCl at 85°C. The mixture was transferred through a C 18 cartridge, washed and rinsed with methanol/water (5:1, v/v). The eluate was evaporated, and residues were dissolved in methanol/water (2: 1, v/v) and analyzed by LC-MS/MS. Analysis was performed on a Phenomenex Luna C18 column using a methanol/water gradient with formic acid as modifier.

Recovery findings

The mean recovery values were between 77% and 70% for M1, 74% and 78% for M-12 at 0.02 and 0.1 mg/kg fortification and 72% and 76% overall, respectively. The detailed results are given in the table below.

Table 6.4.1-9: Validation results of method RM-41M-3 using LC-MS/MS: metconazole (BAS 555 F) metabolites M1 and M12 in kidney

Matrix	Analyte	Mass transition	Fortification level (mg/kg)	No of replicates	Mean recovery (%)	RSD (%)
Kidney	M-1	336→125	0.02	4	77	10
			0.1	6	70	2.3
			Overall	10	72	8.1
	M-12	350→125	0.02	4	74	6.0
			0.1	6	78	3.6
			Overall	10	76	5.1

Linearity
The linearity was tested using four standards at concentrations between of 0.005 to 0.1 µg/mL. For M1 and M12 linear correlations with coefficients of determination (R^2) greater than 0.99 were obtained. Calibration standards were prepared in methanol/water.

Specificity
LC-MS/MS is a highly specific self-confirmatory technique. Due to the high selectivity and specificity of LC-MS/MS an additional confirmatory technique is not necessary.

Interference
No interferences from the reagent blank at the relevant retention times have been reported. No residues were detected in control samples.

Limit of Quantitation
The limit of quantitation (LOQ) is defined as the lowest fortification level successfully tested. During this study, a limit of quantitation of 0.02 mg/kg for metconazole metabolites M-1 and M-12 was obtained in kidney.

Limit of Detection
For metconazole metabolites M1 and M12, the limit of detection (LOD) was 0.01 mg/kg in kidney.

Repeatability
The relative standard deviations (RSD, %) for all fortification levels were ≤10%.

Stability of solutions
The stability of the analytes in working solutions was no subject of this study. Test and reference substance solutions were stored in a refrigerator and were refrigerated during their use in this study. Standards were generally not used for more than one week.

Conclusion
The analytical method RM-41M-3 for the analysis of metconazole metabolites M1 and M12 in kidney uses LC-MS/MS for final determination, with an LOQ of 0.02 mg/kg per analyte. It could be demonstrated that the method generally fulfils the requirements with regard to repeatability, specificity, LOQ and recoveries and is therefore considered suitable for the analysis of metconazole metabolites M1 and M12 in kidney.

Report:	CA 4.1.2/23 [see KCA 6.4.2/1 2006/1046033] [redacted] 2006 a Magnitude of the residues of Metconazole in dairy cattle and meat
Guidelines:	EPA 860.1460
GLP:	yes (certified by United States Environmental Protection Agency)

Executive summary - Validation of method RM-41M-1

Principle of the method

The analytical method RM-41M-1 was validated for the determination of metconazole (BAS 555 F) *cis*- and *trans*-isomers in milk. The study was performed by [redacted]

Residues were extracted from a milk sample using ethyl acetate/methanol mixture (2:1, v/v), filtered, rotary evaporated to an aqueous oily residue and partitioned with methylene chloride/aqueous sodium chloride solution. The organic phase, containing the residues, was evaporated, residues were cleaned up by acetonitrile-hexane partition and acetonitrile was removed by rotary evaporation. Residues were dissolved in toluene for analysis by gas chromatography with a nitrogen-phosphorus detector (GC-NPD) for the determination of metconazole (*cis* and *trans* isomer). Analysis was performed on an Agilent HP-5 column with helium as carrier gas.

Recovery findings

The mean recovery values were between 70% and 110% for both *cis*- and *trans*-metconazole. Details are presented in the table below.

Table 4.1.2-32: Validation results of method RM-41M-1 using GC-NPD: metconazole (BAS 555 F) isomers in milk

Matrix	Analyte	Fortification level (mg/kg)	No of replicates	Mean recovery (%)	RSD (%)
Milk	*Cis*-metconazole	0.02	3	93	2.3
		0.1	6	88	2.1
		Overall	9	89	3.3
	Trans-metconazole	0.02	3	95	1.9
		0.1	6	90	2.4
		Overall	9	92	3.7

Table 4.1.2-33: Validation results of method RM-41M-1 using LC-MS/MS: metconazole (BAS 555 F) isomers in milk

Matrix	Analyte	Mass transition	Fortification level (mg/kg)	No of replicates	Mean recovery (%)	RSD (%)
Milk	*Cis*-metconazole	320→125	0.02	3	85	3.7
	Trans-metconazole	320→125	0.02	3	84	1.3

Linearity
The linearity was tested using four standards at concentrations between of 0.1 to 2.0 µg/mL for GC and between 0.005 and 0.100 µg/mL for HPLC analysis. For BAS 555 F (*cis* and *trans*), linear correlations with coefficients of determination (R^2) greater than 0.99 were obtained. Calibration standards were prepared in acetone for GC and in methanol/water for HPLC analysis.

Specificity
LC-MS/MS was used as confirmatory technique in the positive ionization mode on an Agilent Zorbax SB-C18 column. A water-methanol gradient was used with formic acid as modifier.

Interference
No interferences from the reagent blank at the relevant retention times have been reported. No residues were detected in control samples.

Limit of Quantitation
The limit of quantitation (LOQ) is defined as the lowest fortification level successfully tested. During this study, a limit of quantitation of 0.02 mg/kg for BAS 555 F (each isomer, *cis* and *trans*) was obtained in milk.

Limit of Detection
For BAS 555 F (*cis* and *trans*), the limit of detection (LOD) was 0.01 mg/kg in milk.

Repeatability
The relative standard deviations (RSD, %) for all fortification levels were within the respective repeatability criteria (<10%).

Stability of solutions
The stability of the analytes in working solutions was no subject of this study. Test and reference substance solutions were stored in a refrigerator and were refrigerated during their use in this study.

Conclusion
The analytical method RM-41M-1 for the analysis of metconazole *cis*- and *trans*-isomers in milk uses GC-NPD for final determination, with an LOQ of 0.04 mg/kg (as sum of isomers).
It could be demonstrated that the method generally fulfils the requirements with regard to repeatability, specificity, LOQ and recoveries and is therefore considered suitable for the analysis of metconazole (*cis*- and *trans*-isomer) in milk.

(f) Methods in soil, water, sediment, feed and any additional matrices used in support of ecotoxicology studies

Analytical methods used in ecotoxicological studies, are in general described and validated with the corresponding ecotoxicological study, and therefore presented in the corresponding dossier chapter. In addition, the following methods were developed and validated within stand-alone validation studies.

Validation of method L0019/02 (550/0)

This method was already validated for plant matrices. To support ecotoxicological studies a validation in honey, pollen and nectar has been conducted in addition.

Report:	CA 4.1.2/24 Weber H.,Zetzsch A., 2014a Validation of an analytical method for the determination of Metconazole (cis- and trans-isomer) in honey, pollen and nectar 2014/1028660
Guidelines:	EC 1107/2009 of the European Parliament and of the Council of 21 Oct 2009, EEC 79/117, EEC 91/414, SANCO/825/00 rev. 8.1 (16 November 2010), SANCO/3029/99 rev. 4 (11 July 2000), OECD Guidance Document on Pesticide Residue Analytical Methods (ENV/JM/MONO(2007)17 - 13-Aug-07
GLP:	yes (certified by Freie und Hansestadt Hamburg, Behoerde fuer Soziales, Familie, Gesundheit und Verbraucherschutz, Hamburg, Germany)

Principle of the method

The analytical method L0019/02 (550/0) was developed for the determination of residues of metconazole (*cis*- and *trans*-isomer) in matrices of honey, pollen and nectar. The study was performed by Eurofins Agroscience Services EcoChem GmbH, Hamburg, Germany.

Metconazole residues are extracted with a methanol/water/2N HCl (70:25:5, v/v/v) mixture. An aliquot of extract is cleaned up by liquid/liquid partition with dichloromethane, evaporated to dryness and the residue is re-dissolved in methanol/water (8:2, v/v) before analysis by liquid chromatography with tandem mass spectrometric detection (LC-MS/MS) monitoring two mass transitions of metconazole. Analysis is accomplished using a Luna C18 column and an acetonitrile/water gradient with formic acid as modifier.

The limit of quantification (LOQ) of the method is 0.01 mg kg^{-1} for metconazole (sum of cis- (0.005 mg kg^{-1}) and trans- (0.005 mg kg^{-1}) isomer).

Recovery findings

The method is suitable to determine residues of metconazole in honey, pollen and nectar. Samples are fortified with the analytes at the limit of quantification of 0.005 mg kg^{-1} and 10 times higher (0.05 mg kg^{-1}).

Mean recovery values for both isomers (mean of five replicates per fortification level and analyte) are between 79% and 103% in all matrices tested (see table below), which fulfils the legal requirements.

Table 4.1.2-34: Validation results of method L0019/02: metconazole (*cis*- and *trans*-isomer) in plant matrices

Analyte	Matrix	Fortification level (mg/kg)	No. of Replicates	Mean Recovery (%)	RSD (%)	Overall Recovery (%)	RSD (%)	Mean Recovery (%)	RSD (%)	Overall Recovery (%)	RSD (%)
Transition				**320 → 70**				**320 → 125**			
cis-Metconazole Reg.No. 4079468	Honey	0.005	5	103	2	99	4	102	6	98	6
		0.05	5	95	2			94	3		
	Pollen	0.005	5	86	18	84	12	88	14	85	10
		0.05	5	81	9			81	10		
	Nectar	0.005	5	86	11	84	7	93	12	87	10
		0.05	5	82	7			81	6		
Transition				**320 → 70**				**320 → 125**			
trans-Metconazole *Reg.No. 4079654*	Honey	0.005	5	101	4	98	4	101	5	97	5
		0.05	5	96	2			93	2		
	Pollen	0.005	5	87	14	84	11	91	14	85	11
		0.05	5	80	7			79	8		
	Nectar	0.005	5	89	6	87	6	98	8	90	10
		0.05	5	86	5			83	7		

Linearity
The linearity of the detector response is confirmed by injecting eight standard solutions covering the working range of 0.025 to 5.00 ng mL^{-1}. Good linearity is observed for both *cis*- and *trans*-metconazole ($r \geq 0.995$) using solvent-based standards (diluted in methanol/water, 80/20, v/v).

Specificity
LC-MS/MS is a highly specific self-confirmatory technique. Due to the high selectivity and specificity of LC-MS/MS an additional confirmatory technique is not necessary.
No significant interferences (> 30% of LOQ) are observed at the retention times and mass transitions considered for each analyte.

Matrix effects
Matrix effects on LC-MS/MS detection are investigated by comparing peak areas of solvent standard solutions and peak areas of matrix-matched standard solutions. The mean matrix effects for each matrix do not exceed 11.6% for cis-metconazole and 12.6% for trans-metconazole in honey, pollen and nectar.

Limit of Quantitation
The limit of quantification (LOQ) is defined by the lowest fortification level successfully tested. LOQ is 0.005 mg kg^{-1} for each isomer of metconazole (*cis*- and *trans*-isomer) in honey, pollen and nectar.

Limit of Detection
The limit of detection (LOD) is defined as 30% of the LOQ; equivalent to 0.0015 mg kg^{-1} for each isomer of metconazole (*cis*- and *trans*-isomer).

Repeatability
The relative standard deviations (RSD, %) for all commodities and fortification levels are below 20%.

Standard Stability
Calibration solutions (dissolved in methanol/water (80/20, v/v)) are stable (deviation within ±10.2% for cis-metconazole and ±13.7% for trans-metconazole) for at least 22 days when stored refrigerated at 5 ± 4°C in the dark.

Extract Stability
Mean recovery values of all sample extracts after a storage period of 8 days in a refrigerator in the dark are within a range of 70-110% recovery, hence sample extracts are considered stable for this period of time.

Conclusion
The analytical method L0019/02 for analysis of metconazole (*cis*- and *trans*-isomer) in honey, pollen and nectar uses LC-MS/MS for final determination, with a limit of quantification of 0.01 mg kg^{-1} (as sum of *cis*- and *trans*-isomer).
It could be demonstrated that the method fulfils the requirements with regard to specificity, repeatability, limit of quantification and recoveries and is therefore applicable to correctly and accurately determine residues of metconazole (*cis*- and *trans*-isomer) in honey, pollen and nectar.

Validation of method APL0500/02

Report: CA 4.1.2/25
Obermann M., 2006a
Validation of analytical method APL0500/02: Determination of pesticides in water by HPLC/MS
2006/1024332*)
Guidelines: SANCO/825/00 rev. 7 (17 March 2004)
GLP: yes
(certified by Landesamt fuer Umweltschutz und Gewerbeaufsicht, Mainz, Germany Fed.Rep.)

Principle of the method

Analytical method APL0500/02 (based on BASF method no. APL0500/01) was validated for the analysis of several BASF pesticides in aqueous matrices by LC/MS, to support ecotoxicological studies for dose verification. The study was performed by BASF, Limburgerhof, Germany.
The method is based on dilution of the aqueous samples with acetonitrile/water and acidification with formic acid following by final determination by reversed phase HPLC with MS-detection (external calibration). After dissolving in, the samples are quantitated by HPLC-MS using the m/z 320. Analysis was accomplished using a YMC ProC18 column and an acetonitrile-pure water gradient with formic acid as modifier. The total amount of metconazole is calculated as the sum of the *cis*- and *trans*-isomers.
The limit of quantification (LOQ) of the method is 0.001 mg L^{-1}.

Recovery findings

The method proved to be suitable to determine BAS 555 F in water samples. Samples were fortified at concentrations of 0.001 mg L^{-1} (LOQ) and 0.1 mg L^{-1} (100xLOQ). The analyses yielded acceptable mean recoveries of 98% and 101%. The detailed results are given in the table below.

Table 4.1.2-35: Results of the method validation for the determination of BAS 555 F in water

Matrix	Analyte	Fortification level [mg L^{-1}]	Number of Replicates	Mean Recovery [%]	RSD [%]	Overall Recovery [%]	RSD [%]
Ft-Mix Water (Tap-Water)	metconazole Reg.No.4056343	0.001	5	101	0.9	99	1.7
		0.1	5	98	0.7		
AAP-Water		0.001	1	99	--	--	--
M4-Water		0.001	1	97	--	--	--
OECD-Water		0.001	1	96	--	--	--

RSD = Relative standard deviation

Linearity
The results proved good linearity (r > 0.999) of the detector response in the investigated concentration range of approximately 0.0005 mg L^{-1} to 0.13 mg L^{-1} calibration solutions in acetonitrile/water/formic acid.

Specificity
HPLC/MS is highly specific for the analyte BAS 555 F. The identification and quantification is based on the selected ion monitoring of ESI MS molecular ion signal M320 characteristic for the analyte. Under the described conditions the method is specific for the determination of BAS 555 F in water.
As the method is only used for dose verification of known substances and known nominal concentrations, no additional confirmatory technique is necessary.
No significant matrix interferences were observed in the investigated blank water samples.

Limit of Quantification
The limit of quantification (LOQ) is defined by the lowest fortification level successfully tested.
The method has a limit of quantification (LOQ) of 0.001 mg L^{-1}.

Limit of Detection
The limit of detection (LOD) is defined as 50% of the LOQ; equivalent to 0.0005 mg L^{-1}, corresponding to the lowest calibration level used.

Repeatability
The relative standard deviations (RSD, %) for all fortification levels were below 20%.

Reproducibility
Reproducibility of the method was not determined within the validation study.

Conclusion
The results of the analytical study proved that analytical method APL0500/02 is suitable for the determination of BAS 555 F in water. It could be demonstrated that the method fulfils the requirements with regard to linearity, specificity, accuracy, repeatability, and limit of quantification.

Remark to method APL0500/02
Meanwhile, to this method further analytes have been included and validated, resulting in version 03 of the method (APL0500/03). This version covers all analytes, independent in which version they have been validated. Therefore it could be possible, that for metconazole version 02 or version 03 might be cited in studies. Both is correct, as this is a multi-component method, validated within multiple validation studies.

(g) Methods in water, buffer solutions, organic solvents and any additional matrices resulting from the physical and chemical properties tests

Where necessary, these methods are reported along with the respective studies

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CA 4.2 Methods for post-approval control and monitoring purposes

(a) Methods for the analysis in food and feed of plant and animal origin

The enforcement methods for plants and animals evaluated in the previous Annex I inclusion process were considered as suitable.
During the peer review under Directive 91/414/EEC, several analytical methods using GC-NPD confirmed by GC-MS and their ILV were evaluated and validated for determination of metconazole (*cis*- and *trans*-isomers measured separately) in plant matrices with, for each pair of enantiomers, a LOQ of 0.01 mg/kg in high water content (sugar beet leaves), high starch content (sugar beet roots, cereals) and high fat content (oil seed rape) commodities and 0.03 mg/kg for cereals straw. In addition, the multi-residue method DFG S19 (and DFG cleanup method 5 for fatty/oily matrices) using GC-NPD confirmed by GC-MS and its ILV were evaluated and validated for determination of metconazole (*cis*- and *trans*-isomers measured separately) in plant matrices with, for each pair of enantiomers, a LOQ of 0.01 mg/kg in high water content (peas), high starch content (wheat), acidic (grapes) and high fat content (oilseed rape) commodities. An overview is given in chapter 4.1.2 e).
During the peer review under Directive 91/414/EEC, the multi-residue method DFG S19 (and DFG cleanup method 5 for fatty/oily matrices) using GC-NPD confirmed by GC-MS and its ILV were evaluated and validated for determination of metconazole (*cis*- and *trans*-isomers measured separately) in food of animal origin with, for each pair of enantiomers, an LOQ of 0.01 mg/kg in bovine muscle and fat, milk and eggs. An overview is given in Table 4.2-1.

Based on the residue definition for MRL setting and enforcement, residue analytical methods are required for the parent molecule metconazole (sum of isomers) in food of plant and animal origin.

For animal matrices, an additional analytical method DFG S19 is available with an LOQ of 0.005 mg/kg, for each pair of enantiomers, in liver and kidney and was evaluated during the Article 12 review. However, as those studies are not regarded as peer reviewed, they are summarized here again. Recently, DFG S19 was also validated in milk, egg, meat and fat with the new LOQ of 0.005 mg/kg per isomer. These studies are submitted in this dossier along with their corresponding ILVs.

For plant matrices, enforcement method DFG S19 was validated with the new LOQ of 0.005 mg/kg per isomer in commodities with high water, oil, protein, starch and acid content and is submitted in this dossier chapter together with its ILV.

The conclusions on residue analytical methods for food of plant and animal origin was derived from the EFSA Reasoned Opinion on MRLs (European Food Safety Authority; Review of the existing maximum residue levels (MRLs) for metconazole according to Article 12 of Regulation (EC) No 396/2005. EFSA Journal 2011;9(10):2422. [47 pp.] doi:10.2903/j.efsa.2011.2422. Available online: www.efsa.europa.eu/efsajournal).

General remark
The following recovery and repeatability criteria are required according to the OECD Guidance document on analytical methods (ENV/JM/MONO(2007)17), depending on the fortification levels:

≤1 µg/kg	50 - 120 ± 35%
>1 µg/kg ≤0.01 mg/kg	60 - 120 ± 30%
>0.01 mg/kg ≤0.1 mg/kg	70 - 120 ± 20%
>0.1 mg/kg ≤1.0 mg/kg	70 - 110 ± 15%
>1 mg/kg	70 - 110 ± 10%

Food of animal origin

Analytical methods for the determination of metconazole residues in animal matrices were evaluated in the context of the inclusion in Annex I of Directive 91/414/EEC. Methods evaluated previously are summarized in Table 4.2-1 for the reviewer´s convenience. The respective study is listed as fully peer-reviewed if it is part of the DAR.

Table 4.2-1: Summary of analytical methods for determination of metconazole residues in animal matrices

Method No	DocID	Matrix	Method principle	Target analytes	LOQ	Year	Comments	EU reviewed
DFG S19	MK-240-002 2003/1016623	Animal	GC-NPD & GC-MSD	*Cis*-metconazole *Trans*-metconazole	0.01 mg/kg	1999 2003	Milk, egg, bovine muscle & fat	Yes
DFG S19	MK-240-003	Animal	GC-NPD & GC-MSD	*Cis*-metconazole *Trans*-metconazole	0.01 mg/kg	1999	Milk, egg, bovine muscle & fat	Yes

In context of the re-evaluation of existing MRLs, EFSA concluded that metconazole (sum of isomers) can be enforced in food of animal origin with a combined LOQ of 0.02 mg/kg in muscle, fat, liver, kidney, milk and eggs. However, to cover the requirements of the new guidelines in force, analytical method DFG S19 was validated for analysis of animal matrices and is provided in this document along with its independent laboratory validation. It allows an efficient enforcement of metconazole parent residues using state of the art technology (LC-MS/MS).

Validation of method DFG S19 (Part I)

Report:	CA 4.2/1 Kuhn T., 2010a Metconazole: Validation of the multi-residue enforcement method DFG S19 for the determination of residues in liver and kidney, using LC/MS/MS 2010/1080270
Guidelines:	SANCO/825/00 rev. 7 (17 March 2004), SANCO/3029/99 rev. 4 (11 July 2000), OECD Guidance Document on Pesticide Residue Analytical Methods (ENV/JM/MONO(2007)17 - 13-Aug-07
GLP:	yes (certified by Umweltministerium Baden-Wuerttemberg, Stuttgart)

Principle of the method

The analytical method DFG S19 was validated for the determination of metconazole (BAS 555 F) *cis*- and *trans*-isomers in bovine liver and kidney. The study was performed by PTRL Europe, Ulm, Germany.

Metconazole (*cis*- and *trans*-isomer) residues were extracted from kidney and liver specimens with DFG S19 extraction module E and then filtered. The extracts were purified by gel permeation chromatography (GPC) on Bio Beads S-X3 polystyrene gel with GPC eluent (ethyl acetate/cyclohexane 1/1, v/v) as mobile phase. The eluent was evaporated almost to dryness under a nitrogen stream, and the residues were then re-dissolved in methanol/water (1:1, v/v). The extracts were centrifuged prior to analysis by liquid chromatography with tandem mass spectrometric detection (LC-MS/MS). Results were quantified on peak areas against the linearity of response of external calibration standard solutions. Transition m/z 320 → 70 was used for quantitation and transition m/z 320 → 125 was used for confirmation. Analysis was accomplished on a Phenomenex Luna C_{18} column (with pre-column Phenomenex C_{18} RP) applying a acetonitrile/water gradient using 0.1% formic acid as modifier.

Recovery findings

In both matrices tested, the mean recovery values were between 70% and 110%. The detailed results are given in the table below.

Table 4.2-2: Validation results of method DFG S19: metconazole (BAS 555 F) isomers in bovine matrices

Matrix	Analyte	Mass transition	Fortification level (mg/kg)	No of replicates	Mean recovery (%)	RSD (%)
Kidney	*Cis*-metconazole	320 → 70	0.005	5	89	3.3
			0.05	5	94	2.5
			Overall	10	92	4.0
		320 → 125	0.005	5	90	3.7
			0.05	5	94	3.1
			Overall	10	92	4.0
	Trans-metconazole	320 → 70	0.005	5	88	2.6
			0.05	5	92	2.4
			Overall	10	90	3.3
		320 → 125	0.005	5	88	2.4
			0.05	5	92	3.5
			Overall	10	90	3.8
Liver	*Cis*-metconazole	320 → 70	0.005	5	94	9.7
			0.05	5	88	2.9
			Overall	10	91	7.7
		320 → 125	0.005	5	93	8.6
			0.05	5	87	3.4
			Overall	10	90	7.2
	Trans -metconazole	320 → 70	0.005	5	93	9.1
			0.05	5	87	3.1
			Overall	10	90	7.2
		320 → 125	0.005	5	93	9.3
			0.05	5	87	2.8
			Overall	10	90	7.6

Linearity
Linear calibration functions with 1/x weighting were calculated and plotted by regression analysis. Good linearity was observed in the range of 1.0 to 100.0 ng/mL (7 concentration levels) for both *cis*- and *trans*-metconazole (external reference standard) with correlation coefficients ≥0.99. Calibration solutions were prepared in methanol/water (1:1, v/v).

Specificity
LC-MS/MS is a highly specific detection technique and therefore a confirmatory technique is not required. Analysis is possible at two ion transitions.

Interference
No significant peaks interferences (> 30% of LOQ) were observed at the retention time of the analytes when matrix blank control extracts were injected.

Matrix effects
Matrix effects were tested by evaluating LC-MS/MS response of standards in solvent with standards in matrix. No signal suppression or enhancement was observed for both animal matrices.

Limit of Quantitation
The limit of quantitation was 0.005 mg/kg per analyte in both matrices, resulting from the lowest concentration level successfully tested within recovery experiments.

Limit of Detection
Based on the detectability of the analytes in the final extracts, the limit of detection (LOD) was demonstrated to be about 0.001 mg/kg.

Repeatability
The relative standard deviations (RSD, %) for both commodities and fortification levels were <20%.

Reproducibility
An independent laboratory validation has been conducted and is reported below (DocID 2010/1144332).

Stability of solutions
Stability of the analytes in stock and calibration solutions (up to 2 weeks) and in selected extracts (at least 2 days) was demonstrated when stored refrigerated.

Conclusion
The analytical method DFG S19 for the analysis of metconazole *cis*- and *trans*-isomers in kidney and liver uses LC-MS/MS for final determination, with an LOQ of 0.01 mg/kg (as sum of isomers).
It could be demonstrated that the method fulfils the requirements with regard to specificity, repeatability, LOQ and recoveries and is therefore considered suitable for the analysis of metconazole (*cis*- and *trans*-isomer) in animal matrices for enforcement purposes.

Independent laboratory validation of method DFG S19 (Part I)

Report:	CA 4.2/2 Toledo F., 2010a Determination of Metconazole (BAS 555 F) in animal matrices - Independent laboratory validation 2010/1144332
Guidelines:	SANCO/825/00 rev. 7 (17 March 2004), OECD Guidance Document on Pesticide Residue Analytical Methods (ENV/JM/MONO(2007)17 - 13-Aug-07
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Laendlichen Raum und Verbraucherschutz)

Principle of the method

The analytical method DFG S19 was independently validated for the determination of metconazole (BAS 555 F) *cis*- and *trans*-isomers in bovine liver and kidney. The study was performed by SGS Institut Fresenius GmbH, Taunusstein, Germany.

Metconazole (*cis*- and *trans*-isomer) residues were extracted from kidney and liver specimens with DFG S19 extraction module E and then filtered. The extracts were purified by gel permeation chromatography (GPC) on Bio Beads S-X3 polystyrene gel with GPC eluent (ethyl acetate/cyclohexane 1/1, v/v) as mobile phase. The eluent was evaporated almost to dryness under a nitrogen stream, and the residues were then re-dissolved in methanol/water (1:1, v/v). The extracts were centrifuged prior to analysis by liquid chromatography with tandem mass spectrometric detection (LC-MS/MS). Results were quantified on peak areas against the linearity of response of external calibration standard solutions. Transition m/z 320 → 70 was used for quantitation and transition m/z 320 → 125 was used for confirmation. Analysis was accomplished on a Phenomenex Luna C_{18} column applying an acetonitrile/pure water gradient using 0.1% formic acid as modifier.

Recovery findings

In both matrices tested, the mean recovery values were between 70% and 110%. The detailed results are given in the table below.

Table 4.2-3: Independent laboratory validation results of method DFG S19: metconazole (BAS 555 F) isomers in bovine matrices

Matrix	Analyte	Mass transition	Fortification level (mg/kg)	No of replicates	Mean recovery (%)	RSD (%)
Kidney	*Cis*-metconazole	320 → 70	0.005	5	82	7.4
			0.05	5	89	6.7
			Overall	10	85	7.9
		320 → 125	0.005	5	84	3.2
			0.05	5	88	5.0
			Overall	10	86	4.7
	Trans-metconazole	320 → 70	0.005	5	83	8.0
			0.05	5	84	3.7
			Overall	10	83	6.0
		320 → 125	0.005	5	78	5.9
			0.05	5	87	3.4
			Overall	10	83	7.3
Liver	*Cis*-metconazole	320 → 70	0.005	5	91	8.3
			0.05	5	97	5.8
			Overall	10	94	7.7
		320 → 125	0.005	5	95	7.3
			0.05	5	96	6.7
			Overall	10	95	6.6
	Trans -metconazole	320 → 70	0.005	5	94	9.4
			0.05	5	97	6.3
			Overall	10	96	7.7
		320 → 125	0.005	5	91	10
			0.05	5	95	8.0
			Overall	10	93	9.1

Linearity

Linear calibration functions with 1/x weighting were calculated and plotted by regression analysis. Good linearity was observed in the range of 0.02 to 1.0 ng/mL (6-7 concentration levels) for both *cis*- and *trans*-metconazole (external reference standard) with correlation coefficients ≥0.99. Calibration solutions were prepared in methanol/water (1:1, v/v).

Specificity

LC-MS/MS is a highly specific detection technique and therefore a confirmatory technique is not required. Analysis is possible at two ion transitions.

Interference

The detector signals in all control specimens were below 30% of the detector signals of the specimens fortified at the LOQ. No significant interferences from the specimen matrices were detected at the retention time of interest.

Matrix effects
Already tested in original validation.

Limit of Quantitation
The limit of quantitation was 0.005 mg/kg per analyte in both matrices, resulting from the lowest concentration level successfully tested within recovery experiments.

Limit of Detection
Based on the detectability of the analytes in the final extracts, the limit of detection (LOD) was demonstrated to be about 0.001 mg/kg.

Repeatability
The relative standard deviations (RSD, %) for both commodities and fortification levels were <20%.

Reproducibility
The method was successfully tested in this independent laboratory validation (ILV).

Stability of solutions
The stock and fortification solutions were stored in a refrigerator at ≤-18°C in the dark. The calibration solutions were prepared daily using non matrix-matched calibration solutions. The stability of the calibration solutions was proven by comparing the concentrations of one old solution and one freshly prepared solution by means of a single injection. It was proven that solutions of *cis*- and *trans*-metconazole were stable for at least 22 days covering at least the duration of the laboratory work.

Conclusion
The analytical method DFG S19 for the analysis of metconazole *cis*- and *trans*-isomers in kidney and liver uses LC-MS/MS for final determination, with an LOQ of 0.01 mg/kg (as sum of isomers).
It could be demonstrated that the method fulfils the requirements with regard to specificity, repeatability, LOQ and recoveries and is therefore considered suitable for the analysis of metconazole (*cis*- and *trans*-isomer) in animal matrices for enforcement purposes.

Validation of method DFG S19 (Part II)

Report:	CA 4.2/3 Kuhn T., 2014a Metconazole: Validation of the multi-residue enforcement method DFG S19 for the determination of residues in milk, egg, meat and fat, using LC/MS/MS 2013/1349767
Guidelines:	SANCO/825/00 rev. 8.1 (16 November 2010), SANCO/3029/99 rev. 4 (11 July 2000), EPA 860.1340, OECD Guidance Document on Pesticide Residue Analytical Methods (ENV/JM/MONO(2007)17 - 13-Aug-07
GLP:	yes (certified by Landesanstalt fuer Umwelt, Messungen und Naturschutz Baden-Wuerttemberg, Karlsruhe, Germany)

Principle of the method

The analytical method DFG S19 was validated for the determination of metconazole (BAS 555 F) *cis*- and *trans*-isomers in milk, fat, meat and egg. The study was performed by PTRL Europe, Ulm, Germany.

Metconazole (*cis*- and *trans*-isomer) residues were extracted from animal specimens with DFG S19 extraction module E1 (milk, meat, egg) and E6 (fat). The extracts were purified by gel permeation chromatography (GPC) on Bio Beads S-X3 polystyrene gel with GPC eluent (ethyl acetate/cyclohexane 1/1, v/v) as mobile phase. The eluent was evaporated almost to dryness under a nitrogen stream, and the residues were then re-dissolved in methanol/water (1:1, v/v). Analysis was performed by liquid chromatography with tandem mass spectrometric detection (LC-MS/MS). Results were quantified on peak areas against the linearity of response of external calibration standard solutions. Transition m/z 320 → 70 was used for quantitation and transition m/z 320 → 125 was used for confirmation. Analysis was accomplished on a Phenomenex Luna C_{18} column applying an acetonitrile/water gradient using 0.1% formic acid as modifier.

Recovery findings

In all matrices tested, the mean recovery values were between 70% and 110%. The detailed results are given in the table below.

Table 4.2-4: Validation results of method DFG S19: metconazole (BAS 555 F) isomers in animal matrices

Matrix	Analyte	Mass transition	Fortification level (mg/kg)	No of replicates	Mean recovery (%)	RSD (%)
Milk	*Cis*-metconazole	320 → 70	0.005	5	90	18
			0.05	5	109	3.4
			Overall	10	100	15
		320 → 125	0.005	5	91	18
			0.05	5	108	4.3
			Overall	10	99	15
	Trans-metconazole	320 → 70	0.005	5	93	19
			0.05	5	110	2.3
			Overall	10	102	15
		320 → 125	0.005	5	92	20
			0.05	5	110	2.9
			Overall	10	101	15
Fat	*Cis*-metconazole	320 → 70	0.005	5	86	11
			0.05	5	93	7.1
			Overall	10	89	9.7
		320 → 125	0.005	5	87	11
			0.05	5	92	6.6
			Overall	10	89	9.1
	Trans-metconazole	320 → 70	0.005	5	84	13
			0.05	5	93	7.0
			Overall	10	89	11
		320 → 125	0.005	5	86	13
			0.05	5	93	5.9
			Overall	10	90	9.9
Meat	*Cis*-metconazole	320 → 70	0.005	5	97	4.8
			0.05	5	86	6.5
			Overall	10	91	8.4
		320 → 125	0.005	5	98	6.7
			0.05	5	86	6.5
			Overall	10	92	9.3
	Trans-metconazole	320 → 70	0.005	5	96	5.6
			0.05	5	84	5.3
			Overall	10	91	8.1
		320 → 125	0.005	5	96	6.4
			0.05	5	85	6.0
			Overall	10	90	8.8
Egg	*Cis*-metconazole	320 → 70	0.005	5	110	11
			0.05	5	102	6.3
			Overall	10	106	9.6
		320 → 125	0.005	5	108	13
			0.05	5	102	6.7
			Overall	10	105	10
	Trans -metconazole	320 → 70	0.005	5	110	11
			0.05	5	102	6.0
			Overall	10	106	9.4
		320 → 125	0.005	5	109	12
			0.05	5	102	6.3
			Overall	10	105	10

Linearity

Linear calibration functions with 1/x weighting were calculated and plotted by regression analysis. Good linearity was observed in the range of 0.5 (fat) or 1.0 to 100 ng/mL (at least 5 concentrations) for both *cis*- and *trans*-metconazole (external reference standard) with correlation coefficients ≥0.99. Calibration solutions were prepared in methanol/water (1:1, v/v).

Specificity

LC-MS/MS is a highly specific detection technique and therefore a confirmatory technique is not required. Analysis is possible at two ion transitions.

Interference

Apparent residues or interferences in blank control specimens were below 30% of the LOQ. There were no known interferences from animal commodity components or from reagents, solvents and glassware used.

Matrix effects

Matrix effects (i.e. response of analyte in calibration solutions in solvent versus response in matrix matched calibration solutions) were not significant (i.e. <20%).

Limit of Quantitation

The limit of quantitation was 0.005 mg/kg per analyte in all matrices, resulting from the lowest concentration level successfully tested within recovery experiments.

Limit of Detection

The limit of detection (LOD) of the method was set to 20% of LOQ for each analyte, corresponding to the lowest calibration level used.

Repeatability

The relative standard deviations (RSD, %) for all commodities and fortification levels were ≤20%.

Reproducibility

An independent laboratory validation has been conducted and is reported below (DocID 2014/7000242).

Stability of solutions

Stability of the analytes in stock and calibration solutions (up to 2 weeks) when stored refrigerated was proven in a previously study (see BASF DocID 2010/1080270 above). The stability of the extracts was demonstrated by acceptable recoveries within 70-120%.

Conclusion
The analytical method DFG S19 for the analysis of metconazole *cis*- and *trans*-isomers in animal matrices uses LC-MS/MS for final determination, with an LOQ of 0.01 mg/kg (as sum of isomers).
It could be demonstrated that the method fulfils the requirements with regard to specificity, repeatability, LOQ and recoveries and is therefore considered suitable for the analysis of metconazole (*cis*- and *trans*-isomer) in animal matrices for enforcement purposes.

Independent laboratory validation of method DFG S19 (Part II)

Report:	CA 4.2/4 Schemikau N.,Colorado C., 2015b Metconazole: Independent laboratory validation of the multi-residue enforcement method DFG S19 for the determination of residues in milk, egg, meat and fat, using LC/MS/MS 2014/7000242
Guidelines:	SANCO/825/00 rev. 8.1 (16 November 2010), SANCO 11802/2010 rev. July 2010, SANCO/3029/99 rev. 4 (11 July 2000), OECD-ENV/JM/MONO/(2007)17, EPA 860.1340 (1996)
GLP:	yes (certified by Freie und Hansestadt Hamburg, Behoerde fuer Gesundheit und Verbraucherschutz, Hamburg, Germany)

Principle of the method
The analytical method DFG S19 was independently validated for the determination of metconazole (BAS 555 F) *cis*- and *trans*-isomers in milk, fat, meat and egg. The study was performed by Eurofins Agroscience Services Chem GmbH, Hamburg, Germany.
Metconazole (*cis*- and *trans*-isomer) residues were extracted from milk, egg and meat specimens with DFG S19 extraction module E1 (water/acetone) and module E6 (clean-up solution for GPC) for fat. For module E1, sodium chloride and cyclohexane/ethyl acetate was added for liquid/liquid partition. An aliquot portion of the organic phase was dried with sodium sulphate and concentrated. To the residue obtained, ethyl acetate was added followed by the same volume of cyclohexane. Remaining water was eliminated with a mixture of sodium sulphate and sodium chloride and the solution was filtered. The extract was used for clean-up by gel permeation chromatography. Detection was achieved by liquid chromatography with tandem mass spectrometric detection (LC-MS/MS). Results were quantified on peak areas against the linearity of response of external calibration standard solutions. Transition m/z 320 → 70 was used for quantitation and transition m/z 320 → 125 was used for confirmation. Analysis was accomplished on a Luna C_{18} column applying an acetonitrile/pure water gradient using 0.1% formic acid as modifier.

Recovery findings
In all matrices tested, the mean recovery values were between 70% and 110%. The detailed results are given in the table below.

Table 4.2-5: Independent laboratory validation results of method DFG S19: metconazole (BAS 555 F) isomers in animal matrices

Matrix	Analyte	Mass transition	Fortification level (mg/kg)	No of replicates	Mean recovery (%)	RSD (%)
Milk	*Cis*-metconazole	320 → 70	0.005	5	109	3.3
			0.05	5	109	7.1
			Overall	10	109	5.2
		320 → 125	0.005	5	107	3.9
			0.05	5	109	7.3
			Overall	10	108	5.7
	Trans-metconazole	320 → 70	0.005	5	108	3.3
			0.05	5	107	8.1
			Overall	10	107	5.8
		320 → 125	0.005	5	108	4.6
			0.05	5	108	8.2
			Overall	10	108	6.3
Fat	*Cis*-metconazole	320 → 70	0.005	5	97	5.7
			0.05	5	79	2.0
			Overall	10	88	12
		320 → 125	0.005	5	96	5.4
			0.05	5	78	2.2
			Overall	10	87	12
	Trans-metconazole	320 → 70	0.005	5	96	4.6
			0.05	5	78	2.0
			Overall	10	87	11
		320 → 125	0.005	5	96	4.8
			0.05	5	78	1.7
			Overall	10	87	12
Meat	*Cis*-metconazole	320 → 70	0.005	5	110	12
			0.05	5	98	9.0
			Overall	10	104	12
		320 → 125	0.005	5	109	12
			0.05	5	98	8.8
			Overall	10	103	11
	Trans-metconazole	320 → 70	0.005	5	108	12
			0.05	5	98	9.5
			Overall	10	103	11
		320 → 125	0.005	5	107	13
			0.05	5	98	9.5
			Overall	10	103	12
Egg	*Cis*-metconazole	320 → 70	0.005	5	110	3.2
			0.05	5	94	3.5
			Overall	10	102	9.3
		320 → 125	0.005	5	109	4.2
			0.05	5	94	3.9
			Overall	10	101	9.0
	Trans -metconazole	320 → 70	0.005	5	109	3.3
			0.05	5	92	3.3
			Overall	10	101	9.5
		320 → 125	0.005	5	110	5.4
			0.05	5	93	3.4
			Overall	10	101	9.8

Linearity
The linearity of the detector response of metconazole (BAS 555 F) was demonstrated by single determination of solvent calibration standards at eight concentration levels ranging from 0.50 to 100 ng/mL. The calibration curves obtained for both ion mass transitions in all matrices were linear with coefficients of determination greater than 0.99. Calibration solutions were prepared in methanol/water (1:1, v/v).

Specificity
LC-MS/MS is a highly specific detection technique and therefore a confirmatory technique is not required. Analysis is possible at two ion transitions.

Interference
No significant interference above 30% of LOQ was detected in any of the control specimen extracts of each matrix or the reagent blanks.

Matrix effects
Matrix effects on the detection of metconazole in extracts of matrix were found to be insignificant (<20%). Therefore, solvent standards were used for quantitation.

Limit of Quantitation
The limit of quantitation was 0.005 mg/kg per analyte in all matrices, resulting from the lowest concentration level successfully tested within recovery experiments.

Limit of Detection
The limit of detection (LOD) for all matrices was set at 0.0015 mg/kg, which is 30% of the LOQ, corresponding to the lowest calibration level used.

Repeatability
The relative standard deviations (RSD, %) for all commodities and both fortification levels were <20%.

Reproducibility
The method was successfully tested within this independent laboratory validation (ILV).

Stability of solutions
Final specimen extracts were stored at 5±4°C in the dark until analysis. All standard solutions were stored at 5±4°C in a glass vial under dark conditions. Stability of the analytes in stock and calibration solutions (up to 2 weeks) when stored refrigerated was proven in a previously study (see BASF DocID 2010/1080270 above). The stability of the extracts was demonstrated by acceptable recoveries within 70-110%.

Conclusion
The analytical method DFG S19 for the analysis of metconazole *cis*- and *trans*-isomers in animal matrices uses LC-MS/MS for final determination, with an LOQ of 0.01 mg/kg (as sum of isomers).
It could be demonstrated that the method fulfils the requirements with regard to specificity, repeatability, LOQ and recoveries and is therefore considered suitable for the analysis of metconazole (*cis*- and *trans*-isomer) in animal matrices for enforcement purposes.

Food of plant origin

Analytical methods for the determination of metconazole residues in plant matrices were evaluated in the context of the inclusion in Annex I of Directive 91/414/EEC. Methods evaluated previously are summarized in Table 4.2-6 for the reviewer´s convenience. The respective study is listed as fully peer-reviewed if it is part of the DAR.

Table 4.2-6: Summary of analytical methods for determination of metconazole residues in plant matrices

Method No	DocID	Matrix	Method principle	Target analytes	LOQ	Year	Comments	EU reviewed
DFG S19	2003/7007238	Plant	GC-NPD & GC-MS	*Cis*-metconazole *Trans*-metconazole	0.01 mg/kg	2003	wheat grain, grape, pea and oilseed rape seedt	Yes

In context of the re-evaluation of existing MRLs, EFSA concluded that metconazole (sum of isomers) can be enforced in food of plant origin with a combined LOQ of 0.02 mg/kg in high water content, high fat content, dry and acidic commodities. However, to cover the requirements of the new guidelines in force, analytical method DFG S19 was validated for analysis of plant matrices and is provided in this document along with its independent laboratory validation. It allows an efficient enforcement of metconazole parent residues using state of the art technology (LC-MS/MS).
Furthermore, the CRL Data Pool has been checked on data about alternative enforcement methods. Even the available data is limited, an overview on the existing data regarding QuEChERS, as well as the ChemElut methodology, is presented below. In addition, a validation of the QuEChERS method on oily matrices, originating from the public literature search, is described in detail within this chapter.

Table 4.2-7: CRL Data Pool summary – QuEChERS methodology

Long Overview List

Pesticide	Chr	Matrix Type	Level min	Level max	Rec Median	Rec Mean	CV [%]	# of rec	% Rec (70-120%)	# of Labs
Metconazole			0,002	0,5	**96**	97	10,7	282	**100**	3
	GC	Acidic	0,5	0,5	**93**	93	13	10	**100**	1
	GC	Water containing	0,05	0,5	**93**	96	12,6	21	**100**	1
	LC	Acidic	0,01	0,1	**96**	96	10,7	76	**100**	3
	LC	Dry (cereals, dry pulses)	0,02	0,1	**94**	94	8,7	13	**100**	3
	LC	Fatty, dry (oil seeds, nuts)	0,05	0,05		114		1	**100**	1
	LC	Other	0,02	0,02		84		1	**100**	1
	LC	Sugar containing	0,04	0,1	**101**	103	5,7	12	**100**	3
	LC	Water containing	0,002	0,2	**97**	97	10,5	143	**99**	3
	LC	Water containing, extract rich	0,02	0,1	**92**	94	13,9	5	**100**	2

Table 4.2-8: CRL Data Pool summary – ChemElut methodology

Long Overview List

Pesticide	Chr	Matrix Type	Level min	Level max	Rec Median	Rec Mean	CV [%]	# of rec	% Rec (70-120%)	# of Labs
Metconazole			0,025	0,1	**46**	50	31,8	32	**9**	2
	GC	Acidic	0,1	0,1		71		1	**100**	1
	LC	Acidic	0,025	0,1	**50**	55	31,2	13	**15**	2
	LC	Dry (cereals, dry pulses)	0,025	0,025		38		1	**0**	1
	LC	Other	0,025	0,03		44	7,9	2	**0**	1
	LC	Water containing	0,025	0,03	**50**	52	15,9	12	**0**	1
	LC	Water containing, extract rich	0,025	0,03	**20**	21	12,6	3	**0**	1

Validation of method DFG S19

Report:	CA 4.2/5 Kuhn T., 2014a Metconazole: Validation of the multi-residue enforcement method DFG S19 for the determination of residues in wheat grain, grape, peas, oilseed rape seed and apple using LC/MS/MS 2014/1028662
Guidelines:	SANCO/825/00 rev. 8.1 (16 November 2010), SANCO/3029/99 rev. 4 (11 July 2000), EEC 91/414 Annex II (Part A Section 4), EEC 91/414 Annex III (Part A Section 5), EC 1107/2009 of the European Parliament, EPA 860.1340: Residue Chemistry Test Guidelines - Residue Analytical Method, OECD Guidance Document on Pesticide Residue Analytical Methods (ENV/JM/MONO(2007)17 - 13-Aug-07
GLP:	yes (certified by Landesamt fuer Umwelt, Messungen und Naturschutz Baden Wuerttemberg, Karlsruhe, Germany)

Report:	CA 4.2/6 Richter S., 2014a **Report amendment No. 1** - Metconazole: Validation of the multi-residue enforcement method DFG S19 for the determination of residues in wheat grain, grape, peas, oilseed rape seed and apple, using LC/MS/MS 2014/1263163
Guidelines:	SANCO/825/00 rev. 8.1 (16 November 2010), SANCO/3029/99 rev. 4 (11 July 2000), EEC 91/414 Annex II (Part A Section 4), EEC 91/414 Annex III (Part A Section 5), EPA 860.1340: Residue Chemistry Test Guidelines - Residue Analytical Method, OECD Guidance Document on Pesticide Residue Analytical Methods (ENV/JM/MONO(2007)17 - 13-Aug-07
GLP:	yes (certified by Landesanstalt fuer Umwelt, Messungen und Naturschutz Baden-Wuerttemberg, Karlsruhe, Germany)

Principle of the method

The analytical method DFG S19 was validated for the determination of metconazole (BAS 555 F) *cis*- and *trans*-isomers in plant matrices. The study was performed by PTRL Europe, Ulm, Germany.

Metconazole (*cis*- and *trans*-isomer) residues were extracted from plant specimens with DFG S19 extraction module E1 (apple), E2 (wheat grain, peas), E3 (grape) and E7 (oilseed rape seed).

The extracts of wheat grain, peas and oilseed rape seed were purified by gel permeation chromatography (GPC) on Bio Beads S-X3 polystyrene gel with GPC eluent (ethyl acetate/cyclohexane 1/1, v/v) as mobile phase. The eluent was evaporated almost to dryness under a nitrogen stream, and the residues were then re-dissolved in methanol/water (1:1, v/v).

Analysis was performed by liquid chromatography with tandem mass spectrometric detection (LC-MS/MS). Residues were quantified on peak areas against the linearity of response of external calibration standard solutions. Transition m/z 320 → 70 was used for quantitation and transition m/z 320 → 125 was used for confirmation. Analysis was accomplished on a Phenomenex Luna C_{18} column applying an acetonitrile/water gradient using 0.1% formic acid as modifier.

Recovery findings
In all matrices tested, the mean recovery values were between 70% and 110%. The detailed results are given in the table below.

Table 4.2-9: Validation results of method DFG S19: metconazole (BAS 555 F) isomers in plant matrices

Matrix	Analyte	Mass transition	Fortification level (mg/kg)	No of replicates	Mean recovery (%)	RSD (%)
Apple	*Cis*-metconazole	320 → 70	0.005	5	87	4.9
			0.05	5	82	2.2
			Overall	10	85	5.1
		320 → 125	0.005	5	86	4.9
			0.05	5	82	2.6
			Overall	10	84	4.4
	Trans-metconazole	320 → 70	0.005	5	85	5.1
			0.05	5	80	2.5
			Overall	10	82	5.1
		320 → 125	0.005	5	85	6.1
			0.05	5	81	2.0
			Overall	10	83	5.2
Grape	*Cis*-metconazole	320 → 70	0.005	5	97	5.8
			0.05	5	96	10
			Overall	10	97	7.8
		320 → 125	0.005	5	101	4.6
			0.05	5	96	11
			Overall	10	99	8.3
	Trans-metconazole	320 → 70	0.005	5	100	6.3
			0.05	5	97	12
			Overall	10	99	8.9
		320 → 125	0.005	5	99	6.5
			0.05	5	98	12
			Overall	10	98	9.1
Wheat grain	*Cis*-metconazole	320 → 70	0.005	5	92	8.7
			0.05	5	82	7.3
			Overall	10	87	9.9
		320 → 125	0.005	5	93	8.0
			0.05	5	82	7.9
			Overall	10	87	10
	Trans-metconazole	320 → 70	0.005	5	92	8.9
			0.05	5	83	7.3
			Overall	10	87	9.6
		320 → 125	0.005	5	91	7.3
			0.05	5	82	8.4
			Overall	10	87	9.2

Table 4.2-9: Validation results of method DFG S19: metconazole (BAS 555 F) isomers in plant matrices

Matrix	Analyte	Mass transition	Fortification level (mg/kg)	No of replicates	Mean recovery (%)	RSD (%)
Peas	*Cis*-metconazole	320 → 70	0.005	5	93	5.4
			0.05	5	79	4.1
			Overall	10	86	9.9
		320 → 125	0.005	5	92	5.9
			0.05	5	77	5.2
			Overall	10	85	11
	Trans-metconazole	320 → 70	0.005	5	94	5.7
			0.05	5	81	4.4
			Overall	10	87	9.2
		320 → 125	0.005	5	95	5.5
			0.05	5	81	4.4
			Overall	10	88	10
Oilseed rape seed	*Cis*-metconazole	320 → 70	0.005	5	96	17
			0.05	5	99	6.4
			Overall	10	98	12
		320 → 125	0.005	5	97	19
			0.05	5	100	5.7
			Overall	10	98	13
	Trans -metconazole	320 → 70	0.005	5	95	17
			0.05	5	100	5.6
			Overall	10	98	12
		320 → 125	0.005	5	96	18
			0.05	5	100	6.4
			Overall	10	98	13

Linearity
Linear calibration functions with 1/x weighting were calculated and plotted by regression analysis. Good linearity was observed in the range of 0.5 or 1.25 to 100 ng/mL for both *cis*- and *trans*-metconazole (external reference standard) with correlation coefficients ≥0.99. Calibration solutions were prepared in methanol/water (1:1, v/v).

Specificity
LC-MS/MS is a highly specific detection technique and therefore a confirmatory technique is not required. Analysis is possible at two ion transitions.

Interference
Apparent residues or interferences in blank control specimens were below 30% of the LOQ. There were no known interferences from animal commodity components or from reagents, solvents and glassware used.

Matrix effects
Matrix effects (i.e. response of analyte in calibration solutions in solvent versus response in matrix matched calibration solutions) were not significant (i.e. <20%).

Limit of Quantitation
The limit of quantitation was 0.005 mg/kg per analyte in all matrices, resulting from the lowest concentration level successfully tested within recovery experiments.

Limit of Detection
The limit of detection (LOD) of the method was set to 20% of LOQ for each analyte, corresponding to the lowest calibration level used.

Repeatability
The relative standard deviations (RSD, %) for all commodities and fortification levels were <20%.

Reproducibility
An independent laboratory validation has been conducted and is reported below (DocID 2014/7000243).

Stability of solutions
Stability of the analytes in stock and calibration solutions (up to 2 weeks) when stored refrigerated was proven in a previously study (see BASF DocID 2010/1080270 above). According to SANCO/825/00 rev. 8.1 the stability of the extracts was demonstrated by acceptable recoveries within 70 to 120%.

Conclusion
The analytical method DFG S19 for the analysis of metconazole *cis*- and *trans*-isomers in plant matrices uses LC-MS/MS for final determination, with an LOQ of 0.01 mg/kg (as sum of isomers).
It could be demonstrated that the method fulfils the requirements with regard to specificity, repeatability, LOQ and recoveries and is therefore considered suitable for the analysis of metconazole (*cis*- and *trans*-isomer) in plant matrices for enforcement purposes.

Independent laboratory validation of method DFG S19

Report:	CA 4.2/7 Schernikau N.,Colorado C., 2015c Metconazole: Independent laboratory validation of the BASF method L0259/01 multi residue enforcement method DFG S19 for the determination of residues in wheat grain, grape, peas, oilseed rape seed and apple, using LC/MS/MS 2014/7000243
Guidelines:	SANCO/825/00 rev. 8.1 (16 November 2010), OECD Guidance Document on Pesticide Residue Analytical Methods (ENV/JM/MONO(2007)17 - 13-Aug-07, EPA 860.1340 (1996), SANCO/3029/99 rev. 4 (11 July 2000)
GLP:	yes (certified by Freie und Hansestadt Hamburg, Behoerde fuer Gesundheit und Verbraucherschutz, Hamburg, Germany)

Principle of the method

The analytical method DFG S19 (internal BASF method No. L0259/01) was independently validated for the determination of metconazole (BAS 555 F) *cis*- and *trans*-isomers in plant matrices. The study was performed by Eurofins Agroscience Services Chem GmbH, Hamburg, Germany.

Metconazole (*cis*- and *trans*-isomer) residues were extracted from plant specimens with DFG S19 extraction module E1 (apple), E2 (wheat grain, peas), E3 (grape) and E7 (oilseed rape seed).

The extracts of wheat grain, peas and oilseed rape seed were purified by gel permeation chromatography (GPC).

Analysis was performed by liquid chromatography with tandem mass spectrometric detection (LC-MS/MS). Transition m/z 320 → 70 was used for quantitation and transition m/z 320 → 125 was used for confirmation. No addition or modification to the original method other than optimization of instrumental parameters was done.

Recovery findings

In all matrices tested, the mean recovery values were between 70% and 110%. The detailed results are given in the table below.

Table 4.2-10: Independent laboratory validation results of method DFG S19: metconazole (BAS 555 F) isomers in plant matrices

Matrix	Analyte	Mass transition	Fortification level (mg/kg)	No of replicates	Mean recovery (%)	RSD (%)
Apple	*Cis*-metconazole	320 → 70	0.005	5	79	8.5
			0.05	5	78	7.9
			Overall	10	79	7.8
		320 → 125	0.005	5	78	8.0
			0.05	5	78	7.9
			Overall	10	78	7.5
	Trans-metconazole	320 → 70	0.005	5	73	11
			0.05	5	75	9.2
			Overall	10	74	9.4
		320 → 125	0.005	5	73	10
			0.05	5	75	10
			Overall	10	74	9.7
Grape	*Cis*-metconazole	320 → 70	0.005	5	110	2.4
			0.05	5	95	6.6
			Overall	10	103	8.9
		320 → 125	0.005	5	109	1.0
			0.05	5	95	6.6
			Overall	10	102	8.2
	Trans-metconazole	320 → 70	0.005	5	109	2.1
			0.05	5	93	5.8
			Overall	10	101	9.2
		320 → 125	0.005	5	109	2.1
			0.05	5	93	5.8
			Overall	10	101	9.2
Wheat grain	*Cis*-metconazole	320 → 70	0.005	4	91	5.8
			0.05	5	77	16
			Overall	9	83	14
		320 → 125	0.005	4	91	7.9
			0.05	5	77	16
			Overall	9	83	14
	Trans-metconazole	320 → 70	0.005	4	92	6.5
			0.05	5	77	14
			Overall	9	84	14
		320 → 125	0.005	4	91	6.8
			0.05	5	78	16
			Overall	9	84	14
Peas	*Cis*-metconazole	320 → 70	0.005	5	96	13
			0.05	5	82	7.0
			Overall	10	89	13
		320 → 125	0.005	5	94	14
			0.05	5	83	8.1
			Overall	10	89	13
	Trans-metconazole	320 → 70	0.005	5	93	13
			0.05	5	80	7.3
			Overall	10	87	13
		320 → 125	0.005	5	93	13
			0.05	5	80	7.3
			Overall	10	87	13

Table 4.2-10: Independent laboratory validation results of method DFG S19: metconazole (BAS 555 F) isomers in plant matrices

Matrix	Analyte	Mass transition	Fortification level (mg/kg)	No of replicates	Mean recovery (%)	RSD (%)
Oilseed rape seed	*Cis*-metconazole	320 → 70	0.005	5	83	18
			0.05	5	75	10
			Overall	10	79	15
		320 → 125	0.005	5	83	19
			0.05	5	75	9.3
			Overall	10	79	15
	Trans - metconazole	320 → 70	0.005	5	84	16
			0.05	5	75	9.8
			Overall	10	80	14
		320 → 125	0.005	5	83	15
			0.05	5	74	10
			Overall	10	79	14

Linearity
The linearity of the detector response of metconazole (BAS 555 F) was demonstrated by single determination of solvent calibration standards at eight concentration levels ranging from 0.50 ng/mL to 100 ng/mL. The linearity covers the range from no more than 30% of the LOQ and at least +20% of the highest analyte concentration level detected in a sample. The calibration curves obtained for both ion mass transitions in all matrices were linear with coefficients of determination (R^2) greater than 0.99. Calibration solutions were prepared in methanol/water (1:1, v/v).

Specificity
LC-MS/MS is a highly specific detection technique and therefore a confirmatory technique is not required. Analysis is possible at two ion transitions.

Interference
No significant interference above 30% of LOQ was detected in any of the control specimen extracts of each matrix or the reagent blanks, so that a high level of selectivity was demonstrated.

Matrix effects
Matrix effects on the detection of analytes in extracts of matrix were found to be insignificant (<20%).

Limit of Quantitation
The limit of quantitation was 0.005 mg/kg per analyte in all matrices, resulting from the lowest concentration level successfully tested within recovery experiments.

Limit of Detection
The limit of detection (LOD) for all matrices was set at 0.0015 mg/kg, which is 30% of the LOQ, corresponding to the lowest calibration level used.

Repeatability
The relative standard deviations (RSD, %) for all commodities and fortification levels were <20%.

Reproducibility
The independent laboratory validation confirmed the good results of the method validation reported above (DocID 2014/1028662).

Stability of solutions
Stability of the analytes in stock and calibration solutions (up to 2 weeks) when stored refrigerated was proven in a previous study (see BASF DocID 2010/1080270 above). According to SANCO/825/00 rev. 8.1 the stability of the extracts was demonstrated by acceptable recoveries within 70 to 120%.

Conclusion
The analytical method DFG S19 for the analysis of metconazole *cis*- and *trans*-isomers in plant matrices uses LC-MS/MS for final determination, with an LOQ of 0.01 mg/kg (as sum of isomers).
It could be demonstrated that the method fulfils the requirements with regard to specificity, repeatability, LOQ and recoveries and is therefore considered suitable for the analysis of metconazole (*cis*- and *trans*-isomer) in plant matrices for enforcement purposes.

Additional method data from public literature search

Report: CA 4.2/8
Rajski L. et al., 2013b
Determination of pesticide residues in high oil vegetal commodities by using various multi-residue methods and clean-ups followed by liquid chromatography tandem mass spectrometry
(Journal of Chromatography A, 1304 (2013) 109– 120)
2013/1420081
Guidelines: None
GLP: no

Principle of the method

Metconazole (sum of *cis*- and *trans*-isomers) residues were extracted from homogenized avocado and almond specimens with **QuEChERS** protocol with Z-Sep clean-up: extraction was performed with water and acetonitrile; after shaking with several salts and centrifugation, the extract was mixed with magnesium sulphate and Z-Sep. After evaporation using a nitrogen stream, the residue was re-dissolved in acetonitrile/water and filtered prior to injection into the LC-MS/MS system. Transition m/z 320 → 70 can be used for quantitation and transition m/z 320 → 125 can be used for confirmation. However, only quantitation transition was validated. Analysis was accomplished on an Agilent Zorbax SB reversed-phase C_{18} column applying an acetonitrile/water gradient using 0.1% formic acid as modifier.

Recovery findings

In both matrices tested, the mean recovery values were between 70% and 110%. The detailed results are given in the table below.

Table 4.2-11: Validation results of QuEChERS-based method: metconazole (BAS 555 F) in oily matrices

Matrix	Analyte	Mass transition	Fortification level (mg/kg)	No of replicates	Mean recovery (%)	RSD (%)
Avocado	Metconazole	320 → 70	0.010	5	95	4
			0.050	5	88	2
			Overall	10	n.r.	n r.
Almond	Metconazole	320 → 70	0.010	5	79	2
			0.050	5	92	1
			Overall	10	n.r.	n r.

n.r. Not reported

Linearity
For metconazole (sum of *cis* and *trans*-isomers) good linearity was observed in the range tested (avocado: 0.5-250 µg/L; almond: 0.25-125 µg/L, corresponding to 1-500 µg/kg in the sample, respectively) with correlation coefficients >0.99. Seven calibration points distributed over this range were used.

Specificity
LC-MS/MS is a highly specific detection technique and therefore a confirmatory technique is not required. Analysis is possible at two ion transitions.

Matrix effects
Matrix effects (% = ((slope matrix/slope solvent)-1) x 100)) were not significant (i.e. <20%) in avocado. Medium matrix effects, i.e. enhancement of 27%, were observed in almonds.

Limit of Quantitation
The limit of quantitation was 0.010 mg/kg for metconazole in both matrices tested.

Repeatability
The relative standard deviations (RSD, %) for both commodities and fortification levels were <20%. The detailed values are shown in Table 4.2-13.

Reproducibility
The validated method was successfully employed in the analysis of real (monitoring) avocado and almond samples bought in local shops in Spain.

Stability of Working Solutions
Individual pesticide stock solutions (1-2 g/L) were prepared in acetonitrile and ethyl acetate and were stored in the dark at -20°C. Stability was not assessed. According to SANCO/825/00 rev. 8.1 the stability of the extracts was demonstrated by acceptable recoveries within 70 to 120%.

Conclusion
The QuEChERS-based multi-residue analytical method can be used for the analysis of metconazole (sum of *cis*- and *trans*-isomers) in oily matrices.

To provide supplementary information on a multi residue method, using acetone for sample extraction, the corresponding method summary is listed below.

Report:	CA 4.2/9 Hiemstra M.,Kok A. de, 2007b Comprehensive multi-residue method for the target analysis of pesticides in crops using liquid chromatography-tandem mass spectrometry (Journal of Chromatography A, 1154 (2007) 3–25) 2007/1070965
Guidelines:	None
GLP:	no

Principle of the method

Metconazole (sum of *cis*- and *trans*-isomers) residues were extracted from homogenized plant samples with acetone and partitioned in dichloromethane and light petroleum. After centrifugation, the extracts were evaporated to dryness and re-dissolved in methanol acidified with acetic acid for LC-MS/MS analysis.

In case of non-compliance with the MRL for pesticides with an average recovery lower than 70% obtained with the extraction method describes above, this method was modified by adding sodium sulphate to the acetone for extraction and proceeding in the same way.

Transition m/z 320 → 70 was used for quantitation. Analysis was accomplished on an Alltima C_{18} column applying a methanol/5 mM ammonium formate gradient.

Recovery findings

In all matrices tested, the mean recovery values were between 70% and 110%. The detailed results are given in the table below.

Table 4.2-12: Validation results of multi-residue LC-MS/MS method: metconazole (BAS 555 F) in plant matrices

Matrix	Analyte	Mass transition	Fortification level (mg/kg)	No of replicates	Mean recovery (%)	RSD (%)
Lettuce	Metconazole	320 → 70	0.01	6	95	10
			0.05	6	78	5
			0.10	6	83	5
			Overall	18	n.r.	n r.
Orange	Metconazole	320 → 70	0.01	6	84	7
			0.02	6	88	3
			0.10	6	85	1
			Overall	18	n.r.	n r.
Apple	Metconazole	320 → 70	0.01	6	99	5
			0.02	6	98	2
			0.10	6	101	2
			Overall	18	n.r.	n r.
Cabbage	Metconazole	320 → 70	0.01	6	89	4
			0.02	6	96	3
			0.10	6	93	2
			Overall	18	n.r.	n r.
Grape	Metconazole	320 → 70	0.01	6	89	7
			0.02	6	98	4
			0.10	6	98	5
			Overall	18	n.r.	n r.
Wheat flour	Metconazole	320 → 70	0.01	6	90	1
			0.02	6	86	3
			0.10	6	90	8
			Overall	18	n.r.	n r.

n.r. Not reported

Linearity
For metconazole (sum of *cis* and *trans*-isomers) good linearity was observed in the range tested (1-200 ng/mL) with correlation coefficients >0.99. Seven calibration points distributed over this range were used.

Specificity
LC-MS/MS is a highly specific detection technique and therefore a confirmatory technique is not required. However, only one ion transition was validated.

Matrix effects
Calibration curve response factors (slope) did not change significantly between the different matrices and solvent, thereby demonstrating negligible to moderate matrix effects. During routine analyses of regulatory samples, moderate matrix effects, on average ranging from +10 to -20% were observed for all calibration levels.

Limit of Quantitation
The limit of quantitation was 0.01 mg/kg for metconazole in all matrices tested.

Repeatability
The relative standard deviations (RSD, %) for all commodities and fortification levels were <20%.

Reproducibility
Over a 3-year period (2004-2006), quality control samples were prepared with varying matrices and fortification levels (0.01-0.05 mg/kg). Good recoveries and intra-laboratory reproducibility were obtained:
in 2004, 69 samples were fortified at 0.05 mg/kg, giving a recovery of 93% and RSD of 7%;
in 2005, 124 samples were fortified at 0.01 mg/kg, giving a recovery of 93% and RSD of 11%;
in 2006, 105 samples were fortified at 0.05 mg/kg, giving a recovery of 93% and RSD of 8%
Furthermore, the e accuracy, selectivity and robustness was demonstrated by a 1-year comparison of its analytical results with those obtained from validated GC and LC multi-residue methods applied to more than 3500 routine samples taken from the Dutch market. No false positive or negative results were generated for pesticides in fruits and vegetables above the 0.01 mg/kg levels (LOQ).

Stability of solutions
Individual pesticide stock and standard solutions were prepared in toluene, methanol or acetone and were stored in the dark at -18°C for a maximum period of 5 and 1 year, respectively. All freshly prepared solutions were checked for accuracy (±5%) with those to be discarded. Calibration solutions (serial dilutions of the standard solutions) were determined to be stable at 4°C in the dark for three months.

Conclusion
The multi-residue analytical method for the analysis of metconazole (sum of *cis*- and *trans*-isomers) in plant matrices is suitable for the application in pesticide residue/monitoring programs.

(b) Methods for the analysis in soil and water

Soil

Suitable methods (DocID's 2013/1376999 and 2013/1377001) for the monitoring of metconazole in soil are already described in section M-CA 4.1.2, Methods for Risk Management. These methods are also supposed to be used for post-approval control and monitoring purposes, if needed.

Water

A suitable method (DocID 2015/7000428) for the monitoring of metconazole in water is already described in section M-CA 4.1.2, Methods for Risk Management. This method is also supposed to be used for post-approval control and monitoring purposes.
The corresponding independent laboratory validation of this method is described below.

Independent laboratory validation of method D1501/01 (L0273/01)

Report:	CA 4.2/10 Perez R., 2015b Independent Laboratory Validation of Method D1501/01 (L0273/01): Method for the determination of Enantiomers of BAS 555 F (Reg.No.5836046, Reg.No.4677200, Reg.No.5836047 and Reg.No.5836048) in Surface and Drinking Water by LC-MS/MS 2015/7000429*)
Guidelines:	EPA 850.6100, SANCO/825/00 rev. 8.1 (16 November 2010), OECD-ENV/JM/MONO/(2007)17
GLP:	yes (certified by United States Environmental Protection Agency)

Principle of the method
The objective of this independent laboratory validation (ILV) study was to demonstrate that BASF Analytical Method D1501/01 (L0273/01) could successfully determine residues of metconazole (BAS 555 F) enantiomers M555F000-RS (Reg. No. 5836046), M555F000-SS (Reg. No. 5836047), M555F000-RR (Reg. No. 5836048) and M555F000-SR (Reg. No. 4677200) in surface and drinking water. The study was performed by ADPEN Laboratories, Inc., Jacksonville, USA.
A sample aliquot is directly injected for analysis by LC-MS/MS. Two ion transitions (for quantitation and confirmatory purposes) are observed for the detection.

Recovery findings

The method is suitable to determine residues of the enantiomeric forms of metconazole in surface and drinking water. Water samples are fortified with the analytes at the limit of quantification of 25 ng L^{-1} and ten times higher (250 ng L^{-1}). Mean recovery values (mean of five replicates per fortification level and analyte) are between 74% and 92% for all enantiomers of metconazole (see table below), which fulfils the legal requirements.

Table 4.2-13: Recoveries of Metconazole from Drinking Water and Surface Water

Analyte	Matrix	Fortification level (ng L^{-1})	Number of replicates	Recovery (%)	Mean Recovery (%)	Standard Deviation	% RSD	Overall Recovery (%)
Metconazole (+)trans M555F000-RR (Reg. No. 5836048)	Drinking water (well water)	Primary Quantitation (m/z 320→70)						
		25	5	73 - 90	77	7.0	9.0	76
		250	5	69 - 80	75	4.2	5.6	
		Confirmatory Quantitation (m/z 322→70)						
		25	5	77 - 91	82	5.7	7.0	80
		250	5	72 – 81	78	3.3	4.2	
	Surface water (pond water)	Primary Quantitation (m/z 320→70)						
		25	5	80 – 97	87	6.3	7.2	87
		250	5	81 - 89	86	3.1	3.5	
		Confirmatory Quantitation (m/z 322→70)						
		25	5	84 - 94	90	4.3	4.8	87
		250	5	81 - 86	84	2.3	2.7	
Metconazole (+)cis M555F000-RS (Reg. No. 5836046)	Drinking water (well water)	Primary Quantitation (m/z 320→70)						
		25	5	74 – 86	80	5.3	6.6	79
		250	5	72 – 83	77	4.7	6.1	
		Confirmatory Quantitation (m/z 322→70)						
		25	5	71 - 85	78	5.8	7.4	78
		250	5	73 - 82	79	3.6	4.6	
	Surface water (pond water)	Primary Quantitation (m/z 320→70)						
		25	5	82 - 94	87	4.3	4.9	86
		250	5	82 - 90	85	3.4	4.0	
		Confirmatory Quantitation (m/z 322→70)						
		25	5	83 - 91	88	3.1	3.6	87
		250	5	83 - 93	87	4.3	5.0	
Metconazole (-)cis M555F000-SR (Reg. No. 4677200)	Drinking water (well water)	Primary Quantitation (m/z 320→70)						
		25	5	78 – 92	84	6.0	7.1	80
		250	5	71 – 84	76	5.4	7.1	
		Confirmatory Quantitation (m/z 322→70)						
		25	5	71 - 114	85	17.8	20.9	80
		250	5	69 - 79	75	4.3	5.7	
	Surface water (pond water)	Primary Quantitation (m/z 320→70)						
		25	5	83 – 95	87	4.8	5.5	88
		250	5	82 – 95	89	4.9	5.5	
		Confirmatory Quantitation (m/z 322→70)						
		25	5	85 - 99	92	4.9	5.4	90
		250	5	84 - 90	88	2.4	2.8	
Metconazole (-)trans M555F000-SS (Reg. No. 5836047)	Drinking water (well water)	Primary Quantitation (m/z 320→70)						
		25	5	70 - 81	74	5.4	7.3	75
		250	5	72 - 80	75	3.5	4.6	
		Confirmatory Quantitation (m/z 322→70)						
		25	5	70 - 83	74	5.5	7.5	76
		250	5	72 - 86	79	5.3	6.8	
	Surface water (pond water)	Primary Quantitation (m/z 320→70)						
		25	5	83 – 90	88	3.1	3.5	88
		250	5	83 - 90	87	3.1	3.6	
		Confirmatory Quantitation (m/z 322→70)						
		25	5	81 - 97	88	5.9	6.7	87
		250	5	83 - 95	86	4.7	5.5	

Linearity
Good linearity (r > 0.99) is observed in the range of 0.005 to 0.100 ng mL^{-1} for all mass transitions of each enantiomer of metconazole (RR, RS, SR, SS) using solvent-based standards (prepared in water).

Specificity
Significant interferences (> 30% of LOQ) were not observed at the retention time and mass transitions of the analytes.
LC-MS/MS is a highly specific self-confirmatory technique. Due to the high selectivity and specificity of LC MS/MS an additional confirmatory technique is not necessary.

Matrix Effects
Already tested in original validation study.

Limit of Quantification
The limit of quantification (LOQ) is defined by the lowest fortification level successfully tested, which is 25 ng L^{-1} per enantiomer of metconazole.

Limit of Detection
The limit of detection (LOD) is defined as 20% of the LOQ; equivalent to 5 ng mL^{-1} per enantiomer of metconazole, corresponding to the lowest calibration level used.

Stability of Working Solutions
Already tested in original validation study.

Extract Stability
Already tested in original validation study.

Repeatability
The overall relative standard deviations (mean of the two fortification levels per ion transition and analyte) (RSD, %) are below 20%.

Reproducibility
The method was successfully tested in this independent laboratory validation. Therefore the method applied in M-CA 4.1.2/3 (DocID 2015/7000428) could be confirmed.

Conclusion
BASF Analytical Method D1501/01 (L0273/01) for the analysis of metconazole (BAS 555 F) enantiomers M555F000-RS (Reg. No. 5836046), M555F000-SS (Reg. No. 5836047), M555F000-RR (Reg. No. 5836048) and M555F000-SR (Reg. No. 4677200) in surface water and drinking water uses HPLC-MS/MS for final determination, with a limit of quantification of 25 ng L^{-1} for each enantiomer.
It could be demonstrated that the method fulfils the requirements with regard to specificity, repeatability, limit of quantification and recoveries and is therefore applicable to correctly and accurately determine residues of the enantiomers of metconazole in water matrices.

(c) Methods for the analysis in air

A suitable method (DocID 2014/1045705) for the monitoring of metconazole in air is already described in section 4.1.2, Methods for Risk Management. This method is also supposed to be used for post-approval control and monitoring purposes.

(d) Methods for the analysis in body fluids and tissues

No analytical method is needed for metconazole in body fluids and tissues since it is not regarded as toxic.

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Metconazole

Document M-CA, Section 5

TOXICOLOGICAL AND METABOLISM STUDIES ON THE ACTIVE SUBSTANCE

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Version history[1]

Date	Data points containing amendments or additions and brief description	Document identifier and version number
27/Jan/2016	The following studyheader were included: CA 5.6.1/2 BASF DocID 2006/8000261 CA 5.6.2/2 BASF DocID 2006/8000263 CA 5.6.2/3 BASF DocID 2008/8000121 CA 5.6.2/7 BASF DocID 2012/8000573 CA 5.7.1/2 BASF DocID 2002/8000062 CA 5.7.1/4 BASF DocID 2002/8000063 CA 5.8.2/5 BASF DocID 2006/8000262 CA 5.8.2/7 BASF DocID 2010/8000287 Cross References: CA 5.6.1/3 BASF DocID 2015/1276507 CA 5.6.1/4 BASF DocID 2016/1025262 CA 5.6.2/9 BASF DocID 2016/1025284	Document MCA Section 5 Version 2 (BASF DocID 2016/1030852)

[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 How to revise an Assessment Report

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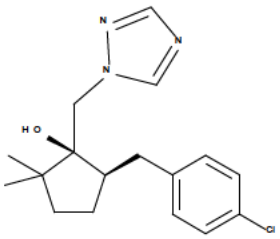
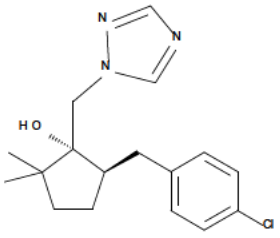
CA 5 TOXICOLOGICAL AND METABOLISM STUDIES ON THE ACTIVE SUBSTANCE

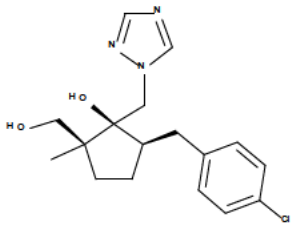
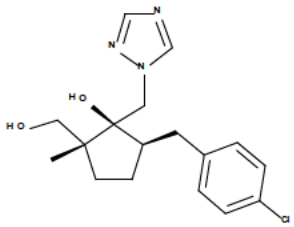
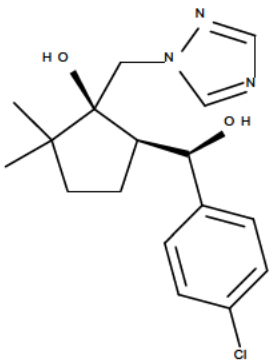
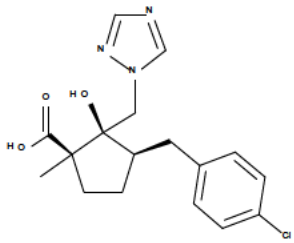
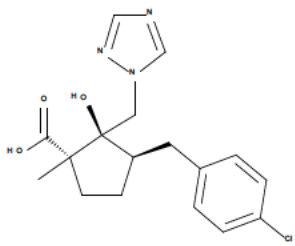
CA 5.1 Studies on Absorption, Distribution, Metabolism and Excretion in Mammals

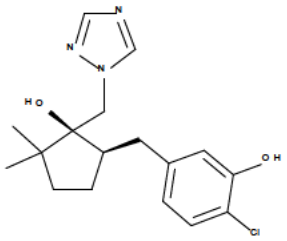
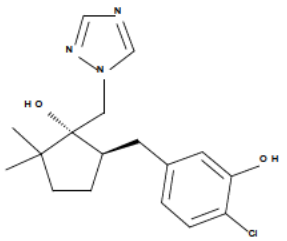
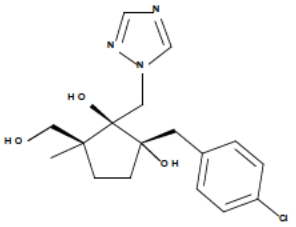
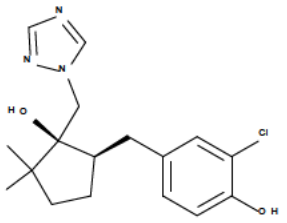
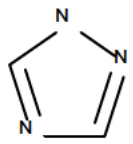
An overview on the metabolites identified in the three rat metabolism studies from 1991 is provided in the table below. In order to increase the readability of the table, information from other test systems have been deleted. If not stated otherwise in the occurrence column, the metabolites indicated for rats have been found in the corresponding *in-vivo* studies (BASF DocID MK-440-004, MK 440-007 and MK-440-009) evaluated in the Annex II approval process.

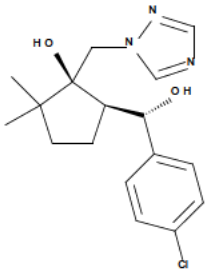
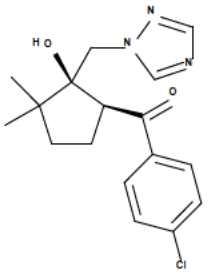
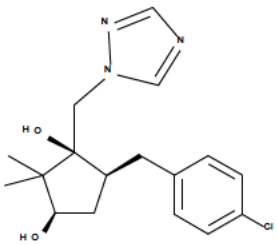
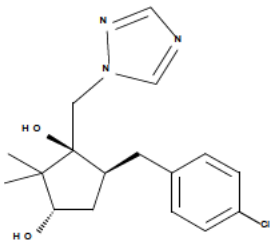
The short metabolite codes, such as M1 or M30, used in this document are consistent with the codes used in the metabolism studies. The formal BASF codes, BASF Reg. No. (Signify a synthesized metabolite) and the CL number used in early metabolism studies are included in the metabolite table (Table 5.1-1) with structures to allow easy cross-reference.

Table 5.1-1: Notations of parent and metabolites of metconazole (BAS 555 F)

Metabolite designation		**Chemical Name**	**CAS-No.**	**Structure**	**Rat matrix (% AD)**
Code	**Other code (Reg. No.)**	**IUPAC Name**			
M555F000 cis	4079468 CL 354801	cis-1-(1H-1,2,4-triazole-1-ylmethyl)-2,2-dimethyl-5-(4-chlorobenzyl)cyclopentanol	115850-27-6		Urine: 1 nd 2 nd 3 nd .Feces: 1 nd 2 nd 3. 2(m)2 (f)
M555F000 trans	4079654	trans-1-(1H-1,2,4-triazole-1-ylmethyl)-2,2-dimethyl-5-(4-chlorobenzyl)cyclopentanol	115850-28-7		Urine nd Feces: nd

Metabolite designation		Chemical Name	CAS-No.	Structure	Rat matrix (% AD)
Code	Other code (Reg. No.)	IUPAC Name			
M555F001 cis	M1 (4111795) CL 359451	(1SR,2SR,5RS)-5-(4-chlorobenzyl)-2-(hydroxymethyl)-2-methyl-1-(1H-1,2,4-triazol-1-ylmethyl)cyclopentanol	155413-23-3		Urine: 1. nd 2. nd 3. 5(m), 14(f) Feces: 1.14 2.12(m)13(f) 3. 21(m) 15 (f)
M555F002 cis	M2 (4111882 CL 359452)	(1SR,2RS,5RS)-5-(4-chlorobenzyl)-2-(hydroxymethyl)-2-methyl-1-(1H-1,2,4-triazol-1-ylmethyl)cyclopentanol	155488-30-5		Urine: 1 .nd 2. nd 3. 5(m), 14(f) A) Feces: 1. 5 2. 4(m) 2(f) 3. 3(m) 3 (f)
M555F011 cis	M11 (4111112) CL 382390	(1RS,5SR)5-[(SR)-(4-chlorophenyl)(hydroxy)methyl]-2,2-dimethyl-1-(1H-1,2,4-triazol-1-ylmethyl)cyclopentanol	153322-43-1		Urine: 1. nd 2. nd 3. nd Feces: 1. nd 2. nd 3. nd
M555F012 cis	M12 (4543815) CL 359138	(1RS,2SR,3RS)-3-(4-chlorobenzyl)-2-hydroxy-1-methyl-2-(1H-1,2,4-triazol-1-ylmethyl)cyclopentanecarboxylic acid			Urine: 1. 5 2. 1(m) 8(f) 3. nd Feces: 1. 10 2. 10(m) 14 (f) 3. 2(m) 7 (f)
M555F013 cis	M13 (4543816) CL 359139	(1SR,2SR,3RS)-3-(4-chlorobenzyl)-2-hydroxy-1-methyl-2-(1H-1,2,4-triazol-1-ylmethyl)cyclopentanecarboxylic acid			Urine: 1. 1 2. nd 3. nd Feces: 1. 4 2. 3 3. nd

Metabolite designation		Chemical Name	CAS-No.	Structure	Rat matrix (% AD)
Code	Other code (Reg. No.)	IUPAC Name			
M555F015 cis	M15 CL 359453	(1RS,5SR)-5-(4-chloro-3-hydroxybenzyl)-2,2-dimethyl-1-(1H-1,2,4-triazol-1-ylmethyl)cyclopentanol	171438-41-8		Urine: 1. nd 2. nd 3. 5(m), 14(f) A) Feces: 1. 1 2. 1(m) 1(f) 3. 3(m) 2(f)
M555F015 trans	6011738	2-chloro-5-{[(1RS,2RS)-2-hydroxy-3,3-dimethyl-2-(1H-1,2,4-triazol-1-ylmethyl)cyclopentyl]methyl}phenol			Urine: 1. nd 2. nd 3. nd Feces: 1. nd 2. nd 3.
M555F018 cis	M18				Urine: 1. nd 2. nd 3. nd Feces: 1. 1 2. nd 3 nd(m) 1(f)
M555F019 cis	M19 (4111113) CL 395838	(1RS,5SR)-5-(3-chloro-4-hydroxybenzyl)-2,2-dimethyl-1-(1H-1,2,4-triazol-1-ylmethyl)cyclopentanol			Urine: 1. nd 2. nd 3. 5(m), 14(f) A) Feces: 1. 6 2. 3(m) 9(f) 3. 8(m) 8(f)
M555F020	M20 87084	1,2,4-(1H)-triazole	288-88-0		Urine: 1. 5 Feces: 1. nd

Metabolite designation		Chemical Name	CAS-No.	Structure	Rat matrix (% AD)
Code	Other code (Reg. No.)	IUPAC Name			
M555F021 cis	M21 4558878 CL 382391	(1RS,5SR)-5-[(RS)-(4-chlorophenyl)(hydroxy)methyl]-2,2-dimethyl-1-(1H-1,2,4-triazol-1-ylmethyl)cyclopentanol	153208-75-4		Urine: 1. nd 2. nd 3. nd Feces: 1. 2 2.1 (m) <1(f) 3. 6(m) 1(f)
M555F030 cis	M30 4110625 CL 382389	(1RS,5SR)-5-(4-chlorobenzoyl)-2,2-dimethyl-1-(1H-1,2,4-triazol-1-ylmethyl)cyclopentanol	153208-73-2		Urine: 1. nd 2. nd 3. nd Feces: 1 nd 2 nd 3. nd
M555F031 cis	M31 5968488	(1RS,3SR,5RS)-5-(4-chlorobenzyl)-2,2-dimethyl-1-(1H-1,2,4-triazol-1-ylmethyl)cyclopentane-1,3-diol			Urine: 1. nd 2. nd 3. nd Feces: 1. nd 2. nd 3. nd
M555F032 cis	M32 5968479	(1RS,3RS,5RS)-5-(4-chlorobenzyl)-2,2-dimethyl-1-(1H-1,2,4-triazol-1-ylmethyl)cyclopentane-1,3-diol			Urine: 1. nd 2. nd 3. nd Feces: 1. nd 2 nd 3 nd

A) As conjugates; the conjugated metabolite was tentatively quantified as 5% AD (male) and 14% AD (female).

CA 5.1.1 Absorption, distribution, metabolism and excretion by oral exposure

Studies submitted in context of the Annex I inclusion according to Directive 91/414

In order to provide information on the adsorption, distribution and excretion in rats, three studies were provided for the previous Annex I listing process and are considered as peer reviewed and still valid. The dosing details and excretion results of the studies are summarized in the table below.

Table 5.1.1-1: Details on dosing in absorption, distribution and excretion studies

Study	Nominal dose [mg/kg bw]		Number of doses	Sex, No. of animals	Samples measured in the study
5.5.1/1 MK-440-006	2	3,5-triazole (*cis*-isomer)	1	3 male 3 female	bile, urine, feces, GI tract
5.5.1/2 MK-440-002	2	Cyclopentyl (cis:trans 79:21)	1	5 male 5 female	urine, feces, tissues
5.5.1/3 MK-440-010	2	Cyclopentyl (*cis*-isomer)	14	6 male 6 female	urine, feces, tissues

The excretion distribution was similar and not affected if the dosed test substance was cis-isomer or a mixture of cis:trans isomer (79:21).

Table 5.1.1-2: Excretion comparison in absorption, distribution and excretion studies

Matrix	5.5.1/1 MK-440-006	5.5.1/2 MK-440-002	5.5.1/3 MK-440-010
^{14}C-Label	3,5-triazole (*cis*-isomer)	Cyclopentyl (cis:trans 79:21)	Cyclopentyl (*cis*-isomer)
Collection time point	0-48 hours	0-72 hours	0-96 hours
Bile male	78.7	Not collected	Not collected
Bile female	83.3	Not collected	Not collected
Urine male	4.3	14.8	14.8
Urine female	12.1	25.9	29.9
Feces male	0.2	80.3	82.2
Feces female	0.3	67.1	65.4

The following observations are taken from the EFSA Conclusion (EFSA 2006):

> "Metconazole cis, is well absorbed (95-97% after 48h); after 48h, up to 83% of radioactivity is eliminated in the bile, while up to 12% is eliminated renally, by 72h about 93-96% of metconazole (cis/trans) is excreted.
> Metconazole is widely distributed, but adrenals, gastrointestinal tract, and liver tended to have the highest amount of the compound."

Three studies were provided for the previous Annex I listing process to provide information on the metabolism of metconazole in rats (BASF DocID MK-440-004, MK 440-007 and MK-440-009), and these studies are considered as peer reviewed and still valid.

Dosing and dose groups
In Table 5.1.1-3, details on dosing are provided for those experiments which were used for metabolism investigations.

Table 5.1.1-3: Details on dosing in studies defining the metabolism of metconazole in rats

Study	Nominal dose [mg/kg bw]	^{14}C-Label	Number of doses	Sex, No. of animals	Samples investigated in the study
MK-440-004	200	3,5-triazole (*cis*-isomer)	1	Male, 6	urine, feces
MK-440-007	2	Cyclopentyl (cis:trans 79:21)	1	5 male 5 female	urine, feces, liver [A]
MK-440-009	164	Cyclopentyl (cis:trans 79:21)	1	5 male 5 female	urine, feces, liver [B], adrenal [A]

[A] Characterization was done of extracts but not identification of metabolites

[B] The extractability of the liver was investigated but no further characterization was done.

The level of each metabolite identified in the rat metabolism studies is included in Table 5.1-1.

In the EFSA Conclusion (EFSA 2006) the following was noted concerning metabolism in rat:

> "There was no indication that the metabolism in the rat of the isomer mix is different from that of the *cis*-isomer. The major metabolites were recovered in the faeces. No significant difference was generally observed between sexes, except for M20 (1,2,4-triazole) mainly in female rats, and M12 (a carboxylated derivative) at a relative high proportion in the urine of males in all metabolism studies.
> The parent compound was extensively metabolised as ≤2% of dose was recovered in the faeces.
> The metabolic breakdown was not affected by isomer ratio, sex, dose or pre-treatment.
> The main metabolites of metconazole in rat are:
> - monohydroxy-metabolites, (M1 and M21)
> - hydroxyphenyl-metabolites (M15 and M19)
> - carboxy-metabolites (M12 and M13)
> - multi hydroxy metabolites (M18)
> - mixed-function metabolites
> - various sulphate conjugates of the abovementioned metabolites (M22)"

CA 5.1.2 Absorption, distribution, metabolism and excretion by other routes

Study submitted in context of the Annex I inclusion according to Directive 91/414

Dermal penetration

In order to provide information on the adsorption, distribution and excretion of metconazole in rats following dermal exposure, one study was provided for the previous Annex I listing process. A nominal dose level of 1.5 mg per animal within a 10.2 cm^2 area of skin (equivalent to 0.147 mg/cm^2) was tested in female Fisher 344 rats. Exposure times of 0, 4 and 8 hours were used and the study duration varied from 0-72 hours. Very low systemic absorption was found with the rate of penetration increasing over the first 24 hours. The study is considered as peer reviewed and still valid.

Comparative in-vitro metabolism studies

According to the new data requirements for active ingredients of plant protection products as set out in Commission Regulation (EU) No. 283/2013 (1 March 2013, OJ L93, 1ff, 3.4.2013), "comparative *in vitro* metabolism studies shall be performed on animal species ... and on human material ...in order to determine the relevance of the toxicological animal data and to guide in the interpretation of findings and in further definition of the testing strategy..." (Section 5, Toxicological and metabolism studies, point 5.1.1., page 22).

In the absence of validated test methods or guidance documents this data requirement is waived in accordance to SANCO Guidance Document SANCO/10181/2013-rev 2.1 (13 May 2013).

CA 5.2 Acute Toxicity

Studies evaluated in the draft monograph by the rapporteur member state Belgium (January, 2004):

Metconazole (BAS 555 F) has been tested in various species and via different routes of administration. Studies were carried out with WL 136184 (95/0.1 cis/trans isomer mixture = cis isomer) and/or BAS 555 F (WL 148271, 80/15 cis/trans mixture, technical grade). All studies are scientifically valid. The studies listed in Table 5.2-1 have been evaluated and peer reviewed during the previous Annex I inclusion process. Brief summaries of the respective studies were extracted from the monograph of metconazole and are presented under the respective chapters.

Table 5.2-1: Summary of already peer-reviewed acute toxicity studies with metconazole

Route/species/sex	Purity (%), cis/trans ratio, batch no.	Dose range / Vehicle	Result	Reference (BASF DocID)
Metconazole cis/trans				
Oral Rat, Fischer 344, m/f	95.3%, cis/trans: 79.8/15.5 batch: 89-01	255, 357, 500, 700, 980 mg/kg bw in corn oil	LD_{50} (m) = 727 mg/kg bw LD_{50} (f) = 595 mg/kg bw LD_{50} (f+m) = 660 mg/kg bw	[redacted] 1990(a) (MK-411-001)
Oral Mouse, CD-1, m/f	95.3%, cis/trans: 79.8/15.5 batch: 89-01	391, 625, 1000, 1600 mg/kg bw in corn oil	LD_{50} (m) = 718 mg/kg bw LD_{50} (f) = 410 mg/kg bw LD_{50} (m+f) = 566 mg/kg bw	[redacted] 1990(a) (MK-411-001)
Dermal Rat, Fischer 344, m/f	95.3%, cis/trans: 79.8/15.5 batch: 89-01	2000 mg/kg bw moistened with water	LD_{50} (m, f) > 2000 mg/kg bw	[redacted] 1990(a) (MK-411-001)
Dermal Rabbit, NZW, m/f	95.3%, cis/trans: 79.8/15.5 batch: 89-01	2000 mg/kg bw moistened with water	LD_{50} (m, f) > 2000 mg/kg bw	[redacted] 1990(a) (MK-411-001)
Inhalation (head-only) Rat, Sprague-Dawley, m/f	95.3%, cis/trans: 79.8/15.5 batch: 89-01	5.588 mg/L air (4 h)	LC_{50} (m, f) > 5.588 mg/L air	[redacted] 1990 (MK-413-001)
Skin irritation Rabbit, NZW, m/f	95.3%, cis/trans: 79.8/15.5 batch: 89-01	0.5 g moistened with water	Not irritating	[redacted] 1990(b) (MK-415-001)
Eye irritation Rabbit, NZW, m/f	95.3%, cis/trans: 79.8/15.5 batch: 89-01	65 mg (0.1 mL bulk volume)	Not irritating	[redacted] 1990(b) (MK-415-001)
Skin sensitization (modified Buehler Test) Guinea pig, Dunkin-Hartley, m/f	95.3%, cis/trans: 79.8/15.5 batch: 89-01	Induction/challenge: 60% as Vaseline paste	Not sensitising	[redacted] 1990(b) (MK-415-001)
Skin sensitisation, (Maximisation Test) Guinea pig, albino Hartley, m	97.4%, cis/trans: 83.7/13.7 batch: AC 9339-114	Intradermal (5 and 10%; 0.1 mL): in mixture Freund's adjuvant /mineral oil Epidermal/challenge: 25% in petrolatum	Not sensitising	[redacted] 1995 (MK-416-001)

Table 5.2-1: Summary of already peer-reviewed acute toxicity studies with metconazole

Route/species/sex	Purity (%), cis/trans ratio, batch no.	Dose range / Vehicle	Result	Reference (BASF DocID)
Metconazole cis				
Oral Rat, Fischer 344, m/f	95.29%, cis/trans: 95.2/0.1 batch: 12	850, 1190, 1666, 2332 mg/kg bw in corn oil	LD_{50} (m) = 1627 mg/kg bw LD_{50} (f) = 1312 mg/kg bw LD_{50} (m+f) = 1459 mg/kg bw	[redacted] 1991 (MK-410-001)
Dermal Rat, Fischer 344, m/f	95.29%, cis/trans: 95.2/0.1 batch: 12	2000 mg/kg bw moistened with water	LD_{50} (m, f) > 2000 mg/kg bw	[redacted] 1991 (MK-410-001)
Skin irritation Rabbit, NZW, m/f	95.29%, cis/trans: 95.2/0.1 batch: 12	0.5 g Moistened with water	Not irritating	[redacted] 1991 (MK-410-001)
Eye irritation Rabbit, NZW, m/f	95.29%, cis/trans: 95.2/0.1 batch: 12	42 mg (0.1 mL bulk volume)	Not irritating	[redacted] 1991 (MK-410-001)
Skin sensitization (Buehler Test) Guinea pig, Dunkin-Hartley, m/f	95.29%, cis/trans: 95.2/0.1 batch: 12	Induction/challenge: 50% as Vaseline paste	Not sensitising	[redacted] 1991 (MK-410-001)

Based on the data available at that time, the following EU agreed endpoints were given in the EFSA Scientific Report (64, 1-71) from 2006:

Acute toxicity

Rat LD_{50} oral:	595 mg/kg bw R22
Mouse LD50 oral	410 mg/kg bw
Rabbit LD_{50} dermal:	> 2000 mg/kg bw
Rat LC_{50} inhalation:	>5.6 mg/L air
Skin irritation:	Not irritant
Eye irritation:	Not irritant
Skin sensitization (M&K/Buehler):	Not sensitizing

Studies submitted in this AIR 3 dossier (not yet peer-reviewed):

The existing acute toxicity data package was corroborated by a complete set of new in vivo acute studies on metconazole cis/trans, which was performed as a request for the registration in a non-EU region. With regard to skin sensitisation, two Buehler tests were conducted on two different batches of metconazole cis/trans (for details of batch/impurity composition please refer to document J). All these studies were performed after the last submission in EU.

In accordance with the data requirements in Commission Regulation (EU) No. 283/2013 (1 March 2013) an in vitro NRU-Phototoxicity study in Balb/c 3T3 cells was not performed with metconazole, since the respective circumstances under which such a study is required are not met. All new studies are submitted within the AIR III process and are listed in Table 5.2-2. Detailed study summaries are presented under the respective chapters.

Table 5.2-2: Summary of not yet peer-reviewed acute toxicity studies with metconazole cis/trans

Route/species/sex	Purity (%), cis/trans ratio, batch no.	Dose range / Vehicle	Result	Reference (BASF DocID)
Oral Rat, Wistar, f	98%, cis/trans:83.7/14.4 batch: 42704	500, 2000 mg/kg bw in olive oil	500 mg/kg bw < LD_{50} < 2000 mg/kg bw	[redacted]., 2005(a) (2005/1005772)
Dermal Rat, Wistar, m/f	98%, cis/trans:83.7/14.4 batch: 42704	2000 mg/kg bw in CMC (0.5%)	LD_{50} > 2000 mg/kg bw	[redacted]., 2005(b) (2005/1005773)
Inhalation (head/nose-only) Rat, Wistar, m/f	98%, cis/trans:83.7/14.4 batch: 42704	5.2 mg/L air (4 h)	LC_{50} > 5.2 mg/L air	[redacted] 2005(c) (2005/1013230)
Skin irritation Rabbit, NZW, m/f	98%, cis/trans:83.7/14.4 batch: 42704	0.5 g	Not irritating	[redacted] 2005(a) (2005/1005775)
Eye irritation Rabbit, NZW, m/f	98%, cis/trans:83.7/14.4 batch: 42704	32 mg (0.1 mL bulk volume)	Not irritating	[redacted], 2005(b) (2005/1005774)
Skin sensitization (Buehler Test) Guinea pig, Dunkin-Hartley, f	98.6%, cis/trans:83.5/15.1 batch: 39513	Induction/challenge (0.5 mL): 50% in CMC (1%)	Not sensitising	[redacted] 2005(d) (2005/1020108)
Skin sensitization (Buehler Test) Guinea pig, Dunkin-Hartley, f	99.1%, cis/trans:85/14.1 batch: 43707	Induction/challenge (0.5 mL): 50% in CMC (1%)	Not sensitising	[redacted] 2005(e) (2005/1020109)

Considering all available studies, metconazole technical (cis/trans) shows moderate acute oral toxicity in rats, justifying a classification with **Acute Tox.4 (H302).** Acute dermal and inhalation toxicity of metconazole is low. It is neither a skin nor an eye irritant in rabbits. Furthermore, metconazole showed no skin sensitising properties in the guinea pig Maximisation test and Buehler test. Overall, the new studies confirmed the results previously reported and evaluated on the cis/trans metconazole.

Under consideration of all available data, the classification of metconazole will not differ under Reg. EC 1272/2008 (CLP) compared to the EU agreed endpoints given in the EFSA Scientific Report (64, 1-71) from 2006. The list of endpoints will be adapted accordingly as shown below:

Acute toxicity (Regulation (EU) N° 283/2013, Annex Part A, point 5.2)

Rat LD_{50} oral	500 mg/kg bw < LD_{50} < 2000 mg/kg bw	**Acute Tox. 4 (H302)**
Rat LD_{50} dermal	> 2000 mg/kg bw	No classification required
Rat LC_{50} inhalation	> 5.2 mg/L air /4h (head-nose)	No classification required
Skin irritation	Non-irritant	No classification required
Eye irritation	Non-irritant	No classification required
Skin sensitisation	Not sensitising (Max. test & Buehler test)	No classification required
Phototoxicity	Not required	No classification required

For the convenience of the reviewer brief summaries of the respective studies as extracted from the monograph of metconazole are provided below together with the full summaries of recently conducted studies.

CA 5.2.1 Oral

Acute oral toxicity, rat and mouse –Metconazole cis/trans (1990(a); BASF DocID MK-411-001)

Guidelines: In compliance with the test method B.1 of directive 92/69/EEC
GLP: Yes
Acceptance: The study was considered acceptable in the EU registration process 2004.

Rat study

Groups of 5 male and 5 female Fischer 344 rats received metconazole (95.3%, cis/trans: 79.8/15.5; batch: 89-01) by gavage at dose levels of 255, 357, 500, 700 and 980 mg/kg bw. The test material was administered as suspensions in corn oil. Animals were observed for a period of 14 days.

Mortality was observed between 2 and 7 days following treatment at dose levels of 500 mg/kg bw (1/5 males and 3/5 females), 700 mg/kg bw (1/5 males and 2/5 females), and 980 mg/kg bw (5/5 males and 5/5 females).

Clinical signs of toxicity in decedents and survivors were first observed between 30 minutes and 2 hours after treatment in all groups and included unkempt appearance, lacrimation, diarrhea and hunched posture. In addition, ataxia, abasia, salivation, and chromodacryorrhea were observed at dose levels of 357 mg/kg bw and higher. Recovery of surviving rats was complete by 11 days after dosing.

Body weight gains in the surviving rats receiving 500 and 700 mg/kg bw were reduced from days 1 to 7, as compared to the animals receiving 255 mg/kg bw. However, all survivors gained weight during the 14-day observation period.

Necropsy findings in decedents showed abnormal livers (pale color, exaggerated lobular pattern), discoloration of the renal medulla, lung congestion, stomach inflammation, and abnormal contents of liquid/gas in the stomach and intestines. At terminal necropsy macroscopic abnormalities in surviving animals considered to be possible effects of treatment included softened, enlarged or discoloured livers, dark and hyperaemic medistinal lymph nodes and distension of the caecum and/or colon.

Conclusion

Under the conditions of this study, the oral LD_{50} for male and female rats was considered to be 727 mg/kg bw and 595 mg/kg bw, respectively. The combined LD_{50} was 660 mg/kg bw for both sexes.

Mouse study

Groups of 5 male and 5 female Albino CD-1 mice received metconazole (95.3%, cis/trans: 79.8/15.5; batch: 89-01) by gavage at dose levels of 391, 625, 1000 and 1600 mg/kg bw. The test material was administered as suspensions in corn oil. Animals were observed for a period of 14 days.

At the top dose level of 1600 mg/kg bw, all 10 mice died within 2-3 days after treatment. At 1000 mg/kg bw, 9 of 10 mice died also within 2-3 days. At 625 mg/kg bw, 2 of 5 male mice and all 5 female mice died between day 3-5. At the low dose of 391 mg/kg bw, a female mouse was found dead at post-treatment day 4.

Common signs of reaction upon treatment were abasia/ataxia, hunched posture, pallor of the skin and eyes and stereotype behavior (circling movements). Hyperactivity and hind-limb spasticity also occurred among mice, especially at the lower dose levels. Prostration, coma, hypothermia or cyanosis were observed, usually as a prelude to death, among mice at all dose levels. Occasionaly, unkempt appearance, piloerection, lethargy, abnormal posture (leaning), stereotype behavior (lateral rocking), tremor and convulsion were also observed. The more common clinical signs generally developed within 5 hours of dosing. Recovery of surviving mice, as judged by external appearance and behavior, was observed during day 4-7.

All surviving mice had gained body weight by day 14.

Among decedents, necropsy findings were darkening or exaggerated lobular pattern of the liver, discolouration of the renal medulla, lung congestion and inflammation and/or abnormal contents of the stomach. Among surviving mice sacrificed on day 14, enlarged or pale livers, distension of the caecum and, in a single animal, hyperaemia of the urinary bladder were observed.

Conclusion

Under the conditions of this study, the oral LD_{50} for male and female mice was considered to be 718 mg/kg bw and 410 mg/kg bw, respectively. The combined LD_{50} was 566 mg/kg bw for both sexes.

Acute oral toxicity, rat - Metconazole cis ([redacted] 1991; BASF DocID MK-410-001)

Guidelines: In compliance with the test method B.1 of directive 92/69/EEC
GLP: Yes
Acceptance: The study was considered acceptable in the EU registration process 2004.

Groups of 5 male and 5 female Fischer 344 rats received cis metconazole (95.29%, cis/trans: 95.2/0.09%; batch: 12) by gavage at dose levels of 850, 1190, 1666 and 2332 mg/kg bw. The test material was administered in corn oil at a constant dose volume of 10.0 mL/kg bw, and animals were observed for a period of 14 days. The applied dose levels were based on a preliminary range-finding study, at that 1 rat/sex was dosed at 400, 750, 1500 and 3000 mg/kg bw. The acute median lethal oral dose in a range-finding study was between 400 and 1500 mg/kg bw.

Mortality occurred in 1 male and 3 females of the 1190 mg/kg bw dose group on day 4. Three males (days 3 and 6) and 3 females (days 2 and 3) died in the 1666 mg/kg bw dose group and 4 males (days 3, 5, 6) and 5 females (days 3, 4, 5) died in the 2332 mg/kg bw dose group.
All treated rats showed unkempt appearance, staining (yellow) of the anogenital fur and a hunched posture, in addition to lethargy, salivation, lachrymation, periorbital encrustation and diarrhoea. Among rats dosed with 1190 mg/kg and above, the principal clinical signs were piloerection, abasia/ataxia or prostration. Tachypnoea and coma were apparent among rats that failed to survive treatment at the high dose-level. The onset of salivation, lachrymation, lethargy, hunched posture and diarrhoea occurred at all dose-levels within 2 hours of dosing. Onset of the other principal reactions to treatment was apparent within 5 hours of dosing. Recovery of rats surviving treatment, as judged by external appearance and behavior, was generally advanced by day 7 but was incomplete until day 15.
Although bodyweight reduction was recorded for several rats surviving treatment at 1190 mg/kg and above during the first week of the observation period, these animals had gained weight by the end of the 14 day-observation period.
The principal necropsy findings among decedents were exaggerated hepatic lobular pattern, areas of pallor/darkening of the liver, darkening of the thymus, spleen and kidneys, abnormal fluid contents of the gastro-intestinal tract and in 4 rats, inflammation of the stomach. Necropsy findings among the rats sacrificed on day 15 were limited to the presence of white/yellow areas on the liver of a single male rat treated at the high dose level.

Conclusion
Under the conditions of this study, the oral LD_{50} for male and female rats was considered to be 1627 mg/kg bw and 1312 mg/kg bw, respectively. The combined LD_{50} was 1459 mg/kg bw for both sexes.

Report: CA 5.2.1/1
[redacted]., 2005a
BAS 555 F - Acute oral toxicity study in rats
2005/1005772
Guidelines: OECD 423, EEC 2004/73 B.1 tris, EPA 870.1100
GLP: yes
(certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Executive Summary

Single doses of 500 and 2000 mg/kg bw of metconazole (98.0%, cis/trans: 83.7/14.4, batch: 42704) preparations in olive oil were administered to 3 treatment groups of three fasted female Wistar rats, each (2000 mg/kg bw in 3 females, 500 mg/kg bw in 6 females each) by gavage in a sequential manner. Animals were observed for 14 days. Two animals of the high dose group died at days 3 and 4. No mortality occurred in the 500 mg/kg bw dose groups. Accordingly, the oral LD_{50} was found to be greater than 500 mg/kg bw and less than 2000 mg/kg bw:

Rat, oral: 500 mg/kg bw < LD_{50} < 2000 mg/kg bw

Clinical signs of the high dose group included impaired and poor general state, dyspnea, abdominal position, staggering, piloerection, smeared fur, lacrimation and red clammy snout and eyelid. No clinical signs and findings were observed in animals treated with 500 mg/kg bw. The mean body weights of the treatment groups increased throughout the study period. No macroscopic pathologic abnormalities were noted in the animals that died or that were examined at the end of the observation period.
Under the conditions of this study the median lethal dose of metconazole (BAS 555 F) after oral administration was found to be greater than 500 mg/kg bw and less than 2000 mg/kg bw in rats.

(DocID 2005/1005772)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:	BAS 555 F (Metconazole)
Description:	Solid; crystalline powder/white
Lot/Batch #:	42704
Purity/content:	98% (cis:trans: 83.7/14.4)
Stability of test compound:	The stability of the test substance in the vehicle was determined indirectly by the concentration control analysis.
2. Vehicle:	Olive oil Ph. Eur./DAB
3. Test animals:	
Species:	Rat
Strain:	Wistar / Rcc:WIST(SPF)
Sex:	female
Age:	approximately 8 -12 weeks
Weight at dosing (range):	172 - 192 g
Source:	RCC Ltd Laboratory Animal Services, Wölferstrasse 4, CH-4414 Füllinsdorf, Switzerland
Acclimation period:	At least 5 days
Diet:	Kliba-Labordiät (Maus / Ratte Haltung “GLP”), Provimi Kliba SA, Kaiseraugst, Basel, Switzerland, ad libitum
Water:	Tap water, ad libitum
Housing:	Single housing in stainless steel wire mesh cages, type DK-III (Becker & Co., Castrop-Rauxel, FRG)
Environmental conditions:	
Temperature:	20 - 24 °C
Humidity:	30 - 70 %
Air changes:	Central air-conditioning
Photo period:	Alternating 12-hour light and dark cycles, artificial light from 6 am to 6 pm

B. STUDY DESIGN AND METHODS

1. Dates of work: 29-Nov-2004 - 1-Mar-2005

2. Animal assignment and treatment:

Single doses of 500 and 2000 mg/kg bw of test material preparations in olive oil were given to 3 administration groups of three fasted animals each (2000 mg/kg bw in 3 females, 500 mg/kg bw in 6 females) by gavage in a sequential manner. Clinical signs and symptoms were recorded several times on the day of administration and afterwards at least once each workday for the individual animals up to 14 days post-administration. A check for any dead or moribund animal was made twice each workday and once on weekends and public holiday. Individual body weights were determined shortly before administration, weekly thereafter and at the end of the study. The animals were sacrificed by CO_2-inhalation and subjected to necropsy including gross pathological examination on the last day of the observation period or as soon as possible after death in case of animals that died before.

II. RESULTS AND DISCUSSION

A. MORTALITY

Two animals of the 2000 mg/kg bw group were found dead on study days 3 and 4, respectively. No mortality occurred in the 500 mg/kg bw dose groups.

B. CLINICAL OBSERVATIONS

Clinical observations of animals treated with 2000 mg/kg bw revealed impaired and poor general state, dyspnea, abdominal position, staggering, piloerection, smeared fur, lacrimation and red clammy snout and eyelid. These signs were observed from hour 4 until study day 5 after administration.
No clinical signs were observed in the 500 mg/kg bw dose group.

C. BODY WEIGHT

The mean body weights of the surviving animals of the administration groups increased throughout the study period.

D. NECROPSY

No abnormalities were observed in the animals that died or in the surviving animals at gross necropsy.

III. CONCLUSION

Under the conditions of this study, the oral LD_{50} in rats administered cis/trans metconazole (BAS 555 F) was determined to be greater than 500 mg/kg bw and less than 2000 mg/kg bw.

CA 5.2.2 Dermal

Acute dermal toxicity, rat - Metconazole cis/trans (1990(a); BASF DocID MK-411-001)

Guidelines: In compliance with the test method B.3 of directive 92/69/EEC
GLP: Yes
Acceptance: The study was considered acceptable in the EU registration process 2004.

Groups of 5 male and 5 female Fischer 344 rats received a single dermal application of metconazole (95.3%, cis/trans: 79.8/15.5; batch: 89-01) at a dose level of 2000 mg/kg bw to the clipped dorsal skin as a dry powder moistened with deionized water for 24 hours under semi-occlusive conditions. Thereafter, test material was removed with warm dilute detergent solution, and animals were observed for a period of 14 day.

No mortalities or clinical signs of toxicity were observed during the study period. No dermal irritation was observed in any animal at the application sites. Bodyweight was not affected by the treatment. No relevant findings were recorded at necropsy.

Conclusion
Under the conditions of this study, the dermal LD_{50} for male and female rats was > 2000 mg/kg bw.

Acute dermal toxicity, rat - Metconazole cis (1991; BASF DocID MK-410-001)

Guidelines: In compliance with the test method B.3 of directive 92/69/EEC
GLP: Yes
Acceptance: The study was considered acceptable in the EU registration process 2004.

Groups of 5 male and 5 female Fischer 344 rats received a single dermal application of cis metconazole (95.29% pure, cis/trans: 95.2/0.09%; batch: 12) at a dose level of 2000 mg/kg bw to the clipped dorsal skin as a dry powder moistened with deionized water for 24 hours under semi-occlusive conditions. Thereafter, test material was removed with warm dilute detergent solution, and animals were observed for a period of 14 day.

No mortalities or clinical signs of toxicity were observed during the study period. Erythema was observed in 1/5 males and 2/5 females on day 2 but was recovered until day 3. Bodyweight was not affected by the treatment. No relevant findings were recorded at necropsy.

Conclusion
Under the conditions of this study, the dermal LD_{50} for male and female rats was > 2000 mg/kg bw.

Acute dermal toxicity, rabbit - Metconazole cis/trans [redacted] 1990(a); BASF DocID MK-411-001)

Guidelines: In compliance with the test method B.3 of directive 92/69/EEC
GLP: Yes
Acceptance: The study was considered acceptable in the EU registration process 2004.

Groups of 5 male and 5 female New Zealand White rabbits received a single dermal application of metconazole (95.3%, cis/trans: 79.8/15.5; batch: 89-01) at a dose level of 2000 mg/kg bw to the clipped dorsal skin as a dry powder moistened with deionized water for 24 hours under semi-occlusive conditions. Thereafter, test material was removed with warm dilute detergent solution, and animals were observed for a period of 14 day.

No mortalities or clinical signs of toxicity were observed during the study period. Sites of application showed no inflammatory reactions but desquamation affected 2 male rabbits during week 2 of the study. Bodyweight was not affected by the treatment. No relevant findings were recorded at necropsy.

Conclusion
Under the conditions of this study, the dermal LD_{50} for male and female rabbits was > 2000 mg/kg bw.

Report: CA 5.2.2/1
[redacted] 2005b
BAS 555 F - Acute dermal toxicity study in rats
2005/1005773
Guidelines: OECD 402, EPA 870.1200, EEC 92/69 B 3
GLP: yes
(certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Executive Summary

In an acute dermal toxicity study groups of 5 male and 5 female Wistar rats were exposed to 2000 mg/kg bw of metconazole (98.0%, cis/trans: 83.7/14.4, batch: 42704) preparation in 0.5% CMC solution in doubly distilled water. The preparation was applied to the clipped skin under semi-occlusive conditions for 24 hours. The animals were observed for 14 days after administration. No mortality occurred in any dose group. Accordingly, the dermal LD_{50} was determined to be greater than 2000 mg/kg bw:

Rat, dermal: LD_{50} > 2000 mg/kg bw

No systemic clinical observations or skin effects were noted in the animals. The mean body weights of the animals increased throughout the study period. No macroscopic pathologic abnormalities were noted in the animals at the end of the study.

DocID (2005/1005773)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:	BAS 555 F (Metconazol)
Description:	Solid; crystalline powder/white
Lot/Batch #:	42704
Purity/content:	98% (cis:trans ratio: 83.7/14.4)
Stability of test compound:	The stability of the test substance in the vehicle was determined indirectly by the concentration control analysis.
2. Vehicle:	0.5% CMC-solution (cleaned sodium carboxymethylcellulose) in doubly distilled water
3. Test animals:	
Species:	Rat
Strain:	Wistar / Rcc:WIST(SPF)
Sex:	male / female
Age:	male animals approx. 8 – 10 weeks, female animals approx. 12 - 14 weeks
Weight at dosing (mean):	males: 254 g, females: 224 g
Source:	RCC Ltd Laboratory Animal Services, Wölferstrasse 4, CH-4414 Füllinsdorf, Switzerland
Acclimation period:	At least 5 days
Diet:	Kliba-Labordiät (Maus / Ratte Haltung “GLP”), Provimi Kliba SA, Kaiseraugst, Basel, Switzerland, ad libitum
Water:	Tap water, ad libitum
Housing:	Single housing in stainless steel wire mesh cages, type DK-III (Becker & Co., Castrop-Rauxel, FRG)
Environmental conditions:	
Temperature:	20 - 24 °C
Humidity:	30 - 70 %
Air changes:	Central air-conditioning
Photo period:	Alternating 12-hour light and dark cycles, artificial light from 6 am to 6 pm

B. STUDY DESIGN AND METHODS

1. Dates of work: 07-Dec-2004 - 11-Mar-2005

2. Animal assignment and treatment:

Five male and five female rats were given a single application of 2000 mg/kg bw of the test material preparation in 0.5% CMC solution in doubly distilled water to the clipped epidermis on the dorsal and dorsolateral parts of the trunk (about 40 cm^2, corresponding to at least 10% of the body surface) for 24 hours. The fur was clipped one day before application of the test substance.
A bandage consisting of four layers absorbent gauze (Ph. Eur. Lohmann GmbH & co. KG) with the calculated amount of the test substance was applied to the test site. The application site was covered with a semi-occlusive dressing (Fixomull Stretch (adhesive fleece), Beiersdorf AG). After the exposure period, the dressing and the bandage were removed and residual test substance was rinsed with warm water.
Scoring of skin findings was performed 30-60 minutes after removal of the semi-occlusive dressing, weekly thereafter and at the end of the study period. Rats were observed for signs and symptoms several times on the day of application and afterwards at least once each workday for a total of 2 weeks. Individual body weights were determined shortly before application, weekly thereafter and at the end of the study. Necropsy with gross-pathology examination on the last day of the observation period was done after killing the animals with CO_2.

II. RESULTS AND DISCUSSION

A. MORTALITY
No mortality occurred.

B. CLINICAL OBSERVATIONS
No systemic clinical observations or skin effects were noted in the animals.

C. BODY WEIGHT
The mean body weights of the animals increased throughout the study period.

D. NECROPSY
No macroscopic pathologic abnormalities were noted in the animals examined at the end of the study.

III. CONCLUSION
Under the conditions of this study, the dermal LD_{50} in rats for BAS 555 F was determined to be greater than 2000 mg/kg bw.

CA 5.2.3 Inhalation

Acute inhalation toxicity, rat - Metconazole cis/trans (1991, BASF DocID MK-413-001)

Guidelines: In compliance with the test method B.2 of directive 92/69/EEC
GLP: Yes
Acceptance: The study was considered acceptable in the EU registration process 2004.

Groups of 5 male and 5 female Sprague Dawley rats (Crl:CD (SD) BR) were exposed to dust of metconazole (95.3%, cis/trans: 79.8/15.5; batch: 89-01) in a head-only inhalation system, at a nominal concentration of 18.702 equivalent to the analytical concentration of 5.588 mg/L (gravimetry) for 4 hours. A concurrent negative control group of 5 animals/sex was treated with filtered air. The mean mass median aerodynamic diameter of the particles in the atmosphere was 3.73 µm ± 1.40 µm.

No mortalities occurred during the study period. Clinical signs of toxicity consisted of piloerection and hunched posture on the day of exposure. Other clinical signs during days 0-12 included transient lethargy, sores on both front paws and unkempt fur.
Treated animals showed a small reduction in body weight gain, which persisted in week 2 of the study in males.
Necropsy findings revealed decreased lung weight of uncertain toxicological importance in the treated males but not the females. No treatment-related macroscopic abnormalities were observed.

Conclusion

Under the conditions of this study, the inhalation LC_{50} for male and female rats was > 5.588 mg/L air.

Report:	CA 5.2.3/1 [redacted] 2005c BAS 555 F - Acute inhalation toxicity study in Wistar rats - 4-hour dust exposure 2005/1013230
Guidelines:	OECD 403, EEC 67/548, EPA 870.1300
GLP:	yes (certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Executive Summary

In an acute inhalation toxicity study, groups of 5 male and 5 female Wistar rats were exposed to dust of cis/trans metconazole (98.0%, cis/trans: 83.7/14.4, batch: 42704) at a concentration of 5.2 mg/L for 4 hours. The animals were observed for 14 days after exposure.

No mortality occurred at the limit concentration of 5.2 mg/L. Accordingly, the acute inhalation LC_{50} for cis/trans metconazole after dust inhalation exposure was determined to be:

LC_{50} (male and female rats): > 5.2 mg/L

Clinical signs of toxicity in animals exposed to 5.2 mg/L comprised visually accelerated respiration, respiratory sounds, crust formation at the nose, apathy, eyelid closure, squatting posture, piloerection, smeared fur, exsiccosis, and reduced general state. Findings were observed from hour 0 of exposure until study day 14. The mean body weights of the male animals decreased during the first post exposure observation week but increased during the second week. The mean body weights of the female animals did not increase adequately during the first post exposure observation week but increased during the second week No gross pathological abnormalities were noted in the animals necropsied at termination of the post exposure observation period, except in one male animal diffuse red discoloration of all lung lobes and edema were observed. Cascade impactor measurements resulted in particle size distributions with a mass median aerodynamic diameters (MMAD) of 4.0 – 4.7 µm and a geometric standard deviation (GSD) of 3.0 – 3.1.

(DocID 2005/1013230)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: BAS 555 F

Description:	Solid; crystalline powder/white
Lot/Batch #:	42704
Purity/content:	98% (cis/trans: 83.7/14.4)
Stability of test compound:	Stable (Expiry date: 01-Nov-2008)

2. Vehicle: Test substance was applied unchanged.

3. Test animals:

Species:	Rat
Strain:	Wistar / RccHan^{TM}:WIST
Sex:	male and female
Age:	Males: approx. 8 weeks; females: approx. 11 - 12 weeks
Weight at dosing (mean):	Males: 283.9 g; females: 202.5 g
Source:	RCC Ltd Laboratory Animal Services; Wölfersraße 4, CH-4414 Füllinsdorf
Acclimation period:	at least 1 week
Diet:	KLIBA mouse / rat laboratory diet 10 mm pellets “GLP”, Provimi Kliba SA, Kaiseraugst, Basel Switzerland, ad libitum
Water:	Drinking water, ad libitum
Housing:	Singly in cages type DK III (Becker, Germany) without bedding.
Environmental conditions:	
Temperature:	20 - 24 °C
Humidity:	30 - 70%
Air changes:	No data. The animals were kept in fully air-conditioned rooms.
Photo period:	Alternating 12-hour light and dark cycles

B. STUDY DESIGN AND METHODS

1. Dates of experimental work: 27-Jan-2005 - 28-Apr-2015

2. Animal assignment and treatment:

For determination of the acute inhalation toxicity (head-nose inhalation, 4-hour-exposure) groups of five male and five female rats were exposed to 5.2 mg/L of the test substance. The test substance was desagglomerated in a mixer under addition of 1 % (w/w) of Aerosil® 200 before introduction into the dust generator, in order to improve dust aerosol formation. After exposure, animals were observed for 14 days. Individual body weights were recorded on arrival, shortly before exposure (day 0) and on days 7 and 14. Additionally, body weight was measured in animals that died from study day 1 onward. A check for overt clinical signs of toxicity or mortality as well as a check for the presence of feed and drinking water was made twice a day on workdays and once daily on weekends and public holidays. Detailed clinical observations were recorded for each animal separately several times during exposure and at least once on each workday of the observation period. At the end of the observation period the surviving animals that were sacrificed with CO_2 were subjected to gross-pathological examination.

3. Statistics/calculations:

The LC_{50} was calculated by Probit analysis by means of a computer program. For results of the type "LC_{50} greater than", "LC_{50} approx.", or "LC_{50} smaller than", the binomial test was used for statistical evaluation. The calculation of the particle size distribution was carried out in the inhalation laboratory on the basis of mathematical methods for evaluating particle measurements.

4. Generation of the test atmosphere and exposure:

The test substance was desagglomerated in a mixer under addition of 1% (w/w) of Aerosil® 200 before introduction into the dust generator, in order to improve dust formation. For each test group the dust was produced inside the dust pre-chamber with a dosing-wheel dust generator (Gericke/BASF) and compressed air and passed into the inhalation system. The concentrations of the dust in the atmosphere were adjusted by varying the apertural width and rotation of the dosing wheel. The exposure systems were located inside an exhaust cabin in an air-conditioned laboratory. A supply air flows (compressed air) of 1.5 m³/h were used for the exposures. The exhaust air flow was set to 1.35 m³/h. An air change of about 27 times per hour can be calculated by dividing the supply air flows by the volume of the inhalation systems. The lower amounts of exhaust air, which were adjusted by means of a separate exhaust air systems, achieved positive pressure inside the exposure systems. This ensured that the mixtures of test substance and air were not diluted with laboratory air in the breathing zones of the animals. The animals were exposed to the inhalation atmospheres for 4 hours plus equilibration time of the inhalation systems (t_{99} about 10 min).

5. Analytical investigation:

The flows of supply and exhaust air were adjusted and continuously measured with flowmeters (Rota). They were recorded four times in about 1-hour intervals. The temperature in the inhalation systems were measured continuously with a digital thermometer and recorded four times in about 1-hour intervals. The humidity in the inhalation system was measured with a dielectric probe four times in about 1-hour intervals. No surveillance of the oxygen content in the inhalation systems was performed. The air change was judged to be sufficient to prevent oxygen depletion by the breathing of the animals, and the concentrations of the test substance used could not have a substantial influence on oxygen partial pressure. The nominal concentration was calculated from the amounts of substance dosed and the supply air flows.

Gravimetric determination of the inhalation atmosphere concentration was performed with a balance Mettler AT 250. Pre-weighed filters were placed into the filtration equipment. By means of the vacuum pump metered volumes of the dust aerosol were drawn through the filter. For each sample the dust aerosol concentration in mg/L was calculated from the difference between the pre-weight of the filter and the weight of the filter after sampling, with reference to the sample volume of the inhalation atmospheres. Mean and standard deviation were calculated for the concentration from the results of the individual measurements. The mean concentration were corrected for the amount of additive used

6. Particle Size Analysis:

Before sampling, the impactor was assembled with pre-weighed glass-fiber collecting discs, and a backup particle filter. The impactor was connected to the vacuum pump and one sample per exposure was taken from the breathing zone of the animals starting not earlier than 30 minutes after the beginning of the exposure. The sample volume was 6 L. After sampling the impactor was taken apart. The collecting discs and the backup particle filter were re-weighed. The amounts of material adsorbed to the walls of the impactor and in the sampling probe (wall losses) were also determined quantitatively. The results from the particle size analysis were not corrected for the additive.

II. RESULTS AND DISCUSSION

A. MORTALITY

No lethality occurred at the tested concentration of 5.2 mg/L during the study period of 14 days. Therefore, the study satisfies the criteria of a limit test.

Table 5.2.3-1: Lethality in rats exposed for 4 hours to BAS 555 F as dust

Test group (mg/L)	Cumulated lethality		Time interval of lethality
	Males	Females	
5.2	0/5	0/5	-

B. CLINICAL OBSERVATIONS

The nature and duration of the observations are indicated in Table 5.2.3-2. All animals recovered within the 14-day observation period, except for visually accelerated respiration that was still observed at day 14 in male animals.

Table 5.2.3-2: Nature and duration of clinical signs observed in rats exposed for 4 hours to BAS 555 F as dust

Test group: 5.2 mg/L	Males	Females
Total number of animals	5	5
Respiration, visually accelerated	h 0-d 14	h 0-d 8
Respiratory sounds	d 6-d 12	-
Nose, crust formation	d 1-d 4	d 1
Eyelid closure (left side)	-	d 1
Exsiccosis	d 1	d 1
Apathy	d 1	d 1
Squatting posture	d 0-d 7	d 0-d 7
Piloerection	d 5-d 6; d 8-d 11	d 5-d 7; d 8
Fur, smeared	d 0-d 1	d 0-d 1
Reduced general state	d 1	-

hn: hour n of exposure; d0: post-exposure on the day of exposure; dn: day n after exposure

C. BODY WEIGHT

The mean body weights of the male animals decreased during the first post exposure observation week but increased during the second week. The mean body weights of the female animals did not increase adequately during the first post exposure observation week but increased during the second week.

D. NECROPSY

No gross pathological abnormalities were noted in the animals necropsied at termination of the post exposure observation period, except in one male animal diffuse red discoloration of all lung lobes and edema were observed.

E. ANALYTICAL MEASUREMENTS

The exposure conditions are summarized in Table 5.2.3-3.

Table 5.2.3-3: Exposure conditions

Test group (mg/L)	Supply air (m^3/h)	Exhaust air (m^3/h)	Temperature (°C)	Relative humidity (%)	Substance flow (g/h)[4]
5.2	1.5	1.35	21.4±0.1	39.5±1.0	108.7

[4] corrected for 1% (w/w) Aerosil.

Test atmosphere concentrations are presented in Table 5.2.3-4.

Table 5.2.3-4: Atmosphere concentrations

Mean achieved (mg/L)	Standard deviation	Nominal (mg/L)
5.2	0.19	72.5

The measurements of particle-size distribution revealed mass median aerodynamic diameters (MMAD) of 4.0 – 4.7 µm with a geometric standard deviation of 3.0 – 3.1 respectively (see Table 5.2.3-5).

Table 5.2.3-5: Particle size distribution

Mean achieved (analytical) atmosphere concentration (mg/L)	Mean mass median aerodynamic diameter (µm)	Inhalable fraction (% <3 µm)	Standard deviation
5.2 (Analysis 1)	4.7	34.0	3.0
5.2 (Analysis 2)	4.0	40.1	3.1

III. CONCLUSION

Under the conditions of this study, the 4 hour inhalation LC_{50} for male and female rats was estimated to be >5.2 mg/L of cis/trans metconazole.

CA 5.2.4 Skin irritation

Skin irritation study, rabbit - Metconazole cis/trans ([redacted] 1990(b); BASF DocID MK-415-001)

Guidelines: In compliance with the test method B.4 of directive 92/69/EEC
GLP: Yes
Acceptance: The study was considered acceptable in the EU registration process 2004.

Groups of 3 male and 3 female NZW rabbits were exposed with 0.5 g undiluted metconazole (95.3%, cis/trans: 79.8/15.5; batch: 89-01) to the clipped dorsal skin on a 6 cm² lint patch, moistened with deionised water, for 4 hours under semi-occlusive conditions. Thereafter, test material was removed with water and animals were examined for signs of dermal irritation (erythema and oedema according to the Draize scoring system) at 0.5, 24, 48 and 72 hours, and 7 days after application.

No signs of skin irritation were observed in any animal at any observation time-point. Mean erythema and edema scores for the 24 - 72 hours period were 0.0 for each animal.

Conclusion
Under the conditions of this study, metconazole cis/trans revealed no skin irritating properties.

Skin irritation study, rabbit - Metconazole cis ([redacted]. 1991; BASF DocID MK-410-001)

Guidelines: In compliance with the test method B.4 of directive 92/69/EEC
GLP: Yes
Acceptance: The study was considered acceptable in the EU registration process 2004.

Groups of 3 male and 3 female NZW rabbits were exposed with 0.5 g undiluted cis metconazole (95.29%, cis/trans: 95.2/0.09%; batch: 12) to the clipped dorsal skin on a 6 cm² lint patch, moistened with deionised water, for 4 hours under semi-occlusive conditions. Thereafter, test material was removed with warm water and animals were examined for signs of dermal irritation (erythema and oedema according to the Draize scoring system) at 1, 24, 48 and 72 hours, and 7 days after application.

No signs of skin irritation were observed in any animal at any observation time-point. Mean erythema and edema scores for the observation period of 24 to 72 hours were 0.0 for each animal.

Conclusion
Under the conditions of this study, cis metconazole revealed no skin irritating properties.

Report:	CA 5.2.4/1 [redacted] 2005a BAS 555 F - Acute dermal irritation / corrosion in rabbits 2005/1005775
Guidelines:	OECD 404, EEC 2004/73 B.4, EPA 870.2500, JMAFF No 12 Nosan No 8147
GLP:	yes (certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Executive Summary

In an acute dermal irritation study, the skin irritation/corrosion potential of cis/trans metconazole (98.0%, cis/trans: 83.7/14.4, batch: 42704) was tested. The intact clipped skin of 3 New Zealand White rabbits was exposed to 0.5 g of the unchanged test substance for 4 hours covered with a semi-occlusive dressing. The cutaneous reactions were assessed immediately after removal of the patch, approximately 1, 24, 48 and 72 hours after removal of the patch and then generally in weekly intervals until day 14 after treatment.

Slight erythema (grade 1) were observed in all animals immediately after removal of the patch and 1 h after patch removal. Erythema reactions were fully reversible within 24 hours in all three animals. No edema reactions were observed in any animal at any time point. Mean scores over 24, 48 and 72 hours for each animal were 0.0 for erythema and for edema, respectively. Based on the findings of this study, the cis/trans metconazole showed no skin irritation potential to rabbits under the test conditions chosen.

(DocID 2005/1005775)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: BAS 555 F

Description:	Solid; crystalline powder/white
Lot/Batch #:	42704
Purity:	98% (cis/trans ratio: 83.7/14.4)
Stability of test compound:	Stable

2. Vehicle: The test substance was administered unchanged.

3. Test animals:

Species:	Rabbit
Strain:	New Zealand white A 1077 INRA (SPF)
Sex:	2 males / 1 female
Age:	ca. 6 months
Weight at dosing:	males: 3.28 and 3.39 kg, female: 3.47 kg
Source:	Centre Lago S. A., 01540 Vonnas, France
Acclimation period:	At least 5 days
Diet:	Kliba-Labordiät (Kaninchen & Meerschweinchenhaltung "GLP"), Provimi Kliba SA, Kaiseraugst, Basel, Switzerland (about 130 g/animal per day)
Water:	Tap water, ad libitum
Housing:	Single housing in stainless steel wire mesh cages with grating, floor area: 3000 cm²

Environmental conditions:

Temperature:	20 - 24 °C
Humidity:	30 - 70 %
Air changes:	Central air-conditioning
Photo period:	Alternating 12-hour light and dark cycles, artificial light from 6 am to 6 pm

B. STUDY DESIGN AND METHODS

1. Dates of work: 30-Nov-2004 - 03-Dec-2004 (End of in-life phase)

2. In-vitro pre-test: No *in vitro* pre-test was conducted.

3. Animal assignment and treatment:
The potential of BAS 555 F to cause acute dermal irritation or corrosion was assessed by a single topical application of 0.5 g of the unchanged test substance for 4 hours to the intact untreated skin of three New Zealand White rabbits using a patch of 2.5 cm x 2.5 cm. The test substance was covered with a test patch (Idealbinde, Pfaelzische Verbandstoff-Fabrik, Kaiserslautern) and Fixomull stretch (adhesive fleece, Beiersdorf AG). The test substance was removed at the end of the exposure period with Lutrol and Lutrol/water (1:1).
At least 24 hours before treatment, the dorsolateral part of the trunk of the animals was clipped.
The cutaneous reactions were assessed immediately after removal of the patch, approximately 1, 24, 48 and 72 hours, on day 7 and maximally up to day 14 after removal of the patch.
Body weights were measured before application of the test substance and after the last reading. The animals were checked for mortality and morbidity twice on working days and once daily at weekends and on public holidays.

II. RESULTS AND DISCUSSION

Slight erythema (grade 1) were observed in all animals immediately after removal of the patch and 1 h after patch removal. Erythema reactions were fully reversible within 24 h in all three animals. No edema reactions were observed in any animal at any time point. Mean scores over 24, 48 and 72 hours for each animal were 0.0 for erythema and for edema, respectively. Individual and mean irritation scores after 4 hour dermal application of BAS 555 F are presented in Table 5.2.4-1.

Table 5.2.4-1: Individual and mean skin irritation scores after 4 hour dermal application of BAS 555 F

Readings	Animal	Erythema	Edema	Additional findings
0 h	01	1	0	
	02	1	0	
	03	1	0	
1 h	01	1	0	
	02	1	0	
	03	1	0	
24 h	01	0	0	
	02	0	0	
	03	0	0	
48 h	01	0	0	
	02	0	0	
	03	0	0	
72 h	01	0	0	
	02	0	0	
	03	0	0	
Mean 24 - 72 h	01	0.0	0.0	
	02	0.0	0.0	
	03	0.0	0.0	
Mean		0.0	0.0	

III. CONCLUSION

Based on the findings of this study, the cis/trans metconazole showed no skin irritation potential to rabbits under the test conditions chosen.

CA 5.2.5 Eye irritation

Eye irritation study, rabbits - Metconazole cis/trans ([redacted] 1990(b); BASF DocID MK-415-001)

Guidelines: In compliance with the test method B.4 of directive 92/69/EEC
GLP: Yes
Acceptance: The study was considered acceptable in the EU registration process 2004.

Groups of 3 male and 3 female NZW rabbits were exposed to cis/trans metconazole (95.3%, cis/trans: 79.8/15.5; batch: 89-01) in powder form, by instillation of 65 mg, corresponding to 0.1 mL of the undiluted test substance bulk volume, into the lower conjunctival sac of one eye of each animal. The other eye of each rabbit served as the negative control. Signs of eye irritation (corneal opacity, iritis, redness and chemosis of the conjunctivae) were recorded at 1, 24, 48 and 72 hours, and 7 days after application.

Instillation of 0.1 mL metconazole cis/trans: 80/15 into the eyes of 6 rabbits resulted in slight initial pain response. Conjunctival irritation reactions not exceeding a beefy-red appearance, chemosis obscuring the greater part of the cornea and an ocular discharge affected all rabbits within 24 h of treatment. Resolution of the conjunctivae effects was advanced by day 3 and complete by day 8. Areas of diffuse opacity were apparent on the cornea of 4/6 rabbits on day 2. Such opacities had completely resolved 7 days after treatment. The iris showed no reactions upon treatment. The iris remained unaffected by the test material. The individual mean eye irritation scores for the 24 -72-hour period are summarised in the Table 5.2.5-2.

Table 5.2.5-1: Individual mean (24-72 hours) eye irritation scores

Animal	Cornea	Iris	Conjunctivae	
			Redness	Chemosis
888 (m)	0.3	0.0	0.7	0.7
933 (m)	0.3	0.0	1.0	0.0
934 (m)	0.0	0.0	1.0	0.3
969 (f)	1.0	0.0	1.3	0.7
970 (f)	0.0	0.0	1.0	0.7
972 (f)	0.7	0.0	1.7	0.7
mean	**0.4**	**0.0**	**1.1**	**0.5**

Mean erythema, chemosis, iris, and corneal opacity scores were 1.1, 0.5, 0.0, and 0.4, respectively.

Conclusion
Under the conditions of this study, metconazole cis/trans is not considered to be an eye irritant.

Eye irritation study, rabbits - Metconazole cis [redacted] 1991; BASF DocID MK-410-001)

Guidelines: In compliance with the test method B.4 of directive 92/69/EEC
GLP: Yes
Acceptance: The study was considered acceptable in the EU registration process 2004.

Groups of 3 male and 3 female NZW rabbits were exposed to cis metconazole (95.29%, cis/trans: 95.2/0.09%; batch: 12) in powder form, by instillation of 42 mg, corresponding to 0.1 mL of the undiluted test substance bulk volume, into the lower conjunctival sac of one eye of each animal. The other eye of each rabbit served as the negative control. Signs of eye irritation (corneal opacity, iritis, redness and chemosis of the conjunctivae) were recorded at 1, 4, 24, 48 and 72 hours, and 7 days after application.

Instillation of 0.1 mL cis metconazole into one eye of each of six rabbits resulted in practically no initial pain response. All rabbits had developed a crimson-red appearance of the conjunctivae, slight chemosis and a slight ocular discharge one hour after treatment. Resolution of the conjunctival reactions was advanced 24 hours after treatment and completed 2 days later. Two rabbits showed small areas of slight, diffuse corneal opacity either 24 hours or 24, 48 and 72 hours after instillation. The iris remained unaffected by the test material. The individual mean eye irritation scores for the 24 -72-hour period are summarised in the Table 5.2.5-2.

Table 5.2.5-2: Individual mean (24-72 hours) eye irritation scores

Animal	Cornea	Iris	Conjunctivae	
			Redness	Chemosis
389 (m)	1.0	0.0	0.7	0.3
421 (m)	0.0	0.0	0.7	0.3
422 (m)	0.0	0.0	0.7	0.3
416 (f)	0.3	0.0	0.7	0.3
417 (f)	0.0	0.0	0.3	0.0
418 (f)	0.0	0.0	0.3	0.0
mean	**0.2**	**0.0**	**0.6**	**0.2**

All overt effects resolved within 7 days of treatment. Mean erythema, chemosis, iris, and corneal opacity scores were 0.6, 0.2, 0.0, and 0.2, respectively.

Conclusion
Under the conditions of this study, cis metconazole is not considered to be an eye irritant.

Report:	CA 5.2.5/1 [redacted]., 2005b BAS 555 F - Acute eye irritation in rabbits 2005/1005774
Guidelines:	OECD 405, EEC 2004/73 B.5, EPA 870.2400, JMAFF No 12 Nosan No 8147
GLP:	yes (certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Executive Summary

In an eye irritation study, the eye irritation/corrosion potential of cis/trans metconazole (98.0%, cis/trans: 83.7/14.4, batch: 42704) was determined by instillation of 0.1 mL bulk volume (about 32 mg) of the unchanged test substance into the conjunctival sac of the right eye of three New Zealand White rabbits. The application of the test substance was performed in a stepwise procedure starting with one animal and supplementing two additional animals. About 1 hour after application the eye was rinsed with tap water.

The ocular reactions were assessed approximately 1, 24, 48 and 72 hours after the administration of the test substance.

Moderate conjunctival redness (grade 2) was observed in all animals 1 hour after application and decreased to slight (grade 1) within 24 hours. Slight conjunctival chemosis (grade 1) and slight discharge (grade 1) were observed in the animals 1 hour after application, only. In addition, circular injected scleral vessels were noted in all animals 1 hour after application. The ocular reactions were reversible in all animals within 48 hours after application. Mean scores calculated for each animal over 24, 48 and 72 hours were 0.0 for corneal opacity and for iris lesions, 0.3 for redness of the conjunctiva and 0.0 for chemosis. Based on the findings of this study, the cis/trans metconazole does not show an eye irritation potential to rabbits under the test conditions chosen.

DocID (2005/1011571)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: BAS 555 F

Description:	Solid; crystalline powder/white
Lot/Batch #:	42704
Purity:	98% (cis/trans ratio: 83.7/14.4)
Stability of test compound:	Stable

2. Vehicle: The test substance was administered undiluted.

3. Test animals:

Species:	Rabbit
Strain:	New Zealand white A 1077 INRA (SPF)
Sex:	one male / two females
Age:	about 3-4 months
Weight at dosing:	2.72 - 2.94 kg
Source:	Centre Lago S.A., 01540 Vonnas, France
Acclimation period:	At least 5 days
Diet:	Kliba-Labordiät, Provimi Kliba SA, Kaiseraugst, Basel, Switzerland (about 130 g/animal per day)
Water:	Tap water, ad libitum
Housing:	Single housing in stainless steel wire mesh cages with grating, floor area: 3000 cm²

Environmental conditions:

Temperature:	20 - 24 °C
Humidity:	30 - 70 %
Air changes:	Central air-conditioning
Photo period:	Alternating 12-hour light and dark cycles, artificial light from 6 am to 6 pm

B. STUDY DESIGN AND METHODS

1. Dates of work: 07-Dec-2004 - 10-Dec-2004

2. In-vitro pre-test: No *in vitro* pre-test was conducted.

3. Animal assignment and treatment:
The potential of BAS 555 F to cause acute eye irritation/corrosion was assessed by instillation of 0.1 mL bulk volume of the undiluted test substance (about 32 mg of the comminuted test substance) into the conjunctival sac of the right eye. The left eye, which remained untreated, served as the negative control. About 1 hour after application of the test substance, the treated eye was rinsed with 3 to 6 mL of hand warm tap water for 1 to 2 minutes using a syringe with a blunt probe. The ocular reactions were assessed approximately 1, 24, 48 and 72 hours and then in weekly intervals maximally up to day 28 after the administration of the test substance.
Body weights were determined shortly prior to application and after the last reading. The animals were checked for mortality and morbidity twice on working days and once daily at weekends and on public holidays.

II. RESULTS AND DISCUSSION

Moderate conjunctival redness (grade 2) was observed in all animals 1 hour after application and decreased to slight (grade 1) within 24 hours. Slight conjunctival chemosis (grade 1) and slight discharge (grade 1) were observed in the animals 1 hour after application, only. In addition, circular injected scleral vessels were noted in all animals 1 hour after application. The ocular reactions were reversible in all animals within 48 hours after application. Mean scores calculated for each animal over 24, 48 and 72 hours were 0.0 for corneal opacity and for iris lesions, 0.3 for redness of the conjunctiva and 0.0 for chemosis. For details regarding the individual and mean scores as well as additional findings see Table 5.2.5-3.

Table 5.2.5-3: Individual and mean eye irritation scores after ocular application of BAS 555 F

Readings	Animal	Cornea		Iris	Conjunctiva			Additional findings
		Opacity	Area involved		Redness	Chemosis	Discharge	
1 h	01	0	0	0	2	1	1	49
	02	0	0	0	2	1	1	49
	03	0	0	0	2	1	1	49
24 h	01	0	0	0	1	0	0	-
	02	0	0	0	1	0	0	-
	03	0	0	0	1	0	0	-
48 h	01	0	0	0	0	0	0	-
	02	0	0	0	0	0	0	-
	03	0	0	0	0	0	0	-
72 h	01	0	0	0	0	0	0	-
	02	0	0	0	0	0	0	-
	03	0	0	0	0	0	0	-
Mean 24 - 72 h	01	0.0		0.0	0.3	0.0		
	02	0.0		0.0	0.3	0.0		
	03	0.0		0.0	0.3	0.0		
Mean		0.0		0.0	0.3	0.0		

49 = scleral vessels injected, circular

III. CONCLUSION

Based on the findings of this study, the cis/trans metconazole does not show an eye irritation potential to rabbits under the test conditions chosen.

CA 5.2.6 Skin sensitisation

Skin sensitisation study, modified Buehler test - Metconazole cis/trans (1990(b); BASF DocID MK-415-001)

Guidelines:	In compliance with the test method B.6 of directive 92/69/EEC
GLP:	Yes
Deviation:	Dermal exposure of 24 h instead of 6 h.
Acceptance:	The study was considered acceptable in the EU registration process 2004.

The evaluation of the sensitizing properties of cis/trans metconazole (95.3%, cis/trans: 79.8/15.5; batch: 89-01) was conducted using a group of 10 male and 10 female guinea pigs (Dunkin-Hartley) together with a control group of 5 male and 5 female animals. Based on the range finding test conducted with 5, 10, 25, 50 and 60% test item concentrations in Vaseline (w/w), the following concentration of test material was selected for the main study: 60% cis/trans metconazole (w/w) in Vaseline for both topical induction and challenge. All concentrations tested in the pre-test were non-irritating to skin but it was not possible to prepare a paste incorporating more than 60% (w/w).
At *induction*, on days 1, 3, 6, 8, 10, 13, 15, 17 and 20 a 16 cm² patch of filter paper loaded with approximately 0.45 mL of the appropriate test item preparation was applied to the shaved skin of each test group animal. Similar patches of filter paper moistened with Vaseline alone were applied to the control group guinea pigs. The same left flank site was used for all induction applications on all test and control group animals. The patches were covered with occlusive tape and held in place by adhesive bandage for 24 hours. Any dermal reactions to the induction procedure were recorded.
Challenge was carried out on day 28. A 16 cm² patch of filter paper, moistened with 0.45 mL of the appropriate dilution of test material, was placed on the shaved area, covered by occlusive tape and held in position by elastic adhesive bandage. Control group animals were treated with the same formulation of test material that was applied to test group animals. After 24 hours the patches and bandages were removed. Dermal reactions to challenge were assessed shortly after removal of the challenge patches, and 24h or 48h after patch removal upon challenge.
The susceptibility of the strain of guinea pigs to induction of skin sensitisation by skin sensitisers was confirmed in a contemporaneous positive control group treated with 2% and 1% (m/v) 2,4-dinitrochlorobenzene in corn oil for the induction and challenge, respectively.

None of the twenty test animals showed any positive response either 24 or 48 hours after removal of the challenge patches.

Conclusion
Under the conditions of this study, metconazole cis/trans is not a skin sensitizer.

Skill sensitisation study, Maximisation test - Metconazole cis/trans ([redacted] 1995, BASF DocID MK-416-001)

Guidelines: In compliance with the test method B.6 of directive 92/69/EEC
GLP: Yes
Acceptance: The study was considered acceptable in the EU registration process 2004.

In the main test, conducted according to the methodology of Magnusson and Kligman, 20 male albino Hartley guinea pigs (Crl:(HA)BR) were assigned to the test group (G2) and 10 animals were assigned to both irritation control (G3) and positive control (G4) groups. Five animals were assigned to both the positive control-irritation control group (G5) and an additional irritation control group (only used in the second challenge procedure).
On day 1 (*intradermal induction*), animals in G2 and G4 received duplicate 0.1 mL intradermal injections of (i) a 1:1 dilution of Freund's Complete Adjuvant (FCA) in sterile water, (ii) a 5% w/v suspension of cis/trans metconazole (97.4%; cis/trans: 83.7/13.7; batch: AC 9339-114) in mineral oil (G2) or a 5% w/v suspension of sulfathiazole in sterile water (G4), and (iii) a 1:1 dilution of a 10% w/v suspension of cis/trans metconazole in FCA and sterile water (G2) or a 1:1 dilution of 10% w/v sulfathiazole in sterile water and FCA (G4) on the shoulder area. At this time, the animals in G3 and G5 received duplicate 0.1 mL intradermal injections of (i) a 1:1 dilution of FCA in sterile water, (ii) the vehicle alone (mineral oil for G3 and sterile water for G5) and (iii) a 1:1 dilution of the respective vehicle in FCA. Six days later, the G2 - G5 animals were pretreated with sodium lauryl sulfate applied topically at the injection sites.
On day 8 (*topical induction*), a 25% w/w mixture of cis/trans metconazole in petrolatum was applied over the injection sites of the animals in G2, a 25% w/w mixture of sulfathiazole in petrolatum was applied over the injection sites of the animals in G4, and petrolatum was applied over the injection sites of the animals in G3 and G5. All induction sites were then occluded for 48 h.
Two weeks after the topical induction application, all animals received a *challenge* dose. A 25% w/w mixture of test material in petrolatum was applied to the right flank and the control material (petrolatum) was applied to the left flank of each animal in G2 and G3. A 10% w/w mixture of sulfathiazole in petrolatum was applied to the right flank and petrolatum alone was applied to the left flank of each animal in G4 and G5. All test and control sites were occluded for 24 hours and then wiped clean. The sites were examined for dermal reactions at 24 and 48 hours after patch removal. One week after the initial challenge application, the animals in G2 and G3 received a second challenge dose. A new group of 5 animals (irritation control group) also received a challenge dose at that time as well. The animals in G4 and G5, however, did not receive a second challenge dose and were terminated on day 29.
In an irritation screening study, cis/trans metconazole was applied topically to the skin of one group of 4 male guinea pigs at concentrations of 1%, 5%, 10% and 25% in petrolatum with each animal receiving two different concentrations of the test material. The test sites were occluded for 24 hours and dermal irritation was evaluated at 24 and 48 hours after removal of the patches. No dermal irritation was observed. The 25% w/v mixture was determined to be the highest non irritating topical concentration tested. A second irritation screening group of 4 male guinea pigs received 0.1 mL intradermal injections of cis/trans metconazole prepared at concentrations of 1%, 5%, 10%, 25% and 50% w/v in mineral oil. These test sites were evaluated for dermal reactions 24 and 48 hours after injection. Moderate to intense erythema reactions were observed. A 5% w/v mixture of the test material was determined to be the highest concentration that did not cause excessive irritation.

After intradermal injections, the irritation was similar for all groups and consisted of scab formation and mild to moderate erythema and edema reactions.
Upon challenge, 4/20 of the test group (G2) animals exhibited dermal reactions (scattered mild redness) at the 24-hour reading time-point. Subsequently, the reactions disappeared at 48 hours in 1/4 animals. None of the 10 irritation control animals (G3) exhibited a dermal reaction to the initial challenge application of the test material. None of the animals in the test (G2) or irritation control (G3) groups exhibited a dermal reaction to the petrolatum following the initial challenge application. Since these results indicated a rather weak equivocal response for the initial challenge, a second challenge application was conducted. For this re-challenge, 1/20 animals in G2 showed the same mild scattered redness reaction at 24-hour reading time-point, which subsequently disappeared 48 hours after patch removal. One animal in the irritation control group (G3) also exhibited a mild dermal reaction while 0/5 additional irritation control animals exhibited a dermal reaction to the test material as a 25% w/w mixture in petrolatum.
All 10 animals in the positive control group (G4) exhibited slight to intense dermal reactions on the test sites treated with sulfathiazole and no dermal reactions on the test sites treated with petrolatum. None of the positive control irritation control animals (G5) exhibited a dermal reaction to the challenge of sulfathiazole or petrolatum.

Conclusion
Based on the low incidence (<30% incidence) of animals in the test group exhibiting dermal reactions at challenge (initial or re-challenge), metconazole cis/trans: 83.7/13.7 is not to be classified as a skin sensitiser under these study conditions.

Skin sensitisation study, Buehler test - Metconazole cis ([redacted] 1991; BASF DocID MK-410-001)

Guidelines: In compliance with the test method B.6 of directive 92/69/EEC
GLP: Yes
Deviation: Dermal exposure of 24 h instead of 6 h
Acceptance: The study was considered acceptable in the EU registration process 2004.

The evaluation of the sensitizing properties of cis metconazole (95.29%, cis/trans: 95.2/0.09%; batch: 12) was conducted using a group of 10 male and 10 female guinea pigs (Dunkin-Hartley) together with a control group of 5 males and 5 females. Based on the range finding test with 5, 10, 25 and 50% test item concentrations in Vaseline (w/w), the following concentration of test material were selected for the main study: 50% cis metconazole (w/w) in Vaseline for both topical induction and challenge. All concentrations tested in the pre-test were non-irritant to the skin, but higher than 50% concentrations were judged unsuitable for administration.
For *induction*, on days 1, 8 and 15 a 16 cm² patch of filter paper loaded with approximately 0.45 mL of the appropriate test item preparation was applied to the shaved skin of each test group animal. Similar patches of filter paper moistened with Vaseline alone were applied to the control group guinea pigs. The same left flank site was used for all induction applications on all test and control group animals. The patches were covered with occlusive tape and held in place by adhesive bandage for 24 hours. Any dermal reactions to the induction procedure were recorded.
Challenge was carried out on day 28. A 4 cm² patch of filter paper, moistened with 0.15 mL of the appropriate test item preparation was placed on the shaved skin, covered by occlusive tape and held in position by elastic adhesive bandage. Control group animals were treated with the same formulation of test material that was applied to test group animals. After 24 hours the patches and bandages were removed. Dermal reactions to challenge were assessed shortly after removal of the challenge patches, and 24 and 48 hours after patch removal.
The susceptibility of the strain of guinea pigs to induction of skin sensitisation by strong (trans-cinnamaldehyde) and weak (ethyl p-aminobenzoate benzocaine) skin sensitisers was confirmed in contemporaneous positive control groups.

None of the twenty test animals showed any positive response either 24 or 48 hours after removal of the challenge patches.

Conclusion
Under the conditions of this study, cis metconazole is not considered to be a skin sensitiser.

Report:	CA 5.2.6/1 [redacted] 2005d BAS 555 F - BUEHLER test in guinea pigs 2005/1020108
Guidelines:	EEC 96/54 B 6, OECD 406, EPA 870.2600, JMAFF No 12 Nosan No 8147
GLP:	yes (certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Executive Summary

For the determination of potential sensitizing properties of BAS 555 F (98.6% pure; cis/trans: 83.5/15.1; batch: 39513) a Buehler test in female Dunkin Hartley guinea pigs (HsdPoc:DH) was conducted with 10 control and 20 test group animals.. Based on the results of a pre-test, the epicutaneous induction and both challenges were performed with a 50% test item preparation in 1% CMC-solution in doubly distilled water at the flanks of the animals. The induction procedure was performed three times in weekly intervals (days 0, 7 and 14) for 6 hours under occlusive conditions. Readings for skin reactions were performed 24 hours after the induction and 24/48 hours after patch removal upon challenge. Regarding epicutaneous induction, the control group animals were not treated with the vehicle (1% CMC-solution in doubly distilled water) since it was not expected to influence the result of the study. At the challenge treatments both control and test groups were treated with the 50% test substance formulation for 6 hours under occlusive conditions. The 1st challenge was performed 14 days after the last induction, and the 2nd challenge was performed 7 days after the 1st challenge. A positive control with a known sensitizer was not included into the study. However, studies with alpha-hexylcinnamaldehyde (techn. 85%) are regularly performed as reliability check in the laboratory.
The epicutaneous induction caused slight (grade 1) erythema in 3/20 animals. The first challenge resulted in skin reactions in 0/10 control and 2/20 test group animals. After the second challenge 0/10 animals of the control and test group showed any positive reactions. The positive control alpha-hexylcinnamaldehyde (techn. 85%) was valid by showing a sensitization rate of 95% in this guinea pig strain.
Based on the results of this study it is concluded that metconazole cis/trans 83.5/15.1 does not have sensitising properties in the Buehler test under the test conditions chosen. None of the animals were considered positive after the second challenge application.

(DocID 2005/1020108)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: BAS 555 F

Description:	Solid
Lot/Batch #:	39513
Purity:	98.6% (cis/trans ratio: 83.5/15.1)
Stability of test compound:	Stable

2. Vehicle / Positive control:

Vehicles:
1% CMC (cleaned sodium carboxymethylcellulose) in doubly distilled water
Positive control: Alpha-Hexylcinnamaldehyde

3. Test animals:

Species:	Guinea Pig
Strain:	Dunkin Hartley, HsdPoc: DH
Sex:	female
Age:	8 - 9 weeks
Weight at dosing (mean):	385 - 521 g
Source:	Harlan Winkelmann, Gartenstr. 27, 33178 Borchen, Germany
Acclimation period:	21 days
Diet:	Kliba Labordiät (Kaninchen/ Meerschweinchen-Haltungsdiät) Provimi Kliba SA, Kaiseraugst, Switzerland, ad libitum
Water:	Tap water, ad libitum
Housing:	Groups of 5 animals were housed in stainless steel wire mesh cages with plastic-coated grating, minimum floor area: 2000 cm²

Environmental conditions:

Temperature:	20 - 24 °C
Humidity:	30 - 70 %
Air changes:	Central air-conditioning
Photo period:	Alternating 12-hour light and dark cycles, artificial light from 6 am to 6 pm

B. STUDY DESIGN AND METHODS

1. Dates of work: 20-May-2005 - 30-June-2005 (end of in life phase)

2. Animal assignment and treatment:
The skin sensitizing potential of BAS 555 F was assessed using the Buehler test. For this, female guinea pigs were randomly allocated to groups. Ten animals were used as control group animals and 20 animals were used in the test group. Based on the results of a pre-test, animals were epicutaneously induced with 50% test substance preparations for 6 hours under occlusive dressing. Epicutaneous challenge was also conducted with a 50% test substance preparations for 6 hours under occlusive conditions. The homogeneity and the stability of the test substance in the vehicle were determined indirectly by the concentration control analysis. The fur was clipped at least 15 hours before each test item application at the appropriate application sites. If necessary, the fur was additionally clipped at least 2 hours before evaluation of the skin reactions.

3. Clinical observation:
A check for any dead or moribund animal was made twice each workday and once on weekends and on public holidays.

4. Body weights:
Individual body weights were determined on day 0 on the last day of observation.

5. Pre-test:
2 x 2 cm gauze patches (6 layers surgical gauze Ph. Eur. from Lohmann GmbH & Co. KG) containing 0.5 mL of the test substance formulation were applied to the skin of the flanks of 3 animals for 6 hours under an occlusive dressing. The dressing consisted of rubberized linen patches (4 x 4 cm from Russka), patches of Idealbinde (5 x 5 cm from Pfälzische Verbandstoff-Fabrik) and Fixomull® Stretch (adhesive fleece) from Beiersdorf AG. A 50% test substance solution was applied on the right flank and a 25% test substance solution was applied at the left flank. Readings were performed 1, 24, and 48 h after removal of the patches.

6. Main study – epicutaneous induction:
Based on the results of the pretest, test group animals received 3 epicutaneous applications of 0.5 mL of a 50% test substance formulation in weekly intervals (day 0, 7, and 14) on the skin of the flank for 6 hours under occlusive conditions. The procedure was the same as described for the pre-test. The control animals were not treated since the 1% aqueous CMC used as formulating agent was not expected to influence the result of the study. Readings were performed 24 h after removal of the patch.

7. Main study – epicutaneous challenge:
The 1st challenge was carried out 14 days after the third induction. A 2nd challenge was performed one week after the 1st challenge. 2 x 2 cm gauze patches (6 layers surgical gauze Ph. Eur. from Lohmann GmbH & Co. KG) containing the test substance formulation were applied to the skin of the flank under an occlusive dressing. The dressing consisted of rubberized linen patches (4 x 4 cm from Russka), patches of Idealbinde (5 x 5 cm from Pfälzische Verbandstoff-Fabrik) and Fixomull® Stretch (adhesive fleece) from Beiersdorf AG. 0.5 mL of the 50% test substance formulation was applied to each animal. The test group and control group were occluded with the test substance formulation for 6 hours. Readings were performed 24 and 48 h after removal of the patch.

8. Evaluation of results
The number of animals with skin findings at 24 and/or 48 hours after the removal of the patch was taken into account for the determination of the sensitization rate. The evaluation "sensitizing" results if at least 15% of the test animals exhibit skin reactions.

9. Positive controls
A positive control (reliability check) with a known sensitizer was not included in this study. However, a separate study with the positive control alpha-hexylcinnamaldehyde (techn. 85%) is regularly performed in the laboratory.

II. RESULTS AND DISCUSSION

A. PRE-TEST

Treatment of the animals with a 50% or 25% test substance preparation in 1% aqueous CMC did not cause any skin reactions 24 or 48 hours after removal of the patches.

B. OBSERVATIONS

No abnormalities were observed during general observation.

C. BODY WEIGHTS

Body weight gain was not adversely affected during the course of the study.

D. SKIN REACTIONS AFTER EPICUTANEOUS INDUCTION

Epicutaneous induction with a 50% test substance preparation caused no skin reactions in any animal at the first and second induction. After the third induction 3/20 animals showed slight erythema (grade 1) 24 hours after patch removal.

E. SKIN REACTIONS AFTER CHALLENGE

The first challenge with a 50% test substance preparation in 1% CMC-solution in doubly distilled water did not cause any skin reactions in animals of the control group 24 and 48 hours after removal of the patches (see Table 5.2.6-1). In the test group 2/20 animals showed slight skin reactions (grade 1) 24 and 48 hours after patch removal. The second challenge with a 50% test substance preparation in 1% CMC-solution in doubly distilled water did not cause any skin reactions in animals of the control and test group 24 and 48 hours after removal of the patches.

Table 5.2.6-1: BAS 555 F - Skin reactions after challenge treatments

	Challenge			
Skin findings	**Control group**		**Test group**	
	24 h	**48 h**	**24 h**	**48 h**
1st Challenge	0/10[#]	0/10	2/20	2/20
2nd Challenge	0/10	0/10	0/20	0/20

[#] x/y = number of positive animals / number of animals tested

F. POSITIVE CONTROL

The positive control alpha-hexylcinnamaldehyde showed a sensitization rate of 95% in the guinea pig strain. The results of the latest study conducted with the positive control are presented in Table 5.2.6-2.

Table 5.2.6-2: Skin reactions after challenge with the positive control

	Challenge			
Skin findings	**alpha-hexylcinnamaldehyde (techn. 85%) 15% in Lutrol® E 400**		**Vehicle Control: Lutrol® E 400**	
	24 h	**48 h**	**24 h**	**48 h**
Control group	0/10[#]	0/10	0/10	0/10
Test group	19/20	16/20	0/20	0/20

[#] x/y = number of positive reactions/number of animals tested (reading at 24 h and/or 48 h after the removal of the patch)

III. CONCLUSION

Based on the results of this study it is concluded that metconazole cis/trans 83.5/15.1 does not have sensitising properties in the Buehler test under the test conditions chosen. None of the animals were considered positive after the second challenge application.

Report:	CA 5.2.6/2 [redacted] 2005e BAS 555 F - BUEHLER test in guinea pigs 2005/1020109
Guidelines:	EEC 96/54 B 6, OECD 406, EPA 870.2600, JMAFF No 12 Nosan No 8147
GLP:	yes (certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Executive Summary

For the determination of potential sensitizing properties of cis/trans metconazole (99.1%, cis/trans: 85.0/14.1; batch: 43707) a Buehler test in female Dunkin Hartley guinea pigs (HsdPoc: DH) was conducted with 10 control and 20 test group animals. Based on the results of a pre-test, the epicutaneous induction and challenge were performed with a 50% test item preparation in 1% CMC-solution in doubly distilled water at the flanks of the animals. The induction procedure was performed three times in weekly intervals for 6 hours under occlusive conditions each. Readings for skin reactions were performed 24 hours after the induction and 24/48 hours after patch removal upon challenge. Regarding epicutaneous induction, the control group animals were not treated with the vehicle (1% CMC-solution in doubly distilled water) since it was not expected to influence the result of the study. At the challenge both control and test groups were treated with the 50% test substance formulation for 6 hours under occlusive conditions. A positive control with a known sensitizer was not included into the study. However, studies with alpha-hexylcinnamaldehyde (techn. 85%) are regularly performed as reliability check in the laboratory.
The epicutaneous induction caused no reactions in any animal. Similarly, after the challenge procedure 0/10 control and 0/20 test group animals showed any skin reactions. The positive control alpha-hexylcinnamaldehyde (techn. 85%) was valid by showing a sensitization rate of 95% in the guinea pig strain.
Based on the results of this study it is concluded that metconazole cis/trans 85.0/14.1 does not have sensitizing properties in the Buehler test under the test conditions chosen.

(DocID 2005/1020109)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: BAS 555 F

Description:	Solid
Lot/Batch #:	43707
Purity:	99.1% (cis/trans ratio: 85.0/14.1)
Stability of test compound:	Stable

2. Vehicle / Positive control: Vehicles:
1% CMC (cleaned sodium carboxymethylcellulose) in doubly distilled water
Positive control: alpha-hexylcinnamaldehyde

3. Test animals:

Species:	Guinea Pig
Strain:	Dunkin Hartley, HsdPoc: DH
Sex:	female
Age:	8 - 9 weeks
Weight at dosing (mean):	400 - 517 g
Source:	Harlan Winkelmann, Gartenstr. 27, 33178 Borchen, Germany
Acclimation period:	21 days
Diet:	Kliba Labordiät (Kaninchen/ Meerschweinchen-Haltungsdiät) Provimi Kliba SA, Kaiseraugst, Switzerland, ad libitum
Water:	Tap water, ad libitum
Housing:	Groups of 5 animals were housed in stainless steel wire mesh cages with plastic-coated grating, minimum floor area: 2000 cm²

Environmental conditions:

Temperature:	20 - 24 °C
Humidity:	30 - 70 %
Air changes:	Central air-conditioning
Photo period:	Alternating 12-hour light and dark cycles, artificial light from 6 am to 6 pm

B. STUDY DESIGN AND METHODS

1. Dates of work: 20-May-2005 - 23-June-2005 (end of in life phase)

2. Animal assignment and treatment:
The skin sensitizing potential of BAS 555 F was assessed using the Buehler test. For this, female guinea pigs were randomly allocated to groups. Ten animals were used as control group animals and 20 animals were used in the test group. Based on the results of a pre-test, animals were epicutaneously induced with 50% test substance preparations for 6 hours under occlusive conditions. Epicutaneous challenge was also conducted with a 50% test substance preparations for 6 hours under occlusive conditions. The homogeneity and the stability of the test substance in the vehicle were determined indirectly by the concentration control analysis. The fur was clipped at least 15 hours before each test item application at the appropriate application sites. If necessary, the fur was additionally clipped at least 2 hours before evaluation of the skin reactions.

3. Clinical observation:
A check for any dead or moribund animal was made twice each workday and once on Saturdays, Sundays and on public holidays.

4. Body weights:
Individual body weights were determined on day 0 on the last day of observation.

5. Pre-test:
2 x 2 cm gauze patches (6 layers surgical gauze Ph. Eur. from Lohmann GmbH & Co. KG) containing 0.5 mL of the test substance formulation were applied to the skin of the flanks of 3 animals for 6 hours under an occlusive dressing. The dressing consisted of rubberized linen patches (4 x 4 cm from Russka), patches of Idealbinde (5 x 5 cm from Pfälzische Verbandstoff-Fabrik) and Fixomull® Stretch (adhesive fleece) from Beiersdorf AG. A 50% test substance solution was applied on the right flank and a 25% test substance solution was applied at the left flank. Readings were performed 1, 24, and 48 h after removal of the patches.

6. Main study – epicutaneous induction:
Based on the results of the pretest, test group animals received 3 epicutaneous applications of 0.5 mL of a 50% test substance formulation in weekly intervals (day 0, 7, and 14) on the skin of the flank for 6 hours. The procedure was the same as described for the pre-test. The control animals were not treated since the 1% aqueous CMC used as formulating agent was not expected to influence the result of the study. Readings were performed 24 h after removal of the patch.

7. Main study – epicutaneous challenge:
The 1st challenge was carried out 14 days after the third induction. A 2nd challenge was not performed as no borderline results were observed. 2 x 2 cm gauze patches (6 layers surgical gauze Ph. Eur. from Lohmann GmbH & Co. KG) containing the test substance formulation were applied to the skin of the flank for 6 hours under an occlusive dressing. The dressing consisted of rubberized linen patches (4 x 4 cm from Russka), patches of Idealbinde (5 x 5 cm from Pfälzische Verbandstoff-Fabrik) and Fixomull® Stretch (adhesive fleece) from Beiersdorf AG. 0.5 mL of the 50% test substance formulation was applied to each animal. The test group and control group were treated with the test substance formulation for 6 h. Readings were performed 24 and 48 h after removal of the patch.

8. Evaluation of results
The number of animals with skin findings at 24 and/or 48 hours after the removal of the patch upon challenge treatment was taken into account for the determination of the sensitization rate. The evaluation "sensitizing" results if at least 15% of the test animals exhibit skin reactions.

9. Positive controls
A positive control (reliability check) with a known sensitizer was not included in this study. However, a separate study with the positive control alpha-hexylcinnamaldehyde (techn. 85%) is regularly performed in the laboratory.

II. RESULTS AND DISCUSSION

A. PRE-TEST

Treatment of the animals with a 50% or 25% test substance preparation in 1% aqueous CMC did not cause any skin reactions 24 or 48 hours after removal of the patches.

B. OBSERVATIONS

No abnormalities were observed during general observation.

C. BODY WEIGHTS

Body weight gain was not adversely affected during the course of the study.

D. SKIN REACTIONS AFTER EPICUTANEOUS INDUCTION

Epicutaneous induction with a 50% test substance preparation caused no skin reactions in any animal after the first, second or third induction.

E. SKIN REACTIONS AFTER CHALLENGE

The first challenge with a 50% test substance preparation in 1% CMC-solution in doubly distilled water did not cause any skin reactions in animals of the control or test group 24 and 48 hours after removal of the patches (see Table 5.2.6-1).

Table 5.2.6-3: BAS 555 F - Skin reactions after challenge

	Challenge			
Skin findings	**Control group**		**Test group**	
	24 h	**48 h**	**24 h**	**48 h**
1st Challenge	0/10[#]	0/10	0/20	0/20

[#] x/y = number of positive animals / number of animals tested

F. POSITIVE CONTROL

The positive control alpha-hexylcinnamaldehyde showed a sensitization rate of 95% in the guinea pig strain. The results of the latest study conducted with the positive control are presented in Table 5.2.6-2.

Table 5.2.6-4: Skin reactions after challenge with the positive control

	Challenge			
Skin findings	**alpha-hexylcinnamaldehyde (techn. 85%) 15% in Lutrol® E 400**		**Vehicle Control: Lutrol® E 400**	
	24 h	**48 h**	**24 h**	**48 h**
Control group	0/10[#]	0/10	0/10	0/10
Test group	19/20	16/20	0/20	0/20

[#] x/y = number of positive reactions/number of animals tested (reading at 24 h and/or 48 h after the removal of the patch)

III. CONCLUSION

Based on the results of this study it is concluded that metconazole cis/trans 85.0/14.1 does not have sensitizing properties in the Buehler test under the test conditions chosen. None of the animals were considered positive after the challenge application.

CA 5.2.7 Phototoxicity

In accordance with the requirements of Commission Regulation SANCO/11802/2010 and regulation (EC) No 1107/2009 an in vitro NRU-Phototoxicity study in Balb/c 3T3 cells was not performed, since the respective trigger value is not met [see M-CA 2.4].

CA 5.3 Short-Term Toxicity

Studies already evaluated during the last EU registration process in 2004: Short-term toxicity of metconazole for both the isomer mix as well as the cis-isomer was evaluated in subacute (rat, dog) and subchronic studies (rat, mouse, dog). These studies are scientifically valid and have been evaluated already by European authorities and Belgium as Rapporteur member state (European Commission Peer Review Program) and were considered to be acceptable. All studies are listed in Table 5.3-1 and Table 5.3-2.

Table 5.3-1: Summary of already peer-reviewed oral subacute studies with metconazole

Study	Purity (%), cis/trans content, batch no.	NOAEL [mg/kg/day]	LOAEL [mg/kg/day]	Critical effects	Reference (BASF DocID)
28-Day, oral Fischer rat 0, 30, 100, 1000, 3000 ppm	96.0%, cis/trans: 79.7/16.3 batch: 88-10	9.1 (100 ppm)	90.5 (1000 ppm)	Reduction: body weight, food consumption Increase: ALT, AST, γ-GT, liver weight, liver pallor/ enlargement, hepatocellular vacuolation/ hypertrophy	[redacted] 1990 (MK-420-002)
28-Day, oral Fischer rat 0, 30, 100, 300, 1000, 10000 ppm	98.0%, cis/trans: 97/1 batch: ST87-181	27.3 (300 ppm)	89.3 (1000 ppm)	Reduction: body weight, food consumption Increase: liver weight, hepatocellular vacuolation	[redacted] 1991 (MK-420-003)
28-Day, oral Beagle dog 0, 100, 1000, 7000 ppm	95.3%, cis/trans: 79.8/15.5 batch: 89-01	3.7 (100 ppm)	37.8 (1000 ppm)	Reduction: body weight, food consumption Increase: liver weight, ALP	[redacted] 1991 (MK-123-013)

Table 5.3-2: Summary of already peer-reviewed oral subchronic studies with metconazole

Study	Purity (%), cis/trans content, batch no.	NOAEL [mg/kg/day]	LOAEL [mg/kg/day]	Critical effects	Reference (BASF DocID)
90-Day, oral CD1 mouse 0, 30, 300, 2000 ppm	95.3%, cis/trans: 79.8/15.5 batch: 89-01	4.6 (30 ppm)	50.5 (300 ppm)	Reduction: cholesterol Increase: AST/ALT/AP, liver/spleen weight, hepatocellular vacuolation/ hypertrophy	[redacted] 1991 (MK-425-003)
90-Day, oral Fischer rat 0, 30, 100, 300, 1000, 3000 ppm	94.5%, cis/trans: 76.5/18 batch: 88-10	19.2 (300 ppm)	64.3 (1000 ppm)	Reduction: food consumption, body weight, Increase: liver weight, ALT, γ-GT, hepatocellular centrilobular hypertrophy, mild hypochromic microcytic anaemia	[redacted] 1991 (MK-425-002)
90-Day, oral Fischer rat 0, 50, 150, 450, 1350 and 4050 ppm	98.0%, cis/trans: 97/1 batch: 88-05	28.8 (450 ppm)	88.6 (1350 ppm)	Reduction: feed consumption, body weight, RBC Increase: liver/spleen weight, AST/ALT, γ-GT, hepatocellular vacuolation	[redacted] 1992 (MK-425-004)
90-Day, oral Beagle dog 0, 60, 600, 6000 ppm	95.3%, cis/trans: 79.8/15.5 batch: 89-01	2.6 (60 ppm)	24.5 (600 ppm)	Reduction: feed consumption and body weight gain in females	[redacted] 1991 (MK-425-001)
1-year, oral Beagle dog 0, 30, 300, 1000, 3000 ppm	95.3%, cis/trans: 79.8/15.5 batch: 89-01	10 (300 ppm)	36.5 (1000 ppm)	Reduction: body weight gain Increase: AP	[redacted] 1992 (MK-427-002)

Submission of not yet peer-reviewed studies in this AIR3-Dossier:
No new sub-acute or sub-chronic studies with the active ingredient have been performed.

Summary of subacute and subchronic studies in rat, mouse, and dog

Both the isomer mix and the cis isomer of metconazole were tested in subacute (rat, dog) and subchronic studies (rat, mouse, dog).

In the 28-day studies on metconazole cis/trans and metconazole cis in the rat, similar toxicological effects were detected at the top dose, and included decrease in food consumption and body weight, haematological perturbations, and clinical chemical modifications such as increased transaminase activities (AST, ALT), gamma-glutamyl transpeptidase, and alkaline phosphatase activity, the latter indicating a hepatotoxic effect. In a separate mechanistic study with metconazole cis (see also M-CA 5.8.2), it was also demonstrated that cytochrome P450-dependent enzymes were induced in the rat liver at doses corresponding to the lowest observed adverse effect levels (1000 ppm, about 90 mg/kg bw/day). The effect was corroborated by organ gravimetric findings (liver weight increase) and by histopathology (centrilobular hypertrophy) at 1000 ppm and above. In addition, subtle signs of liver necrosis and centrilobular vacuolation were observed, the latter indicating fatty degeneration.

At the top dose of both metconazole cis/trans (3000 ppm or 261.2 mg/kg bw/day) and metconazole cis (10000 ppm or 720.6 mg/kg bw/day), the decreased number of RBC, corpuscular volume and mean cellular haemoglobin concentrations (associated with increased reticulocyte counts with the cis-isomer) were indicative of slight microcytic hypochromic anaemia. In addition, local irritant or inflammatory effects in the fore-stomach were present in most animals. Administration of metconazole during 90 days in the rat revealed the same toxicological effects as seen in the subacute studies. In short, mild anaemia, increased transaminase, γ-GT and AP activities, increased liver and spleen weights, necropsy findings and histological lesions indicating hepatotoxicity (fatty vacuolation) and enzyme induction (centrilobular hypertrophy), signs of haemosiderosis in both liver and spleen (pigment deposit), and adrenal cortex vacuolation were the most relevant findings.

Adrenal effects in the rat occurred at high dose levels. Generally, increased relative organ weights were observed and/or cortical vacuolation. In all studies, the highest dose tested was shown to exceed the maximal tolerable concentrations by severity of clinical signs, markedly reduced food consumption and reduced body weight, as well as local irritant properties as observed by fore-stomach irritation/ulceration. All these findings are associated with pain and distress that are a known causes of stress. Thus, there is a strong indication of a stress-induced secondary response on the adrenal due to a generally poor health status of the animals.

With regard to other observed effects in rat studies on the organs of the endocrine system, respective organ weight decreases (testes/seminal vesicles/prostate, uterus/ovaries) were associated with the impaired health state of the animals as evidenced by significant body weight reduction of approximately 20 - 60% with a corresponding body weight gain reduction that is even higher. According to the guidance document for histologic evaluation of endocrine and reproductive tests in rodents, such findings are considered to be an unspecific effect rather than a consequence of an endocrine mechanism. As shown for seminal vesicles and prostate, decreased body weight gains of ≥ 10% results in weight and size reduction is accompanied by weight or morphology changes in testes or epididymides (OECD Series on Testing and Assessment No. 106). (see also M-CA 5.8.3)

Contrary to the acute oral toxicity studies, where the isomer mix was slightly more toxic (see M-CA 5.2), the isomer mix showed comparable toxicity and similar endpoints than the cis/trans mixture when comparing NOAELs of 28-day and 90-day studies in the rat. For both the cis isomer and the cis/trans mixture liver, spleen, and the adrenals were detected as target organs.
In the 90-day mouse study, both biochemical (CYP liver enzyme induction in two separate mechanistic studies, see also M-CA 5.8.2), as well as toxicological effects were similar to those found in the rat. The overall lowest relevant NOAEL from the available subacute and subchronic studies was derived from the 90-day mouse study at 30 ppm (equivalent to 4.6 mg/kg bw/day) based on increased liver and spleen weights with macroscopic and microscopic correlates, and corroborated by clinical chemistry findings observed at 300 ppm (equivalent to 50.5 mg/kg bw/day).
In the dog, the subchronic toxicity profile was comparable with that observed in rodents, with liver and spleen effects at the top dose in both sexes. Based on decreased food consumption and body weight loss at 600 ppm (24.3 mg/kg bw/day) in the 90-day study, the NOAEL in the females was as low as 60 ppm, corresponding to 2.6 mg/kg bw/day. In the one-year feeding study, the NOAEL (300 ppm, or 10 mg/kg bw/d) was set on the basis of increased alkaline phosphatase activity and decreased body weight gain. Since in the 1 year-study, no effects were observed up to 300 ppm (also at the 90 day sampling time), and this dose-level was higher than the NOAEL in the 90 day study, the 90 day NOAEL was not taken into account. Of note was the emergence of lens degeneration in top-dose females in both 90 day and 1 year feeding studies. With regard to adrenal effects in dogs, organ weight increases were observed only at the top-dose of the 90 day study but not in the 1 year study. This effect is not corroborated by gross necropsy and histology findings, however corresponds with a decrease of food consumption and body weight as well as marked body weight gain reductions in males and females, respectively, which is indicative of stress. Furthermore, the finding of thymus involution seen in 3/5 dogs at the top dose of the 90-day study was a well described feature of stress and is considered to demonstrate an intact glucocorticoid production of the adrenal (Harvey and Sutcliffe, 2010 a, DocID 2010/1233292). Thus, the adrenal findings are assessed to be a physiological adaptation to stress rather than toxicity-induced effects. Regarding effects of metconazole in dog studies on other organs of the endocrine system, non-significantly increased testes weights in top dose males of the 90-day study (6000 ppm or 225.2 mg/kg bw/d) and non-significantly increased ovary weights in top dose females of the 1-year dog study (3000 ppm or 113.7 mg/kg bw/d) were not considered to be relevant in the absence of histopathological correlates. Additionally, isolated weight increases of the thyroids were observed in both sexes at the mid and high dose (600 ppm or 24.3 mg/kg bw/d and 6000 ppm or 206.6 mg/kg bw/d). However the thyroid organ weight changes were not statistically significant, except in females of the top-dose when adjusted to the terminal body weight, and without corresponding relevant gross necropsy and histopathology findings. In the absence of other pathological findings in this organ the increased thyroid weights are of unclear toxicological significance.

Based on the available studies, the following endpoints are provided:

Short-term toxicity

Target organ / critical effect	Rat: liver (weight increase, hypertrophy, AST/ALT/γ-GT increase), mild hypochromic microcytic anaemia, adrenal (weight increase, cortical vacuolation at high dose levels) Mouse: liver (weight increase, hypertrophy, AST/ALT increase), slight microcytic anaemia at highest dose Dog: reduction in body weight gain, increased AP; lens degeneration at higher dose levels	No classification
Relevant oral NOAEL	90-day rat: 19.2 mg/kg bw per day 90-day mouse: 4.6 mg/kg bw/d 1-year dog: 10 mg/kg bw per day	
Relevant dermal NOAEL	No data - not required	
Relevant inhalation NOAEL	No data - not required	

For convenience of the reviewer brief summaries of the respective studies with a special focus on the effects on endocrine organs are presented under the respective chapter.

CA 5.3.1 Oral 28-day study

Rat – 28 days

28-day toxicity study in rat (dietary administration) - metconazole cis/trans (1990, BASF DocID MK-420-002)

Guidelines: In compliance with the test method B.7 of directive 92/69/EEC
GLP: Yes
Acceptance: The study was considered acceptable in the EU registration process 2004.

Material and Methods:
Groups of 7 males and 7 females Fischer 334 rats were fed with diets containing 30, 100, 1000, and 3000 ppm metconazole (96.0%; cis/trans ratio: 83/17, batch: 88-10) for 4 weeks. Achieved doses for males were 2.7, 9.1, 90.5 and 261.2 mg/kg bw/day and for females 3.1, 10.1, 97.0 and 287.4 mg/kg bw/day, respectively.

Findings:
For tabulation of results please refer to the table presented in the monograph (2004).

No mortality was observed. Clinical signs of toxicity included food spillage at 3000 ppm indicating palatability problems. Feed consumption was altered at 1000 ppm (males) and above (both sexes) leading to reduced body weights and body weight gains from week 1-4 (3000 ppm, both sexes) and from week 2-4 (1000 ppm, males). Haematuria was observed in the top-dose males. Haematology findings included mild hypochromic microcytic anaemia as illustrated by the decreased corpuscular volume (3000 ppm) and decreased haemoglobin concentration at 1000 ppm (males) and above (both sexes). Moderate thrombocytopenia was observed in both sexes at the top-dose. Increased leukocyte (females) and monocyte counts (both sexes) were observed at the top-dose, but in the absence of further observations in differential counts, the finding is probably without toxicological relevance.
The compound was hepatotoxic as illustrated by the elevated transaminase and alkaline phosphatase activities (both sexes) at the top-dose. In addition, increased γ-GT were apparent at 1000 ppm and above in animals of both sexes. The slightly increased bilirubin levels in the top-dose females was possibly subsequent to the observed anaemic events (increased Hb catabolism). Further changes at the top-dose included serum glucose decrease (both sexes), and increased albumin, BUN, creatinine, Ca^{2+}, and inorganic phosphorus levels (females). Protein level was increased (females), and cholesterol was decreased at 1000 ppm (males) and above (both sexes).
The observed absolute weight decreases of the heart, kidneys (both sexes) and testes at ≥ 1000 ppm, and of brain (both sexes), ovaries and adrenals (females) at the top dose were considered secondary to the body weight drop at termination. Further alterations included increased relative liver weights at 1000 ppm and above (both sexes). The increased spleen weights at the top dose could be in line with the observed platelet modifications.

At 100 ppm and above, liver enlargement was observed in the females. This finding, as well as pale appearance was observed at 1000 ppm and above in animals of both sexes. At the top dose, indications of stomach irritation were observed, as well as testes wastage.
The gross pathological findings in the liver were confirmed by either slight to moderate (1000 ppm) and moderate to very severe (3000 ppm) fatty degeneration, and dose-dependent severe diffuse (males) or centrilobular (females) hypertrophy. The observed liver enlargement/pallor at 100 ppm was not corroborated by any histological finding. Occasional liver focal necrosis was observed at the top dose. Further observations at the top-dose included: slight to moderate reduction in spermatocyte presence, slight (males) to very slight (females) vacuolation of the adrenal cortex, and moderate hyperkeratosis in the fore-stomach which appeared grossly abnormal.
Paying particular attention to the organs of the endocrine system, the effects of cis/trans metconazole are summarised below:

Table 5.3.1-1: Effects on organs of the endocrine system by cis/trans metconazole administration for 28 days

Sex	Effect dose (ppm / mg/kg bw/day)	Organ affected	Weight effect	Gross pathology	Histopathology	Terminal body weight / Food consumption
Males	3000 / 261	Testes	reduced abs. weight (-44%**)	reduction in size (5/7)	reduced sperma-togenesis (7**/7)	reduction: -34%** / -32%**
		Adrenals	-	-	cortical vacuolation (6**/7)	
	1000 / 91	Testes	reduced abs. weight (-14%*)	-	-	reduction: -14%** / -12%**
Females	3000 / 287	Ovaries	reduced abs. weight (-50%**)	cyst (1/7)	-	reduction: -19%** / -18%**
		Adrenals	reduced abs. weight (-29%**)	-	cortical vacuolation (7**/7)	

* $p < 0.05$, ** $p < 0.01$

Treatment related absolute weight reduction of the testes was observed in animals of both sexes at 3000 ppm as well as in males at 1000 ppm. As a consequence of the severely reduced testes weight at the top dose, reduced size was observed during gross pathology and reduced spermatogenesis in all animals as a histopathological correlate. Cortical vacuolation of the adrenals was seen in animals of both sexes at 3000 ppm. Only in females reduced absolute was observed without an effect on the relative adrenal weight. All organ findings correlated to severe body weight reductions as a consequence of markedly reduced food consumption at dose levels of 1000 ppm and 3000 ppm. Furthermore, stomach irritation as a sign of local toxicity was observed at 3000 ppm in animals of both sexes is also regarded as a sign of pain and distress. Thus, the effects of cis/trans metconazole on the organs of the endocrine system are considered to be a stress-induced secondary response to general impaired state of health as a consequence of malnutrition 1000 ppm and stomach irritation at 3000 ppm in animals of both sexes.

Conclusion:
The NOAEL for this study was established at 100 ppm (equivalent to 9.1 mg/kg bw/day) based on reduced body weight and food consumption as well as liver weight increase with corresponding macroscopic and microscopic changes corroborated by clinical chemistry findings observed at 1000 ppm (equivalent to 90.5 mg/kg bw/day).

Rat – 28 days

28-day toxicity study in rat (dietary administration) - metconazole cis (1991, BASF DocID MK-420-003)

Guidelines: In compliance with the test method B.7 of directive 92/69/EEC
GLP: Yes
Acceptance: The study was considered acceptable in the EU registration process 2004.

Material and Methods:
Groups of 7 males and 7 females Fischer 334 rats were fed with diets containing 30, 100, 300, 1000, and 10000 ppm metconazole (98% pure; cis/trans ratio: 99/1; batch: ST87-181) for 4 weeks. Achieved doses for males were 2.7, 9.2, 27.3, 89.3, and 720.6 mg/kg bw/day and for females 3.0, 9.5, 29.8, 100.7 and 784.3 mg/kg bw/day, respectively.

Findings:
For tabulation of results please refer to the table presented in the monograph (2004).

One top-dose female was killed moribund on day 15, and had similar necropsy findings as the other animals of this dose level. The top-dose animals spilled their feed, leading to body weight loss during week 1, and reduced body weight gain until week 4. They were emaciated, unkempt, showed alopecia (males), and had discoloured urine. Decreased feed intake was observed at 1000 ppm in males and above in animals of both sexes at all sampling times. At 300 ppm, a single statistically significant drop of 7% was noted only during week 3 in the males. Body weight and body weight gain was impaired at 1000 ppm and above on week 1 - 4 in animals of both sexes. On week 1 - 3 at 100 ppm (females) and at 300 ppm (both sexes), sporadic decreases observed were low (below 5%) and not dose-related.

At the top-dose animals of both sexes showed signs of a microcytic hypochromic anaemia, associated with increased levels of RBC-precursor cells like reticulocytes and erythroblasts (normoblasts), indicating a regenerative process. The aetiology was likely a combination of haemolysis, haemorrhage or Fe-deficiency due to decreased feed intake. The high incidence of platelets was reported as an instrumental artefact (interference between nucleated RBC precursors and leukocytes, and of cell debris with platelets), since no abnormally high platelet or leukocyte number were count on smears. The increased neutrophil content was in line with both regenerative anaemia, and acute inflammation events (gastric inflammation). Total protein and albumin levels were increased, and albumin/globulin ratio decreased in the top-dose females, reflecting both an effect on liver and kidney physiology (see also increased plasma urea nitrogen). Liver toxicity was demonstrated by the huge increase in both transaminase and alkaline phosphatase and γ-GT activities in all top-dose animals. The elevated bilirubin level could be a result of both hepatotoxicity and anaemia.

Both absolute and relative (adjusted for terminal bodyweight) liver weight increase was observed at 300 ppm and above in females, and at the top dose in males. Testes weight was decreased at 1000 ppm (absolute) and above (absolute and relative). Statistically significant decreases of absolute but not relative adrenal weight was observed at 300 ppm (males) and at 1000 ppm (both sexes) but not at the top-dose.

Other decreases (absolute) or increases (relative) of organ weights were explained by the body weight decrease at termination. At necropsy, top-dose animals showed enlarged and pale livers, dark kidneys, and fore-stomach irritation lesions. Liver pallor was also observed at 300 ppm and above in males and at 1000 ppm in animals of both sexes. Small testes were observed at 300 ppm and above.
Histological evidence of liver toxicity was observed in the top-dose animals, and occasionally at 1000 ppm, including both fibrotic or necrotic areas and single-cell necrosis, and both micro- and macrovesicular fatty degeneration. In addition, marked parenchymal hypertrophy (indicating enzyme induction) and hyperplasia (increased mitoses) were observed. The liver weight increase and gross lesions observed at 300 ppm were not corroborated by histological findings. Therefore, the effects at 300 ppm could be considered as adaptive and not toxic changes.
Renal toxicity at the top-dose was evidenced by cortical tubular vacuolation, basophilic change, pigmentation, dilatation, and protein casts. Tubular necrosis and acidophilic change was observed at one occasion in females.
In the fore-stomach, papillomatous hyperplasia with associated inflammatory lesions were observed at the top-dose. At this dose-level, further effects were noted on male gonads (testicular and seminal vesicle degeneration), on the immune system (lymphoid depletion in spleen and mesenteric lymph nodes) and on the adrenals (increased cortical vacuolation). While liver, adrenals glands, and kidney were obviously detected as target organs for systemic toxicity, and stomach for local toxicity, it is probable that effects on gonads and lymphoid system were subsequent to feed restriction and associated stress at the top-dose.
Paying particular attention to the organs of the endocrine system, the effects of cis metconazole are summarised below:

Table 5.3.1-2: Effects on organs of the endocrine system by cis metconazole administration for 28 days

Sex	Effect dose (ppm / mg/kg bw/day)	Organ affected	Weight effect	Gross pathology	Histopathology	Terminal body weight / Food consumption
Males	10000 / 721	Testes	reduced abs. / rel. weight (-89%** / -71%**)	reduction in size (5/7); small seminal vesicles (5/7)	tubular atrophy and aspermatogenesis (7**/7); seminal vesicles atrophy (6**/7)	reduction: -62%** / -56%**
		Adrenals	reduced abs. weight (-7%), increased rel. weight (+146%**)	no	cortical vacuolation (7/**7)	
	1000 / 89	Testes	reduced abs. weight (-7%**)	reduction in size (1/7); small seminal vesicles (2/7)	no	reduction: -8%** / -10%**
		Adrenals	reduced abs. weight (-14%**)	no	no	
	300 / 27	Testes	no	small seminal vesicles (2/7)	no	reduction: -5% / -5%
		Adrenals	reduced abs. weight (-10%**)	no	no	
Females	10000 / 784	Ovaries	no	reduction in size (1/7)	no	reduction: -52%** / -48%**
		Adrenals	reduced abs. weight (-6%), increased rel. weight (+100%**)	no	cortical vacuolation (7**/7)	
		Uterus	no	thinning (1/7)	atrophy (1/7)	

* $p < 0.05$, ** $p < 0.01$

In males, macroscopic findings in testes (≥ 300 ppm) were corroborated by histopathological findings (atrophy and aspermatogenesis) only at 10000 ppm, correlating with organ weight reductions at 1000 ppm (absolute) and 10000 ppm (absolute and relative). Any changes at the top dose on the organ weights adjusted for terminal bodyweight were associated with the severe body weight decrease at termination and were therefore not applicable. Additionally, reduced absolute adrenal weight were recorded in males at ≥ 300 ppm without any changes on the relative weights At 10000 ppm adrenal weight reduction was only marginal, leading to highly significant weight increase when adjusted to the terminal body weight. A histopathological correlate for the adrenal weight effects in males was only found at the top dose where adrenal cortical vacuolation was observed in all animals.
In females at 10000 ppm reduction in ovary size and uterus thinning/atrophy was recorded in 1/7 animals. Additionally, marginal adrenal weight reduction led to a significant weight increase when adjusted to the terminal body weight. Adrenal weight effects at 10000 ppm correspond with adrenal cortical vacuolation in all animals.
All organ findings in animals of both sexes correlated to the dramatic terminal body weight reduction particularly at the top dose as a consequence of impaired food consumption. Thus, the effects of cis metconazole on the organs of the endocrine system are a stress-induced secondary response to general impaired state of health as a consequence of malnutrition evident by the body weight loss and reduced food consumption as well as by stomach irritation and reduction of body fat.

Conclusion:
The NOAEL for this study was established at 300 ppm (equivalent to 27.3 mg/kg bw/day) based on reduced body weight and food consumption as well as liver weight increase with corresponding macroscopic and microscopic changes observed at 1000 ppm (equivalent to 89.3 mg/kg bw/day).

Dog – 28 days

28-day toxicity study in dog (dietary administration) - metconazole cis/trans (1991, BASF DocID MK-123-013)

Guidelines: Protocol not in compliance with test method B.7 of directive 92/69/EEC
GLP: Yes
Deviation: At the beginning of the study (week -1), the body weight variation in the female group was > 20%, thus compromising body weight measurements at later sampling times (week 1-4); No histopathological examinations were performed
Acceptance: The study was considered to be a range-finding study for the 90-day oral study

Material and Methods:
Groups of 2 males and 2 females Beagle dogs were fed with diets containing 100 or 1000 ppm (day 1-29), and 10000 (day 1-2)/ 8000 (day 3-4)/7000 ppm (day 5-29) metconazole (95.3% pure; cis/trans ratio: 83.7/16.3, batch: 89-01) for 28 days. Achieved doses for males were 3.7, 37.8, and 170.5 mg/kg bw/day and for females 4.0, 41.2, and 178.4 mg/kg bw/day, respectively. In a preliminary palatability study the test substance has been administered at increasing doses of 200, 400, 800, 1600, 2400, 3200, 4800, 6400, 9600 ppm (dose-levels increased approximately each 3 days if no apparent treatment effects were seen). Critical decline of body weight occurred at 4800 ppm and above, feed consumption was markedly reduced at 9600 ppm, and the majority of the animals showed red areas on duodenum and colon.

Findings:
No mortality was observed. Emesis was noted in one female 5 hours after dosing at 10000 ppm. Food consumption was reduced in males and females treated with the top-dose by 48% and 36%, respectively. In the top-dose males, body weight decreases of 15% (week 1), 20% (week 2), 23% (week 3) and 24% (week 4) were recorded. Body weight losses in the females at this dose-level were hardly interpretable (variation too high at the start of the study), but when comparing to week –1, body weight losses were noted at the top-dose whereas animals gained weight in the other dose-groups. At termination, body weight in males treated with the top dose was decreased by 27% as compared to the control animals. Urine of top-dose animals showed an increased specific gravity, a more intense colour, and a lower pH when compared to controls. No relevant haematology findings were observed. Clinical chemistry revealed increased alkaline phosphatase activities at the top dose (14 to 23-fold on week 2, 16- to 17-fold on week4).

At 1000 ppm a trend toward higher values was also noted, but the increases were weak (by a factor of 0.5 - 0.7).
In the top-dose females absolute and relative weight increases were noted in the spleen (by 26% and 22%, respectively), and in the liver (by 26% and 27%, respectively). In top dose males, elevation of relative spleen weight (+39%) and liver weight (+10%) was noted. Finally, in the females absolute and relative thyroid weight increases of 60% and 41%, respectively, were observed at 1000 ppm and of 65% and 62%, respectively, at the top-dose. No relevant findings were observed at gross pathology. No histopathology was performed.

Conclusion:
The MTD for this study was established at 7000 ppm (170-178 mg/kg bw/day).

CA 5.3.2 Oral 90-day study

Mouse – 90 days

90-day toxicity study in mice (dietary administration) - metconazole cis/trans ([redacted] 1991; BASF DocID MK-425-003):

Guidelines:	Partly in compliance with test method B.26 of directive 87/302/EEC
GLP:	Yes
Deviation:	Raw data or summary tables of clinical signs and ophthalmology not provided
Acceptance:	The study was considered acceptable in the EU registration process 2004

Material and Methods:
Groups of 12 males and 12 females Crl:CD1 (ICR)BR mice were fed with diets containing 30, 300, and 3000 (day 1-7)/2000 (day 7-90) ppm metconazole (purity 95.3%; cis/trans ratio: 83.7/16.3, batch: 89-01) for 90 days. Achieved doses for males were 4.6, 50.5, and 341.1 mg/kg bw/day and for females 6.4, 60.7, and 438 mg/kg bw/day, respectively.

Findings:
For tabulation of results please refer to the table presented in the monograph (2004).

One female dosed at 3000 ppm was in a poor condition at the end of week 1, but was replaced with an animal of the same weight from week 2 (to be dosed 2000 ppm). In the 30 ppm group, one male was humanely killed (week 11) because of sore, swollen shoulders/hind paws, hunched posture and thin appearance, and one male was found dead as a result of urinary tract lesions. Other fatalities included 3 males and 1 female of the control group and 2 males at 30 ppm, which died subsequent to blood sampling problems at termination, and thus these mortalities are not considered to be treatment-related.
No information on clinical signs, urinalysis or ophthalmology was provided. Feed consumption was impaired in all animals treated with 2000 ppm. As a consequence, body weight was decreased at this dose-level during the whole treatment period, leading to a significant drop in body weight gain.
The decreased values of haematocrit, mean cell volume and cell haemoglobin (concentration) at the top-dose were indicative of a slight microcytic anaemia. In addition, increased leukocyte counts, with increased neutrophil but decreased lymphocyte fraction were observed in the top-dose females. Other statistically significant deviations from control groups at the lower dose-levels were considered irrelevant, in the absence of dose-response relationship. The observed increase of transaminase activities (ALT/AST) at 300 ppm (males) and above (both sexes) and of alkaline phosphatase at the top-dose was indicative of liver toxicity. The marginal increase of AST at 30 ppm was probably substance-related, but not considered adverse, in the absence of other relevant effects at this dose. Bilirubin levels were decreased at 300 ppm (males) and above (both sexes). The decreased cholesterol levels at 300 ppm and above maybe related to an alteration of the lipid metabolism.

Creatinine was increased in males at 300 ppm and above, possibly reflecting an effect on the kidney function, although this was not confirmed by neither organ weight nor histopathology. In addition, slightly decreased protein levels were detected in males at 300 ppm and above.

Finally, electrolyte concentrations appeared to be disturbed at the top-dose, as illustrated by increased Ca^{2+} and K^{+} (females) and inorganic phosphate (males), and decreased Cl^{-} (males). Modifications at the lower doses in the absence of effects at the top-dose, were considered not relevant.
At 300 ppm and above, dose-related increased liver weight (both sexes) and increased spleen weight (females) was observed. In males at the top dose the spleen weight increase was marginal. Decreased absolute and increased relative weights of other organs at the top-dose were in line with the observed terminal body weight drop and not considered a primary substance-related effect.
At the top dose, both liver and spleen were enlarged. In addition, spleen enlargement was detected in one female at 300 ppm. The macroscopic findings were confirmed histopathologically. In the liver, hypertrophy/vacuolation was observed at 300 ppm and above, while slight to moderate multifocal leukocyte infiltration was present at the top-dose. At 300 ppm, liver hypertrophy/vacuolation was graded mainly slight to moderate, while the finding was moderately severe to severe at the top-dose. The vacuolation (mainly fine at 300 ppm, more crude at the top-dose) was considered to reflect a fatty degenerative event (oil red O-staining). In addition, the presence of single cell necrosis and pigmented Kupffer cells confirmed the accentuation of degenerative changes at the top-dose.

Paying particular attention to the organs of the endocrine system, the effects of cis/trans metconazole are summarised below:

Table 5.3.2-1: Effects on organs of the endocrine system by cis/trans metconazole administration for 90 days to mice

Sex	Dose level (ppm / mg/kg bw/day)	Organ affected	Weight effect	Gross pathology	Histopathology	Terminal body weight / Food consumption
Males	3000-2000 / 341	Testes	increased rel. weight (+25%**)	small seminal vesicle (1/12)	no	reduction: -17*** / -9%
		Adrenals	increased rel. weight (+91%**)	no	no	
		Thyroid	no	no	cystic follicle (4/12)[&]	
Females	3000-2000 / 438	Ovaries	reduced abs. weight (-27%*)	no	no	reduction: -12%* / -14%**
		Adrenals	no	enlarged (1/12)	cortical vacuolation (5/12)[§]	

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$
[§] = incidence comparable to concurrent control group animals
[&] = some incidences were also observed in concurrent control group males

In males of the top-dose increased relative testes weight was recorded without any histopathological correlate, and in contrary to the weight increase macroscopically one incidence of reduced size of seminal vesicle was observed. Additionally, increased relative weight of adrenals was observed but without macro- or microscopic correlates. Furthermore, some histopathological findings were recorded in thyroids, but they were also observed in concurrent control group males and no organ weight effect or macroscopic correlate were observed.
In females of the top-dose reduced absolute ovary weights were recorded, but without micros- or macroscopic correlates. Additionally, some macroscopic (enlargement) and microscopic (cortical vacuolation) findings were observed in the adrenals. However, the histopathological incidences were also observed in the concurrent control females and no effect on the organ weight was determined.

All organ findings correlated to significant body weight reductions greater than 10% as a consequence of reduced food consumption at the top dose.
Overall the effects of cis/trans metconazole on the organs of the endocrine system are considered to be a stress-induced secondary response to general impaired state of health.

Conclusion:
The NOAEL for this study was established at 30 ppm (equivalent to 4.6 mg/kg bw/day) based on increased liver and spleen weights with corresponding macroscopic and microscopic changes corroborated by clinical chemistry findings observed at 300 ppm (equivalent to 50.5 mg/kg bw/day).

Rat – 90 days

90-day toxicity study in rat (dietary administration) - metconazole cis/trans (1991; BASF DocID MK-425-002)

Guidelines: In compliance with test method B.26 of directive 87/302/EEC
GLP: Yes
Acceptance: The study was considered acceptable in the EU registration process 2004

Material and Methods:
Groups of 10 males and 10 females Fischer rats were fed with diets containing 30, 100, 300, 1000, and 3000 ppm metconazole (94.5% pure; cis/trans ratio: 81/19; batch: 88-10) for 90 days. An additional 7-week recovery experiment was set up, where 10 rats/sex/dose received metconazole in the diet at dose levels of 0 or 3000 ppm. Achieved doses for males were 1.9, 6.4, 19.2, 64.3, and 192.7 mg/kg bw/day and for females 2.1, 7.2, 22.1, 71.4, and 208 mg/kg bw/day, respectively.

Findings:
For tabulation of results please refer to the table presented in the monograph (2004).

No mortality was observed throughout the study period. Feed spillage and evident emaciation was observed in all top-dose animals. During ophthalmoscopic examination, increased lacrimation and eye orbit alopecia was observed in most top-dose animals of both sexes (intermediate dose-levels not examined). Both body weights and feed consumption were decreased during the whole treatment period at 1000 ppm (male) and above (both sexes). The differences in body weight were attenuated during the recovery period but still remarkable at all top-dose animals at study termination.
In the top-dose males, a significant increase in ketone-bodies was observed (+770%), which was related to inanition. In addition, urine showed a more intense colour, which persisted after recovery. In the male recovery group statistically significantly increased osmolarity (+51%) and glucose level (+48%), and decreased volume was noted, while in the female recovery group, increased osmolarity (+24%) and volume decrease (-19%) was noted. The significance remains doubtful in the absence of these modifications during treatment. The changes at the 1000 ppm and above were consistent with a mild hypochromic microcytic anaemia. At the top-dose, platelets were decreased, with increased variation in size, reflecting platelet anisocytosis. In the males, this was accompanied by a marginal fibrinogen clottability deficiency. However, APTT was apparently decreased, and no clinical signs of coagulation deficiency were observed. The increased number of leukocytes (and lymphocytes) in the females was only highly significant at the highest dose; however, differential count did not reveal any pertinent disturbance in the blood formula, and no atypical cells were observed. Protein fractions were disturbed in the top-dose animals, with increased β-globulin fraction as the most consistent modification. Other parameter changes (total protein, albumin, albumin/globulin fraction) were of uncertain significance, as they were of small magnitude, inconsistent between sexes, or gave different results between measuring methods.

The decreased glucose, cholesterol and triglyceride levels at the top-dose were in line with feed deprivation status. The increased transaminase (ALT and AST), alkaline phosphatase and γ-GT levels at 1000 ppm and 3000 ppm were consistent with the effect of the substance on the liver.
The liver was detected as the target organ, both absolute and adjusted liver weights were increased at 1000 ppm and above. Macroscopic effects were noted in the liver at 1000 ppm and above, and occasionally at 300 ppm. Histopathological evaluation revealed microvesicular, diffuse or periportal fatty vacuolation in both sexes, which was graded slight at 300 ppm (males) and at 1000 ppm (females), moderate at 1000 ppm (males) and at 3000 ppm (females) and severe at 3000 ppm in males. After recovery, about half of the animals still showed slight parenchymal vacuolation. Centrilobular hypertrophy was detected at 1000 ppm (males) and above (both sexes), and in one animal at 300 ppm. At the top-dose, the presence of Fe^{3+} - containing pigment within Kupffer cells was observed in both sexes, but was more pronounced in the females. Persistence of hypertrophy in two males and Kupffer-cell pigmentation in two females was demonstrated in the recovery group.

At the top dose, the increase of adjusted splenic weight was highly significant. The weight effects at the other doses in spleen were not always dose-related, or were not corroborated by histological findings. Other weight changes were considered subsequent to terminal body weight changes. The macroscopic alterations in spleen were restricted to the top-dose. Histopathological spleen evaluations revealed decrease of both red and white pulp, and aggregates of pigmented macrophages in the marginal zone of top-dose animals, indicating haemosiderosis.
Macroscopic stomach alterations with correlating fore-stomach focal hyperplasia or hyperkeratosis were seen in mainly the male top-dose animals. On occasions, top-dose females exhibited sub-mucosal inflammatory changes (polymorphonuclear leukocyte infiltration / oedema).

Paying particular attention to the organs of the endocrine system, the effects of cis/trans metconazole are summarised below:

Table 5.3.2-2: Effects on organs of the endocrine system by cis/trans metconazole administration for 90 days to rats followed by a recovery period of 50 days

Sex	Dose level (ppm / mg/kg bw/day)	Organ affected	Group	Weight effect	Gross pathology	Histopathology	Terminal body weight / Food consumption
Males	3000 / 193	Testes	T	reduced abs. weight (-8%**)	dark discoloration(1/10); pallor (1/10); small seminal vesicles (8/10)	focal mineralisation (2/10)	reduction: T: -35%**; R: -24%** / T: -33%**; R: -7%*
			R	reduced abs. weight (-14%*)	reduction in size and pale focus (1/10)	-	
		Prostate	T	n. d.	reduction in size (7/10)	-	
			R	n. d.	-	-	
		Adrenals	T	reduced abs. / rel. weight (-23%** / -11%*),	-	cortical vacuolation (10***/10)	
			R	reduced abs. weight (-20%**)	-	cortical vacuolation (10/10)	
	1000 / 64	Testes	T	-	reduction in size (1/10); reddening of seminal vesicles (1/10)	injection injury of seminal vesicles (1*/10)	reduction: -9%** / -12%**
		Adrenals	T	reduced abs. weight (-10%**)	-	-	
	300 / 19	Testes	T	-	reduction in size (1/10); flaccid (1/10)	tubular atrophy (1*/10); focal mineralisation (1/10)	reduction: -1.4% / -2%
		Adrenals	T	reduced abs. weight (-6%* /)	-	-	

Sex	Dose level (ppm / mg/kg bw/day)	Organ affected	Group	Weight effect	Gross pathology	Histopathology	Terminal body weight / Food consumption
Females	3000 / 208	Ovaries	T	reduced abs. weight (-25%**)	-	-	reduction: T:-19%**; R: -11%** / T: -22%**; R: -3%
			R	no	reddening (3/10)	no	
		Adrenals	T	reduced abs. weight (-24%**)	no	cortical vacuolation (6***/10)	
			R	reduced abs. weight (-11%*)	dark (1/10)	no	
		Uterus	T	n. d.	thinning (8/10)	atrophy (9***/10)	
			R	n. d.	no	distention (1/10)	

* p < 0.05, ** p < 0.01, *** = p < 0.001
T = treatment group; R = recovery group; n. d. = not determined

Absolute but not relative testis weight reductions were observed at 3000 ppm. Macroscopic findings at this top dose consisted of small seminal vesicles. After the recovery period, organ weight reduction were recorded at the top-dose, but only one animal showed a correlating macroscopic finding (reduction in size), and no relevant histopathological effects were noted. Other macroscopic or microscopic findings in testes at the lower doses in the absence of testis weight effects were only single cases and comparable to those seen in the concurrent control animals, and are thus, considered incidental. In the prostate only macroscopic findings were observed at 3000 ppm, however without histopathological correlation and were not seen in the recovery group.
A dose-related significant reduction in absolute adrenal weight was observed in males at 300 ppm and above, a slight significant decrease in relative weights was only observed at 3000 ppm. In top-dose males the adrenal weight effect was corroborated by histopathological findings (moderate adrenal cortical vacuolation).
In females significantly reduced absolute adrenal weight was only reported at the top dose of 3000 ppm, but no effect on the relative weights was observed. Histopathologically, very slight cortical vacuolation was noted in top dose females.
Significantly reduced absolute ovary weight was restricted to the top-dose, was not confirmed by any macro- or microscopic findings, and was not observed at the end of the recovery period. Macroscopic uterus findings (thinning) were confirmed by histopathological evaluation (athrophy), however the severity grade was markedly reduced during recovery period.

All organ effects were reversible within 7 weeks with the exception of the adrenal findings at the top-dose, where the effect on reduced absolute adrenal weight (both sexes) and cortical vacuolation (males only) persisted in both sexes until the end of the recovery period.
Nevertheless, the adrenal findings present at the end of the recovery period also correlated with markedly reduced body weight reductions induced by low food consumption.
Overall, the effects of cis/trans metconazole on the organs of the endocrine system at the top dose are considered to be a stress-induced secondary response to general impaired state of health as a consequence of malnutrition evident by marked body weight reductions and reduced food consumption as well as by stomach irritation.

Conclusion:
Contrary to the previous evaluation in the monograph (2004), the NOAEL for this study is not established at 100 ppm but at 300 ppm (equivalent to 19.2 mg/kg bw/day) based on decreased body weight and food consumption, mild hypochromic microcytic anaemia, and liver effects (increased organ weight, centrilobular hypertrophy, slightly increased ALT and γ-GT) observed at 1000 ppm (equivalent to 64.3 mg/kg bw/day).
The effects seen at 300 ppm (slight hepatocellular vacuolation) are not considered to be adverse.

90-day toxicity study in rat (dietary administration) - metconazole cis (1992; BASF DocID MK-425-004)

Guidelines: In compliance with test method B.26 of directive 87/302/EEC
GLP: Yes
Acceptance: The study was considered acceptable in the EU registration process 2004.

Material and Methods:
Groups of 10 males and 10 females Fischer rats were fed with diets containing 50, 150, 450, 1350, and 4050 ppm metconazole (98% pure; cis/trans ratio: 99/1; batch: 88-05) for 90 days. An additional 7-week recovery experiment was set up, where 10 rats/sex/dose received cis metconazole in the diet at dose levels of 0 or 4050 ppm. Achieved doses for males were 3.2, 9.7, 28.8, 88.6, 264.6 mg/kg bw/day and for females 3.7, 11.0, 33.0, 96.8, and 267.3 mg/kg bw/day, respectively.

Findings:
For tabulation of results please refer to the table presented in the monograph (2004).

No mortality was observed throughout the study period. A dose-dependent increase of feed spillage incidence was observed from 450 ppm (female) and 1350 (male) on. Body weight loss and thin appearance was visible at the top-dose in either sex. In the top-dose males, crusting eyes were additionally observed. At the top-dose a slightly increased incidence of chromodacryorrhea was observed, possibly subsequent to the poor general state of the animals at this dose.
Feed consumption was altered in all top-dose animals during the whole treatment, and at several sampling times at 1350 ppm, the effect being slightly more pronounced in the males. After cessation of the treatment, the feed consumption rebounded from the first week until control level. Body weight decrease was observed throughout treatment duration in both sexes at 1350 ppm and above. The effect was damped but still remarkable until recovery on week 20. No urinalysis was performed.
At 1350 ppm (females) and above (both sexes) RBC parameters demonstrated the presence of a mild hypochromic microcytic anaemia, which was partly compensated. The increased leukocyte (and lymphocyte) count in the top-dose females was not confirmed by findings during differential counting.

After recovery, MCV and MCH (both sexes), were still lower, and RDW (females) and PDW (males) still higher than the control group values. However, RBC count (both sexes), Hct and RBC diameter (males) were increased compared to control values, indicating animals were partially recovered from the anaemic state at the top-dose.
Most enzymatic modifications (increased AST, ALT, γ-GT, AP) were observed at the top-dose, although moderate increases were also noted at 1350 ppm in the males (transaminases) or in the females (γ-GT). Both AP- (males) and γ-GT (females) modifications were still detectable after the recovery period at the top dose. Decreased levels of both cholesterol and triglycerides at 1350 ppm (males) and above (both sexes) may indicate an effect of lipid metabolism. Slight increases of total protein, albumin and bilirubin levels were demonstrated at the top-dose. The altered protein level findings were further investigated by electrophoresis, leading to the observation of decreased α1- and α2-fractions at the top-dose (males) and increased β-globulin fractions at 1350 ppm (males) and above (both sexes).

At the top-dose, an increase of adjusted splenic weight was observed in both sexes. For both liver and spleen the effects were considered substance related in the presence of histological abnormalities observed at study termination.
At necropsy most top-dose animals appeared small and showed reduced abdominal fat. Liver gross lesions (pallor, marked liver lobes, enlargement) were detected at 1350 ppm (males) and above (both sexes) with an isolated case at 450 ppm (males). Fore-stomach or junctional ridge thickening was evident at the top-dose, with some cases of ulcers at 1350 ppm (males). Spleen was characterized by a roughened surface in top-dose animals. Small spleen was also apparent in the female recovery group although this was not reported in the 13 week-treatment group.
Hepatocellular fatty degeneration was illustrated by the increased incidence of microvesicular vacuolation at 1350 ppm and above, which tended to persist in the female animals after recovery. The finding was in line with observed modifications of lipid clinic-chemical parameters at these dose-levels. The observed centrilobular hypertrophy or pallor at the highest dose-levels was an indication of possible enzymatic induction. In the spleen, both red pulp effects (decreased haemotopoietic activity) and white pulp effects (aggregates of pigment-loaded macrophages) were observed at the top-dose.
The inflammatory and hyperplastic changes at 1350 ppm (males) and above (both sexes) were indicative of the irritating effect of the test article on the (fore-) stomach.

Paying particular attention to the organs of the endocrine system, the effects of cis metconazole are summarised below:

Table 5.3.2-3: Effects on organs of the endocrine system by cis metconazole administration for 90 days to rats followed by a recovery period of 7 weeks

Sex	Dose level (ppm / mg/kg bw/day)	Organ affected	Group	Weight effect	Gross pathology	Histopathology	Terminal body weight / Food consumption
Males	4050/ 265	Testes	T	reduced abs. weight (-11%**)	small seminal vesicles (7/10)	reduced secretion of seminal vesicles (2/10), focal mineralisation§ (2/10)	reduction: T: -38%**; R: -18%** / T: -36%**; R: -2%
			R	no	no	focal mineralisation§ (1/10)	
		Prostate	T	n. d.	small prostate (8/10)	no	
			R	n. d.	no	interstitial oedema (3/10)	
		Adrenals	T	reduced abs. weight (-19%**),	enlargement (1/10)	no	
			R	reduced abs. weight (-9%*); increased rel. weight (+10%*)	no	cortical vacuolation (10**/10)	
	1350 / 89	Adrenals	T	reduced abs. weight (-10%**)	no	no	reduction: -10%** / -11%**
	450 / 29	Adrenals	T	reduced abs. weight (-10%**)	no	no	reduction: -3% / -5%
	150 / 10	Adrenals	T	reduced abs. weight (-8%*)	no	no	comparable to control: 103% / 103%
Females	4050 / 267	Ovaries	T	reduced abs. weight (-29%**)	no	no	reduction: T:-20%**; R: -5% / T: -18%**; (R: +5%)
			R	no	parovarian cyst § (1/10)	parovarian cyst § (1/10)	
		Adrenals	T	reduced abs. / rel. weight (-21%** / -13%*)	no	no	
			R	no	no	no	
		Uterus	T	n. d.	thinning (10/10)	atrophy (9***/10)	
			R	n. d.	thinning/small (2/10), small unilateral dark area (1/10); distention (1/10)	thrombus (1/10); dilatation (1/10)	
	1350 / 97	Uterus	T	n. d.	thinning (3/10)	atrophy (3**/10)	reduction: -6%* / -6%
	450 / 33	Adrenals	T	no	haemorrhagic (1/10)	cortical vacuolation and haemorrhage (1*/10)	comparable to control: 99% / 105%
		Ovaries	T	no	parovarian cyst § (2/10), red foci (1/10)	parovarian cyst§ (1/10)	
	150 / 11	Ovaries	T	no	fat nodule (3/10); parovarian cyst§ (4/10), pale nodule (1/10), red focus (1/10)	necrotic fat nodule (3*/10), follicular cyst (1/10)	comparable to control: 101% / 102%

* p < 0.05, ** p < 0.01, *** = p < 0.001
T = treatment group; R = recovery group; n. d. = not determined
§ similar incidences were observed in concurrent control animals (macro- and microscopy)

Absolute but not relative testis weights were reduced at the top dose. Prostate and seminal vesicles appeared small at the top-dose. However, the small appearance of both prostate and seminal vesicles was not corroborated by any histological abnormality.
At the top dose a decrease of absolute (both sexes) adrenal weights was observed, whereas adjusted adrenal weight was increased in males and decreased in females at this dose. At the lower dose-levels absolute adrenal weight decrease was observed in males only without a dose-response, the magnitude was low, and concomitant histological lesions were not reported. The observed cortical vacuolation of the adrenals in the male recovery group at the top dose in the absence of similar findings in the group without recovery was unexplained.
Uterus thinning was apparent at 1350 ppm and above, and the effect persisted in 2/10 females after recovery. At the top-dose, uterus wall atrophy confirmed the thin appearance of this organ at necropsy. Hence, it is probable that the findings were subsequent to the malnutrition status of the animals. Macroscopic and microscopic findings in the ovaries were considered to be not relevant, since either only single incidences were observed, no dose response relationship was seen, or comparable incidences were observed also in concurrent control animals.
Overall, the effects on the organs of the endocrine system at doses of 1350 ppm and higher are considered to be a stress-induced secondary response to general impaired state as a consequence of malnutrition evident by the body weight drop and reduced food consumption as well as by fore-stomach ulcerations and reduced body fat.

Conclusion:
The NOAEL for this study was established at 450 ppm (equivalent to 28.8 mg/kg bw/day) based on reduced body weight and food consumption as well as on increased liver and spleen weights with correlating hepatic macroscopic and microscopic changes corroborated by clinical chemistry findings observed at 1350 ppm (equivalent to 88.6 mg/kg bw/day).

Dog – 90 days

90-day toxicity study in dog (dietary administration) - metconazole cis/trans ([redacted] 1991; BASF DocID MK-425-001)

Guidelines: Partly in compliance with test method B.27 of directive 87/302/EEC
GLP: Yes
Deviation: Raw data or summary tables of clinical signs and ophthalmology not provided
Acceptance: The study was considered acceptable in the EU registration process 2004.

Material and Methods:
Groups of 5 males and 5 females Beagle dogs were fed with diets containing 60, 600, and 6000 ppm metconazole (95.3% pure; cis/trans ratio: 85/15; batch 89-01) for 90 days. Achieved doses for males were 2.5, 24.4, and 225.2 mg/kg bw/day and for females 2.6, 24.3, and 206.6 mg/kg bw/day, respectively.

Findings:
For tabulation of results please refer to the table presented in the monograph (2004).

No mortality was observed throughout the study period. Thin appearance and fur staining was observed from week 7-13 in 2/5 top-dose females as stated in the report. No raw data information was available. Feed consumption, calculated over the whole treatment period, was decreased at 600 ppm (females) and above (both sexes). The decrease occurred mainly during week 1-4 of treatment and was more pronounced in the females than in the males. Consequently, body weights of top-dose animals were depressed throughout the whole treatment period, leading to diminished body weight gains at termination. At the top-dose, decreases of body weight gain amounted up to -60% (male) and -98% (female), while at 600 ppm, a trend toward lower values was noticeable (-29% in females), compared to controls.
Ophthalmology raw data were not available, but in the report, it was stated that all top-dose animals showed lenticular degeneration (cataract). The observed opacities were considered test-article related.
In 3/5 females of the top-dose bilirubin was contained in the urine. There was also a tendency towards increased blood cells in the urine in this group.
At termination, the most prominent changes at the top-dose included reduced RBC counts, Hb content and haematocrit values in both sexes. In conjunction with increased reticulocyte counts, these were considered signs or regenerative anaemia. In the males, platelet count was also increased, but signs of functional blood clotting parameters were inconclusive. Leukocytosis, mainly by increased neutrophil content was also observed in this dose-group. No change was seen in the myelogram, excluding possible test-article related effects in bone marrow. The changes found at the other doses were considered biologically irrelevant, in the absence of dose-responsiveness.

In the top-dose animals, and on the two sampling times, both albumin and albumin/globulin-ratios were consistently lower. Activities of AST, AP and γ-GT were increased on these occasions. The decreased glucose level in the top-dose females was reflected by a malnutrition state.
At the top-dose, both absolute and relative liver (males) and spleen weight (females) were increased. Lenticular degeneration of the lens (characterised by swelling and ballooning of the lens fibres) was evident in all top-dose animals with females being more severely affected than the males. Mottled or pale liver was observed in the top-dose animals. In the females, spleen was enlarged. In histopathological examination the findings corresponded to (mostly periportal) hepatocellular hypertrophy. In the spleen residual blood was present after exsanguination, and haematopoiesis was more pronounced than in the controls. Liver and spleen findings were consistent with the clinic-chemical and haematological modifications. Additionally, renal tubular cell pigmentation (both sexes) and vacuolation (females) was increased at the top-dose. However, primary nephrotoxicity was not suggested, as no clinic-chemical parameters were altered, and further histological signs were absent. Urinary bladder findings at the top-dose were reported as being secondary to catheterization during urine sampling.

Paying particular attention to the organs of the endocrine system, the effects of cis/trans metconazole are summarised below:

Table 5.3.2-4: Effects on organs of the endocrine system by cis/trans metconazole administration for 90 days to dogs

Sex	Dose level (ppm / mg/kg bw/day)	Organ affected	Weight effect	Gross pathology	Histopathology	Body weight / Food consumption
Males	6000 / 225	Adrenals	increased abs. / rel. weight (+12% / +26%)	no	no	reduction: -11% / -14%*
		Testes	increased rel. weight (+26%)	no	segmental hypoplasia (1/5)	
		Thyroids	increased rel. weight (+22%)	no	cyst§ (3/5)	
	600 / 24	Thyroids	increased abs. / rel. weight (+45% / +31%)	slightly enlarged (1/5)	cyst§ (1/5)	comparable to control 111% / 105%
Females	6000 / 207	Adrenals	increased abs. / rel. weight (+16% / +48%)	no	no	reduction: -21%* / -31%***
		Thyroids	increased abs. / rel. weight (+20% / **+52%*)**	no	no	
	600 / 24	Thyroids	increased abs. / rel. weight (+13% / +23%)	no	cyst (2/3) §	reduction: -8% / -14%*

* $p < 0.05$, ** $p < 0.01$
§ comparable incidences were observed in concurrent control animals

At 600 ppm and above, there was an increase of absolute and relative thyroid weight in both sexes, however not significant except for the relative weight increase in females at the top dose (6000 ppm). This finding was not corroborated by macroscopic and microscopic observations. In the absence of other pathological findings in this organ the increased thyroid weights are of unclear toxicological significance.
Increased relative testis weights at the top dose were not statistically significant and in the absence of any macroscopic and microscopic correlates are not considered to be relevant.

At 6000 ppm the adrenal weight was slightly but non-statistically significantly increased in both sexes and neither necropsy nor histopathological findings were indicative of adverse effects. The observed organ weight changes at the top dose were seen in the presence of marked reductions of food consumption and decreased body weight. Thymus involution seen in 3/5 top dose dogs was considered to be a finding associated with stress as a consequence of decreased food intake and decreased body weight gain. The occurrence of thymic involution is a well described feature of stress pathology to demonstrate secretory competency of the adrenal to produce glucocorticoids (Harvey and Sutcliffe, 2010 a, DocID 2010/1233292) and thus, shows functionality of the adrenal. Overall, the effects on the organs of the endocrine system at the highest dose by metconazole cis/trans are considered to be a stress-induced secondary response to malnutrition, which was evident by decreased body weight gain due to decreased food intake.

Conclusion
The NOAEL for this study was established at 60 ppm (equivalent to 2.6 mg/kg bw/day) based on reduced feed consumption and body weight gain observed in females at 600 ppm (equivalent to 24.3 mg/kg bw/day).

Dog – 1 year

1-year toxicity study in dog (dietary administration) - metconazole cis/trans (1992; BASF DocID MK-427-002)

Guidelines: In compliance with the test method B.27 of directive 87/302/EEC
GLP: Yes
Acceptance: The study was considered acceptable in the EU registration process 2004.

Material and Methods:
Groups of 4 males and 4 females Beagle dogs were fed with diets containing 30, 300, 1000, and 3000 ppm metconazole (95.3% pure; cis/trans ratio: 85/15; batch 89-01) for 90 days. Achieved doses for males were 1.1, 12.0, 38.5, and 110 mg/kg bw/day and for females 1.1, 10, 36.5, and 113.7 mg/kg bw/day, respectively.

Findings:
For tabulation of results please refer to the table presented in the monograph (2004).

One high-dose moribund male was euthanized following a history of diarrhea (sometimes containing blood), serious feed consumption decrease and subsequent body weight loss. At necropsy, pale pancreas, ileo-caecal junction / colon mucosa redness and ileum mucosa thickening was observed, together with mesenteric lymph node enlargement and dark colour. The dog showed severe chronic ileum enteritis and caecum/colon chronic inflammatory cell infiltration. However, body weight of this animal was 18% less than the mean group weight at commencement, thus it is not obvious if the poor general state was only related to test substance intake.

At the top-dose, 1 male and 1 female showed opaque eyes from week 26 and week 39 respectively. Food consumption, body weight and body weight gain were globally not changed in either sex over the whole treatment period. However, the males showed a dose-dependent body weight gain reduction when calculated over the first 13 weeks at 1000 ppm and above. Lens opacity was increased in both sexes at top-dose on week 13. At termination the increase compared to controls was evident in the males, but not the females, although the severity of the lesion in the top-dose groups increased as the study progressed. In 1 male and 1 female the lesion was observed in association with uveitis.
Urinalysis revealed no relevant findings. Platelet count was consistently elevated in top-dose animals on weeks 13, 26 and 52. The leukocyte count was also increased in males at all sampling times, sometimes in association with increased neutrophils. Other increases in RBC, Hct or haematocrit of males at lower doses on week 13, 26 or 52 (reaching statistical significance occasionally) were considered non-relevant, in the absence of dose-responsiveness, or when observed at pre-dose sampling time. There was a tendency towards decreased albumin and A/G-ratios in the top-dose animals, although statistical significance in both sexes was only achieved on week 13. The effects seen at the lower doses were spurious, in the absence of dose-dependency. Alkaline phosphatase activity was very significantly and dose-dependently increased in both sexes and on all sampling times, and attained high significance at 1000 ppm and above. On week 52 weak increases were also observed at 30 ppm and 300 ppm, which were not considered of toxicological relevance, since neither evidence of other enzymatic alterations, suggestive of hepatotoxic effects, nor histological findings in the liver were reported at these doses. Rather, the effect may be secondary to induction phenomena (e.g. AP is known to be responsive to induction by glucocorticoids in the dog). Finally weakly increased γ-GT-values (week 13, females) and CPK-values (week 26, males) were noted at the top-dose, but not at termination.
At the top-dose both absolute and relative liver (both sexes) and spleen (males) weight was increased. Minimal weight increases (10-12%) were observed in kidney (males) and thyroid (both sexes), but were probably irrelevant, in the absence of dose-response and other parameters indicating effects in these organs.
Eye lens degeneration was detected in top-dose animals, varying from minor, multifocal degeneration (in 2 males and 1 female) to severe degeneration (lens swelling/liquefaction in males or adhesion and iris cyst in females) and apparent lens thinning (in 1 male and 1 female). The severe cases were reported in association with eye opacity at necropsy. Liver lesions included hepatocyte hypertrophy and vacuolation at the top-dose, and Kupffer cell pigmentation at 1000 ppm (females) and above (both sexes). The cell hypertrophy was predominantly mid-zonal or periportal, and the finding was in line with the observed liver weight increase. Evidence of treatment-related siderosis was found in Kupffer cell pigmentation, which appeared dose-related, both in incidence and in severity. The increased haematopoiesis and pigmentation (severity) in the spleen of top-dose animals was indicative of the increased RBC turnover and breakdown. Other findings included increased minor focal trachea metaplasia incidence and increased caecum inflammatory lesions, although the aetiology of these findings were unexplained.

Paying particular attention to effects on organs of the endocrine system the findings are summarised in the table below:

Table 5.3.2-5: Effects on organs of the endocrine system by cis/trans metconazole administration for 1 year to dogs

Sex	Dose level (ppm / mg/kg bw/day)	Organ affected	Weight effect (adjusted to mean necropsy weight) compared to control	Gross pathology	Histopathology	Terminal body weight / Food consumption
Males	3000 / 110	Thyroids	+12%	no	cyst (1/4)	comparable to control: 99% / 105%
Females	3000 / 114	Ovaries	+45%	enlarged (2/4)	corpora lutea (4/4)	comparable to control: 99% / 99%
		Thyroids	+14%	no	no	

* $p < 0.05$, ** $p < 0.01$

A slight, not significant adjusted thyroid weight increase at the top dose (3000 ppm) in both sexes was considered to be irrelevant in the absence of a dose response relationship and without any related macroscopic and histopathological observations.
Relative ovary weight was increased (45%) at the top dose (3000 ppm), but it was noted that considerable variation in weight was also observed at lower doses, probably reflecting various stages of oestrus cycle of individual animals: some ovaries contained large follicles while others contained corpora lutea. The slightly increased incidence of corpora lutea at the top-dose was not considered to be of pathological alert.
Overall, no treatment-related effects on the organs of the endocrine system could be assigned to metconazole cis/trans.

Conclusion
The NOAEL for this study was established at 300 ppm (equivalent to 10 mg/kg bw/day) based on significantly increased alkaline phosphatase activity and decreased body weight gain observed at 1000 ppm (equivalent to 36.5 mg/kg bw/day).

CA 5.3.1 Other routes

Since the vapour pressure of metconazole is low ($2.1x10^{-8}$ Pa at 25°C, thus < 10^{-2} Pa), and no mortality was observed in the acute inhalation study, no sub-chronic inhalation study was conducted.

Furthermore, as metconazole showed no dermal toxicity when administered acutely, the waiving of the conduct of short-term dermal toxicity study was justified.

CA 5.4 Genotoxicity Testing

The genotoxic potential of metconazole cis/trans: 80/15 was investigated *in-vitro* in the Ames test and in a chromosomal aberration assay in CHO cells, and *in-vivo* in the mouse bone marrow micronucleus test and in an unscheduled DNA synthesis in rat liver cells [Table 5.4-1]. The genotoxic potential of metconazole 95% cis was also investigated in a battery of both *in-vitro* and *in-vivo* tests [Table 5.4-2]. All the tests were negative with the exception of the chromosomal aberration assay performed with metconazole cis/trans: 80/15 in CHO cells, where structural chromosomal aberrations were induced in the presence of S9-mix, in both main and repeat experiments. Overall, both metconazole cis/trans: 80/15 and metconazole 95% cis are considered not genotoxic.

Table 5.4-1: Summary of peer-reviewed metconazole cis/trans (80/15) mutagenicity studies

Study/strain/species	**Purity (%), cis/trans content, batch no.**	**Test conditions**	**Results**	**Reference (BASF DocID)**
Ames mutagenicity test; TA 98, TA 100, TA 1535, TA 1537, TA 1538, and WP2 uvrA	95.3%, cis/trans:79.8/15.5, 89-01	plate incorporation, solvent: acetone, ± S9 mix, up to 5000 µg/plate	Negative	Brooks T.M. and Wiggins D.E. 1990; (MK-435-001)
In vitro cytogenetics: chromosome aberration in CHO-K1 cells	95.3%, cis/trans:79.8/15.5, 89-01	solvent: acetone –S9: 24h/24h: 1.56-12.5 µg/mL, 48h/48h: 0.625-5 µg/mL +S9: 3h/24h: 6.25-50 µg/mL, 3h/48h: 4.375-35 µg/mL	–S9 mix: negative + S9 mix: 24h, **positive** at 50 µg/mL 48h: negative	Brooks T.M. and Wiggins D.E. 1991; (MK-435-002)
UDS, rat (SD) hepatocytes	97.9%, cis/trans:83.7/13.7, AC 9339-114	400, 1000, 2000 mg/kg bw in 0.5% CMC by gavage treatment: 4h and 16h	Negative	[redacted] 1995; (MK-435-008)
In vivo chromosome aberration: Mouse micronucleus test, CD-1 mice	97.9%, cis/trans:83.7/13.7, AC 9339-114	400, 1000, 2000 mg/kg bw in 0.5% CMC by gavage sacrifice: 24h, 48h, 72h	Negative	[redacted] 1995; (MK-435-009)

Table 5.4-2: Summary of peer-reviewed metconazole cis 95% mutagenicity studies

Study/strain/species	Purity (%), cis/trans ratio, batch no.	Test conditions	Results	Reference (BASF DocID)
Ames mutagenicity test; TA 98, TA 100, TA 1535, TA 1537, TA 1538, and WP2 uvrA	95.29%, cis/trans:95.2/0.1, Batch 12	plate incorporation, solvent: acetone, ± S9 mix, up to 5000 µg/plate	Negative	Brooks T.M. and Wiggins D.E. 1991; (MK-435-003)
Gene mutation assay in mouse lymphoma cells L5478Y/TK$^{+/-}$	95.29%, cis/trans:95.2/0.1, Batch 12	Solvent: acetone ± S9 mix: 12.5-90 µg/mL	Negative	Clements J. 1991; (MK-435-007)
In vitro cytogenetics: chromosome aberration in human lymphocytes	95.29%, cis/trans:95.2/0.1, Batch 12	Solvent: acetone ± S9 mix (3h/24h): 93.75 - 750 µg/mL - S9 mix (24h/24h): 5.86 - 93.75 µg/mL - S9 mix (48h/48h): 5.86 - 46.88 µg/mL	Negative	Brooks T.M. and Wiggins D.E. 1991; (MK-435-005)
UDS, rat (SD) hepatocytes	95.29%, cis/trans:95.2/0.1, Batch 12	443, 1400 mg/kg bw in 0.5% CMC by gavage treatment: 12-13 h, 2-3 h	Negative	[redacted]. 1991; (MK-435-004)
In vivo chromosome aberration: Mouse micronucleus test, CD-1 mice	95.29%, cis/trans:95.2/0.1, Batch 12	2 doses/day: 75, 150, 300 mg/kg bw in 0.5% CMC by gavage sacrifice: 24h, 48h after 2nd dosing	Negative	[redacted] 1991; (MK-435-006)

Based on the studies previously peer-reviewed, the following EU agreed endpoints are given in the EFSA conclusion of metconazole (EFSA scientific report, 64, 1-71, 2006):

Genotoxicity	In-vitro: positive in CHO (cis/trans): clastogenic with S9 at 24h but not at 48h sampling time, neither without S9 In-vivo: negative in mouse bone marrow micro-nucleus test and liver UDS

Studies submitted in this AIR 3 dossier (not yet peer-reviewed):
No new studies were submitted. In accordance with the actual requirements the following conclusion on list of endpoints (point 5.4) is proposed for this dossier:

Genotoxicity (Regulation (EU) N° 283/2013, Annex Part A, point 5.4)

In vitro studies	Ames test: Negative Mouse Lymphoma Assay: Negative Chromosome aberration assay in human lymphocytes: Negative Chromosome aberration assay in CHO cells: clastogenic with S9 at 24h but not at 48h sampling time, neither without S9	
In vivo studies	Mouse micronucleus assay: Negative UDS assay: Negative	
Photomutagenicity	Not required	
Potential for genotoxicity	No evidence for genotoxic potential	

For convenience of the reviewer brief summaries of the studies were extracted from the monograph and are provided under the respective chapters.

Comparison with CLP criteria

According to the criteria of the CLP (Regulation 1272/2008/EC), a mutation means a permanent change in the amount or structure of the genetic material in a cell. The term ‘mutation’ applies both to heritable genetic changes that may be manifested at the phenotypic level and to the underlying DNA modifications when known (including specific base pair changes and chromosomal translocations). The term ‘mutagenic’ and ‘mutagen’ will be used for agents giving rise to an increased occurrence of mutations in populations of cells and/or organisms.
In a series of *in vitro* and *in vivo* tests with metconazole several endpoints of potential genotoxicity were measured such as gene mutation in bacterial and mammalian cells, chromosomal aberration and DNA damage/repair. Metconazole cis/trans has been tested negative in an Ames assay using bacterial cells. Results of an *in vitro* chromosome aberration test using mammalian cells were equivocal. However, the non-clastogenicity of metconazole cis/trans confirmed in an *in vivo* micronucleus assay in mouse bone marrow cells and the *in vivo* UDS test using rat hepatocytes was also negative. All available genotoxicity studies with the metconazole cis isomer, including an Ames test, a mouse lymphoma assay in mammalian cells, an *in vitro* chromosome aberration in human lymphocytes, an *in vivo* UDS assay in rat hepatocytes, and an *in vivo* micronucleus test in mouse bone marrow, were negative.

Conclusion on classification and labelling

Overall, based on the available studies metconazole was evaluated to have no genotoxic potential. In conclusion, in comparison with the criteria on classification and labelling for mutagenicity, metconazole is not subject to classification for “mutagenicity” according to Regulation 1272/2008/EC.

CA 5.4.1 In vitro studies

Gene mutation test in bacterial cells

***S. typhimurium* TA98, TA100, TA1535, TA1537 and TA1538 and *E. coli WP2 uvrA*, plate incorporation assay, Metconazole cis/trans (Brooks T.M. and Wiggins D.E. 1990; BASF DocID MK-435-001)**

Guidelines: In compliance with the test method B.13-14 of directive 2000/32/EC.
GLP: Yes
Acceptance: The study was considered acceptable in the EU registration process 2004.

Metconazole (95.3% pure, cis/trans ratio: 83.7/16.3; Batch 89-01) was tested in a standard plate incorporation assay using bacterial tester strains TA 98, TA 100, TA 1535, TA 1537 and TA 1538 of Salmonella typhimurium and the strain WP2 uvrA of Escherichia coli. The assay was performed with and without an S9 liver fraction from rats pretreated with Aroclor 1254. All five tester strains received the test material in acetone at dose levels of 0 (acetone), 31.25, 62.5, 125, 250, 500, 1000, 2000 and 5000 (limit dose) µg/plate with and without metabolic activation. Positive controls tested in the presence of metabolic activation included 5 µg/plate of 2-aminoanthracene administered to TA 1535, 10 µg/plate of benzo(a)pyrene to TA 98, TA 100, TA 1538 and WP2 uvrA, and 20 µg/plate of Neutral Red to TA 1537. Positive controls tested in the absence of metabolic activation included: 2 or 5 µg/plate of sodium azide to TA 1535 and TA 100, respectively; 25 µg/plate of 9-aminoacridine to TA 1537; 20 µg/plate of potassium dichromate to WP2 uvrA, and 5 µg/plate of 2-nitrofluorene to TA 98 and TA 1538. Three replicate plates were used for each dose condition, and the assay was conducted twice.
A preliminary test was first carried out to assess the cytotoxicity of the test material, its solubility in the top agar and for any effect on the pH of the test system. Reproducible dose-related increases or values of 2.5-fold the control values or greater were considered to indicate a mutagenic response.

The stability of test material in the vehicle acetone has been determined analytically.
Cis/trans metconazole formed oil globules in the top agar at 5000 µg/plate dose level; however, no evidence of cytotoxicity was observed at any dose level. The results obtained from both trials showed no positive responses or dose-related increases in revertant frequencies in the presence or absence of metabolic activation. The positive control materials elicited positive responses indicating that the test system was capable of detecting base-pair frame-shift mutations and that the metabolic activation system was functioning properly.

Conclusion:
Metconazole cis/trans: 80/15 did not induce reverse gene mutation in the selected bacterial strains, under the experimental conditions described.

***S. typhimurium* TA98, TA100, TA1535, TA1537 and TA1538 and *E. coli WP2 uvrA*, plate incorporation assay, Metconazole cis (Brooks T.M. and Wiggins D.E. 1991; BASF DocID MK-435-003)**

Guidelines: In compliance with the test method B.13-14 of directive 2000/32/EC.
GLP: Yes
Acceptance: The study was considered acceptable in the EU registration process 2004.

Metconazole (95.29% pure, cis/trans ratio: 99.9/0.1, Batch 12) dissolved in acetone was tested for mutagenicity towards *S. typhimurium* TA98, TA100, TA1535, TA1537 and TA1538 and *E. coli* WP2 uvrA in the presence or absence of an S9 microsomal fraction obtained from a liver homogenate from rats pre-treated with Aroclor 1254. The following concentrations were tested: 31.25, 62.5, 125, 250, 500, 1000, 2000 or 5000 µg/plate. In each of the bacterial mutation assays control plates were set up with the solvent alone and with an appropriate positive control compound. All tests were carried out in triplicate. Two replicate assays were carried out. The standard plate incorporation assay was used. The positive control compounds were in absence of S9: potassium dichromate (*E. coli*), sodium azide (TA1535 and TA100), 9-aminoacridine (TA1537), 2-nitrofluorene (TA1538 and TA98) and in presence of S9: benzo(a)pyrene (*E. coli*, TA1538, TA98 and TA100), 2-aminoanthracene (TA1535) and neutral red (TA1537). A preliminary cytotoxicity assay was first carried out to assess the cytotoxicity of the test material, its solubility in the top agar and for any effect on the pH of the test system. Reproducible dose-related increases or values of 2.5 x control values or greater were considered to indicate a mutagenic response. The criteria for a valid assay were fulfilled.

The test compound formed a precipitate on the top agar at 1000 µg per plate and above showing that it was not totally miscible in the aqueous test system at these treatment levels. Microscopical evaluation of the background lawn showed no evidence of cytotoxicity at amounts up to 5000 µg/plate either in the presence or in the absence of S9. The addition of cis metconazole at amounts up to 5000 µg/plate to agar layer cultures of *S. typhimurium* TA98, TA100, TA1535, TA1537 and TA1538 and *E. coli* WP2 uvrA did not increase the reverse mutation frequency in any of the strains either in the presence or in the absence of rat liver S9. Positive controls gave the expected results.

Conclusion:
Cis metconazole did not induce reverse gene mutation in the selected bacterial strains, under the experimental conditions described.

Gene mutation test in mammalian cells

Mouse lymphoma cells L5178Y/TK$^{+/-}$ using a fluctuation assay - Metconazole cis (Clements J. 1991; BASF DocID MK-435-007)

Guidelines: In compliance with the test method B.17 of directive 2000/32/EC
GLP: Yes
Acceptance: The study was considered acceptable in the EU registration process 2004.

Metconazole (95.29% pure, cis/trans ratio: 99.9/0.1, Batch 12) was assayed for its ability to induce mutation at the tk-locus in mouse lymphoma L5178Y cells using a fluctuation protocol. The study consisted of a cytotoxicity range-finder followed by 2 independent experiments, each conducted in the absence and presence of metabolic activation by an Aroclor 1254-induced rat liver S9 fraction. Concentrations tested in the range-finder both with and without S-9 were 1.58, 5.00, 15.8, 50.0, 158, and 500 µg/mL (a precipitating dose). DMSO served as the vehicle and negative control. Dosing solutions were prepared fresh on the day of dosing. Based on the cytotoxicity observed in the range-finder, the concentrations in the first mutation assay ranged from 12.5 to 125 µg/mL with and without S-9. The concentrations in the independent repeat were adjusted to account for sharp toxicity curve seen in first experiment and ranged from 20 to 70 µg/mL without S-9 and from 12.5 to 90 µg/mL with S-9 activation. Concurrent negative and positive controls (4-nitroquinoline-1-oxide [NQO] at 0.05 and 0.1 µg/mL without S-9 and benzo(a)pyrene [BaP] at 2 and 3 µg/mL with S-9 activation were also included in both studies. All treatments were prepared in duplicate cultures, except for positive controls (single cultures only).
Approximately 10^7 cells/treatment were exposed to the test or control articles for 3 hours. After cleansing, the cultures were maintained through a two-day expression period. Concentrations were selected for viability and selection (5-TFT resistance) plating based on observations on recovery and growth of the cultures through the expression period. Plates were incubated for 1 to 2 weeks and clones were counted and classified as ‘large’ or ‘small.’ In addition, for the negative and positive controls, the number of small and large colonies/well were scored, the mutant frequencies for small and large colonies was estimated, and the proportion of small mutant colonies calculated.
The assay was considered valid if mutant frequencies in the solvent control cultures were within historical control ranges and statistically significant increases in mutation were induced by the positive control compounds NQO (without S9) and BaP (with S9). The test substance was considered to be mutagenic if the validity of the assay was confirmed, the mutant frequency at one or more doses was significantly greater than that of the solvent control, if there was a significant dose-relationship as indicated by the linear trend analysis, and if the effects described above are reproducible. For the solvent and positive controls, the wells containing both small and large colonies were scored. Thus the small and large colony mutant frequencies could be estimated and the proportion of small mutant colonies could be calculated.

In the first experiment, the top dose plated yielded 0.2% and 86.6% relative survival, in the absence and presence of S9, respectively. In the second experiment, the top doses plated yielded 30.3% and 35.1% relative survival in the absence and presence of S9, respectively.
In the absence of S9, no statistically significant increases in mutant frequency were observed in experiment 1 or 2 following treatment with cis metconazole at any dose level. In the presence of S9, one statistically significant increase in mutant frequency was observed at 50 µg/mL in experiment 1 (less than 2-fold) but not at the higher dose of 75 µg/mL or at any lower doses. A significant linear trend was also observed in this experiment. However, when tested at these doses, and to higher doses in experiment 2, no significant increases in mutant frequency were observed and there was no significant linear trend. Therefore, the findings in experiment 1 were not reproducible and thus of no biological significance. Positive controls gave the expected results.

Conclusion:
Under the conditions employed in this study cis metconazole is not mutagenic in this test system.

In vitro chromosome aberration assay in mammalian cells

Chromosome aberration assay in Chinese hamster ovary cells - Metconazole cis/trans (Brooks T.M. and Wiggins D.E. 1991; BASF DocID MK-435-002)

Guidelines: Partly in compliance with the test method B.10 of directive 92/69/EEC or 2000/32/EC.
GLP: Yes
Deviation: Not all experiments were repeated; no short term exposure (3 h) was performed in the absence of S9 mix.
Acceptance: The study was considered acceptable in the EU registration process 2004.

Metconazole (95.3% pure, cis/trans ratio: 83.7/16.3; batch 89-01 technical was tested for its ability to induce chromosomal aberrations in-vitro in Chinese Hamster ovary (CHO) cells. Cultured cells were harvested for chromosome preparation at 24 and 48 hours after the start of the treatment with the test substance. The treatment time was 3 hours in the presence of metabolic activation (Aroclor 1254-treated rat liver S9 fraction), whereas a continuous exposure of 24 or 48 hours was employed in the absence of metabolic activation. Using inhibition of mitotic index (>50%) as the indicator of toxicity, the following doses were evaluated for chromosomal aberrations in the absence of metabolic activation: 12.5, 6.25 and 1.56 µg/mL at the 24-hour harvest; and 5, 2.5 and 0.625 µg/mL at the 48-hour harvest. In the presence of the S9 mix, dose levels of 50, 25 and 6.5 µg/mL were evaluated for the 24-hour sample time, and dose levels of 4.3, 17.5 and 35 µg/mL were evaluated for the 48-hour harvest. Higher doses induced significant toxicity to cell cultures. Concurrent untreated, solvent (acetone), and positive controls (20 µg/mL methyl methanesulfonate (MMS) and 25 µg/mL benzo(a)pyrene (BaP)) were also tested. In each experimental group 200 metaphases were scored for chromosomal aberrations.

The stability of the test material in the vehicle acetone has been determined analytically. In the absence of S9 mix, cell cultures exposed to cis/trans metconazole did not induce any increases in chromosomal aberrations at any dose level at either the 24- or 48-hour harvest interval. After metabolic activation, an increase in the chromosomal damage was observed at the 24-hour harvest at the highest concentration of 50 µg/mL. However, the magnitude of response was small, and the test of linear trend was positive in only one of the two trials, and thus was not reproducible. At the 48 hour harvest time, there was no evidence of any increase in chromosomal aberration at any dose level in the presence of metabolic activation. The MMS and BaP induced statistically significant increases in chromosomal aberrations, thus validating the test system.
In conclusion, as a result of the non-reproducibility of the trend analysis, the weak clastogenic responses only at 24 hours in CHO cells in the presence of metabolic activation were considered equivocal. Moreover, as described below, the non-clastogenicity of metconazole was confirmed in the micronucleus assay under *in vivo* conditions.

Conclusion:
Metconazole cis/trans: 80/15 induced chromosomal aberrations in cultured CHO cells in the presence of S9 mix after 24 but not 48 hours. No damage was observed in the absence of S9 mix under the experimental conditions described.

Chromosome aberration assay in human lymphocytes - Metconazole cis (Brooks T.M. and Wiggins D.E. 1991; BASF DocID MK-435-005)

Guidelines: Partly in compliance with the test method B.10 of directive 92/69/EEC or 2000/32/EC.
GLP: Yes
Deviation: Not all experiments were repeated.
Acceptance: The study was considered acceptable in the EU registration process 2004.

Metconazole (95.29% pure, cis/trans ratio: 99.9/0.1, Batch 12) was tested in cultured human lymphocytes in the presence or absence of an S9 liver fraction from Aroclor 1254-treated rats. Human blood samples were obtained from 3 healthy, male donors. All tests were carried out in duplicate. Each experiment included an untreated control, a solvent (acetone) control and a positive control (without S9: mitomycin C, MMC; with S9: cyclophosphamide, CPA).
Where possible, 200 metaphases (100/culture) were scored for each dose group. The mitotic index of each dose group was assessed at each sample time by counting the number of metaphases in a total of 1000 cells. All exposure times were set up with a top dose of 750 µg/mL, the highest concentration due to solubility. Doses selected for metaphase analysis were based on the mitotic index values.
Experiments without S9 mix were performed with 3 h exposure and 24 hours sampling time, testing concentrations of 93.75, 375 and 750 µg/mL; with 24 h exposure and 24 h sampling time, testing concentrations of 5.86, 23.44, 46.88 and 93.75 µg/mL; and with 48 h exposure, 48 h sampling time, testing concentrations of 5.86, 11.72, 23.44 and 46.88 µg/mL.
Experiments with S9 mix (10% final) were performed with 3 h exposure and 24 h sampling time, testing concentrations of 93.75, 375 and 750 µg/mL.

In a rang-finding study after 3 hours exposure without S9, no effect on the mitotic index (MI) was observed. After 24 hours exposure, cytotoxicity was observed at 46.88 and 93.75 µg/mL with MI of 50% and 15%, respectively, as compared with the solvent control. After 48 hours exposure, mitotic inhibition of 71%, 85% and 31% was observed at 11.72, 23.44 and 46.88 µg/mL, respectively. In the presence of S9 mix, MI of 59%, 78% and 58% at 93.75, 375 and 750 µg/mL, respectively, was observed.
Exposure of human lymphocytes to cis metconazole for 3, 24 or 48 hours in the absence of S9 mix or for 3 hours in the presence of S9 mix did not show any increase in metaphase chromosome damage compared to the negative and solvent controls. The positive controls, MMC and CPA induced significant levels of chromosomal aberrations.

Conclusion:
Cis metconazole does not induce structural chromosomal aberrations in cultured human lymphocytes either in the presence or in the absence of S9 mix, under the experimental conditions described.

CA 5.4.2 In vivo studies in somatic cells

***In vivo* mammalian bone-marrow micronucleus test**

Mouse bone marrow micronucleus test, oral (gavage) - Metconazole cis/trans ([redacted] 1995; BASF Doc ID MK-435-009)

Guidelines: In compliance with the test method B.12 of directive 2000/32/EEC.
GLP: Yes
Acceptance: The study was considered acceptable in the EU registration process 2004.

Five CD-1 mice/sex/dose/time point were exposed to cis/trans metconazole (97.9% pure; cis/trans ratio: 85.5/14; batch AC 9339-114) dissolved in 0.5% carboxymethyl cellulose, at the nominal dose levels of 0, 400, 1000 and 2000 mg/kg bw by gavage. Dosing volume was 20 mL/kg bw. Triethylenemelamine (1 mg/kg bw) was used as positive control and was administered by i.p. injection. Animals were sacrificed 24, 48 and 72 hours after dosing (24 hours after dosing with the positive control). The ratio of polychromatic erythrocytes (PCE) to normochromatic erythrocytes (NCE), determined in approximately 1000 erythrocytes, was established as an indication of bone marrow toxicity. The number of micronucleated PCE (MPCE) and micronucleated NCE (MNCE) per 2000 PCE was determined.
Criteria for determining positive response include the requirement of the test article to produce a positive dose-response trend and a statistically significant increase over that of the concurrent vehicle control in the number of MPCE at one or more dose levels, or if no positive dose-response trend was induced, at least two consecutive test doses should produce a statistically significant increase in the number of MPCE. The doses selection was based on the results of a previous range-finding test with 3 mice/sex/dose at 125, 250, 500, 750, 1000 and 2000 mg/kg bw.

In the dose-finding experiment, none of the treated animals lost more than 10 % bodyweight. No deaths were observed at any dose levels. Clinical symptoms were observed only at the highest dose level (2000 mg/kg bw): 1/3 of males had ataxia and piloerection on days 0-3, 1/3 of females had ataxia and piloerection on day 0 and 1/3 had only piloerection on day 1.
In the main experiment, none of the treated animals lost more than 10% bodyweight. There were no statistically significant increases in the number of MPCE in the treated groups at any harvest time in either sex compared to the concurrent vehicle control groups. However, a reduction in the PCE/NCE ratio compared to the concurrent vehicle control was observed in both males and females, at all dose levels and harvest times (ranging from 16 to 87%), demonstrating that the test compound reached the target bone marrow cells and induced cellular toxicity. The positive control depressed the PCE/NCE ratio and showed a statistically and biologically significant increase in MPCE. The validity criteria in this assay were fulfilled.

Conclusion:
Under the experimental conditions chosen metconazole cis/trans was negative in the micronucleus assay.

Mouse bone marrow micronucleus test, oral (gavage) - Metconazole cis (1991; BASF DocID MK-435-006)

Guidelines: In compliance with the test method B.12 of directive 2000/32/EEC.
GLP: Yes
Acceptance: The study was considered acceptable in the EU registration process 2004.

Five CD-1 mice/sex/dose/time point were administered cis metconazole (95.29% pure, cis/trans ratio: 99.9/0.1, Batch 12) dissolved in 0.5% carboxymethyl cellulose, as 2 daily doses of 0, 162.5, 325 and 650 mg/kg bw by gavage. Dosing volume was 25 mL/kg bw. Cyclophosphamide (CPA; 80 mg/kg bw) was used as positive control and was administered orally as a single dose. Animals were sacrificed 24 and 48 hours after the second dose of metconazole or vehicle and 24 hours after dosing with the positive control. As cytotoxicity was so great that analysis was precluded, the main study was repeated using 2 daily doses of 75, 150 and 300 mg/kg bw.
The ratio of polychromatic erythrocytes (PCE) to normochromatic erythrocytes (NCE), determined in approximately 1000 erythrocytes, was established as an indication of bone marrow toxicity. The number of micronucleated PCE (MPCE) and micronucleated NCE (MNCE) per 2000 PCE was determined.
Criteria for determining positive response include the requirement for a statistically significant increase in the frequency of MPCE occurred for at least one dose at one sampling time, increase of the MPCE frequency above the historical vehicle control range, and a corroborating evidence, for example, increased but statistically insignificant frequencies of MPCE at the other doses or sampling time, or dose response profiles has to be obtained. The doses were based on the results of a previous range-finding test with 3 mice/sex/dose receiving the test substance on 2 consecutive days at the doses of 232.1, 357.0, 549.3, 845.0, 1300 and 2000 mg/kg bw.

In the range-finding experiment, mortalities occurred at 845 mg/kg bw (1 male and 1 female), at 1300 mg/kg bw (3 males and 2 females) and at 2000 mg/kg bw (3 males and 3 females). The LD_{50} calculated from these data was approximately 975 mg/kg bw (x2). A dose equivalent to 50-80% of the LD_{50} was considered acceptable as a maximum dose level, thus 650 mg/kg bw (about 67% of LD_{50}) was initially chosen as an appropriate upper dose level for the micronucleus study.
The 1st main study was attempted using 162.5, 325 and 650 mg/kg bw. Animals were sacrificed 14 and 48 hours after the last application and slides checked prior to analysis. Excessive bone marrow toxicity at the high and intermediate dose levels resulted in frequencies of PCE which were so low as to prohibit analysis. In the 2nd main study dose levels of 75, 150 and 300 mg/kg bw were applied. Mice treated at the two highest doses exhibited PCE/NCE ratios which were 11-35% (150 mg/kg) and 9-19% (300 mg/kg) of control, thus indicating bone marrow was affected. The numbers of MPCE were, however, similar to those seen in controls and were not significantly different by $\chi 2$-analysis. The positive control showed a statistically and biologically significant increase in MPCE. The criteria for a valid assay were fulfilled.

Conclusion:
Under the given experimental conditions, cis metconazole was negative in the micronucleus assay.

In vivo unscheduled DNA synthesis test

UDS *in vivo*/*in vitro*, rat hepatocytes - Metconazole cis/trans ([redacted] 1995; BASF DocID MK-435-008)

Guidelines: In compliance with the test method B.39 of directive 2000/32/EC
GLP: Yes
Acceptance: The study was considered acceptable in the EU registration process 2004.

Groups of 3 male Sprague-Dawley rats/time point were treated with 400, 1000 and 2000 mg/kg bw cis/trans metconazole (97.9% pure; cis/trans ratio: 85.5/14; batch AC 9339-114) dissolved in 0.5% carboxymethyl cellulose by gavage. Dosing volume was 20 mL/kg bw. The rats were treated for 4 or 16 hours prior to sacrifice. Negative control was obtained by treating with the vehicle. Positive controls were obtained by treating by gavage with methyl methanesulfonate (MMS, 100 mg/kg bw, 2 hours prior to sacrifice for the 4 hours harvest) or 2-acetylaminofluorene (2-AAF, 50 mg/kg bw, 18 hours prior to sacrifice for the 16 hours harvest). The hepatocytes were isolated by the liver perfusion method. After culturing, the cells were radiolabeled with ^{3}H-thymidine and the hepatocytes were processed for autoradiography. Three slides/animal with at least 50 cells/slide were analysed. For each dose level, the average net nuclear grain count (NNG) and the percentage of cells with NNG $\geq$ 5 were calculated. The results of each dose were considered significant if the average NNG count was increased by at least 5 grain counts over the control or more than 25% of the cells scored showed a NNG $\geq$ 5. The criteria for a positive response was the requirement for the test article to cause a dose-related response and to exhibits a significant increase over its concurrent vehicle control in at least one dose, or in the absence of a dose-response, at least two successive doses should exhibited increase over the concurrent vehicle control data. The doses selection was based on the results of a previous range-finding toxicity test with 3 male rats/dose at 250, 500, 750, 1000 and 2000 mg/kg bw.

In the range-finding study, the rats treated with 250 mg/kg bw continued to gain weight during the 3-day observation period at the same rate as the rats treated with the vehicle. The rats in the other treated groups also gained weight, except at a slower rate than the rats treated with the vehicle. None of the treated rats died. There were no visible clinical symptoms during the observation period.
In the main experiment, the NNG counts for rats treated at 400, 1000 and 2000 mg/kg bw were -2.0, -1.69 and -1.48, respectively, at the 4-hour harvest time. At the 16-hour harvest, rats treated with the same concentrations had NNG counts of -2.33, -2.32 and -1.76, respectively. None of the treated animals had average NNG counts greater than 0, nor did any animal have greater than 2.22% of nuclei scored with greater than or equal to 5 NNG. These findings indicate that no significant increase in the NNG count resulted from treatment with cis/trans metconazole. Positive controls induced significant positive responses for UDS (average NNG counts of 14.05 and 11.7, for MMS and 2AAF, respectively) and the negative control values were well within the historical control range. The percentage of nuclei in S-phase ranged from 0.54 to 1.89 in the metconazole-treated rats compared to 0.45 to 1.00 in the vehicle control. There was no apparent dose-related response and this finding was not considered significant. Since all treated and control groups showed $\geq$ 0.2% of their nuclei in S-Phase synthesis, the data indicated that the negative response was not due to the inhibition of DNA synthesis at these dose levels. The criteria for a valid assay were fulfilled.

Conclusion:
Under the conditions of study, cis/trans metconazole was negative in the *in vivo*/*in vitro* UDS assay in rats.

UDS *in vivo*/*in vitro*, rat hepatocytes - Metconazole cis ([redacted] 1991; BASF DocID MK-435-004)

Guidelines: In compliance with the test method B.39 of directive 2000/32/EC
GLP: Yes
Acceptance: The study was considered acceptable in the EU registration process 2004.

Groups of 6 male Wistar rats/time point were treated with 443 and 1400 mg/kg bw cis metconazole (95.29% pure, cis/trans ratio: 99.9/0.1, Batch 12) dissolved in 0.5% carboxymethyl cellulose by gavage. Dosing volume was 10 mL/kg bw. The rats were treated for 12-13 hours (1st experiment) or 2-3 hours (2nd experiment) prior to sacrifice. Negative control was obtained by treating the animals with the vehicle. Positive controls were obtained by administering 2-acetyl-aminofluorene (2-AAF, 50 mg/kg bw, for the 12-13 hours sacrifice) or dimethylnitrosamine (DMN, 10 mg/kg bw, for the 2-3 hours sacrifice) by gavage. The hepatocytes were isolated by the liver perfusion method. Cultures were made from 5 animals in each dose group, cells were radiolabeled with ^{3}H-thymidine and the hepatocytes were processed for autoradiography. Three slides/animal (50 cells/animal) were analysed. For each dose level, the average net nuclear grain count (NNG) and the percentage of cells with NNG grains of $\geq$ 5 (cells in repair) were calculated. A test chemical at a particular dose level would be considered clearly positive in this assay if the chemical yielded at least 5 NNG and 20% or more of the cells responding a dose-related increase in both NNG and the percentage of cells in repair would also be required. Cytoplasmic and nuclear grain count values would be considered in relation to the overall net grain count.
The doses selection was based on the results of a previous range-finding toxicity test with 3 male rats/dose at 250, 500, 750, 1000 and 2000 mg/kg bw.

In the range-finding study, 2/3 rats of the top-dose were killed in extremis. Based upon the pattern of mortality, an LD_{50} of 1782 mg/kg bw was calculated. A dose of 1400 mg/kg bw (approximately 79% of the LD_{50}) was chosen as an appropriate upper dose level for the main study. A second dose of 443 mg/kg bw, approximately one ½ log of the top dose was also selected.
In the main experiment, treatment with 443 or 1400 mg/kg bw yielded NNG values less than 0, producing mean NNG values over the 2 experiments in the range of –2.6 to –3.3, well below the level required for a positive response. Furthermore, no cultures from cis metconazole-treated animals gave any cells in repair. Positive controls induced significant positive responses for UDS (average NNG counts of 12.7 and 12.3 as well as 11.6% and 7% of cells in repair, for 2AAF and DMN, respectively) and the negative control values were less than 0. The criteria for a valid assay were fulfilled.

Conclusion:
Under the conditions of study, cis metconazole was negative in the *in vivo*/*in vitro* UDS assay in rats.

CA 5.4.3 In vivo studies in germ cells

No genotoxic activity was detected in somatic mammalian cells, therefore, it is not necessary to conduct a germ cell mutagenicity test.

CA 5.5 Long-Term Toxicity and Carcinogenicity

Long-term toxicity and carcinogenicity potential of metconazole cis/trans were evaluated in rats and mice.
Similar non-neoplastic toxicological endpoints were reported in the rat and in the mouse, comprising body weight effects, as well as increased adrenal gland, liver, and spleen weights. Furthermore, adrenal cortex vacuolation and clinicochemical findings such as decreased cholesterol and triglyceride were observed.
In both rat and mouse the effects on the liver comprised increase liver weights, vacuolation, centrilobular hypertrophy associated with CYP450-dependent liver enzyme induction, and hepatocellular foci. Metconazole showed liver tumours only in the mouse treated for 22 months at 300 ppm (43.6 mg/kg bw/d) and above. Liver activation did not lead to treatment-related induction of tumours in rats. Thus, the compound was considered carcinogenic in the mouse but not in the rat.
Severely increased transaminases (ALT and AST) at the highest dose tested (1000 ppm) in mice were indicative of excessive hepatotoxicity, exceeding the maximum tolerable dose.
The tumour mechanism was investigated in mechanistic studies to address the essential key events leading to the hepatocellular adenoma and carcinoma in mice. The observed findings of specific hepatic CYP activation (mainly via CYP2B) and significantly increased transient proliferation of hepatocytes at the carcinogenic LOAEL of 300 ppm clearly indicate that the mechanism of liver tumour formation is primarily via a CAR (Constitutive Androstane Receptor)-mediated mode of action. Thus, for the mouse liver tumours a phenobarbital-like mode of action, which is not considered to be relevant to human health risk assessment was confirmed.
In the absence of a genotoxic potential it was concluded by the experts during the classification and labelling process, that a classification of metconazole as a carcinogenic substance was not required (see Regulation (EC) No. 1272/2008 as amended by Commission Regulation (EC) No. 790/2009 (1st ATP)). This evaluation is considered to be still valid and no further studies are required.

Studies presented in the original monograph (2004) and the addendum of the monograph (2006): Chronic/carcinogenicity studies with rats and mice were performed. These studies have been evaluated by European authorities and Belgium as Rapporteur member state (European Commission Peer Review Program) and were considered acceptable. Mechanistic studies reported in the original dossier can be found in chapter M-CA 5.8.2 of this dossier.

Table 5.5-1: Critical effects on long-term exposure/carcinogenicity of metconazole cis/trans

Study Species, Dose levels, (test substance, cis/trans ratio, purity, batch)	**NOAEL (mg/kg/day)**	**LOAEL (mg/kg/day)**	**LOAEL Critical effects**	**Reference and year, BASF DocID**
Chronic (2-year), dietary Fischer 344 rat 10, 100, 300, 1000 ppm (WL148271, 79.8% cis:15.5% trans, 95.3% purity, batch 89-01	Toxicity: 4.3 (100 ppm)	Toxicity: 13.1 (300 ppm)	↑ liver/spleen weights, hepatocellular vacuolation/hypertrophy	[redacted] 1992 (a); MK-427-003
Carcinogenicity (2-year), dietary Fischer 344 rat 100, 300, 1000 ppm (WL148271, 79.8% cis :15.5% trans, 95.3% purity, batch 89-01)	Toxicity: 4.6 (100 ppm)	Toxicity: 13.8 (300 ppm)	liver: centrilobular hypertrophy, pigment deposit; adrenal cortex vacuolation	[redacted] 1992 (b); MK-428-001
	Carcinogenicity: 46.5 (1000 ppm, HDT)	-	No carcinogenic effects.	
Carcinogenicity (22-month), dietary Crl:CD-1(ICR)BR mouse 30, 300, 1000 ppm ((WL148271, 79.8% cis :15.5% trans, 95.3% purity, batch 89-01)	Toxicity: 4.4 (30 ppm)	Toxicity: 43.6 (300 ppm)	↓cholesterol/triglycerides ↑AST/ALT, ↑liver weight, liver vacuolation/hypertrophy/ single cell necrosis/sinusoidal hypercellularity/pigmentation, spleen atrophy	[redacted], 1992 (b); MK-428-002
	Carc: 4.4 (30 ppm)	Carc.: 43.6 (300 ppm)	liver adenoma/carcinoma (females)	

HDT: highest dose tested

Based on the studies previously peer-reviewed, the following EU agreed endpoints are given in the EFSA scientific report of metconazole (2006; 64, 1-71):

Target/critical effect:	Liver toxicity (hepatocellular vacuolation/ in mouse and rat; liver hypertrophy/necrosis, ALT/AST increase in mouse); spleen atrophy in mouse; reduced cholesterol/triglyceride levels (mouse); adrenal corticomedullary pigmentation (mouse)
Lowest relevant NOAEL	4.3 mg/kg bw/d (rat)
Carcinogenicity:	Mouse: liver cell adenoma/carcinoma; Phenobarbital-like mechanism (enzymatic induction, subsequent to hepatocellular necrosis and cell renewal) Rat: not carcinogenic

Submission of not yet peer-reviewed studies in this AIR3-Dossier:
No new long-term or carcinogenicity studies have been performed. Further mechanistic data is available from a subacute study in mice (DocID 2004/1032005), that has been submitted and reviewed already during the classification and labelling process of metconazole. This study has been evaluated by RMS Belgium but was not reported in the DAR or Addendum. Thus, the detailed study summary of this mechanistic study is provided in M-CA 5.8.2.

Overall, the major target organ in rats and mice is the liver, resulting in weight increases, vacuolation, hypertrophy, and cell foci. These effects led to increased liver tumours in mice. The rat showed no carcinogenic effects.
Brief study summaries of the long-term and carcinogenicity studies are given below.
Additionally, with regard to the carcinogenicity study in the mouse, relevant effects in the mouse liver are presented and discussed in further detail. An overall assessment of the mode of action of liver tumours in mice and a comparison with CLP criteria can be found at the end of this chapter M-CA 5.5.

No new long-term toxicity or carcinogenicity studies are submitted in this supplementary dossier. Based on the discussion and evaluation of mouse and rat liver effects and mouse liver tumours, as presented below, the conclusion for relevant endpoints for the current renewal of approval is as follows:

Long-term effects (target organ/critical effect)	Liver toxicity (liver weight increases, hepatocellular vacuolation/hypertrophy, and cell foci in mouse and rat; ALT/AST increase in mouse, single cell necrosis/sinusoidal hypercellularity/pigmentation in mouse); spleen atrophy, reduced cholesterol/triglyceride levels	
Relevant long-term NOAEL	Chronic (2-year rat): 4.3 mg/kg bw/d (100 ppm)	
Carcinogenicity (target organ, tumour type)	Mouse: Increased liver adenoma/carcinoma (female). Identified key events from mechanistic studies and effects on liver (i.e. liver weight increase, liver hypertrophy/vacuolation, specific hepatic CYP induction (mainly CYP2B), transient hepatocellular proliferation, cell foci, multifocal hyperplasia) suggest a phenobarbital-like mode of action (CAR-mediated), which is not relevant for humans Rat: no carcinogenic effects	No classification
Relevant NOAEL for carcinogenicity	18-month mouse: 4.4 mg/kg bw/d (30 ppm)	

Chronic toxicity studies

Rat

Metconazole cis/trans / WL148271: A two year chronic toxicity feeding study in rats ([redacted], 1992 (a); BASF DocID MK-427-003)

Guidelines: Protocol in compliance with test methods B.32 of directive 92/69/EEC
GLP: Yes
Acceptance: The study was accepted.

Materials and Methods:
Metconazole (WL148271; purity 95.3%; 79.8% cis:15.5% trans, batch 89-01) was administered at dietary concentrations of 0, 10, 100, 300, and 1000 ppm to Fischer 344 (SPF) rats. The number of treated rats/sex was 40 (vehicle control) and 20 (test article) for the terminal 2 year sacrifice, or 20 (vehicle control) and 10 (test article) for the interim 1 year sacrifice. Achieved test article intake was 0.4, 4.3, 13.1, 43.9 mg/kg bw/day in males and 0.5, 5.3, 16.0, and 53.8 mg/kg bw/day in females.

Findings:
For tabulation of detailed findings please refer to the effect tables presented in the monograph (2004).

There was no treatment-related increase in mortality. Survival rates after 104 weeks increased with increasing dose-levels. Lowest survival (even lower than historical controls) was noted in the study controls. Intermittent deaths were mostly caused by mononuclear cell leukaemia, pituitary and uterine tumours, and chronic nephropathy, which commonly occur in old rats. There were no significant differences in death cause among the study groups. There were no treatment-related clinical observations.
All top-dose animals of the 2 year-sacrifice group showed an initial body weight drop by 8-10% (males, wk 1-10) and by 6-7% (females, wk 1-7), which was sustained until week 104. Overall, body weight gains during the 104-week treatment period were decreased by 9% for males and 6% for females at 1000 ppm, as compared to controls. At the top-dose, consistent decreases in food consumption were observed in the starting phase of the treatment (up to week 7 in males and week 13 in females), but differences during the last 20 weeks of treatment were slight or unremarkable. There were no treatment-related effects on body weight or food consumption in the other treatment groups.
The transient, slight reductions in red blood cell parameters (i.e. haemoglobin concentration, erythrocyte mean diameter, and mean corpuscular haemoglobin) in females at 1000 ppm following 13 weeks of treatment in the 104-week study are consistent with similar reductions in red blood cell parameters observed for females at 1000 ppm in the 90-day study. Although these modifications were subtle, the signs of anaemia were also described in the short-term studies and are therefore considered to be treatment-related. The mild monocytosis in the top-dose females (wk 51, 77, 104) was of questionable toxicological significance, as no abnormal cell types were reported, and no treatment-related leukaemia were seen.

An evaluation of clinical chemistry parameters showed a statistically significant increase in gamma glutamyl transferase activity (γ-GT) in males at week 26 and in females at weeks 26, 52, and 77 in the 1000 ppm group, as compared to controls. At the top-dose, statistically significant decreases in a number of parameters (serum cholesterol and triglycerides (both sexes), bilirubin (both sexes), small decreased in albumin (females), and slight reductions in alkaline phosphatase (males) and alanine aminotransferase (both sexes.)) were observed during one of the intermediate analysis intervals but not at termination. The increase of γ-GT enzyme activity, as well as the decreased lipid parameters was coherent with observed hepatic lesions in histopathology. On the other hand, the (slight) decreases of the transaminase ALT and phosphatase activity (AP) remained unexplained.
Increased urine osmolarity values and decreased urine volumes were observed in top-dose males during weeks 51, 77 and 104. However, the significance of these modifications was unclear due to the absence of nephrotoxicity both at intermediate and final sacrifice.
Statistically significant increased relative liver weights were observed at 300 ppm (+5%) and 1000 ppm (+20%) in males at week 52, and at the top-dose (1000 ppm) in females at termination (+12%). Relative spleen weights were increased at 300 ppm (males: +39%)) and 1000 ppm (males: +56%; females: +21%) at week 104; and already at 52 weeks a marginal but statistically significant relative spleen weight increase (+9%) was observed in top dose females.
At intermediate sacrifice, gross liver lesions (mottled appearance, enlargement, and at one occasion fatty appearance) were apparent in males at 300 ppm or 1000 ppm. Increased hepatocellular lipid vacuolation and centrilobular hypertrophy was observed in males at 300 ppm and in both sexes at 1000 ppm. Furthermore, at 52 weeks some evidence of cytotoxicity was noted by increased slight mononuclear cell infiltrate in the liver of top dose males and females, and the incidence of inflammatory necrotic foci was also slightly increased at this dose in males. However, no signs of necrotic inflammatory foci were observed at termination.
At termination, lipid vacuolation and centrilobular hypertrophy was noted in females and males at the top dose. Top dose males also showed increased hepatic pigment deposition in the liver, that might be indicative of slight haemosiderosis, as subtle haematological disturbances were noted, and this finding was also reported in earlier studies.
In this study only a slight increase in clear-cell hepatocellular foci was reported in top dose male animals. This finding also occurs spontaneously in untreated Fischer rats, however at the same dose level in the carcinogenicity study clear cell foci were much more prominently increased and were considered as a consequence of the sustained liver activation. In correlation to the increased spleen weights an increased incidence of splenic histiocytic foci was noted for both sexes at the 1000 ppm dose level at termination, which may be a sign for increased haematopoiesis due to increased erythrocyte break-down.
The findings in the liver (increased weights, centrilobular hypertrophy, vacuolation) were also reported in short-term studies and were associated with liver activation and induction of metabolizing enzymes (see also mechanistic study in chapter MCA 5.8.2). The liver effects were more pronounced in males.

Conclusion:
In this chronic toxicity study target organs were the liver and the spleen. The toxicity NOAEL was found to be at 100 ppm (4.3 mg/kg bw/d) based on increased liver and spleen weight, as well as hepatocellular hypertrophy and vacuolation at 300 ppm (LOAEL, 13.1 mg/kg bw/d)

Executive summary of the analytical method used within the analytical phase (MK-427-003)

Principle of the method

The analytical method used in study MK-427-003 was validated for the determination of *cis*-metconazole (BAS 555 F) in animal test diet. The study was performed by SITTINGBOURNE RESEARCH CENTRE, UK.

Residues were extracted from test diet samples by Soxhlet extraction with 30% acetone in hexane. The extracts were diluted to a known volume with hexane. For control samples as well as samples fortified at a low concentration, an acetonitrile/hexane partition step was performed prior to analysis. Measurements are based on the peak due to the *cis*-isomer alone, and results are expressed in terms of the test item as received, no purity correction being applied. Residues were analyzed by gas-liquid chromatography (GLC) coupled to a nitrogen thermionic detector (TID). Analysis was performed on an Ultrabond II column.

Recovery findings

The mean recovery values were between 70% and 110% for metconazole. The detailed results are given in the table below.

Table 5.5-2: Validation results of method applied in study MK-427-003 using GLC-TID: *cis*-metconazole (BAS 555 F) in animal test diet

Matrix	Analyte	Detector settings	Fortification level (mg/kg)	No of replicates	Mean recovery (%)	RSD (%)
Animal test diet	*Cis*-metconazole	Nitrogen thermionic detector, 300°C	10	60	102	6.1
			100	10	104	5.8
			300	6	101	4.9
			1000	12	107	16.6
			1000 [a]	11 [a]	103 [a]	7.0 [a]
			Overall [a]	87 [a]	102 [a]	6.0 [a]

[a] Excluding one recovery value of 155%

Linearity

The linearity was tested using standards at concentrations between of 1.0 to 4.0 µg/mL. Calibration solutions were prepared in hexane and contained about 2 to 4% by volume of the extract from control diet to improve the performance of the method.

Specificity

Final detection has been applied by nitrogen thermionic detection (TID). As only known analytes of known concentration were analyzed for dose verification purposes, no additional confirmatory technique is necessary.

Interference

No relevant interferences from the reagent blank at the relevant retention times have been reported.

Limit of Quantitation
The limit of quantitation (LOQ) is defined as the lowest fortification level successfully tested. During this study, a limit of quantitation of 10 mg/kg for *cis*-BAS 555 F was obtained in animal test diet.

Repeatability
The relative standard deviations (RSD, %) for all fortification levels were below 20%.

Stability of solutions
The stability of the analytes in working solutions was tested within this study. Test substance solutions were stable for up to one month when stored in the dark at ambient temperature.

Conclusion
The analytical method used in study MK-427-003 for the analysis of *cis*-metconazole in animal test diet uses GLC-TID for final determination, with an LOQ of 10 mg/kg.
It could be demonstrated that the method generally fulfils the requirements with regard to repeatability, specificity, LOQ and recoveries and is therefore considered suitable for the analysis of *cis*-metconazole in animal test diet.

Carcinogenicity studies

Rat

Metconazole cis/trans / WL148271: A two year oncogenicity feeding study in rats ([redacted], 1992 (b); BASF DocID MK-428-001)

Guidelines: Protocol in compliance with test methods B.32 of directive 92/69/EEC
GLP: Yes
Acceptance: The study was accepted.

Materials and Methods:
Metconazole (WL148271; purity 95.3%; 79.8% cis:15.5% trans, batch 89-01) was administered at dietary concentrations of 0, 100, 300, and 1000 ppm to 50 Fischer rats per sex and group for 24 months. Achieved test article intake was 4.6, 13.8, 46.5 mg/kg bw/day in males and 5.5, 16.6, 56.2 mg/kg bw/day in females.

Findings:
For tabulation of detailed findings please refer to the effect tables presented in the monograph (2004).

There were no treatment-related effects on mortality. Survival rates at study termination for the animals receiving 0, 100, 300 and 1000 ppm were 60%, 56%, 50% and 66%, respectively, for males, and 66%, 60%, 62% and 68%, respectively, for females. The most common causes of death were pituitary tumours, disseminated mononuclear cell leukaemia and severe chronic nephropathy in both treated and untreated animals. All these findings occur commonly in ageing untreated control rats of the F344 strain and there was no significant differences in the causes of death between control and treated animals. There were no treatment-related clinical observations. Ophthalmology revealed a slightly increased incidence of keratitis in top-dose females (12/20) compared to controls (9/20).
Mean body weights were statistically significantly reduced during the initial 13 weeks of the treatment period for males at 1000 ppm and throughout the treatment period for females at 1000 ppm, as compared to controls. Body weight gains were slightly reduced at both the week 0 to 13 interval and the week 16 to 104 interval for males and females at the top dose, with the largest differences occurring during the first week of treatment. Overall, body weight gains in the 1000 ppm group were decreased by 6% for males and by 9% for females during the 104-week treatment period. Food consumption was statistically significantly decreased during treatment weeks 1 to 8 for males, and at numerous intervals during the first 72 weeks for females at 1000 ppm. There were no treatment-related effects on body weight or food consumption in the other treatment groups

Haematological evaluations showed minimal but consistent statistically significant decreases of erythrocyte mean diameters in top-dose males (all sampling times) and females (wk 52). Changes in erythrocyte morphology (microcytosis, anisocytosis and spherocytosis) were observed for males in the 1000 ppm group, suggestive of a slight haemolytic anaemia. Clinical chemistry and urinalysis were not performed in the study. The organ weight analysis at terminal sacrifice showed statistically significant increased weights of liver and spleen (males and females), as well as of kidneys and adrenals (males) in top dose animals only.

Histopathological evaluation showed increased incidences of hepatocellular (macrovesicular lipid) vacuolation at 1000 ppm, which was more pronounced in males. Furthermore, increased hepatocellular (centrilobular) hypertrophy and pigment deposition was seen in males at 300 and 1000 ppm, as well as statistically significantly increased clear cell (both sexes) and eosinophilic foci (males) in the liver at 1000 ppm, as compared to controls. Liver hypertrophy and vacuolation were consistently observed in other rat feeding studies and are associated with liver activation and liver enzyme induction (see also mechanistic study in chapter MCA 5.8.2).
Pigment deposition in the liver of mid and high dose males was also a change seen in the 90-day rat study after exposure to 3000 ppm of metconazole cis/trans, where it was demonstrated to be Fe3+ (see also M-CA 5.3: Esdaile, 1991; MK-425-002). This may be associated with haematological disturbances. Although erythrocyte parameters were not measured in this carcinogenicity study, morpho1ogica1 observations of erythrocyte microcytosis in top dose males would suggest anaemia in this dose group. No other evidence of increased erythrocyte destruction was seen histologically. Since no specific stain was performed to identify the origin of the pigments in this study they could also represent an aging pigment (lipofuscin). However, the evidence from the subchronic study provides quite strong indication that it may be haemosiderin.
The increased incidence of both c1ear cel1 and eosinophilic foci, more strongly observed in males in this study, may represent a hepatocellular proliferative response to the compound. Both increased numbers of clear cell foci and eosinophilic foci have been associated with chronic enzyme induction and are typically induced after treatment with phenobarbital, the model compound for a CAR-mediated liver inducer in rodents (Whysner et al., 1996, BASF DocID 1996/1005220).
The incidence of adrenal cortical vacuolation was increased in males at 300 or 1000 ppm. This change is considered to be reflected by the increased adrenal weights in males at 1000 ppm. In contrary to the discussion in the study report and the previous evaluation in the DAR the effect on the adrenals are not regarded as a consequence of direct toxicity, since the findings (adrenal cortical vacuolation and increased adrenal weights) are also typical of a secondary response to stress (Harvey and Sutcliffe, 2010; DocID 2010/1233293). In this study observed forestomach lesions were consistent with irritation and inflammation and are considered to induce pain and distress. In the forestomach of treated male rats a non-dose related increased incidence of histological changes associated with erosion and ulceration was seen, although the incidence was not considered to be related to treatment by the study author. An increase of inflammatory and hyperplastic changes in the non-glandular stomach of males at 100 ppm and above was reported without a clear dose-response. The relative incidences (46.4-48.4%) of erosions and ulcers at the intermediate doses (but not at the top-dose) exceeded slightly those of the in-house historical controls (4-46%).

It has to be noted that this carcinogenicity study was run in parallel to the rat chronic toxicity study, which did not report adrenal changes at similar dose levels and no stomach lesions were detected (see above MK-427-003). However, similar stomach lesions were present in both subacute and subchronic rat studies, hence, a causal relationship with treatment is probable (see also chapter M-CA 5.3). It cannot be explained why the two studies differed, group size may be a factor accounting for the difference between studies. However, the overall profile of adrenal changes after metconazole treatment does not indicate direct adrenal toxicity (for detailed discussion please refer to chapter M-CA 5.8.3).
In the kidney, a slight increase in the severity (but not the incidence) of chronic renal nephropathy was observed in males at 300 ppm and 1000 ppm. Parathyroid hyperplasia was observed in males at 300 ppm and above and corresponded to secondary hyperparathyroidism, probably subsequent to the renal insufficiency.
All top-dose animals showed focal histiocytes in the spleen, which can be a sign of increased erythrocyte break-down. Thus, the effect may be associated with the haematological disorders, since an accumulation of pigmented macrophage aggregates was described in short-term feeding studies.
A marginal increase of focal interstitial-cell hyperplasia in the testes was reported at the top-dose, but the effect is not necessarily adverse, since no reproductive impairment was described in the 2-generation rat studies and no gonadal tumours appeared. Furthermore, the spontaneous rate of testis interstitial cell tumors is quite high in the Fisher 344 rat strain used and the observed incidence of testis interstitial cell tumors was within the range of historical controls as reported by National Toxicology Program/NTP (Haseman et al., 1990; BASF DocID MK-905-011).
There were no treatment-related neoplastic findings. At 300 ppm, an increased incidence of pituitary adenoma (pars distalis) was observed in males. At the top-dose, the incidence was similar to that of controls, and the incidence at any dose was within the incidence range which could be expected for F344-rats of this age (Haseman et al., 1998; BASF DocID 1998/1006954).
An increased incidence of mononuclear cell leukaemia was observed at 100 ppm and 300 ppm (both sexes) and at the top-dose (females). The incidence was slightly outside the range of in-house historical controls, but within the historical control incidence as reported by National Toxicology Program/NTP (Haseman et al., 1990; BASF DocID MK-905-011). Considering the high spontaneous incidence of both pituitary and hematopoietic tumours in this rat strain and the lack of a dose-response relationship, metconazole is regarded to be devoid of carcinogenic properties in the rat.

Conclusion:
In this 2-year carcinogenicity toxicity study, target organs were the liver (pigment deposit and centrilobular hypertrophy) and the adrenals (cortical vacuolation). Based on these findings the NOAEL for chronic toxicity was set at 100 ppm (4.6 mg/kg bw/d). Because no treatment-related effects on the types or incidences of neoplasia were observed at any concentration, the NOAEL for carcinogenicity was set at 1000 ppm (46.5 mg/kg bw/d), the highest dose tested.

Executive summary of the analytical method used within the analytical phase (MK-428-001)

Principle of the method

The analytical method used in study MK-428-001 was validated for the determination of *cis*-metconazole (BAS 555 F) in animal test diet. The study was performed by SITTINGBOURNE RESEARCH CENTRE, UK.

Residues were extracted from test diet samples by Soxhlet extraction with 30% acetone in hexane. The extracts were diluted to a known volume with hexane. For control samples as well as samples fortified at a low concentration, an acetonitrile/hexane partition step was performed prior to analysis. Measurements are based on the peak due to the *cis*-isomer alone, and results are expressed in terms of the test item as received, no purity correction being applied. Residues were analyzed by gas-liquid chromatography (GLC) coupled to a nitrogen thermionic detector (TID). Analysis was performed on an Ultrabond II column.

Recovery findings

The mean recovery values were between 70% and 110% for *cis*-metconazole. The detailed results are given in the table below.

Table 5.5-3: Validation results of method applied in study MK-428-001 using GLC-TID: *cis*-metconazole (BAS 555 F) in animal test diet

Matrix	Analyte	Detector settings	Fortification level (mg/kg)	No of replicates	Mean recovery (%)	RSD (%)
Animal test diet	*Cis*-metconazole	Nitrogen thermionic detector, 300°C	100	10	104	5.8
			300	6	101	4.9
			1000	12	107	16.6
			1000 [a]	11 [a]	103 [a]	7.0 [a]
			Overall [a]	87 [a]	103 [a]	5.9 [a]

[a] Excluding one recovery value of 155%

Linearity

The linearity was tested using standard solutions at concentrations of 1.0 to 5.0 µg/mL. For *cis*-BAS 555 F, no information regarding linearity of calibration standard measurements was stated. Calibration solutions were prepared in hexane and contained about 2 to 4% by volume of the extract from control diet to improve the performance of the method.

Specificity

Final detection has been applied by nitrogen thermionic detection (TID). As only known analytes of known concentration were analyzed for dose verification purposes, no additional confirmatory technique is necessary.

Interference
No relevant interferences from the reagent blank at the relevant retention times have been reported.

Limit of Quantitation
The limit of quantitation (LOQ) is defined as the lowest fortification level successfully tested. During this study, a limit of quantitation of 100 mg/kg for *cis*-BAS 555 F was obtained in animal test diet.

Repeatability
The relative standard deviations (RSD, %) for all fortification levels were below 20%.

Stability of solutions
The stability of the analytes in working solutions was tested within this study. Test substance solutions were stable for up to one month when stored in the dark at ambient temperature.

Conclusion
The analytical method used in study MK-428-001 for the analysis of *cis*-metconazole in animal test diet uses GLC-TID for final determination, with an LOQ of 100 mg/kg.
It could be demonstrated that the method generally fulfils the requirements with regard to repeatability, specificity, LOQ and recoveries and is therefore considered suitable for the analysis of *cis*-metconazole in animal test diet.

Mouse

WL 148271: 91 Week oral (dietary administration) carcinogenicity study in the mouse ([redacted], 1992 (b), BASF DocID MK-428-002)

Guidelines: Protocol in compliance with test methods B.32 of directive 92/69/EEC
GLP: Yes
Acceptance: The study was accepted.

Materials and Methods:
Metconazole cis/trans (WL148271; purity 95.3%; 79.8% cis:15.5% trans, batch 89-01) was administered at dietary concentrations of 0, 30, 300, and 1000 ppm to groups of 51 Crl:CD-1 (ICR) BR mice per sex for 22 months. Achieved test substance intake was 4.4, 43.6, and 144.9 mg/kg bw/day in males and 5.2, 53.0, and 179.2 mg/kg bw/day in females. Satellite groups (12/sex/group) were treated at the same concentrations, and were sacrificed after 51 weeks of treatment.

Findings:
Survival rates at week 91 for the animals receiving 0, 30, 300 and 1000 ppm were 47%, 44%, 42% and 43%, respectively, for males, and 51%, 54%, 69% and 56%, respectively, for females.
The survival after 90 weeks was not adversely affected by the treatment. In high-dose female decedents, there was an increased incidence of liver tumours. During the second half of the study (weeks 62 to 90 for males, and weeks 52 to 90 for females) there was an increase of swollen abdomens in the top-dose animals only. There were no other treatment-related clinical observations.
Food consumption for males was decreased at the top-dose in the first week of treatment. Small differences with control group were observed until the end. For females, food consumption was statistically significantly decreased during weeks 1 to 13 and weeks 56 to 88 at the 1000 ppm group. Mean body weights were statistically significantly decreased due to a body weight loss following 1 week of treatment for males and females at 1000 ppm.
Slight and consistent, but not statistically significant reductions in body weight were observed throughout the study in top dose males (generally below 10%). In females slightly decreased body weights were observed at the intermediate dose and top-dose from week 1 to week 88(-8% and -13%, respectively).
Mean body weights gains were statistically significantly decreased due to a body weight loss following 1 week of treatment for males (-1.7 g, $p < 0.01$) and females (-1.0g, $p<0.001$) at 1000 ppm, as compared to controls. Mean body weight gains during weeks 0 to 52 were significantly decreased 23% for males and 32% for females at 1000 ppm, as compared to controls, and overall body weight gain during the treatment period was significantly reduced by 25% for males and by 32% for females in the 1000 ppm group, as compared to controls.
At termination, an increased total leukocyte count was observed at 300 ppm (males) and above (both sexes). At the top-dose a concomitant increase of both neutrophils and lymphocytes was noted in males and females. The findings at low and intermediate dose on week 52 (decrease of lymphocyte count) were considered irrelevant in the absence of dose-effect relationship.

Clinical chemistry parameters at week 52 and/or week 91 revealed highly increased alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities (up to 4.4-fold of control in males and up to 11.4-fold in females) at the top dose of 1000 ppm in all animals (more pronounced in females), indicating marked hepatotoxicity at this dose level, which exceeds the maximum tolerated dose (see Table 5.5-4). At the mid dose of 300 ppm, only small increases in serum ALT and AST activity below two fold of the control were induced (1.1- to 1.9-fold) in females, which is a similar response also seen with other liver enzyme–inducing compounds in rats. (Hall et al., 2012, BASF DocID 2012/1365642). This is also supported by liver histopathology findings (see below). Total cholesterol was decreased at intermediate sacrifice in males and females at 300 ppm and above and triglyceride levels were decreased on week 52 and 91 at 300 ppm and above.

All top-dose animals showed markedly increased liver weights, and reduced spleen weights, both at interim and terminal kill. The liver weight increase was much more pronounced at the top dose and the effect was most prominent at terminal sacrifice (Table 5.5-4). The slight liver weight increase observed at 300 ppm (mainly in females at interim kill) was also considered related to treatment, since body weight differences with controls were unremarkable at that dose.

The most notable necropsy findings in the liver of top-dose animals included enlargement and thickening or patchy/focal paleness both at interim and final sacrifice. At termination, irregular surface appearance, and presence of multiple masses and nodules was obvious at that dose. Top-dose groups also showed pale/mottled spleen, while small spleen was observed at 300 ppm (females) and above (both sexes).

Liver histopathology findings (see Table 5.5-5) consisted of increased incidences of vacuolation and hypertrophy in both sexes at 300 ppm and above. Parenchymal hypertrophy was consistent with the observed induction of CYP450 enzymes in the mouse (see also M-CA Section 5.8.2). There was a dose-related increase in single cell necrosis/sinusoidal hypercellularity/pigmentation reported at the mid and top dose in both sexes, with females being more sensitive. Due to the combined analysis of pigmentation, sinusoidal hypercellularity, and single cell necrosis it is difficult to distinguish between the three (see Table 5.5-6). In addition, increased hyperplastic proliferation of oval cells and biliary duct cells (both sexes), and multifocal parenchymal hyperplasia was observed in top-dose animals with a slight prevalence in females.
Decreased spleen weights, spleen atrophy and concomitant prominent appearance of connective tissue (trabeculae and stroma) was observed at 300 ppm and above, which may also be secondary to statistically significantly decreased terminal body weights (-6% in males, -19% in females) particularly at the top dose. Furthermore, pigment deposition in the corticomedullar part of adrenals was also observed at 300 ppm and above. In contrast to the previous evaluation in the Monograph (2004) it is suggested that the pigment deposition is related to the endogenous aging process in the mouse (Maronpot RR, 1999; Pathology of the mouse) and the association with treatment is therefore, questionable.

Table 5.5-4: Liver clinical chemistry parameters and organ weight: selected findings – interim and main study

Study details	Endpoint (% of control)	Effect level
Interim sacrifice	ALT↑ (249%*** m, 651%*** f)	1000 ppm (m, f)
	AST↑ (1000 ppm: 163%***m, 309%*** f; 300 ppm: 121%* f)	1000 ppm (m, f), 300 (f)
	Relative liver weights↑ (1000 ppm: 151%*** m, 242%*** f; 300 ppm: 121% *** f)	300 ppm (f) 1000 ppm (m, f)
Terminal sacrifice	ALT↑ (1000 ppm: 442%*** m, 1140%*** f; 300 ppm: 194%*** f) AST↑ (1000 ppm: 353%*** m, 413%*** f; 300 ppm: 116%*** f)	1000 ppm (m, f) 300 (f)
	Relative liver weights↑ (300 ppm: 122% m, 128% f; 1000 ppm: 247%*** m, 335% ***f)	300 ppm (m, f) 1000 ppm (m, f)

Statistical evaluation *: p≤0.05, ***: p≤0.001

Table 5.5-5: Histopathology: selected liver findings- interim (I) study and main (M) study, all animals

		Males				Females			
Dose level [ppm]		**0**	**30**	**300**	**1000**	**0**	**30**	**300**	**1000**
Liver									
No. examined	I	12	12	12	12	12	12	12	12
	M	51	51	51	51	51	51	51	51
Hepatocellular vacuolation	I	3	4	5	6	5	4	10	10
	M	11	10	20	37	11	12	36	44
Hepatocellular hypertrophy	I	0	0	9	11	0	0	3	10
	M	0	0	13	44	0	0	8	38
Single cell necrosis / sinusoidal hypercellularity / pigmentation	I	0	0	2	9	0	0	3	11
	M	0	1	10	37	0	0	18	35
Multifocal hepatocellular hyperplasia	I	0	0	0	1	0	0	0	9
	M	0	1	0	31	0	0	0	44

Table 5.5-6: Severity grading of single cell necrosis/sinusoidal hypercellularity/pigmentation of the liver findings in interim (I) and main (M) group rats

		Males				Females			
Dose level [ppm]	**Group**	**0**	**30**	**300**	**1000**	**0**	**30**	**300**	**1000**
Liver									
No. examined	I	12	12	12	12	12	12	12	12
	M	51	51	51	51	51	51	51	51
Single cell necrosis / sinusoidal hypercellularity / pigmentation	I	0	0	2	9	0	0	3	11
	- minimal			1	6			3	5
	- slight			1	3				5
	- moderate			-	-				1
	[mean grade]			[1.5]	[1.5]			[1.0]	[1.6]
	M	0	1	10	37	0	0	18	35
	- minimal		-	5	18			11	13
	- slight		1	3	16			6	11
	- moderate		-	2	3			1	2
	[mean grade]		[2.0]	[1.7]	[1.6]			[1.4]	[1.6]

[] mean severity grading; histopathological findings were graded minimal (Grade 1), slight (Grade 2), moderate (Grade 3), marked (Grade 4), moderately severe (Grade 5), and severe (Grade 6) massive/severe (Grade 5)

With regard to neoplastic findings, increased liver adenoma were present at the interim kill at the top dose in females (see Table 5.5-6). In the main study a dose-related increased incidence of liver adenoma was seen in females at 300 ppm and above (males and females). Liver carcinoma were slightly increased at mid and top-dose in males without a dose-response relationship and markedly increased in females only at the top dose (see Table 5.5-7). Liver tumour incidences in males were in all cases within the in-house historical control data for adenoma, and just outside the historical range for carcinoma in the mid and high dose. In females liver adenoma at 300 ppm and above and liver carcinoma at 1000 ppm were outside the historical control range. Therefore, metconazole was considered to be carcinogenic in female mice at dose levels of 300 ppm and above.

Table 5.5-6: Number of animals with liver tumours in the 91-week carcinogenicity study in CD-1 mice–Interim study (I), all animals

	Males				**Females**			
Dose level [ppm]	**0**	**30**	**300**	**1000**	**0**	**30**	**300**	**1000**
Liver								
No. examined	12	12	12	12	12	12	12	12
Hepatocellular adenoma	1	1	1	1	0	0	0	8
Hepatocellular carcinoma	0	0	0	0	0	0	0	1

Table 5.5-7: Number of animals with liver tumours in the 91-week carcinogenicity study in CD-1 mice–Main study, all animals

	Males				**Females**			
Dose level [ppm]	**0**	**30**	**300**	**1000**	**0**	**30**	**300**	**1000**
Liver								
No. examined	51	51	51	51	51	51	51	51
Hepatocellular adenoma	10	16	15	34	0	1	4	42
Adenoma Historical control[#]	*11-37*				*0-1*			
Hepatocellular carcinoma	4	4	7	7	0	1	0	19
Carcinoma Historical control[#]	*1-6*				*0*			
Hepatocellular adenoma and carcinoma	12	16	18	37	0	2	4	44

[#] *Historical control data from the testing facility: 4 studies (91-weeks) conducted between 1986-1996 (see DocID 2015/1228506)*

Conclusion:

In the chronic toxicity study with metconazole in mice the liver (hypertrophic and hyperplastic events) and spleen (atrophy) were identified as the target organs. Based on these effects the toxicity NOAEL was set at 30 ppm (4.4 mg/kg bw/d). The presence of liver adenoma at medium and high doses and of liver carcinoma at the high dose in female mice was considered to be treatment-related and led to the NOAEL for carcinogenicity at 30 ppm (4.4 mg/kg bw/d).

Executive summary of the analytical method used within the analytical phase (MK-428-002)

Principle of the method

The analytical method HUK 579/25-02F was validated for the determination of metconazole (BAS 555 F) *cis*- and *trans*-isomers in animal test diet. The study was performed by HAZLETON UK, UK.

Residues were extracted from test diet samples by Soxhlet extraction with acetone. Afterwards, the sample was made to a final volume of 100 mL. Control samples as well as samples fortified at a low concentration, concentrated and cleaned-up. Therefore, samples were evaporated to dryness under nitrogen. Acetonitrile was added and the sample mixed to ensure dissolution of the test item. Hexane added, the sample is shaken, and the hexane layer is transferred to another test tube and the process was repeated a second time. Afterwards, the hexane layers were combined. Acetonitrile is then added to the tube containing the combined hexane washes. The acetonitrile combined extract was evaporated to dryness under nitrogen. Acetone is then added and the tube was mixed to ensure dissolution of the test item. Residues were analyzed by gas liquid chromatography (GLC) coupled to a nitrogen-phosphorus detector (NPD). Analysis was performed on a CP Sil 8 CB column.

Recovery findings

The mean recovery values were between 70% and 110% for *cis*- and *trans*-metconazole. The detailed results are given in the table below.

Table 5.5-8: Validation results of method HUK 579/25-02F using GLC-NPD: metconazole (BAS 555 F) *cis*- and *trans*-isomers in animal test diet

Matrix	Analyte	Detector settings	Fortification level (mg/kg)	No of replicates	Mean recovery (%)	RSD (%)
Animal test diet	*Cis*-metconazole	Nitrogen-phosphorus detector 300°C	30	3	93	0.6
			1000	3	97	1.7
			Overall	6	95	2.6
	Trans-metconazole		30	4	86	2.1
			1000	4	97	1.2
			Overall	6	92	6.2

Linearity

The linearity was tested using three matrix standards at concentrations between of 30 to 3000 µg/g. Matrix calibration standards were prepared by fortifying control test diet with a respective volume of fortification solutions. Fortified control test diet was extracted by Soxhlet extraction as described above. For BAS 555 F (*cis* and *trans*), no information regarding linearity of calibration standard measurements was stated. Matrix calibration solutions were prepared in acetone.

Specificity
Final detection has been applied by nitrogen-phosphorus detection (NPD). As only known analytes of known concentration were analyzed for dose verification purposes, no additional confirmatory technique is necessary.

Interference
No interferences from the reagent blank at the relevant retention times have been reported.

Limit of Quantitation
The limit of quantitation (LOQ) is defined as the lowest fortification level successfully tested. During this study, a limit of quantitation of 30 mg/kg for BAS 555 F (each isomer, *cis* and *trans*) was obtained in animal test diet.

Limit of Detection
For BAS 555 F (*cis* and *trans*), the limit of detection (LOD) was 0.2 mg/kg in animal test diet.

Repeatability
The relative standard deviations (RSD, %) for all fortification levels were below 20%.

Conclusion
The analytical method HUK 579/25-02F for the analysis of metconazole *cis*- and *trans*-isomers in animal test diet uses GLC-NPD for final determination, with an LOQ of 30 mg/kg (per isomers).
It could be demonstrated that the method generally fulfils the requirements with regard to repeatability, specificity, LOQ and recoveries and is therefore considered suitable for the analysis of metconazole (*cis*- and *trans*-isomer) in animal test diet.

Mode of action analysis for liver tumour formation and human relevance assessment:

Metconazole causes liver tumours in the mouse carcinogenicity study.
A MOA for phenobarbital (PB)-like (CAR-mediated) rodent liver tumour formation has been hypothesized during the last EU evaluation process of metconazole. The results presented in the 14-day mechanistic study in mice (for details see study summary in M-CA 5.8.2) established a threshold dose level for the key precursor events considered to be critical at early and late stages in the progression of metconazole's non-linear mode of action for induction of mouse liver tumours.

Sequence of key events and chronological time points:
The proposed sequence of key events and chronological time points in the mouse liver tumour mode of action are as follows (Elcombe et al., 2014, BASF DocID 2014/1326892):

Increased relative liver weights → hepatocellular hypertrophy → CYP Induction specific to mode of action (as indicated by liver weight increase and hypertrophy) → hepatocellular proliferation → clonal expansion leading to altered foci/multifocal hyperplasia → liver tumours

Demonstration of the MOA and considerations of possible alternative MOAs:
Principally the key events associated with the rodent specific CAR-mediated MOA for hepatocarcinogenicity were observed in long term rodent studies with metconazole. Both mice and rats responded to the compound with similar key events listed above. In case of the rat, however, the event cascade ended with the increased frequency of eosinophilic and clear cell foci, whereas in the mouse study liver tumours were induced. An overview of the MOA key events related to the mouse liver tumour formation is presented in Table 5.5-9.

Liver weight increase and hypertrophy were consistently observed in subacute, subchronic and chronic studies and were indicative of liver activation. In a 14-day mechanistic study specific CYP-enzyme activities as well as cell proliferation were investigated.
In the absence of significant effects on specific liver surrogate enzymes, indicative for alternative nuclear receptor activation other than PB-like MOA via CAR, metconazole does not appear to act via peroxisome proliferation (no induction of CYP4A) or AhR (only slight induction of CYP1A). The predominant induction of hepatic CYP2B (CAR-dependent) and to a lesser extent CYP3A (due to typical crosstalk with PXR) by metconazole, as evidenced by increased enzyme activities and proteins, is associated with a PB-like mode of action for the mouse liver tumour formation.
An increase in hepatocellular proliferation was observed at the carcinogenic LOAEL and above.
In terms of a genotoxic MOA, metconazole has been shown to be overall negative in a battery of genotoxicity tests in vitro and in vivo (see M-CA 5.4). Furthermore, oxidative DNA damage was also excluded, since no increased 8-OHdG adducts were observed in mice liver after 14-day treatment with metconazole (see M-CA 5.8.2).
The top dose (1000 ppm) was associated with markedly increased ALT/AST, indicating excessive hepatotoxicity exceeding the maximum tolerated dose. At the first glance one may consider cytotoxicity as a possible alternative MOA for the development of hepatocellular tumours. However, a detailed analysis of the data (see Table 5.5-6) indicates that this is unlikely for the following reasons:

- The dose-responses of 'Single cell necrosis / sinusoidal hypercellularity / pigmentation' (SSP), a complex of findings which –according to the study authors - 'probably reflected the various stages of degeneration/necrosis' caused by metconazole, and the hepatocellular tumour incidences were different. While only a doubling in the incidence of SSP in high dose main group females was noted, the incidence of hepatocellular tumors (adenoma and carcinoma) rose 11-fold. In main group males an approximate 4-fold increase in SSP incidence was accompanied by a doubling of tumor incidences, only.
- The mean severity of SSP at the mid and high dose level is essentially identical in males and females and cannot account for differences in tumour incidence.
- For 20 of the 44 hepatocellular tumour bearing high dose females (i.e. females #s 456-459, 461-463, 468, 473, 476, 483, 485, 488, 489, 501, 502 and 504) no SSP was diagnosed.

Prominent multifocal parenchymal hyperplasia was observed in top dose mice with a slight prevalence in females and is a late key event of the liver tumour formation for a phenobarbital-like mechanism.

In conclusion, based on the sequence of key events involved, metconazole shows a non-linear (threshold) CAR-mediated (phenobarbital-like) mode of action leading to increased incidences in mouse liver tumours.

Table 5.5-9: MOA key events related to CD-1 mouse liver tumours after treatment with metconazole cis/trans: temporality and dose response relationship

Key events - effects	Study type /temporal occurrence of effect	Effect dose levels (ppm)	
		LOAELs	NOAEL
Increased relative liver weights	3, 7, 14, days	300 and 1000 (HDT)	30
	28 days (cis)	300 (only concentration tested)	n.a.
	90 days	300 and 2000 (HDT)	
	52 weeks	300 and 1000	30
	91 weeks	300 and 1000	30
Liver hypertrophy	14 days	300 and 1000 (HDT)	30
	28 days (cis)	300 (only concentration tested)	
	90 days	300 and 2000 (HDT)	
	52 weeks	300 and 1000	30
	91 weeks	300 and 1000	30
Hepatic CYP induction: CYP2B↑/CYP3A↑ (enzyme activities & proteins)	7 days	300 and 1000 (HDT)	**30**
	28-days (cis)	300 (only concentration tested)	n.a.
Hepatic cell proliferation (increased PCNA labeling index)	3, 7, 14, days	300 and 1000 (HDT)	30
Hepatocellular foci/multifocal hyperplasia	52 weeks	1000 (HDT)	300
	91 weeks	1000 (HDT)	300

Key events - effects	Study type /temporal occurrence of effect	Effect dose levels (ppm)	
		LOAELs	NOAEL
Liver tumours: adenoma/carcinoma	52 weeks	1000 (HDT)	300
	91 weeks	300 and 1000 (HDT)	30

n.a. not applicable, HDT: highest dose tested

Human relevance assessment:
The MOA in mice, as discussed above, consists of key events that are dose-concordant and occur in a logical temporal sequence. Furthermore, they appear consistently across multiple studies. Other potential MOAs have been considered and were excluded.
A hepatocarcinogenic response in rodents for compounds which have data to support a PB-like MoA is considered to be not relevant to humans (Holsapple et al., 2005, BASF DocID 2006/1047813 and Elcombe et al., 2014, BASF DocID 2014/1326892). Based on the available epidemiological studies in the literature there was no evidence for a specific role of phenobarbital in human liver cancer risk (La Vecchia and Negri, 2013, BASF DocID 2013/1420420).
On this basis, the mouse liver tumours associated with administration of metconazole would not pose a cancer hazard to humans.

Comparison with CLP Criteria

According to the criteria of the CLP (Regulation 1272/2008/EC), Carcinogen means a substance or a mixture of substances which induce cancer or increase its incidence. Substances which have induced benign and malignant tumours in well performed experimental studies on animals are considered also to be presumed or suspected human carcinogens unless there is strong evidence that the mechanism of tumour formation is not relevant for humans.

Chronic toxicity/carcinogenicity studies with metconazole were conducted in two species (rats and mice). Liver tumours were observed only in female mice with a dose response relationship and a carcinogenic LOAEL at 300 ppm (4.4 mg/kg bw/d).
To elucidate the non-genotoxic mode of action responsible for the increased incidence of liver tumours in mice, mechanistic studies were performed. Further indications for such a mode of action were observed in the liver based on identification of precursor key events from subchronic and carcinogenicity studies. In summary, taking all available data into consideration the underlying mode of action for liver tumour formation of metconazole in the CD-1 mice is considered be a phenobarbital-like (CAR-mediated) mode of action, which is a rodent specific effect with no relevance to human health.
In conclusion, based on the assessment of all available data metconazole is not subject to classification for carcinogenicity according to Regulation 1272/2008/EC.

Conclusion on classification and labelling

The available data on carcinogenicity of metconazole do not meet the criteria for classification according to Regulation (EC) 1272/2008 and are therefore conclusive but not sufficient for classification.

CA 5.6 Reproductive Toxicity

Studies previously evaluated during the last Annex I inclusion process:
The metconazole studies evaluated in the draft monograph of the Rapporteur Member State Belgium (January, 2004) consisted of several 1-generation (pilot) reproduction studies in rats, a two-generation study in rats, and a series of developmental toxicity studies in rats and rabbits using metconazole cis/trans and/or cis (Table 5.6-1). Further evaluations of the developmental studies were made in the final Addendum to the DAR (January, 2006). These studies have been evaluated by European authorities and Belgium as Rapporteur Member State (European Commission Peer Review Program) and were considered to be acceptable.

One rabbit developmental toxicity was conducted according to the most recent OECD 414 testing guideline ([redacted] 1997 a).
Additionally, further developmental toxicity studies in rabbits exist. Some aspects of these teratology studies in rabbits performed with metconazole in New Zeeland White rabbits from the Interfauna source were questionable. Interpretation difficulties emerged when comparing data between studies on the same compound, and at the same doses. However, despite the variability encountered, some pertinent effects were detected. In the evaluation, endpoints either emerging at the top-dose, showing a dose-responsive trend, or effects showing replication between different studies were highlighted and discussed. A summary of these additional rabbit developmental toxicity studies is presented in Table 5.6-2.

Table 5.6-1: Summary of already peer-reviewed reproductive and developmental toxicity studies with metconazole cis and cis/trans

Study species, Dose levels	Test Substance (Purity (%), cis/trans content, batch no)	NOAEL [mg/kg bw/d]	LOAEL [mg/kg bw/d]	Critical effects	Reference (BASF DocID)
Reproductive Toxicity rat					
1-Generation, diet, rat (Sprague Dawley) 0, 50, 500, and 1500 ppm (~ 0, 2.9/3.6, 28.0/35.8, 89.9/116 mg/kg bw/d, m/f)	cis/trans (batch 89-01, purity: 95.3%, cis/trans: 79.8/15.5% - nominal 80:15)	Parental/repr.: 2.9/3.6 (50 ppm)	Parental/repr.: 28.0/35.8 (500 ppm)	food and water consumption ↓, bw gain↓, slightly delayed gestation, dystocia (1/10 mortalities)	[redacted] 1991a (PART I; MK-430-002)
		Offspring: 2.9/3.6 (50 ppm)	Offspring: 28.0/35.8 (500 ppm)	pre-birth loss, pup loss at birth↑, body weight gain (PND 0-42)↓,	
1-Generation, diet, rat (Sprague Dawley) 0, 500,750 ppm	Comparative cis versus cis/trans (cis isomer: purity not mentioned, assumed 95% cis, batch ST89/411; cis/trans isomer see info above part I)	-	Parental/repr.: ≥ 500 ppm	food and water consumption ↓, bw gain↓, slightly delayed gestation, dystocia↑ (cis/trans at 750 ppm)	[redacted] 1991a (PART II; MK-430-002)
		-	Offspring: ≥ 500 ppm	pre-birth loss, live litter size↓, pup loss at birth↑, pup weight↓ (PND 21),	
1-Generation, diet, rat (Sprague Dawley) 0, 1, 2, 8, 32 mg/kg bw/d	cis/trans (batch 89-01, purity: 95.3%, cis/trans: 79.8/15.5% - nominal 80:15)	Parental/repr.: 8 (100-149/35-93 ppm)	Parental/repr.: 32 (394-570/141-378 ppm)	food consumption↓ and bw gain↓ during gestation, gestation length↑ (slight)	[redacted] 1991 a (MK-430-001)
		Offspring: 32 (394-570/141-378 ppm)	Offspring: > 32 (>394-570/141-378 ppm)	no effects	
2-Generation study/Main study, diet, rat (Sprague Dawley) 0, 2, 8, 32, 48 mg/kg bw/d	cis (batch 12, purity: 95.2%, cis/trans: 95.2/0.09%)	Parental/repr.: 8 (54-195/41-127 ppm)	Parental/repr.: 32 (219-780/160-511 ppm)	bw↓ and bw gain↓ (F0/F1)↓, gestation length↑ (F0/F1)	[redacted] 1992 a (MK-430-003)
		Offspring: 8 (54-195/41-127 ppm)	Offspring: 32 (219-780/160-511 ppm)	post-implantation survival↓ (F2), pup bw gain (PND1-25)↓;	

Developmental toxicity rat					
Teratogenicity, gavage (GD 6-15), rat (Sprague Dawley) 0, 12, 30, 75 mg/kg bw/d Preliminary study: 0, 37.5, 75, 150, 300 mg/kg bw/d	cis/trans (batch 89-01, purity: 95.3%, cis/trans: 79.8/15.5% - nominal 80:15)	Maternal: 12	Maternal: 30	bw gain↓	[redacted] 1991b (MK-432-005)
		Fetal: 30	Fetal: 75	post-implantation loss↑ (early and late), foetal/litter weight↓	
		Developmental: 12	Developmental: 30	skeletal ossification variations↑ (lumbar/cervical ribs)	
Teratogenicity, gavage (GD 6-15), rat (Sprague Dawley) 0, 6, 24, 60 mg/kg bw/d Preliminary studies: 1st: 0, 6, 12, 24, 48 mg/kg bw/d 2nd: 0, 6, 60, 80 mg/kg bw/d	cis (batch 12, purity: 95.2%, cis/trans: 95.2/0.09%)	Maternal: 24	Maternal: 60	wc↑, fc↓, bw gain↓,	[redacted] 1992b (MK-432-009) Preliminary studies: 1st: [redacted] 1992c (MK-432-008) 2nd: [redacted] 1992d (MK-432-006)
		Fetal/dev.: 24	Fetal/dev.: 60	post-implantation loss↑ (early and late), placental wt↑, fetal wt↓, litter size↓,	
Developmental toxicity, rabbit					
Teratogenicity, gavage (GD 6-28), rabbit (New Zealand White) 0, 5, 10, 20, 40 mg/kg bw/d Preliminary study: 0, 1, 5, 10, 20, 40 mg/kg/d	Cis/trans (batch AC 10575-61, purity: 98.3%, cis/trans ratio: 84.2/13.7%)	Maternal: 20	Maternal: 40	fc↓, bw loss, reduced Hb↓, Hct↓, MCV↓, platelet counts↑, AP↑, liver weight↑ (abs. & rel.)	[redacted] 1997a (MK-432-015)
		Fetal: 20	Fetal: 40	small increase in post implantation loss↑ (early and late); foetal wt↓ (n.s.), litter size↓ (n.s.)	
		Developmental: 40 (HDT)	Developmental: -	-	

n.s. non-significant
bw: body weight
fc: food consumption
PND: post-natal day

Table 5.6-2: Summary of additional developmental toxicity studies (treatment during GD 7-19)

Study doses (mg/kg bw/day)	Code Cis:trans Purity (%), batch no.	NOAEL (mg/kg/day)	LOAEL (mg/kg/day)	Critical effects	Reference (BASF DocID)
Preliminary Teratogenicity (3 isomers), gavage (GD 7-19), rabbit (New Zealand White) cis; (-)cis: 0, 10, 28, 80 mg/kg bw/d trans: 0, 10, 20, 40 mg/kg bw/d	cis (batch: 3454/078, purity: 96.9%); (-) cis (batch: 3454/081, purity: 91%); trans (batch: 88-08, purity: 97.0%)	Maternal: dis: ≤10 (-)cis: 10 trans: 40 (HDT)	Maternal: cis:10 (-)cis:28 Trans: -	cis: clinical signs↑ (-)cis: fc ↓, bw gain↓ trans:no effects	[redacted] 1990a (MK-432-002)
		Fetal: cis: 10 (-)cis: 28 trans: 40 (HDT)	Fetal : cis: 28, (-)cis: 80 trans: -	cis:post-implantation loss↑, litter size↓, litter weight↓ (-)cis: embryo/fetal deaths↑, post-implantation loss↑, litter weight↓ trans: no effects	
Teratogenicity, gavage (GD 7-19), rabbit (New Zealand White) Main (PartI): 0, 4, 10, 25, 62.5 mg/kg bw/d Additional (PartII): 0, 2, 4, 10 mg/kg bw/d Preliminary: 0, 10, 30, 90 mg/kg bw/d	cis/trans (batch 89-01, purity: 95.3%, cis/trans: 79.8/15.5% - nominal 80:15)	Main: Maternal: 10	Main: Maternal: 25	fc↓, bw gain↓	[redacted] 1991c (MK-432-003)
		Main: Fetal: 4	Main: Fetal: 10	↑post-implantation loss↑ (late)	
		Main: Developmental: 10	Main: Developmental: 25	↑hydrocephaly, ↑amelia/peromelia	
		Additional: Maternal: 10	Additional: Maternal: -	No maternal toxicity	
		Additional: Fetal: 10	Additional: Fetal: -	No foetal toxicity	
		Additional: Developmental: 4	Additional: Developmental: 10	↑ hydro/cebocephaly (slight increase)	

Study doses (mg/kg bw/day)	Code Cis:trans Purity (%), batch no.	NOAEL (mg/kg/day)	LOAEL (mg/kg/day)	Critical effects	Reference (BASF DocID)
Teratogenicity, gavage (GD 7-19), rabbit (New Zealand White) First study: 0, 2, 4, 10, 40 mg/kg bw/d Preliminary study: 0, 2, 4, 10, 25, 40 mg/kg bw/d	cis (batch 12, purity: 95.2%, cis/trans: 95.2/0.09%)	Maternal: 4	Maternal: 10	fc↓, bw↓, bw gain↓	[redacted] 1992a (MK-432-007)
		Fetal: 10	Fetal: 40	Post-implantation loss (late)↑, litter size↓, litter weight↓, fetal weight↓ (n.s.)	
		Developmental: 10	Developmental: 40	hydrocephaly↑	
Teratogenicity, gavage (GD 7-19), rabbit (New Zealand White) Second study: 0, 0.5, 1, 2, 10, 40 mg/kg bw/d (split doses)	Cis (95.2% purity, batch 12)	Maternal: 10	Maternal: 40	fc↓, bw gain↓	[redacted] 1992b (MK-432-010)
		Fetal: 10	Fetal: 40	post-implantation loss (late)↑,litter size↓, litter/foetal weight↓	
		Developmental: 10	Developmental: 40	forelimb flexures/malrotated hindlimbs↑	

Based on the studies previously peer-reviewed, the following EU agreed endpoints are given in the EFSA scientific report of metconazole (2006; 64, 1-71):

Reproductive toxicity	
Reproduction target / critical effect:	2G rat (cis): ↓bw gain (F1 pups), ↑gestation length, ↓ post-implantation survival (F2)
Lowest relevant reproductive NOAEL / NOEL:	Pup toxicity: 8 mg/kg bw/d (↓bw gain) Reproduction: 8 mg/kg bw/d (↑gestation length, ↓ post-implantation survival in F2)
Developmental target / critical effect:	Increase in post-implantation loss, decrease in foetal size and litter weight , increase in placental weight
Lowest relevant developmental NOAEL / NOEL:	NOAEL maternal: 24 mg/kg bw/d (↓feed consumption, ↓bw gain) NOAEL foetal: 24 mg/kg bw/d (↑embryonic deaths, ↑post-implantation loss) NOAEL development: 6 mg/kg bw/d (increased incidence of bilateral hydroureter) **R63**

Submission of not yet peer-reviewed studies in this AIR3-Dossier:
A 2-generation reproduction toxicity study in rats and another 1-generation mechanistic study in pregnant rats including hormone measurements during late gestation were performed with metconazole technical (cis/trans isomer). This 2-generation reproduction study was performed according to the most recent OECD 416 guideline (2001), thus, including the investigation of (reproductive) organs, sperm parameters as well as sexual maturation parameters in the offspring. Furthermore, developmental toxicity study in rats was investigated according to the most recent OECD 414 guideline (2001) with treatment of the cis/trans mixture during gestation days (GD) 6-19.
Furthermore, a developmental toxicity study in New Zealand White rabbits via the dermal route of administration was conducted with metconazole cis/trans.
These studies were conducted for non-EU authorities by another metconazole-supporting company. Detailed study summaries were made available to BASF and are part of this submission. The mechanistic study in pregnant rats is presented in chapter M-CA 5.8.2.

In general, similar effects were seen in this 2-generation study with cis/trans as compared with the existing 2-generation study with the cis isomer or the 1-generation studies with cis or cis/trans. Similarly, comparable findings were observed in the newest developmental toxicity study in rats with longer treatment duration (GD 6-19) as compared to treatment confined to the period of embryo/fetal organogenesis in the previous studies (GD 6-15); the extended treatment period did not have an impact on the severity or magnitude of effects.
There was no treatment-related effect observed in rabbits with regard to foetal and developmental toxicity after dermal application (see Table 5.6-3).

Table 5.6-3: Summary of not yet peer-reviewed reproductive and developmental toxicity studies with metconazole cis/trans

Study species, Dose levels	**Test Substance (cis/trans ratio, purity, batch)**	**NOAEL (mg/kg/day)**	**LOAEL (mg/kg/day)**	**Critical effects**	**Reference (BASF DocID)**
Reproductive Toxicity					
2-Generation/Main study, oral, rat (Sprague Dawley) 0, 30, 150, 750 ppm (~ 0, 2, 11, 53 mg/kg bw/d)	cis/trans (batch 9Z521, purity: 98.99% cis/trans: 83.13/15.86)	Parental/reproductive: ~11 (150 ppm)	Parental/reproductive: ~53 (750 ppm)	P&F1: bw↓ and bw gain↓, fc↓, liver weight↑, centri-lobular hepatocyte hypertrophy & fatty change↑, gestation length↑, dystocia and associated maternal deaths, gestation index↓	Summary: [redacted] 2015 (a); Study author: [redacted] 2002 (2015/1087913; 2006/8000261)
		Offspring: ~11 (150 ppm)	Offspring: ~53 (750 ppm)	litter size (F1/F2: day 0, ns)↓, pup bw↓ (F2: day 0,14,21), viability index day 0↓ (F2)	

Developmemtal Toxicity					
Teratogenicity, gavage (GD 6-19), rat (Sprague Dawley) 0, 1, 4, 16, 64 mg/kg bw/d	cis/trans (batch 9Z521, purity: 98.99% cis/trans: 83.13/15.86)	Maternal: 16	Maternal: 64	fc↓, bw↓, bw gain↓	Summary: [redacted] 2015a; Study author: [redacted] 2002 (2015/1087909; 2006/8000263 and 2008/8000121)
		Fetal: 16	Fetal: 64	post-implantation loss↑ (early and late resorptions), litter size↓, foetal weight↓, placental weight↑	
		Dev: 16	Dev: 64	skeletal ossification variations↑ (lumbar/cervical ribs), VSD and small VSD↑	
Teratogenicity, dermal (GD 6-28), rabbit (New Zealand White) 0, 30, 90, 270 mg/kg bw/d	cis/trans (batch AS2122b, purity: 99.7%, cis/trans: 84.2/ 15.5)	Maternal: 90	Maternal: 90	Bw↓, bw gain↓, fc↓, defecation↓, deaths (1 abortion and 1 early delivery), dermal irritation	Summary: [redacted] 2014; Study author: [redacted] 2012 (2015/1087908; 2012/8000573)
		Foetal/developmental: 270 (HDT)	Foetal/developmental: (HDT)	-	

ns:not significant
HDT: highest dose tested

Overall, considering all available toxicological data (including the new information from the additional study summaries) the previously derived NOAELs for reproduction toxicity are not impacted by the results of the new studies.
The conclusion for relevant endpoints for the current re-registration is as follows:

Reproductive toxicity		
Reproduction target / critical effect	Parental toxicity: bw↓ and bw gain↓ Reproductive toxicity: gestation length↑, dystocia and associated maternal deaths, gestation index↓, post-implantation survival↓ Offspring toxicity: decreased number of live fetuses, ↓bw gain	No classification
Relevant parental NOAEL	8 mg/kg bw/d	
Relevant reproductive NOAEL	8 mg/kg bw/d	
Relevant offspring NOAEL	8 mg/kg bw/d	
Developmental toxicity		
Developmental target / critical effect	Rat: Maternal toxicity: bw↓ Fetal/developmental toxicity: increased skeletal ossification variations (lumbar/cervical ribs) and also at high dose additionally; at high dose levels visceral variations (ventricular septal defects), postimplantation loss↓ (early and late), live litter size↓, fetal weight↓ Rabbit: Maternal toxicity: fc↓, bw↓ Fetal/developmental toxicity: small non-significant increase in post implantation loss↑ (early and late) [redacted] study, 1997a); low incidence of hydrocephaly (cis/trans additional study)	**Repr. Cat. 2 (H361d)**
Relevant maternal NOAEL	Rat:12 mg/kg bw/d Rabbit: 10 mg/kg bw/d ([redacted] study,1997a)	
Relevant developmental NOAEL	Rat:12 mg/kg bw/d Rabbit: 20 mg/kg bw/d ([redacted] study,1997a) Rabbit: 4 mg/kg bw/d (cis/trans additional study)	

For the convenience of the reviewer, brief study summaries are provided under the respective chapters and a more detailed tabulation of effects was presented where considered necessary for the discussion and overall evaluation.

Summary of reproductive toxicity and developmental toxicity

- **Reproduction studies in rats**

2-generation reproduction studies in rats are available with metconazole cis and cis/trans. Furthermore two 1-generation studies were conducted with cis/trans metconazole and another 1-generation study investigated the comparative effects of both cis and cis/trans material.
During the previous EU renewal process a 2-generation reproduction study in rats was only available with the cis isomer ([redacted] 1992a). This study was considered suitable to also assess possible effects on reproduction and fertility following exposure to the cis/trans isomer for several reasons. Both acute and repeated toxicity studies performed on the two compounds revealed very similar toxicological effects between the cis and cis/trans isomer. Further evidence came from the 1-generation reproduction studies available for both cis and cis/trans metconazole.
The first pilot study [redacted] 1991a (Part I); treatment: 4 weeks prior pairing through weaning of the F1 offspring (day 21 post-partum)) was conducted with metconazole cis/trans mixture at dietary concentrations of 50, 500 and 1500 ppm (equivalent to 0, 2.9/3.6, 28.0/35.8, 89.9/116 mg/kg bw/d in males/females). The top-dose (1500 ppm: 89.9/116 mg/kg bw/d) induced clear adverse effects on water and food consumption, body weight gain and prolonged gestation with associated signs of dystocia, poor condition, and subsequent maternal mortality and/or total litter losses and total resorption resulting in 1 surviving litter. At the mid dose (500 ppm) similar effects to those seen at 1500 ppm were observed but to a lesser degree. Effects in the offspring at the lower doses consisted of reduced body weight gains after day 21 post-partum in both sexes.
The results of the comparative cis and cis/trans 1-generation study ([redacted] 1991a (Part II); treatment: gestation day 6 through weaning of F1 (postnatal day 21)) demonstrated that dose-related responses in several maternal and offspring parameters (i.e. reduced maternal body weight gains, prolonged gestation, and reduced pup weights) were observed for both compounds at 500 and 750 ppm (top dose). However, the cis/trans mixture tended to show greater reductions in maternal body weight gains and increased mortality associated with delayed parturition (at 750 ppm) as compared to the cis isomer, whereas the cis-isomer showed greater reductions in pup survival as compared to the mixture. It was concluded that at comparable dietary concentrations, the cis/trans mixture produced more maternal toxicity than the cis compound, while the cis isomer produced more toxicity to offspring than the mixture. Thus, the mixture was anticipated to pose less risk to offspring than the cis isomer at maternally toxic doses.
The results from the third pilot 1-generation study ([redacted] 1991; treatment: 15 days prior pairing up to week 8 of F1-pups) conducted with cis/trans metconazole (0, 1, 2, 8, and 32 mg/kg bw/d in diet) were used to set the dose levels for the main 2-generation rat reproduction study. At the top-dose (32 mg/kg bw/d) a slight reduction in body weight gain and food consumption as well as slightly prolonged gestation length were observed in the dams as the sole effects, while litter size, offspring viability and pup bodyweight were unaffected.
The main 2-generation reproduction study ([redacted] 1992a) performed in rats with the metconazole cis-isomer (0, 2, 8, 32, and 48 mg/kg bw/d in diet) showed no effects on rat mating performance and fertility in either parental generation up to the highest dose tested (48 mg/kg bw/d). Increased gestation length, slight reductions in litter sizes due to reduced post-implantation survival and reduced pup weight gain (day 1-12 post-partum) were observed at the two higher doses, but pup survival was not affected. The NOAELs for parental, reproduction and offspring toxicity was set at 8 mg/kg bw/day.

Meanwhile, the study summary of a 2-generation reproduction study in rats with metconazole technical (cis/trans mixture; 0, 30, 150, and 750 ppm) was made available to BASF. This study ([redacted] 2002) was conducted according to the most recent OECD 416 guideline (2001). Adverse findings were primarily restricted to the highest dose tested (750 ppm ~ 49.4/53.2 mg/kg bw/d for parental P males/females and 53.0/55.5 mg/kg bw/d for F1 males/females). The reproductive effects seen in this study with cis/trans metconazole, even though at a lower top dose (750 ppm:~53 mg/kg bw/d), confirmed the finding of delayed parturition and dystocia associated with maternal deaths in both P and F1 generation, which was already previously seen in the first 1-generation study ([redacted] 1991a (Part I)) on the cis/trans mixture at 1500 ppm (116 mg/kg bw/d). This led to decreased gestation indices at the top dose for both generations. At the highest dose level, marginally but significantly prolonged estrous cycle (only in females of the P generation) and slightly increased duration of gestation (both generations) were observed. Absence of effects on the estrous cycle in the second generation and in the 2-generation study with the cis isomer raises however, doubts on the treatment-relationship of this effect. With regard to reproductive performance, the mating and fertility index, as well as spermatogenesis in parental male rats was not affected by treatment with metconazole and the females that delivered normally weaned pups successfully. Furthermore, sexual maturation (preputial separation and vaginal opening) was comparable for all groups. No relevant treatment-related abnormalities on the reproductive organs, pituitary, thyroid, and adrenals were noted in this study. The top dose in this 2-generation study with cis/trans showed considerable parental toxicity as evidenced by reduced body weight, body weight gain, and food consumption as well as increased liver weights associated with histopathological findings (centrilobular hepatocyte hypertrophy and fatty change). The liver findings were also seen in the subacute and subchronic rat studies in the Fisher rat and are in line with discussed mechanism of CYP liver enzyme induction and activation by metconazole. With regard to offspring, the viability index of F2 pups was significantly decreased on the day of birth at the top dose and F2 pup weight was significantly decreased throughout lactation. Based on these findings the NOAEL for parental, reproduction and offspring toxicity was set at 150 ppm (~11 mg/kg bw/d), confirming the NOAEL of 8 mg/kg bw/d for reproduction toxicity previously set based on the 2-generation study with the cis isomer ([redacted] 1992a).

In order to address the mechanism by which prolonged duration of gestation and maternal death associated with parturition difficulties occurred at the high dose of cis/trans metconazole, a mechanistic 1-generation study in rats was performed investigating hormone measurements during late gestation (GD 19 or 21) and liver CYP protein analysis (treatment: 3 weeks pre-mating and throughout gestation until necropsy on GD 19 or 21, females were mated with untreated males) at the same dose levels used in the main 2-generation study with cis/trans (30, 150 and 750 ppm corresponding to 1.8, 8.9, and 43 mg/kg bw/day; [redacted] 2002, study summary in chapter M-CA 5.8.2).

In this supplemental study parental toxicity was obvious at 750 ppm (decreased body gain and food consumption, increased liver and ovary weights). A significant inhibition of increases in the 17ß-estradiol/progesterone (E/P) ratio were observed on gestation days 19 and 21 only at the high dose of 750 ppm, which was also the top dose used in the 2-generation study with metconazole cis/trans. Decreases in the E/P ratio at the top dose were mainly attributed to the decreased serum 17ß-estradiol concentrations on gestation days 19 and 21, which was significantly changed only on gestation day 21. Progesterone concentrations in this 750 ppm group tended to be slightly but not statistically significantly higher than that of the control group on gestation day 21 with large standard deviations. It was suggested that corpora lutea may not have entered the stage of functional regression yet and may thus, still be active for progesterone production during late pregnancy, which was possibly in line with the finding of higher-than-the-control ratio of corpora lutea containing PCNA-positive lutein cells. However, the large variations of the measured serum progesterone levels make it difficult to draw a final conclusion. Additionally, increased hepatic CYP proteins of isozymes, which are involved in the estradiol metabolism in the liver were observed and the study author hypothesized that the inhibition of increases in the E/P ratio at this top dose may be due to an excessive acceleration of 17ß-estradiol metabolism.
The effect on difficult delivery and slight delayed onset of parturition may be associated with aromatase inhibition. Fundamental species differences exist regarding hormonal regulation during pregnancy and parturition. While in rodents and rabbits sustained corpora luteal activity is required to maintain progesterone secretion throughout gestation, in guinea pigs and in humans, the corpora lutea is only active during early pregnancy and thereafter the placenta takes over the progesterone production. Likewise, the placenta is also the primary source for estrogen production in pregnant guinea pigs and humans, while the ovaries are the sole source of estrogen in pregnant rats. (Mitchell and Taggert, 2009a; BASF DocID 2009/1132342). Towards the end of the gestation, an increase in serum estradiol concentrations and a decline in progesterone is required to trigger parturition in rats (Fang et al., 1996a; BASF DocID 1996/1005200). Disturbances of these serum hormone levels may be the cause for parturition difficulties in rats. The effect of slightly extended gestation length and dystocia can therefore be considered as rat-specific without relevance to humans.
In order to elucidate the mode of action of metconazole as an aromatase inhibitor an aromatase assay using recombinant CYP19 enzyme was performed and activity of rat versus human enzyme was investigated (study details see M-CA 5.8.2, Mentzel, 2015a).
Metconazole cis/trans (IC50 (human): 7.21 x 10-7M, IC50 (rat): 1.57 x 10-7 M) inhibited both rat and human aromatase activities, but the aromatase inhibition was considerably more pronounced for the rat enzyme than for the human enzyme with a highly significant difference of 4.6-fold between the enzymes from the two species with a highly significant difference of 4.6-fold between the enzymes from the two species. The cis isomer resulted in a similar response as for the cis/trans mixture, whereas the trans isomer showed a weaker aromatase inhibiting response. In comparison with IC_{50} values of another azole substance letrozole, which is a known aromatase inhibitor designed to inhibit human aromatase, a potency distinction is evident. Metconazole is 103-fold (rat)/799-fold (human) less potent on aromatase inhibition than letrozole. These differences may also be reflected in the severity and magnitude of the typical azole-related effects in vivo.

Taken together, in addition to foetotoxicity as noted by post-implantation losses resulting in decreased number of live litter size (effects, which are also confirmed in the developmental toxicity studies in rats), reproductive toxicity was reported at the top doses with metconazole as evidenced by delayed parturition and dystocia related to maternal deaths (more pronounced for cis/trans). The effects in pregnant rats only occurred in the presence of considerable maternal toxicity (primarily evidenced by impaired food consumption, body weight and body weight gain) at the respective top doses and are associated with aromatase inhibition. The observed slightly extended gestation length and dystocia are considered to be rat-specific without relevance to humans.
No effects on fertility were observed in both main 2-generation studies with the cis isomer and the cis/trans mixture. Since the latest 2-generation toxicity study in rats with cis/trans (2002) confirmed the findings already observed in the previously evaluated generation studies and resulted in a comparable NOAEL (~11 mg/kg bw/d), there is no reason to change the overall NOAEL of 8 mg/kg bw/day already previously established for parental/reproductive and offspring toxicity.

- **Developmental toxicity studies in Rats**

Three studies in rats have been conducted with both the cis/trans mixture and the cis-isomer. The most recent rat prenatal developmental toxicity study (2002) was conducted with metconazole technical (cis/trans) according to the latest OECD 414 guideline with treatment during the major part of gestation period (GD 6-19).
In the other two studies, treatment of pregnant rats during gestation days 6-15 (organogenesis) with the metconazole cis/trans mixture (1991b; 0, 12; 30, and 75 mg/kg bw/d) and the cis-isomer (1992b; 0, 6, 24, 60 mg/kg bw/d) resulted in maternal toxicity at 30 and 75 mg/kg bw/d (decreased body weight gain) and at 60 mg/kg bw/d (decreased food consumption and bodyweight gain), respectively. At the respective top doses embryo/foetotoxicity consisted of an increase in post-implantation loss (both early and late resorptions) associated reduced foetal/litter weights and litter size, and increased placental weights (measured only in the study on the cis isomer). There was no treatment-related increase in malformations observed. Two cases of hydrocephaly in two litters where observed at the top dose of 75 mg/kg bw/d in the cis/trans study and are most probably subsequent to the high maternal toxicity at this dose. Skeletal ossification variations observed at the highest doses tested comprised changes of the axial skeleton, particularly increased incidence of lumbar ribs and cervical ribs; these variations fitted into the picture of a developmental delay. Likewise, the slight occurrence of the visceral variation of ventricular septal defects (VSD) and small VSDs in the study conducted with the cis/trans mixture also represent reversible developmental delay. Additionally, in the Whole Embry Culture (WEC) in rats, an in-vitro embryo-toxicity assay for the independent evaluation of effects on growth and differentiation, metconazole preparations tested (cis/trans, cis, and trans isomer) showed general embryotoxicity with the potency ranking of cis/trans > cis > trans (see Chapter M-CA 5.8.2).

The effects seen with the cis/trans mixture in the most recent rat prenatal developmental toxicity study (2002; 0, 1, 4, 16, and 64 mg/kg bw/d), even though at a marginally lower top dose than (1991b) (75 mg/bw/d), confirmed the findings of increased post-implantation loss (both early and late resorptions), reduced litter size, and fetal weights at the top dose only. The top dose in the study (64 mg/kg bw/d) was maternally toxic. At this top dose increased incidences in skeletal ossification variations, predominantly cervical ribs and lumbar ribs, and a treatment-related increase in VSDs and small VSDs were also reported. In agreement with an expert opinion by (2005) (as referenced to in the study summary of the study, see 2015a) the VSDs and small VSDs seen represent reversible developmental delays and are associated with decreased fetal weights. Slight increased incidences of dilated renal pelvis and ureter were observed at the top doses, and the incidences were covered by historical control data. In one study (1992c) increased bilateral hydroureter was observed at necropsy only, but it was not confirmed during histopathological evaluation. Therefore, this was not considered treatment-related. In general, increased hydronephrosis and hydroureter were considered as a sign of delayed development in association with the reduced foetal weights at the top doses.
In general, the longer treatment period of GD 6-19 in the study (2002) compared to GD 6-15 in the other two studies (1991b and 1992b) did not result in additional or enhanced responses.

In conclusion, a similar effect pattern was seen across the three studies. The study (2002) did not result in a relevantly different maternal or developmental NOAEL (16 mg/kg bw/d) or LOAEL (64 mg/kg bw/d) than derived on the basis of the previously existing study suite (NOAEL of 12 mg/kg bw/d for maternal/developmental effects based on 1991b), and also did not identify additional endpoints of concern.

- **Developmental Toxicity studies in rabbits**

<u>Developmental toxicity studies acc. to OECD 414 (2001) with metconazole technical (cis/trans)</u>
Two developmental toxicity studies in New Zealand White rabbits were conducted with metconazole cis/trans according to the recent guideline OECD 414 (2001), one oral developmental toxicity study (1997a) and one dermal developmental toxicity study (2012; study summary: 2014a).
In the oral developmental toxicity study (1997a; 0, 5, 10, 20, and 40 mg/kg bw/d) administration of 40 mg/kg bw/d metconazole cis/trans (98% purity) was associated with a small increase in post-implantation loss, as evidenced in the statistically significant increase of does with resorptions. At this top dose minimal and non-statistically significant increases in resorptions (early and late), with associated small, but non-statistically significant reductions in live litter size and fetal bodyweight were also noted in the presence of maternal toxicity. The NOAEL for maternal toxicity was established at 20 mg/kg bw/d based upon reduced food consumption and small reductions in body weight gain during late gestation, small reductions in hematocrit, hemoglopbin, MCV, and increases in platelet counts and AP, and increased relative liver weights at the top dose. The values for all changes in embryonal-fetal viability and growth were within the historical control ranges of the testing facility. Therefore, they should not be considered as treatment related.

However, since similar effects (increased late resorptions (not early) and decreased litter size) were observed at the same dose level (40 mg/kg bw/d) in the preliminary range-finding study, the fetal toxicity NOAEL was set at 20 mg/kg bw/d in agreement with the study report (in the monograph (2004) the NOAEL was set at 10 mg/kg bw/d). No malformations or variations in the fetuses were attributable to treatment with the substance. Thus, the developmental NOAEL was 40 mg/kg bw/d (top dose).
There were in total two craniofacial malformations observed in the [redacted] 1997a main study: One hydrocephalus at 10 mg/kg/day and one hydrocephalus at 40 mg/kg/day. In the range-finder study one fetus at the top-dose (40 mg/kg bw/d) was found with a domed head associated with extreme dilation of lateral ventricles. Since this malformation occurs spontaneously in control animals (0-3 fetuses were recorded with marked/extreme dilated lateral brain ventricles (hydrocephaly) in the historical control data provided between 1994-1996), and there was no clear dose-response relationship, it was not considered related to treatment in this study.
Based on the results of this study ([redacted] 1997a), conducted under the most recent OECD guideline (2001) with prolonged treatment interval in New Zealand White rabbits [Hra:(NZW)SPF], a maternally toxic dosage of 40 mg/kg/day was associated with borderline increases in resorption and reductions in fetal body weight. The incidences of malformed fetuses in this study were low in control and treated groups. The low incidences of digit malformations that occurred sporadically in previous rabbit studies were not replicated in this newer study (no fetus in this study had a digit malformation), supporting that the previously observed fetal malformations were unrelated to the test substance.

In a recently conducted dermal developmental toxicity study with metconazole cis/trans ([redacted] 2012; summary provided to BASF and submitted with this dossier; 0, 30, 90, 270 mg/kg bw/d) maternal toxicity was evident at the top dose of 270 mg/kg bw/d (decreased body weight gains along with food consumption, defecation, and skin irritation). In the absence of treatment-related effects on fetal survival, fetal body weight (combined sexes), and fetal morphology (external, visceral, and skeletal examinations), the highest dose tested was the NOAEL for fetal/developmental toxicity. Overall, the malformations in this dermal study were equally distributed among control and treated groups and were considered spontaneous in origin. The fetal incidence of malformations ranged up to 2.5% in the control group. The observed malformations were not considered to be test substance-related as they did not occur in a dose-related manner, were noted similarly in the concurrent control group, and/or the mean litter proportions were within the ranges of the developmental historical control data. Among these findings two fetuses from the same litter in the mid dose group (90 mg/kg/day) and 1 fetus in the high dose group (270 mg/kg/day) had hydrocephaly. However these incidences were within the historical control range and were therefore, not regarded as treatment related.

Thus, the newer studies conducted in NZW rabbit with metconazole cis/trans after oral (gavage) and dermal administration revealed no adverse effects towards developmental parameters, and the developmental NOAEL was established at the top-doses, 40 mg/kg bw/d and 270 mg/kg bw/d, respectively.
The single incidences of hydrocephalus in the [redacted] (1997a) study at 10 and 40mg/kg bw/d and in the dermal metconazole study at 90 and 270 mg/kg bw/d were well within the historical control range and therefore, the data do not support the position that these changes are related to metconazole exposure and are considered to be incidental.

Several triazoles have been associated with cranio-facial anomalies, possibly via a common mechanism of toxicity related to altered morphogenesis of the first branchial arch (Menegola et al., 2006a; BASF DocID 2006/1051893). Malformations commonly observed with triazoles include cleft palate and occasionally other cranio-facial anomalies like hypognathia, exophtalmus, macroglossia. These defects involve a mechanism of aberrant neural crest cell migration from the hindbrain into the branchial arches (Menegola et al., 2003a; BASF DocID 2004/1041214 and Menegola et al., 2005a; BASF DocID 2005/1044180). However, this mechanism of action for the cranio-facial anomalies is not involved in the development of the brain. Neural crest cell migration occurs prior to neural tube closure, while changes related to hydrocephalus development occur after closure of the neural tube. Therefore, hydrocephalus findings observed with metconazole exposure are unlikely to be related to this mechanism.
With regard to metconazole, hydrocephalus was only seen at low incidences and was not convincingly dose-related across the available studies.

Additional developmental toxicity studies conducted in rabbits of the NZW strain (Interfauna, UK Ltd.) with metconazole cis/trans and cis
The original 4 main rabbit developmental toxicity studies on metconazole cis/trans and metconazole cis were all carried out by [redacted] using the NZW rabbit strain from Interfauna. Two of these rabbit teratology studies were performed with metconazole cis/trans ([redacted] 1991c main study (Part I) and additional study (Part II)) and two main studies were conducted with metconazole cis ([redacted] 1992a and [redacted] 1992b)
This Interfauna source of animals as compared to the source used in the studies carried out by [redacted] ([redacted] 1997a) showed striking disparity in the incidence and types of fetal observations. The reasons for this difference are not clear, however it can be noted that in the HRC studies the maternal animals generally showed lower pregnancy rates and higher rates of postimplantion loss. Furthermore, the overall incidence and types of malformations observed in control and treated groups were higher as compared to the newer study ([redacted] 1997a) conducted according to the recent testing guideline. These limitations in the older studies made the interpretation of the developmental data difficult. There was a lack of a clear dose-response relationship in the incidence of fetal malformations and no clear pattern of abnormalities was observed. Some severe malformations were present in fetuses from some control animals as well as in fetuses from treated animals.

Two of the 4 additional main rabbit teratology studies were conducted with cis/trans batch number 89-01: one main study ([redacted] 1991c Part I; 0, 4, 10, 25, 62.5 mg/kg bw/d) and one additional study ([redacted] 1991c Part II; 0, 2, 4, 10 mg/kg bw/d). In the first main study clear fetotoxic and developmental effects were only observed at maternotoxic dose levels, whereby the top dose of 62 mg/kg bw/d showed marked signs of maternal toxicity (reduced food consumption and body weight gain). At 62 mg/kg bw/d significantly increased post-implantation loss (late resorptions) and concomitant reduced litter size, and severely reduced fetal/litter weights were observed, and all of these values were outside the historical control ranges. At the next lower dose of 25 mg/kg bw/d similar effects on maternal toxicity were noted but effects were not as severe as at the top dose. Thus, the maternal NOAEL was set at 10 mg/kg bw/d. in this main study ([redacted] 1991c Part I). Minimal fetotoxic effects at the lower doses (< 25 mg/kg bw/d) were without a dose-response relationship and the values for postimplantation loss, late resorptions, and live litter size were well within the historical control range at all these doses. Developmental toxicity at the top dose was evidenced by an increased incidence of structural defects (skeletal variations).

There was an increased incidence of hydrocephaly (4 fetuses in 4 litters) at 25 mg/kg bw/d which may be associated with maternal toxicity, and the incidence was clearly outside the historical control range.
At the lower doses up to 10 mg/kg bw/d the findings of the main study (1991c Part I) were inconsistent with the additional study (1991c Part II). Overall, increased incidence of malformed fetuses at 10 mg/kg bw/d was higher in the additional study than seen at the same dose in the previous study. Conversely, the number of malformations at 4 mg/kg bw/d was lower in the additional study than seen at the same dose in the previous study. Two fetuses from two litters at 10 mg/kg bw/d were found with hydrocephaly in the additional study and none in the main study, and one fetus at 4 mg/kg bw/d in the main study but none in the additional study showed hydrocephaly associated with cebocephaly. Thus, effects in these two studies were not reproducible at similar dose levels, which makes the interpretation of results difficult.
The newer rabbit developmental toxicity study (1997a) on metconazole cis/trans with a longer treatment period during gestation days 6-28 did not show any treatment-related malformations. Fetotoxic effects (small increase in postimplantation loss) were less severe at the maternotoxic dose level of 40 mg/kg bw/d (top dose) in the study (1997a). The purity as well as the impurity profile of the batch used for this most recent oral rabbit developmental study (AC105575-61) was within the levels of the specification of metconazole cis/trans technical. (for details please refer to confidential document JCA)
Compared to that, batch 89-01 used for the two older rabbit teratology studies with cis/trans metconazole was of lower purity. Furthermore, when comparing the impurity profile of both batches, there was one major significant difference of an impurity present at about 96-fold higher levels in batch 89-01 as compared to batch AC105575-61. This impurity No. (for details please refer to confidential document JCA) was even contained at 3-fold higher amounts above the specified impurity limit in batch 89-01. The levels of impurity contained in batch AC105575-61 used for the most recent oral rabbit teratology study were comparable with the analyses of the 5 batches of the active substance, representing the production material.
Thus, it cannot be excluded that the presence of this impurity at high amounts in batch 89-01, which is not representative of the specification, may be responsible for the effects seen in these two older main studies and may also account for the non-reproducible results at the same dose levels in the two studies.

Two further rabbit development studies were conducted on metconazole cis: the first study (1992a; 0, 2, 4, 10, 40 mg/kg bw/d) and the second study (1992b; 0, 0.5, 1, 2, 4, 10 and 40 mg/kg bw/d).
In the first study (1992a), metconazole cis provoked maternal toxicity at 10 mg/kg bw/d and above, as illustrated by decreased feed consumption, and body weight decrease. It was noted that these effects were not observed at 10 mg/kg bw/d, but only at the top-dose (40 mg/kg bw/d) in the second experiment. Effects on maternal endpoints were more prominent in the first experiment.
At the top-doses in the presence of clear maternotoxic effects, both studies revealed an effect on fetal toxicity with increased incidences of late embryonic deaths and post-implantation losses, and decreased litter size/weight and fetal weight. When comparing fetal endpoints in the two experiments it also appeared that these effects were more prominent in the first one. The incidence of hydrocephaly was increased above the historical control values at 40 mg/kg bw/d in the first experiment, whereas only one case (which is within the historical control range) was found at the same dose in the second experiment.

In the preliminary study (1992a; 0, 2, 4, 10, 25 and 40 mg/kg bw/d) increased incidences of hydrocephaly were observed at 25 mg/kg bw/d (3 fetuses in 2 litters affected) but no case was reported at the top dose.
A single incidences of hydrocephaly was observed in the control group of the second experiment (1992b) and at some (1 case at 1, 10, and 40 mg/kg bw/d) but not all dose levels. These single findings were however found within the historical control range, were or were not reproduced at the same dose levels in the preliminary study and the first main experiment (1992b) and were thus, not interpreted as a result of treatment in the absence of dose-responsiveness.
The fact that "split-dosing" (i.e. dosing volume was separated in two equal doses) was performed in the second study may partially explain why the results as seen in the first study were not replicated.
When taking into account the data of the first experiment, the maternal NOAEL of the rabbit studies with metconazole cis was considered 4 mg/kg bw/d, based upon decreased food consumption, body weight, and body weight gain. The fetal NOAEL was set at 10 mg/kg bw/d, based upon the embryonic deaths, increased postimplantation loss, and decreased litter size/weight and fetal weight at the top dose (40 mg/kg bw/d). Contrary to the previous evaluation in the monograph, the developmental NOAEL based on the cis metconazole studies is set at 10 mg/kg bw/d (not at 4 mg/kg bw/d) due to an increased incidence of hydrocephaly (3 fetuses in 3 litters) at the top dose (40 mg/kg bw/d) in the first experiment in the presence of maternal toxicity. The single incidences of hydrocephaly sporadically seen at doses up to and including 10 mg/kg bw/d were not considered relevant for NOAEL setting.

General conclusion on developmental studies in both rats and rabbits
With regard to the rat, the effects seen with the cis/trans mixture in the newest rat prenatal developmental toxicity study (2002), which was conducted according to the latest OECD 414 guideline with treatment during the major part of gestation period (GD 6-19), confirmed the fetal (post-implantation loss, decreased fetal weights and litter weights/size) and developmental findings (increased skeletal and visceral variations representing reversible developmental delay) observed in the previous two studies (1991b and 1992b) in the presence of maternal toxicity at the top dose. In general, the longer treatment period of GD 6-19 compared to GD 6-15 in the other two main studies did not result in additional or enhanced responses and a similar effect pattern was seen across all 3 studies. Overall, the maternal and developmental NOAEL was set at 12 mg/kg bw/d in the rat.

A total of five main oral rabbit developmental studies have been performed with metconazole cis or cis /trans. The early rabbit studies that were conducted with cis/trans and cis isomers prior to the new guideline (treatment during GD 7-19) indicated the emergence of various malformations without a clear dose response relationship and increased incidences of malformations above concurrent control at the top doses. However, except for the top doses, that showed clear maternal toxicity, the percentages of malformed foetuses were within the historical control range and there was no specific type of defects identified.
The critical finding in the rabbit was a slightly increased occurrence of hydrocephaly. The findings of hydrocephaly occurred without a dose-effect relationship, or were single incidences which are covered by the historical control data. Moreover, some effects were not replicated in additional studies at the same dose levels, making the interpretation of this finding difficult.

No developmental toxicity and thus no increased incidences of hydrocephaly above the historical control range was reported in the most recent oral developmental toxicity study ([redacted] 1997a) and also not in the dermal developmental toxicity study ([redacted] 2014 a).

Increased incidences of hydrocephaly above the historical control range were reported at doses showing clear to severe maternal toxic effects (at ≥ 25 mg/kg bw/d). In only one of the four additional rabbit studies a slightly increased incidence of hydrocephaly above the historical control range (2 fetuses in 2 litters affected) was observed at the LOAEL of 10 mg/kg bw/d in the absence of maternal toxicity, i.e. in the [redacted] study, 1991c (additional study (Part II)) with metconazole cis/trans. This increased incidence was not seen in any of the other studies, where either no or a single case of hydrocephalus was reported at the same dose level (see Table 5.6-4). In addition, this study was conducted with a batch containing an impurity at 3-fold increased levels as compared to the specified amount. The overall incidence of hydrocephaly was low at 10 mg/kg bw/d: 4 out of 714 foetuses from 3 out of 5 studies. When all studies are considered, including preliminary studies, an overall incidence of 5 out of 953 foetuses in 4 out of 9 studies was reported at 10 mg/kg bw/d.

Table 5.6-4: Summary of incidences of hydrocephaly in rabbit oral developmental toxicity studies

Study report author, year, and BASF DocID	**Test substance**	Dose level (mg/kg bw/d)										
		0	**0.5**	**1**	**2**	**4**	**5**	**10**	**20**	**25**	**40**	**62.5**
[redacted] 1997a MK-432-015	*cis/trans*	0	-	-	-	-	0	1 (1)	0	-	1(1)	-
[redacted] 1991b MK-432-005 (main study, Part I)	*cis/trans*	0	-	-	-	1 (1)	-	0	-	4(4)	-	0
[redacted] 1991b MK-432-005 (additional study, Part II)	*cis/trans*	0	-	-	0	0		2 (2)	-	-	-	-
[redacted] 1992a MK-432-007 (First main study)	*cis*	0	-	-	0	0	-	0 (0)	-	-	3(3)	-
[redacted] 1992b MK-432-010 (Second Main Study)	*cis*	1 (1)	0	1 (1)	0	-	-	1 (1)	-	-	1 (1)	-

Number of litters (foetuses) affected
- = test dose level not investigated 0 = zero incidence

Based on the most recent rabbit study ([redacted] 1997a), which was conducted with a representative batch and according to the recent testing guideline with robust numbers of animals, the developmental NOAEL was derived at 20 mg/kg bw/d due a small increase in postimplantation loss in the presence of maternal toxicity. Hydrocephalus was seen in all rabbit studies, but at a very low incidence and the incidence was not clearly dose-related. Although in the [redacted] study (1997a) no treatment-related occurrence of hydrocephaly above the historical control range was observed, the partly inconclusive results of the additional rabbit studies were still considered. Even though the significance of the finding of hydrocephaly at 10 mg/kg bw/d is unclear, the overall NOAEL for developmental toxicity in rabbits was set at 4 mg/kg bw/d based on a slightly increased incidence of hydrocephaly above historical control range at 10 mg/kg bw/d in one rabbit study. This endpoint is also the overall lowest endpoint of the metconazole toxicological database used for deriving the references values (see Document N).

Comparison with CLP criteria

For the purpose of classification for reproductive toxicity according to the criteria of the CLP (Regulation 1272/2008/EC), substances are allocated to one of two categories. Within each category, effects on sexual function and fertility, and on development, are considered separately. In addition, effects on lactation are allocated to a separate hazard category.

Category 1: Known or presumed human reproductive toxicant

Substances are classified in Category 1 for reproductive toxicity when they are known to have produced an adverse effect on sexual function and fertility, or on development in humans or when there is evidence from animal studies, possibly supplemented with other information, to provide a strong presumption that the substance has the capacity to interfere with reproduction in humans. The classification of a substance is further distinguished on the basis of whether the evidence for classification is primarily from human data (Category 1A) or from animal data (Category 1B).

Category 1A: Known human reproductive toxicant

The classification of a substance in Category 1A is largely based on evidence from humans.

Category 1B: Presumed human reproductive toxicant

The classification of a substance in Category 1B is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects. However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in Category 2 may be more appropriate.

Category 2: Suspected human reproductive toxicant

Substances are classified in Category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development, and where the evidence is not sufficiently convincing to place the substance in Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification. Such effects shall have been observed in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects.

Metconazole was tested in several 1-generation studies and in two 2-generation studies in rats, as well as in several developmental toxicity studies in rats and rabbits with the cis isomer and the cis /trans mixture.
In the rat generation studies, fetotoxicity (post-implantation loss resulting in decreased number of live litter size) and reproductive toxicity (slightly increased gestation length and dystocia related to maternal deaths (more pronounced for cis/trans)) was observed. These effects in pregnant rats only occurred in the presence of considerable maternotoxic effects at the respective high/top doses. A mechanistic 1-generation study in rats investigated the mechanism by which slightly prolonged duration of gestation and dystocia occurred at the high dose of cis/trans metconazole. In this supplemental study a significant inhibition of increases in the 17ß-estradiol/progesterone (E/P) ratio were observed on gestation days 19 and 21 only at the high dose of 750 ppm, which was also the top dose used in the 2-generation study with metconazole cis/trans. Decreases in the E/P ratio at the top dose were mainly attributed to decreased serum 17ß-estradiol concentrations on gestation days 19 and 21, and are indicative of aromatase inhibition.

The effects on slightly increased gestation lengths and dystocia are probably due to aromatase inhibition. There exist some characteristic differences in the hormonal control of parturition between rats and humans. While in rodents and rabbits sustained corpora luteal activity is required to maintain progesterone secretion throughout gestation, in guinea pigs and in humans the corpora lutea is only active during early pregnancy and thereafter the placenta takes over the progesterone production. Likewise, the placenta is also the primary source for estrogen production in pregnant guinea pigs and humans, while the ovaries are the sole source of estrogen in pregnant rats. (Mitchell and Taggert, 2009a; BASF DocID 2009/1132342). Towards the end of the gestation, an increase in serum estradiol concentrations and a decline in progesterone is required to trigger parturition in rats (Fang et al., 1996a; BASF DocID 1996/1005200). Disturbances of these serum hormone levels may be the cause for parturition difficulties in rats.
The effects on dystocia and extended gestation lengths in rats are thus, most likely due to aromatase inhibition and are considered as rat-specific without relevance to humans.
Based on the results in the generation studies, the NOAEL of 8 mg/kg bw/day was established for parental/reproductive and offspring toxicity.
In the absence of effects on fertility in both main 2-generation studies (Willoughgy, 1992a and [redacted] 2002) with the cis isomer and the cis/trans mixture no classification for fertility was proposed or considered necessary.

In the rat, metconazole cis/trans and metconazole cis (treatment during GD 6-15) induced fetal and developmental toxicity at maternal toxic doses ([redacted] 1991b and [redacted] 1992b). The toxicity of both compounds towards dams (decreased food consumption, body weight gain) and fetuses (post-implantation loss, decreased fetal weights and litter weights/size) at the top dose was similar. There was no treatment-related increase in malformations observed in the rat. Two cases of hydrocephaly where observed at the top dose of 75 mg/kg bw/d in the cis/trans study and are most probably subsequent to the high maternal toxicity at this dose. At the top doses skeletal ossification variations comprised changes of the axial skeleton, particularly increased incidence of lumbar ribs and cervical ribs; these variations fitted into the picture of a developmental delay. Likewise, the slight occurrence of the visceral variations of ventricular septal defects (VSD) and small VSDs in the study conducted with the cis/trans mixture also represent reversible developmental delay.

The effects seen with the cis/trans mixture in the newest rat prenatal developmental toxicity study (2002), which was conducted according to the latest OECD 414 guideline with treatment during the major part of gestation period (GD 6-19), confirmed the fetal and developmental findings observed in the previous two studies at the top dose in the presence of maternal toxicity. In general, the longer treatment period of GD 6-19 compared to GD 6-15 in the other two main studies did not result in additional or enhanced responses and a similar effect pattern was seen across all 3 studies. Overall, the maternal and developmental NOAEL was set at 12 mg/kg bw/d in the rat.

The results of the rabbit oral teratology study (1997a) with metconazole cis/trans, which was conducted with a representative batch and according to the recent testing guideline with robust numbers of animals, showed borderline increases in resorption and reductions in fetal body weight at the top dose of 40 mg/kg/day in the presence of maternal toxicity. However, no treatment-related increase in developmental toxicity was observed in the study (1997a) up to the highest dose tested (40 mg/kg bw/d). In a dermal developmental toxicity study in rabbits (2012) no treatment-related effects on fetal survival, fetal body weight (combined sexes), and fetal morphology (external, visceral, and skeletal examinations) were observed up to the highest dose tested (270 mg/kg bw/d).

A total of 4 additional main rabbit developmental toxicity studies were conducted with metconazole cis and/or cis/trans. These additional rabbit studies were performed using New Zealand White rabbits from Interfauna, UK Ltd. This strain of rabbits showed striking disparity in the incidence and types of fetal observations. The reasons for this difference are not clear, however it can be noted that in these studies the maternal animals generally showed lower pregnancy rates and higher rates of postimplantion loss. Furthermore, the overall incidence and types of malformations observed in control and treated groups were higher as compared to the newer study (1997a). In these additional studies clear fetotoxic and developmental effects were only observed at maternotoxic dose levels. The critical finding in the rabbit was a slightly increased occurrence of hydrocephaly. The findings of hydrocephaly occurred without a dose-effect relationship, or were single incidences which are covered by the historical control data. In only one of the four additional rabbit studies a slightly increased incidence of hydrocephaly above the historical control range (2 fetuses in 2 litters affected) was observed in the absence of maternal toxicity (at 10 mg/kg bw/d). This slightly increased incidence was not seen in any of the other studies, where either no or a single case of hydrocephalus was reported at the same dose level. Thus, the toxicological significance of the finding at 10 mg/kg bw/d is unclear. Generally, slightly increased incidences of hydrocephaly above the historical control range were reported at doses showing clear to severe maternal toxic effects.

In conclusion, these findings do support the existing classification with **Repr 2 (H361d)** following the reasoning of developmental toxicity at a maternally toxic doses, which is not entirely attributed to maternal toxicity.

Conclusion on classification and labelling
The harmonised classification with Repr. 2 (H361d) is justified.

CA 5.6.1 Generational studies

Rat, Metconazole WL148271 (cis/trans), preliminary 1-Generation study, 0, 50, 500 and 1500 ppm in diet ([redacted] 1991a, part I; BASF DocID MK-430-002)

Guidelines: Preliminary study
GLP: Yes
Acceptance: The study was accepted.

Materials and Methods:
10 rats (Crl: CD(SD) BR VAF/Plus)/sex/dose were administered Metconazole WL148271 (Batch: 89-01, purity: 95.3%, cis/trans ratio: 83.7/16.3%, nominal 85:15) in the diet at levels of 0, 50, 500 and 1500 ppm (equivalent to 0, 2.9/3.6, 28.0/35.8, 89.9/116 mg/kg bw/d in males/females). F0 animals were treated for 4 weeks prior pairing continuing throughout to termination, at or around the time of weaning of the F1 offspring (approximately 11 weeks for F0 males and pregnant females). The F1 offspring remained with the dam until weaning (day 21 post-partum), the dam was then sacrificed and selected offspring retained until 6 weeks of age in order to assess susceptibility of weanling rats to dietary inclusions of metconazole cis/trans. Selection of an F1 generation was impossible at 1500 ppm, since only 1 litter survived weaning. The study is accepted as additional information.

Findings:

Relevant parental and litter observations are presented in Table 5.6.1-1.

Parental data:

F_0 generation:

At 1500 ppm, there were 7 mortalities; these all occurred during the perinatal period. Six of these occurred after signs of delayed parturition (presumed day 23 of pregnancy) and poor condition (pallor, pilo-erection, lethargy, cold and loss of body tone). The 7th died on day 2 post-partum (pp) after the birth of a stillborn litter. In addition, one dam which totally resorbed her litter showed signs consistent with the mortalities at a similar stage of pregnancy. At 500 ppm, 1 pregnant female was also sacrificed after signs of delayed parturition (presumed d24 of pregnancy), with clinical signs similar to those seen at 1500 ppm. In addition, 1 dam which reared young to weaning showed piloerection at a similar stage of pregnancy to the mortalities. There were no mortalities or specific signs of reaction to treatment observed among any ♂ animals of the F0 generation, nor among females at 50 ppm.
For males, mean water consumption at 1500 ppm was lower than controls weeks 1 to 4. For females at 1500 ppm, intake was consistently lower than control. At the lower doses, consumption values were only slightly lower than controls with no clear dose-relationship.
Mean feed consumption was decreased in top-dose animals during the first week of treatment. Recovery occurred thereafter and intake regained parity with the controls. At 500 ppm, feed intake was marginally lower in all animals during week 1, but again showed recovery to regain parity with the controls. A dose-related decreased body weight gain was observed at 500 ppm (females) and above (both sexes).

Bodyweights were most noticeably affected during the first week of treatment when overall weight loss was recorded for both males (-11 g) and females (-15 g) at 1500 ppm compared with gains of 34 and 21 g in the respective controls. Although mean weight gain was restored, parity with control mean overall weight was never regained at either 500 or 1500 ppm. For all pregnant dams at 1500 ppm, bodyweight gain during pregnancy was retarded and continued to diverge from control through to day 20 of pregnancy. At 500 ppm, bodyweight gain was slightly retarded during days 0 to 14 of pregnancy, although good recovery occurred thereafter with overall gain comparable to that of control. Calculated food conversion ratios were indicative of inferior efficiency of food utilisation for males at 1500 ppm and females at 1500 and 500 ppm, the effect being most marked in the first week of treatment.
Terminal examinations revealed no relevant findings.

F1 generation:
There were no clinical signs observed and no effects on mortality. For males at 500 ppm, there was an indication of a slight reduction in mean food consumption during weeks 5 and 6. Although mean bodyweight gains for both males and females at 50 and 500 ppm were essentially similar or superior to control from birth to day 21 *pp*, thereafter values were slightly but consistently retarded and continued to diverge from control in a dose-related manner up to day41 (week 6). Food conversion ratios were essentially similar to controls. Terminal examinations revealed no relevant findings.

Reproductive parameters

F_0 generation:
No relevant findings were recorded during pre-mating. A 100% pregnancy rate was achieved in all groups, and mating performance, as assessed by the type of vaginal smear recorded on the day of conception and the median pre-coital time, showed no consistent treatment or dose-relationship.
At 1500 ppm, 6/10 dams were sacrificed/found dead with signs of prolonged pregnancy (presumed d23) and at 500 ppm, 1/10 dams was sacrificed with signs of prolonged pregnancy (day 24).

Table 5.6.1-1: Reproduction and litter parameters following treatment with metconazole cis/trans in the preliminary 1-generation rat study

Dose level [ppm]	**0**	**50**	**500**	**1500**
Intake F0 (mg/kg bw/d) m/f	0/0	2.9/3.6	28.0/35.3	89.9/116
Parental data F0				
No. of animals mated	10	10	10	10
Died/sacrificed	-	-	1 (dystocia, GD24)	6 (dystocia, GD23), 1 (PND2, total litter loss prior to death)
Total resorption				1
Total litter loss at birth				1
Rearing young to weaning	10	10	9	1
Body weight gain – males (g)				
• Week 0-4	112	115	107	**60** (-46%)**
• Week 0-10	181	194	177	**148** (-18%)**
Body weight gain – females (g)				
• Week 0-4	69	64	**45** (-35%)**	**20** (-71%)**
• Week 0-10	126	138	118	**57** (-55%)**
Food consumption week 1-males (g)	198	496	180	**171* (-14%)**
Food consumption week 1 – females (g)	134	143	124	**105* (-22%)**
Gestation length	21.6	22.1	22.4	22.3
Litter observation				
Intake F1 (mg/kg bw/d) m/f	0/0	6.8/6.5	67.3/66.2	§
Implantations (F0)	16.0	17.4	16.7	15.0§
Live litter size				
• At birth	14.5	15.9	14.1	11.0§
• Day 4 pre-cull (% cumulative pup loss)	14.3 (2.9%)	15.7 (4.4%)	13.3 (9.4%)	11.0§
Fetal weight (F1):				
• At birth	6.1	5.8	6.2	5.7§
• Day 4 (pre-cull)	9.9	10.1	9.9	9.0§
• Day 4 (post-cull)	9.8	10.1	9.8	9.1§
Body weight gain (% of control)				
• Day 0-42, m/f		-4%/-4%	**-12%*/-7%***	§
• Day 21-42, m/f		-10%/**-10%****	**-16%*/-10%****	§

Statistical evaluation: *p≤0.05, **p≤0.01
m: male, f: female
§: Mean values not applicable – only 1 litter survived to weaning

Offspring data:
At 1500 ppm, in addition to the 6 dams which died or were sacrificed around parturition, 2 dams had total litter loss on day 0/1 *pp*, of which 1 subsequently died on day 2 *pp*. The 9th dam, totally resorbed her young. No litter losses were observed in the other groups.
For the 1 surviving litter at 1500 ppm, litter and mean pup weights from birth to d 21 *pp* were lower than controls. However, the group size is too low for any meaningful comparison. Apart from a slightly higher percentage cumulative pup loss at 500 ppm on day 4 *pp* (pre-cull), there were no clear, consistent effects of treatment on litter values/sex ratios at 500 or 50 ppm. A slight retardation in body weight gain was observed at 50 ppm, which was more pronounced at 500 ppm (statistically significant in both sexes). However, achieved food intakes in F1 were twice as high at these dose levels. Terminal examinations revealed no relevant findings.

Conclusion
Administration in the diet of 1500 ppm metconazole cis/trans induced clear adverse effects on food and water consumption and bodyweight gain in both sexes of the F0 generation, particularly in early treatment stages. This dose also had profound effects on the ability of dams to successfully complete parturition and led to the sacrifice/death of 7/10 pregnant animals. It was considered that this dose would be inappropriate for further investigation. At 500 ppm, similar responses to those seen at 1500 ppm were evident in the F0 adults, although much milder in degree, i.e. only 1/10 mortalities in pregnant females.

Rat, Metconazole WL148271 (cis/trans) and WL136147 (cis), preliminary 1-Generation study, oral, 0, 500, and 750 ppm in diet ([redacted] 1991a, part II; BASF DocID MK-430-002)

Guidelines: Preliminary study
GLP: Yes
Acceptance: The study was accepted.

Materials and Methods:
10 rats (Crl: CD(SD) BR VAF/Plus)/sex/dose were administered either metconazole WL148271 (Batch: 89-01, purity: 95.3%, cis/trans ratio: 83.7/16.3%, nominal 85:15) or WL 136184 (Batch: ST89/411, purity: not specified, assumed 95% cis) in the diet at levels of 0, 500 and 750 ppm. F0 animals were treated from day 6 of pregnancy throughout to termination, at or around the time of weaning of the F1 offspring (approximately 11 weeks for F0 males and pregnant females). On day 22, parents and pups were sacrificed and examined for abnormalities. Histopathological examination was not performed.

Findings:

For tabulation of results please refer to the table presented in the monograph (2004).

Parental data:
During days 22 to 23 of pregnancy, there were several mortalities in treated groups, the highest incidence occurring at 750 ppm of the cis/trans mixture. Signs included piloerection, cold and pale extremities, lethargy and hunched posture; these were generally noted for animals just prior to death/sacrifice (from day 22) and were considered to be associated with delayed parturition. The single mortality at 500 ppm cis/trans mixture was comparable (incidence and signs) with that in the previous preliminary study at this dose. Similar signs of reaction to treatment were also observed amongst surviving dams at 750 ppm 95% cis isomer.
Both cis/trans isomer mix and 95% cis isomer provoked a consistent decrease of water intake at all doses after d7 of pregnancy; showing a slight dose-relationship by days 16-19.
Feed intake was slightly lower than controls and tended to follow a dose-relationship with comparability between materials. For both materials, there was a noticeable dose-related decrease of mean bodyweight gain during d6-20, with the cis/trans mixture showing the greatest divergence from control at the same dose as the cis material. On d8, top-dose animals had the same weight (cis) or even lost weight (cis/trans) compared to d6. For all test groups, the difference from the control was still clearly evident at d20 of pregnancy and continued throughout lactation, although bodyweights tend to converge for both materials.
The duration of pregnancy tended to increase with dosage, and appeared slightly longer in the groups treated with the cis/trans mixture than in those treated with the 95% cis isomer. 3/5 mortalities at 750 ppm of the cis/trans mixture have been sacrificed on day 23 of pregnancy with signs of dystocia. For the 5 dams with total litter loss, the duration of pregnancy was 23/24 days.
It was stated in the report that occasional macroscopic changes detected at necropsy of F0 were not related to treatment (data not shown).

Offspring data:
Incidences of total litter loss seemed to follow a dose-related pattern and were comparable for both materials at the same levels. The losses occurred before or by day 2 *post-partum (pp)*. The small number of litters reared to weaning limits the confidence with which conclusions can be drawn from the data, particularly at 750 ppm for both materials. Although the implantation rate was similar for all groups, mean live litter size at birth for treated dams was noticeably lower than the controls and was due to both higher pre-birth losses and pup losses at birth. This higher pup mortality continued to day 4 *pp* in a dose-related pattern with the 95% cis isomer showing a marginally higher rate than the cis/trans material, but after culling, further pup loss was negligible for all groups through to weaning. However, litter sizes for treated groups remained lower than control with the 95% cis isomer groups showing slightly inferior values compared to the cis/trans group.
For both materials at 750 ppm, mean pup weight at birth was slightly lower than controls; from day 8 to weaning, pup weight at both 500 and 750 ppm showed a dose-related decrease with the cis groups marginally lower than the cis/trans groups. Reflecting the slightly lower litter sizes and mean pup weights, litter weights for the cis material were consistently lower than the mix at the same dose from day 4 post cull and tended to follow a dose-relationship.
At 750 ppm 95% cis isomer there was 1 incidence of hydrocephaly. Whereas, in isolation, this and other occasional findings observed at macroscopic examination of F1 offspring (data not shown) did not indicate a clear effect of treatment, it was remarked that this malformation was present in a further developmental study.

Conclusion:
The results obtained generally showed a dose-related toxicological response at 500 and 750 ppm for both metconazole WL148271 (85:15 cis/trans mixture) and metconazole WL136148 (95% cis isomer). However, when comparing these materials the mixed isomers tended to show a greater response for maternal parameters, while the cis isomer appeared to show a more pronounced effect on the F1 offspring.

Rat, Metconazole WL148274 (cis/trans), 2nd preliminary 1-generation study, 0, 1, 2, 8 and 32 mg/kg bw/d in diet ([redacted] 1991a; BASF DocID MK-430-001)

Guidelines: Preliminary study, used as range finder for main study
GLP: Yes
Acceptance: The study was accepted.

Material and Methodes:
6 rats (CD strain of Sprague-Dawley origin)/sex/dose were administered metconazole WL148271 (Batch: 89-01, purity: 95.3%, cis/trans ratio: 83.7/16.3%, nominal 85:15) in the diet at levels that were adjusted at intervals to maintain calculated chemical intakes of 1, 2, 8 or 32 mg/kg bw/d. The actual dietary level ranges (ppm) during the 9 week feeding period were 12-18; 25-37; 100-149 and 394-570 for males, and 4-12; 9-24; 35-93; 141-378 for females. The achieved chemical intakes were usually in the range of ±10% of target values. The F0 animals were treated for 15 days prior pairing. Treatment was continued throughout mating, gestation and lactation and until 8 weeks of age of the F1 litters. Control animals received untreated diet throughout the same period. The study is considered to provide additional information.

Findings:

Parental toxicity:

F0 generation:
There were no relevant findings observed on clinical signs and mortality. Body weight gain of females in all treated groups was slightly lower than that of controls during the gestation period, with females receiving 32 mg/kg bw/d being the most affected (-16%). These weight deficits were recovered during the lactation period. Food consumption was slightly reduced in females during gestation in all treated groups; the effect was somewhat more marked at 2 and 32 mg/kg bw/d, but dose-relationship was lacking. Food consumption was similar in all groups during the lactation period. Food conversion efficiency of females during the second week of treatment tended to be slightly reduced at 8 and 32 mg/kg bw/d.
Water consumption was occasionally slightly increased in males in all treated groups, but no dose-relationship was apparent, thus the finding was not relevant. Water consumption in females was unaffected by treatment. There were no relevant findings noted during terminal examinations.

F1 generation:
There were no relevant findings observed on clinical signs and mortality. Body weight gain and food consumption was not affected by treatment. Water consumption in females showed a slight increase in all treated groups, but the effect was not dose-related. Necropsy of F1 animals at 8 weeks of age revealed no effects that were considered to be related to treatment.

Reproduction parameters: (see Table 5.6.1-2)

F0 generation:
No effect was observed on oestrous cycles, mating performance and fertility up to the highest dose tested. Gestation length was within the normal range of 22 to 23 days for all females. At the top-dose, mean gestation length tended to be increased by approximately half a day. Parturition was unaffected and gestation index was maximal in all groups.

Table 5.6.1-2: Selected data on reproduction and offspring parameters following treatment with metconazole cis/trans (MK-430-001)

Dose levels (mg/kg bw/d)		**0**	**1**	**2**	**8**	**32**
Reproduction Parameters						
No. of females/pregnants		6/6	6/6	6/6	6/6	6/6
Mating index (%)	F0 (m/f)	100/100	100/100	100/100	100/100	100/100
Fertility index (%)	F0 (m/f)	100/100	100/100	100/100	100/100	100/100
Gestation index (%)	F0	100	100	100	100	100
Oestrous cycle (%) • Regular[1]	F0	100	83	100	100	100
• Irregular[1]		0	17	0	0	0
Mean gestation length (days)	F0	22.3	22.2	22.3	22.2	22.8
Offspring data						
Implantation sites	F1	17.2	16.7	15.7	16.3	16.3
Litter size (day1 pp)	F1	15.8	15.3	14.5	15.0	15.0
Post-implantation survival index (%)	F1	92	91	91	92	92
Live birth index (%)	F1	98	99	100	99	92
Viability index (D4 pp)	F1	100	97	97	99	99

pp: post partum
[1] Regular: 4 or 5 days of oestrous cycle; irregular: at least one cycle of two, three or six to nine days

Offspring data: (see Table 5.6.1-2)

● **F1 generation:** There was no indication of a treatment-related effect on the number of implantation sites and total numbers of offspring at birth from day 1 post-partum (pp) to weaning. The lowered live birth index (-6% of control) at the top-dose was mainly attributable to one litter. Pup body weights and sex ratio were unaffected by treatment. There were no relevant findings noted during terminal examinations.

Conclusion:
It was concluded from this preliminary study that dietary concentrations of metconazole WL148271 (cis/trans), resulting in test article intakes of up to 32 mg/kg bw/d, would be suitable for use in a main 2-generation study in the rat. This was based upon the slight body weight changes during gestation and slightly increased gestation lengths as the sole effects.

Rat, Metconazole WL136184 cis, 2-generation main study, 0, 2, 8, 32 and 48 mg/kg bw/d in diet ([redacted] 1992a; BASF DocID MK-430-003)

Guidelines: Protocol in compliance with test methods B.35 of directive 88/302/EEC
GLP: Yes
Acceptance: The study was accepted.

Materials and Methods:
Rats (CD strain of Sprague-Dawley origin) were administered metconazole cis (WL136184, 95.2% purity, 95.2% cis : 0.09% trans, batch 12) in the diet at levels that were adjusted at intervals to maintain calculated chemical intakes of 2, 8, 32 or 48 mg/kg bw/d throughout 2 generations. The actual dietary level ranges (ppm) during the 20 week feeding period were 13-49, 54-195, 219-780, 336-1173 for males and 10-32, 41-127, 160-511, 238-755 for females. The achieved chemical intakes were usually in the range of ±10% of target values.
Control animals received untreated diet throughout the same period. The F0 generation, which comprised 32 rats/sex/dose, received 14 weeks treatment before pairing to produce F1 litters from which 32 male and 32 female offspring were selected to form the F1 generation. Treatment of F1 animals continued until termination after the breeding phase. Both sexes received 14 weeks treatment after selection before pairing to produce F2 litters. All adult animals were subjected to a detailed necropsy on post-natal day 25 (weaning) and reproductive organs were weighed and retained, histological examination was performed on the reproductive organs, from the control and highest treatment levels animals.

Findings:

Parental toxicity: (see Table 5.6.1-3)

F0 generation:
The clinical condition and appearance of F0 animals were unaffected by treatment. Two males and 3 females died. One control male died on wk7 (severely cannibalised) and one top-dose male was found dead during week 23 (cause of death unknown). One female of group 8 mg/kg/d was sacrificed on day 3 post-partum (parturition difficulties, bleeding from vagina and vagina dilated). At the top dose two females were sacrificed, one on day 3 post-coitum (teeth and nasal damage) and another one on day 24 post-coitum (parturition difficulties, bleeding from vagina with subsequent loss of 6 foetuses). It was uncertain if the deaths of the top-dose animals on week 23 and day 24 post-coitum were treatment-related.
Body weights and body weight gain of males showed no statistically significant differences between groups. In top dose females, body weight was decreased at the end of the treatment period before pairing and on gestation day 20; body weight gain decreased during the maturation and gestation periods, but the animals recovered during the lactation period. Food consumption and food conversion efficiency was unaffected by treatment. There were no relevant findings observed in reproductive organ weights or gross pathology and histopathology.

F1 generation:

The clinical condition and appearance of F1 animals were similar in all groups. Two males and 4 females died or were sacrificed, but necropsy findings indicate no treatment-related effects. One control female was sacrificed on day 25 post- coitum after prolonged parturition. Two males of group 8 mg/kg bw/d were sacrificed during week 12 (poor condition) and week 15 (teeth overgrown, underactive). One female of group 8 mg/kg bw/d and one of group 32 mg/kg bw/d were sacrificed on day 23 post-coitum for the same reason (prolonged parturition). Another female of the 32 mg/kg bw/d was found dead on days 3 post-partum (accidental).

At selection (week 0), F1 animals receiving 32 or 48 mg/kg bw/d were lighter than the controls. This difference persisted until the end of treatment at least at the top dose in the males. In the females, the difference was remarkable throughout gestation, and until day 7 of lactation. While inter-group differences in body weight gain were insignificant in the males, decreased bw gain was observed in top-dose females during maturation (wk 0-14), but not during gestation or lactation. There was no relevant effect on food consumption in males and females. Food conversion efficiency was unaffected by treatment.

At necropsy of F1 adults terminal body weights were significantly reduced in females at 32 mg/kg bw/d and in males and females at the top dose. Relative and absolute ovarian weights were increased at 32 and 48 mg/kg bw/d, but histopathology revealed no corroborative findings.

There were no macroscopic abnormalities observed that were considered to be related to treatment, except for four top-dose males, that showed small/dark testes or epididymis. In histology reduced sperm content (epididymis) and degeneration of tubular germinal epithelium were observed. However, the finding was not corroborated by modifications of organ weight, and fertility was not altered, thus the toxicological relevance of the finding is questionable.

Reproduction parameters:

F0 generation:

There was a slight increased incidence of irregular oestrus cycle (16%) or acyclicity (13%) at the top-dose compared to controls (6% and 3%, respectively). The incidences were higher than historical control mean rates, but within the ranges (see Table 5.6.1-3). At this dose and at the next lower dose (32 mg/kg bw/d) gestation length was slightly prolonged with values for gestations length > GD 23-25 outside of historical control ranges. The female mating index at 8 mg/kg bw/d (84%) was lower than usually achieved and at the top dose (90%) just outside the range of historical control data of the laboratory. The male and female fertility index (72%) for the F0 8 mg/kg bw/d group was marginally outside the historical control data range. However, in the absence of a clear dose-response relationship these effects were not considered to be related to treatment. Parturition difficulties associated with mortality (see above) were observed in one female at the 8 mg/kg bw/d group and one female at the top dose. Due to these single incidences at isolated dose levels this finding was not considered relevant. Furthermore, gestation indices were similar in all groups.

F1 generation:

The oestrous cycle of F1 adults appeared to be normal. Gestation length of females receiving 32 and 48 mg/kg bw/d was slightly, but statistically significantly, increased with values at the top dose being outside of the historical control range.

The F1 male and female mating index and fertility index was slightly lower in the control group and the lowest dose-group than in the higher dose groups. In the absence of a dose-response relationship, this finding was considered incidental. Other litter parameters were not affected. There was dystocia observed in single animals at the 8 and 32 mg/kg bw/d group but also in the control group, and was therefore not treatment-related. Furthermore, gestation indices were unaffected by treatment. Ovary weights were significantly increased in F1 females. However, in the absence of any histopathological correlate, the weight changes were not considered to be relevant.

Table 5.6.1-3: Parental toxicity and reproductive parameters in the F0 and F1 generation after treatment with metconazole cis

Dose levels (mg/kg bw/d)		**0**	**2**	**8**	**32**	**48**
Parental data						
No. of females/pregnant		32/27	32/23	32/26	32/26	32/25
Deaths/sacrificed	F0	1 (m)	-	1 (f)	-	2 (f)
	F1	1 (f)	-	2 (m), 1 (f)	2 (f)	-
BW [g] • wk 14 • GD 0 • GD 20	F0 (f)	 349 352 505	 347 337 486	 349 348 506	 339 345 486	 335 (-4%) 337 (-4.3%) 472 (-6.5%)
• Wk 0 • Wk 14 • GD 0 • GD 20 • Lact.D 7	F1 (f)	103 376 365 518 415	103 375 374 528 415	100 382 384 538 421	**95** (-7.8%)** 356 (-5.3%) 364 509 412	**95** (-7.8%)** 342 (-9%) **340*** **484**** 386 (-7%)
• Wk0 • Wk 22	F1 (m)	108 765	111 739	108 793	**100* (-7.4%)** 778	**102* (-5.6%)** 724 (-5.4%)
BW gain (% of control) • Wk 7-14 • Gd 0-20	F0 (f)					 **-16%*** **-11.8%***
• Wk 0-14	F1 (f)					**-9.5%****
Terminal Body weight (g)	F1 (m/f)	763/417	736/414	789/411	774/**390***	**710*/382****
Ovary weight • absolute • relative (%)	F1 (f)	 0.132 0.0322	 0.133 0.0325	 0.145 0.0357	 **0.155*** **0.0398****	 **0.158**** **0.0419****
Reproduction Parameters						
Mating index (%)	F0 (m/f)	97/97	84/84	97/97	94/94	90/90
	F1 (m/f)	81/91	84/90	90/97	100/100	97/100
HCD#	*F0*	*Females: 92-100% (mean= 97.3%), males: 83-100% (mean: 96.3%)*				
	F1	*Females: 92-100% (mean= 97.3%), males: 83-100% (mean: 96.5%)*				
Fertility Index (%)	F0 (m/f)	84/84	72/72	81/81	81/81	81/81
	F1 (m/f)	53/63	61/68	70/75	84/84	78/81

Dose levels (mg/kg bw/d)		**0**	**2**	**8**	**32**	**48**
HCD #	*F0*	*Females: 75-96% (mean= 85.6 %), males: 75-96% (mean= 84.7%)*				
	F1	*Females: 65-96% (mean= 85.0 %), males= 68-96% (mean= 84.2%)*				
Gestation Index (%)	F0	100	96	96	100	96
	F1	90	100	96	93	100
HCD#	*F0 /F1*	*87-100% (mean: 97.9%) / 87-100% (mean:98.0%)*				
Oestrous cycle (%)	F0	88	91	97	88	72
• Regular[1]	F1	91	74	84	78	88
HCD#	F0/F1	*63-96% (mean = 80.8%) / 63-96% (mean = 81.7%)*				
• Irregular[1]	F0	6	3	0	6	16
	F1	0	13	6	6	6
HCD #		*F0: 0-18% (mean = 7.3%); F1: 0-18% (mean = 7.9%)*				
• Extended[1]	F0	3	6	3	0	0
	F1	3	0	0	3	3
HCD #		*F0: 0-8% (mean = 1.2%); F1: 0-8% (mean = 1.4%)*				
• Acyclic[1]	F0	3	0	0	6	13
	F1	6	13	9	13	3
HCD #		*F0: 0-25% (mean = 10.7%); F1: 0-25% (mean = 9.1%)*				
Mean gestation length (days)	F0	23.1	23.0	23.0	23.2	23.4
	F1	22.9	22.8	22.7	23.2	23.3
Gestation length (%)						
• < GD 23		15	41	28	12	4
• = GD 23		59	32	40	46	42
• > GD 23	F0	26	27	32	42	**44***
• < GD 23		37	47	46	4	0
• = GD 23		53	48	46	67	42
• > GD 23	F1	10	5	8	**30****	**58*****
HCD#		*GD<23: 0-45%; GD 23: 16-67%; GD >23: 0-32%*				

Statistical evaluation: *p≤0.05, **p≤0.01, ***p≤0.001
#Historical control data (HCD) as presented in the study report
[1] Regular: 4 or 5 days of estrous cycle; irregular: At least one cycle of two, three or six to nine days; extended estrous: At least four consecutive days of estrous; acyclic: At least ten days without estrous

Offspring data: (see Table 5.6.1-4)

F1 generation:

The numbers of implantation sites were similar in all groups. Post-implantation survival at the top dose was significantly reduced, resulting in a slight (non-significant) reduction in litter size. Live birth index was slightly (not statistically significantly) reduced, but subsequent survival of offspring was unaffected by treatment. The sex ratio was unaffected by treatment.

While bodyweights of offspring at birth were unaffected by treatment, subsequent bodyweight gain of pups dosed at 8, 32 or 48 mg/kg bw/d were significantly decreased when calculated until postnatal day 25. The reductions in body weight gain at 8 mg/kg bw/d and above were small (5-7%).

Physical development was assessed by the timing of onset and completion of pinna unfolding, hair growth and tooth eruption, which were unaffected by treatment. The onset of eye opening in offspring receiving 32 or 48 mg/kg bw/d occurred slightly earlier than in the control group, but was just outside the lower limit of the background data (range: 12.6-14.0 days, mean 13.5 days (n= 21 studies). During necropsy at termination slightly increased unilateral or bilateral hydronephrosis or unilateral hydroureter was observed at the top-dose.

F2 generation:

The numbers of implantation sites were similar in all groups. Post-implantation survival at 32 and 48 mg/kg bw/d was statistically significantly reduced as compared to the controls, resulting in slightly smaller mean litter sizes (non-significant). The live birth index was slightly but not significantly reduced at 48 mg/kg bw/d, but subsequent survival of offspring was unaffected by treatment. The sex ratio was unaffected by treatment.

The pup bodyweights at birth were unaffected by treatment, but bodyweight gain up to postnatal day 25 at 48 mg/kg bw/d was statistically significantly reduced in both sexes when compared to control animals. With regard to the measure physical development parameters the onset of eye opening occurred slightly but statistically significantly earlier at 32 and 48 mg/kg bw/d as compared with the control group. However, the effect was within the lower limit of background control data. During necropsy at termination in F2 offspring, unilateral hydronephrosis was observed occasionally in control animals and at the treated dose groups. Thus, the effect of ureter or kidney dilatation was not considered to be treatment-related.

Table 5.6.1-4: Litter observation in the F1 and F2 offspring following treatment with metconazole cis

Dose levels (mg/kg bw/d)		**0**	**2**	**8**	**32**	**48**
Offspring data						
Implantation sites	F1	15	15	15.1	15.1	15.1
	F2	14.7	15.7	15.3	15.7	15.7
Live litters born	F1	27	22	25	26	24
	F2	18	21	23	25	26
Litter size (day 1 pp)	F1	13.6	13.6	14.4	13.8	12.0
	F2	14.3	14.4	14.2	13.1	12.6
Post-implantation survival index (%)	F1	89	89	94	90	**78****
	F2	93	90	92	**83****	**79****
Live birth index (%)	F1	97	100	98	99	93
	F2	100	95	98	97	**94***
Viability index D4 pp (%)	F1	99	98	99	99	98
	F2	99	90	100	98	98
Pup bw [g] • day 1 pp • day 4 pp • day 25 pp	F1 (m/f)	7.3/6.8 11.1/10.5 91.7/86.7	7.2/6.6 10.6/9.8 89.5/84.6	7.1/6.7 10.7/10.1 86.9/82.6	6.9/6.6 10.7/10.1 86.5/82.3	6.9/6.6 10.6/9.9 85.7/80.4
• day 0 pp • day 4 pp • day 25 pp	F2 (m/f)	7.2/6.8 11.1/10.7 94.1/88.8	6.6/6.4 10.3/9.9 90.1/85.6	7.0/6.5 10.8/10.1 90.9/85.0	7.0/6.6 11.1/10.4 92.2/85.8	6.7/6.5 10.3/10.1 **85.6/83.1**
Pup bw gain (% of control) • day 1-25 pp	F1 (m/f)			**-5.5%*/ -5.1%***	**-5.7%**/ -5.3%***	**-6.6%***/ -7.6%****
	F2 (m/f)					**-9.2%***/ -6.6%****
Physical development: Onset eye opening (days)	F1	13.1	13.0	13.1	**12.6***	**12.5***
	F2	13.3	13.4	13.0	**12.8***	**12.8***

Dose levels (mg/kg bw/d)		0	2	8	32	48
Necropsy:						
Unilateral hydronephrosis	F1	0.7 (1)	1.0 (1)	2.5 (3)	2.8 (2)	7.4 (6)
	F2	3.5 (4)	3.3 (4)	2.7 (5)	3.2 (4)	1.6 (3)
Bilateral hydronephrosis	F1	0.7 (1)	0.0 (0)	0.0 (0)	0.0 (0)	1.1 (1)
Unilateral hydroureter	F1	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	3.2 (2)

Statistical evaluation: *p≤0.05, **p≤0.01, ***p≤0.001
pp: post partum

Conclusion:

Metconazole WL136184 (95% cis) caused alterations of some reproduction parameters and slight foetal systemic toxicity at maternal toxic doses.
The NOAEL for parental toxicity was set at 8 mg/kg bw/d based on decreased body weights at 32 mg/kg bw/d in F1. Likewise, the NOAEL for reproduction toxicity was set at 8 mg/kg bw/d based on increased gestation length and decreased post-implantation survival in F2 at 32 mg/kg bw/d). The offspring NOAEL (8 mg/kg bw/d) was based on decreased body gain of F1 pups until weaning at 32 mg/kg bw/d.

Executive summary of the analytical method used within the analytical phase (MK-430-003)

Principle of the method
The analytical method used in study MK-430-003 was validated for the determination of *cis*-metconazole (BAS 555 F) in animal test diet mixes. The study was performed by HUNTINGDON RESEARCH CENTRE LTD., UK.
Residues were extracted from diet mixes by shaking a representative sub-sample with acetone. The extract was filtered and the filtrate was concentrated or diluted with hexane. Residues were analyzed by gas chromatography (GC) with a nitrogen thermionic detector (TID). Analysis was performed on an Ultrabond 20M column.

Recovery findings
The mean recovery values were between 70% and 110% for *cis*-metconazole. The detailed results are given in the table below.

Table 5.6.1-5: Validation results of the analytical method applied in study MK-430-003 using GC-TID: *cis*-metconazole (BAS 555 F) in animal test diet mixes

Matrix	Analyte	Detector settings	Fortification level (µg/kg)	No of replicates	Mean recovery (%)	RSD (%)
LAD II diet	*Cis*-metconazole	Nitrogen thermionic detector, 300°C	13.3	6	103	6
			341	6	101	10
			Overall	12	102.1	7.9

Linearity
The linearity was tested using five standards at concentrations up to 30 µg/mL. For *cis*-BAS 555 F, linear correlations were obtained. Calibration solutions were prepared in hexane.

Specificity
Final detection has been applied by nitrogen thermionic detection (TID). As only known analytes of known concentration were analyzed for dose verification purposes, no additional confirmatory technique is necessary.

Interference
No relevant interferences from the reagent blank at the relevant retention times have been reported.

Limit of Quantitation
The limit of quantitation (LOQ) is defined as the lowest fortification level successfully tested. During this study, a limit of quantitation of 0.01 mg/kg for *cis*-BAS 555 F was obtained.

Repeatability
The relative standard deviations (RSD, %) for all fortification levels were below 20%.

Conclusion
The analytical method used in study MK-430-003 for the analysis of *cis*-metconazole in animal test diet mixes uses GLC-TID for final determination, with an LOQ of 0.01 mg/kg.
It could be demonstrated that the method generally fulfils the requirements with regard to repeatability, specificity, LOQ and recoveries and is therefore considered suitable for the analysis of *cis*-metconazole in animal test diet mixes

2-generation study in rats with metconazole cis/trans
A 2-generation study in rats (study author: [redacted] 2002; BASF DocID 2006/8000261) with metconazole cis/trans (KNF-474m) was conducted for non-EU authorities by another metconazole-supporting company. A detailed study summary was made available to BASF (prepared by Takahashi, 2015a; BASF DocID 2015/1087913) and is part of this submission.

Report:	CA 5.6.1/1 [redacted] 2015a Summary of the Laboratory Report: IET 00-0146 - KNF-474m: Reproduction toxicity study in rats 2015/1087913
Guidelines:	OECD 416 (2001)
GLP:	yes UK Department of Health

Report:	CA 5.6.1/2 [redacted] 2006 a KNF-474m: Reproductive toxicity study in rats 2006/8000261
Guidelines:	EPA 870.3800, OECD 416
GLP:	yes (certified by Ministry of Agriculture, Forestry and Fisheries of Japan, Japan)

Executive Summary
Metconazole cis/trans (Batch: 9Z521, purity: 98.99%, cis/trans ratio: 84/16) was evaluated for reproductive toxicity potential in a two-generation study in Crj:CD(SD)[IGS] rats. Dietary concentrations of 0, 30, 150 or 750 ppm were administered for 10 weeks before pairing, throughout pairing, gestation and lactation and until termination in both generations (24 males and 24 females per group both in the P and F1 generation). F2 offspring received treated diets until termination on Day 25-27 of age.
No signs of general toxicity effects of test substance treatment were noted in any parameters of both P and F1 parental males and females in the 30 and 150 ppm groups. In the 750 ppm group, food intake was reduced in P females during gestation days 14-20, and in F1 males and females during the pre-mating period. Reduced body weight gains were noted for 2 and 4 weeks after initiation of treatment for P and F1 males, respectively; and throughout the pre-mating period (treatment weeks 1-10) and at week 10 for P and F1 females, respectively. A significantly reduced body weight gain was also observed in P females during gestation (GD 0-20) but not during lactation (PND 1-21), where these animals showed a slight but statistically significant increase in body weight gain compared to controls.

Treatment-related effects were also observed in organ weights in the 750 ppm group. Relative liver weights of P and F1 males and females and absolute and relative ovary weights of P and F1 females were significantly increased. Relative spleen weights of F1 females showed a tendency to increase, as compared to controls. Histopathological examination of these organs revealed centrilobular fatty change of hepatocytes in males and hepatocellular hypertrophy in females, and congestion of the spleens (F1 females only) in the 750 ppm group. No histopathological changes which may be related to the increased organ weight were noted in the ovary. The relative weight of seminal vesicles increased significantly in F1 males in the 750 ppm group; however, this change was considered to be incidental because histopathological alteration were neither observed in this organ nor in other male reproductive organs.

For reproductive performance of P and F1 parental rats, no effects of test substance treatment were noted in any parameters tested in the 30 and 150 ppm groups. In the 750 ppm group, although sexual development, mating performance, and spermatogenesis in parental male rats were not affected, the following alterations were noted in parental female rats: slightly but significantly prolonged oestrous cycle length in P females, prolonged duration of gestation in P females, five P and four F1 maternal deaths during delivery, and significant decreases in the gestation index in both P and F1 females.
The mating index, fertility index, and mean number of implantation sites of P and F1 females in this group were comparable to those in the control group, and the females that delivered without difficulties weaned pups successfully.
For the growth of F1 and F2 pups, no effects of test substance treatment were noted in any parameters in the 30 and 150 ppm groups. In the 750 ppm group, the live birth index of F2 pups was significantly decreased. Mean pup body weights in this group decreased significantly on lactation days 0, 14, and 21 in F2 males and females. Relative spleen weights of F1 male and female and of F2 female weanlings in the 750 ppm group increased significantly without any histopathological alterations.

Based on the results of this two-generation study in the rat, the NOAEL for general parental toxicity in the P and F1 males and females, as well as the NOAEL for reproductive performance in the P and F1 adults were 150 ppm (approx.. 11, i.e. 9.79, 10.78, 10.63, and 11.21 mg/kg/day for P males, P females, F1 males, and F1 females, respectively). The NOAEL for the growth of the F1 and F2 offspring was 150 ppm.
Based on the results of this two-generation study in the rat, the LOAEL for general toxicity in the P and F1 males and females, as well as the LOAEL for reproductive performance in the P and F1 adults were 750 ppm (approx.. 53, i.e. 49.4, 53.2, 53.0, and 55.5 mg/kg/day for P males, P females, F1 males, and F1 females, respectively) [Highest Dose Tested (HDT)]. The LOAEL for the growth of the F1 and F2 offspring was 750 ppm [HDT].

(DocID 2015/1087913)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: Metconazole (Code No. KNF-474m)
Description: solid (crystalline) / white
Lot/Batch #: 9Z521
Purity: 98.99% (83.13:15.86, cis:trans)

2. Vehicle: Basal diet

3. Test animals:
Species: Rat
Strain: Crl:CD(SD) [IGS]
Sex: Male/Female
Age (at mating): 5 weeks (P generation)
Weight (at start of dosing): 139 – 154 g (males); 111 – 123 g (females)
Source: Charles River Japan, Inc., Japan
Acclimation period: 7 days
Diet: Certified pulverized feed MF Mash, Oriental Yeast Co., Ltd., Japan
Water: Well water passed through a rapid filtration unit with sand filter and an absorption unit with charcoal filter and sterilized with sodium hypochlorite, ad libitum
Housing: Animals were housed in suspended wire-mesh stainless steel cages during acclimatization, pre-mating growth, and post-mating (males)/post-lactation (females) periods. Animals were housed in aluminium cages with wire-mesh floors and fronts during the mating period. Post-mating females were housed in aluminium boxes with nesting materials until weaning of their pups.
Animals were housed by sex, up to 5 per cage, or during mating, in pairs (1 male with 1 female). Females were housed individually after mating and with litter until weaning. Litters were then housed mixed sex during maturation.

Environmental conditions:
Temperature: 22 - 26°C
Humidity: 40 - 70%
Air changes: at least 10 per hour
Photo period: 12 h light / 12 h dark

B. STUDY DESIGN AND METHODS

1. Dates of experimental work: 27-Feb-2001 to 12-Nov-2001

2. Animal assignment and treatment:
A total of 114 males and 114 females were received from the supplier. Ninety-six males and 96 females selected after the quarantine/acclimatization period were assigned to four groups in such a way to equalize group means and standard deviations of body weight. These animals were designated as P parental animals.
Groups of 24 male and 24 female rats were fed dietary concentrations of 0, 30, 150 or 750 ppm of metconazole for 10 weeks before pairing, throughout pairing, gestation and lactation and until termination after F1 litters were weaned. In each group, 24 males and 24 females were selected from F1 weanlings at 21-27 days of age to become the F1 parental animals. The selection was performed using litters born during a six-day period including the day of the largest number of parturitions. The number of F1 weanlings selected at random from each litter was one or two per sex. Selected F1 parental animals were fed the relevant concentration of test diet continuously from the start of the F1 generation, for 10 weeks prior to pairing and until termination after F2 litters were weaned. F2 offspring received treated diets from weaning until termination on Day 25-27 of age. Control groups of each generation and their offspring were given untreated diet throughout.
The dosages used in this study were selected based on the results of a preliminary study (IET 00-0060). In the study, 8 male and 8 female Crj:CD(SD)[IGS] rats per group were given diet containing the test substance at a concentration of 0, 30, 100, 300, or 1000 ppm for 3 weeks before mating and until weaning of F1 pups. No test substance treatment effects were noted in parental animals or pups in the 30 and 100 ppm groups. In the 300 ppm group, the duration of gestation in parental females was slightly prolonged. In the 1000 ppm group, food consumption, body weights, and body weight gains of parental animals were affected adversely, and 2 parental females died at parturition. The number of pups delivered and mean pup body weights for both sexes were significantly decreased in the 1000 ppm group. Therefore, considering that a dose level of 1000 ppm of the test substance is too high for parental rats and pups, a dietary concentration of 750 ppm was selected for the high-dose group in this study. The low and intermediate dietary levels of 30 and 150 ppm, respectively, represent a five-fold interval in concentration.
Vaginal smears were taken daily from all P and F1 females for 2 weeks prior to pairing to establish the duration and regularity of the oestrous cycle. After the scheduled treatment period before pairing, females showing pro-oestrus vaginal smears were paired with males within the same dose groups and cohabited overnight. Care was taken to avoid pairing siblings (F1 generation). Next morning after pairing, the trays beneath cages were examined for copulation plugs and a vaginal smear was prepared from each female and examined for the presence of spermatozoa. The day on which evidence of mating was found was designated Day 0 of gestation. The mated rats were then separated.
Daily vaginal smears taken from females before necropsy were used to determine the stage of the oestrous cycle at termination. Females showing metoestrus or dioestrus stage were euthanized.

3. Test substance preparation and analysis:
For each dose level, the specified amount of the test substance was mixed in a mortar with a small part of the basal feed. Then, the premix was stirred into the remaining part of the basal feed in a mixer to obtain a test diet of the prescribed concentration. Test diet batches were prepared once every four weeks during the pre-mating growth period and once every two weeks during the breeding period. At the first preparation, homogeneity and concentration of the test substance were analyzed for all test diets. At second preparation and onward, concentrations of the test substance in the diets were confirmed for all test diets immediately after preparation.

4. Statistics:
The data on the body weights, body weight gains, and food consumption of parental animals, the number of implantation sites, testicular sperm head counts, epididymal sperm counts, the number of pups delivered, organ weights of parental animals and weanlings, and pup body weights were evaluated as follows: Equality of variances was first evaluated by Bartlett's test (α=0.05). When group variances were homogenous, a parametric analysis of variance in one-way classifications (α=0.05) was used to determine if any statistical differences exist among groups. If the analysis of variance was significant, Dunnett's test or Scheffe's test (α=0.05, 0.01, or 0.001) was performed. When Bartlett's test indicated that the variances were not homogeneous, Kruskal-Wallis test (α=0.05) was used for detecting any statistical differences among groups and if significant, Dunnett-type mean rank test or Scheffe-type mean rank test (α=0.05, 0.01, or 0.001) was performed.
The data on the number of primordial follicles in the ovary were evaluated between the control group and the high-dose group as follows: Equality of variances was first evaluated by F test (α=0.05) and if group variances were homogeneous, Student's t test (α=0.05 or 0.01) was used. When group variances were not homogeneous, Aspin-Welch's test was used (α=0.05 or 0.01).
Fisher's exact probability test (α=0.05, 0.01, or 0.001) was used for the data on the incidence of clinical findings, the incidence of normal oestrous cycle, mating, fertility, and gestation indices, incidences of gross pathological and histopathological findings collected from parental animals, and sex ratios of pups.
Mann-Whitney's U-test (α=0.05 or 0.01) was used for the data on the sexual development, oestrous cycle length, the number of days until mating, duration of gestation, percentage motile of epididymal sperm and the incidence of morphologically normal epididymal sperm in parental animals, and viability indices, incidences of clinical and gross pathological findings in pups.

C. METHODS

1. Observations:
<u>P and F1 parental animals</u>
Parental animals were examined for clinical signs and mortality twice daily (once daily on holidays) by cage-side observation. In addition, a more detailed weekly physical examination was also performed. All animals found dead or killed in extremis during the study were subjected to a complete necropsy. Individual body weights of P males were recorded in at the start of treatment, weekly throughout the treatment period, and on the day of necropsy. P females were weighed at the start of treatment, weekly until mating, on gestation days 0, 7, 14 and 20, on lactation days 0, 7, 14 and 21, and on the day of necropsy. Following selection, F1 animals were weighed at the same frequency as P animals.

For the P generation, food consumption was recorded for each week of treatment before pairing. For each P female, food consumption was recorded for the periods of gestation days 0-7, 7-14 and 14-20 and lactation days 0-7, 7-14, and 14-21. Following selection, food consumption of F1 animals was recorded at the same frequency as P animals.
From gestation day 20, P and F1 females were inspected daily for evidence of parturition. Parturition was monitored and the numbers of live and dead pups recorded together with any observed difficulties.

F1 and F2 pups
All pups were examined daily for clinical signs and mortality (cage-side observations) during the lactation period. Complete external examinations were also performed when they were weighed. Pups found dead during the lactation period were necropsied immediately after discovery. On lactation day 4, litters were adjusted to 8 offspring by random culling, leaving, whenever possible, 4 male and 4 female pups in each litter. Culled pups were necropsied on lactation day 4.
For each litter delivered normally, the total number of pups delivered (the sum of live and dead pups) was counted on lactation day 0. Sex ratio was calculated based on the numbers of males and females in each litter on lactation day 0. The number of pups surviving in each litter was counted on lactation days 0, 4, and 21. Pups were weighed on lactation days 0, 4, 7, 14, and 21. On lactation day 0, pups were weighed by sex as a group of males or females in a litter. On lactation day 4 and thereafter, pups were weighed individually. Group mean pup body weights were calculated using a litter as a sample unit.
Pups were weaned on lactation day 21. Selection of the offspring to form the F1 generation was made on Day 21-27 of age when they were separated from unselected littermates. Pups not selected for the F1 generation and F2 pups were euthanized and necropsied at 25-27 days of age.
For all selected F1 generation animals, sexual maturation was assessed daily from day 35 of age in males until preputial separation and in females from day 27 until vaginal opening. Body weight was recorded for males on the day of completion of separation and for females on the day of vaginal opening.
Because of no observed delay in the sexual maturation in the selected F1 offspring, measurements of the anogenital distance in the F2 pups were omitted.

2. Terminal studies:
All P and F1 adult rats were subjected to a detailed necropsy. All macroscopic abnormalities were recorded. Reproductive and other specified organs (adrenals, brain, kidney, liver, pituitary, spleen and thyroid) were weighed and preserved in appropriate fixative. P and selected F1 males were killed after 18 weeks of treatment. P and selected F1 Females that littered and reared offspring to weaning were killed after 18 and 19 weeks of treatment, respectively. Other females (that failed to mate, failed to produce a viable litter or their litter died before Day 21) were also killed at this time.
Sperm analysis was conducted on all P and selected F1 adult males immediately after scheduled sacrifice, generally using the right-sided testis and epididymis. The number of homogenisation-resistant spermatids was determined with a haemocytometer. Epididymal sperm motility and sperm count were assessed using a computer assisted sperm analyser. Sperm morphology was evaluated by light microscopy.
For the P and F1 females, each uterine horn was examined and the number of implantation sites counted.

From each F1 and F2 litter, 1 male and 1 female weanling were selected for organ weight analysis (brain, spleen, thymus and uterus). These organs along with reproductive organs were retained for possible histopathological examination. Unselected weanlings, as well as pups culled on lactation day 4, were also subject to a full macroscopic examination.
F1 and F2 pups that were found dead during the lactation and post-weaning periods were also necropsied immediately after discovery.
Histopathology was conducted for 10 randomly selected P and F1 parental animals in the control and high-dose groups, which successfully weaned their pups. Their following organs and tissues were examined: pituitary, adrenals, and reproductive organs, ie. ovaries, oviducts, uterus, vagina, testis, epididymis, seminal vesicles, coagulating glands, and prostate. For the testis, special attention was paid to spermatogenesis. The epididymis was examined at the levels of the caput, corpus, and cauda. For the ovaries from F1 parental females, the numbers of primordial follicles were also counted. Parental pairs that did not show any evidence of pregnancy, parental females that died during delivery or showed abnormal parturition, and dams that lost all pups during the lactation period were also examined histopathologically for their pituitary, adrenals, and reproductive organs. In addition, livers from males and females in P and F1 generation, and seminal vesicles from males and ovaries and spleens from females in F1 generation were examined histopathologically for all parental animals in all groups including the control group. The spleens from the F1 and F2 pups selected for the organ weight measurement in all groups including the control group were examined histopathologically.

II. RESULTS AND DISCUSSION

A. TEST SUBSTANCE ANALYSES

The analyses for homogeneity demonstrated that the coefficients of variation of mean test substance concentrations in each treated group diet ranged from 1.4% to 5.7%, indicating that the test substance was homogeneously distributed in the test diets. In the analyses for concentration, the test substance was detected in the samples from the treated groups at levels ranging from 93% to 107% of the nominal concentrations, indicating that the test diets were properly prepared. No test substance was detected in any control diets.

B. OBSERVATIONS IN THE PARENT (P) GENERATION (see Table 5.6.1-8)

Adult data

Mortality

There were no deaths among males in the P generation. Five P females in the 750 ppm group died during delivery.

Clinical signs

There were no treatment-related signs.

Body weight and body weight gain

In the 750 ppm group, mean body weights of P males at treatment weeks 1 and 2 were significantly lower than those in the control group (-5%). Mean body weights of P females at treatment weeks 6 through 10 (up to -5.3%) and on gestation days 14 and 20 (up to -6.4%) in the 750 ppm group were significantly lower than those in the control group.

Mean body weight gains of P males and females in the 750 ppm group were significantly lower than those in the control group at treatment weeks 1 and 2 (up to -16.7% for P males and -12.1% for P females) and of P females at treatment weeks 1 through 10 (-8.4%) and on during the gestation period (-13%). During the lactation period (day0- 21), the mean body weight gain of P females in the 750 ppm group was significantly higher than that in the control group, which may be explained by lower body weights on the day of delivery (-6%), but a recovery thereafter.

Food consumption

Male food consumption in the P generation was unaffected by treatment. For P females receiving 750 ppm, food consumption was significantly reduced during gestation days 14-20 (-7.9%).

Achieved test material intake

Mean achieved test material intakes are presented in Table 5.6.1-6.

Table 5.6.1-6: P generation mean test material intakes (mg/kg bw/day)

Dose level (ppm)	Males		Females	
	Pre-mating Weeks 1-10	Post-mating Weeks 12-17	Pre-mating Weeks 1-10	Gestation Days 0-20 and Lactation Days 0-21
Mean				
30	1.97	1.33	2.14	3.22
150	9.79	6.34	10.78	16.39
750	49.4	32.9	53.2	80.0
Range				
30	1.48-2.88	1.26-1.39	1.70-2.88	1.58-6.32
150	7.05-14.55	5.94-6.75	8.48-14.50	7.84-32.10
750	35.7-72.7	31.3-34.1	43.4-71.9	38.9-151.7

Estrous cycles prior to pairing

In the 750 ppm, two P females did not show oestrous repeatedly. Moreover, the mean oestrous cycle length in the 750 ppm group was significantly prolonged as compared to control (4.4 days compared to 4.1 days). Historical control data of four studies[1] conducted between 2002 and 2012 *[see KCA 5.6.1/3 2015/1276507]* reported mean oestrous cycle length of 4.0 to 4.1 days for P females.

[1] It should be noted that data on estrous cycle length, days until mating, preputial separation and vaginal opening had not been collected before 2002 because the test guidelines from regulation authorities did not require these parameters at that time. Therefore the time-period of the historical data set for the mentioned parameters (2002 - 2012) deviates from the other parameters that were collected from 1997 to 2012.

Mating performance and fertility
Mating performance and fertility were unaffected by treatment. The number of days until mating was also unaffected by treatment.

Gestation index and duration of gestation
Gestation index in the 750 ppm group was significantly lower than that in the control group (66.7% compared to 100%) due to reduced number of live litters (14/21 compared to 24/24). The reduction of the number of live litters at 750 ppm is based on increased number of dams that died during delivery.
Duration of gestation in the 750 ppm group was significantly prolonged as compared to control (23.0 days compared to 22.2 days). Historical control data from a total of 9 studies conducted from 1997-2012 *[see KCA 5.6.1/3 2015/1276507]* reported mean values for duration of gestation of 22.1 to 22.4 days.

Sperm assessment
There were no treatment-related effects.

Organ weights
At 750 ppm, the relative liver weight of P males was significantly higher than that in the control group (+6%). P females in the 750 ppm group showed statistically significant increases in the absolute (+11.7%) and relative (+12.8%) liver weights when compared with the corresponding controls.

Compared with the control groups P females in the 750 ppm group showed significantly increased absolute (51.4 g compared to 60.5 g) and relative (0.01648% compared to 0.01991%) ovary weights, which were however within the historical control range. Historical control data of 9 studies conducted from 1997-2012 *[see KCA 5.6.1/3 2015/1276507]* for mean absolute ovary weights range from 49.3 to 61.1 g for individual weights and from 119.5 to 132.3 g for total weights of bilateral ovaries. The historical control range for the mean relative weights to body weight is as follows: 0.01551% to 0.0199% for individual weights, and 0.0369% to 0.0409% for total weights of bilateral ovaries.

Macroscopic findings
There were no macroscopic findings attributable to treatment with metconazole. Five females found dead during delivery also had no treatment-related gross abnormalities.

Microscopic findings
In histopathological examination of 10 randomly selected pairs of P parental males and females in the control and 750 ppm groups, no treatment-related abnormalities were noted in the reproductive organs, pituitary, and adrenals. Examination of animals that did not yield successful reproduction results also showed no treatment-related abnormalities.
Livers of P males in the 750 ppm group showed a significantly increased incidence of centrilobular fatty change of hepatocytes, and livers of P females in the same group did a significantly increased incidence of centrilobular hypertrophy of hepatocytes when compared with the corresponding controls.

Litter data: F1 litters (see Table 5.6.1-9)

Clinical signs

There were no treatment-related signs.

Litter size and offspring survival

Litter size on day 0 was slightly but not significantly decreased. Offspring survival was unaffected by treatment.

Sex ratio

Sex ratios were unaffected by treatment.

Offspring body weight

In the 750 ppm group, mean body weights of both F1 male and female pups were slightly but not significantly lower on lactation days 14 and 21 than those in the control group (up to –6.5%). Male and female body weights of 750 ppm group were well within historical control data (total of 9 studies conducted between 1997 and 2012; *[see KCA 5.6.1/3 2015/1276507]*.

In detail, male body weights on lactation day 14 were 39.0 and 37.5 g in the control and 750 ppm group, respectively. Females yielded body weights of 38.1 and 35.9 g in the control and 750 ppm group, respectively. Historical control data reported mean values for F1 body weights of 35.1 to 40.5 g for males and 33.7 to 39.0 g for females on day 14 of lactation.

Male body weights on lactation day 21 were 63.3 and 60.5 g in the control and 750 ppm group, respectively. Females yielded body weights of 61.4 and 57.4 g in the control and 750 ppm group, respectively. Furthermore, historical control data reported mean values for F1 body weights of 58.0 to 68.3 g for males and 55.3 to 65.4 g for females on day 21 of lactation.

Terminal studies: F1 offspring (see Table 5.6.1-9)

Organ weights

In the 750 ppm group, relative spleen weights of F1 male and female pups were significantly higher than those in the control group. Absolute brain weights of F1 male and female pups were significantly lower than those in the control group; however, no significant differences were noted in the relative weights of their brains.

Macroscopic findings

There were no macroscopic findings attributable to treatment with metconazole.

Microscopic findings

There were no treatment-related microscopic findings in the spleen of F1 male and female weanlings.

B. OBSERVATIONS IN THE F1 GENERATION (see Table 5.6.1-9)

Adult data

Mortality

There were no deaths among males in the F1 generation. Four F1 females in the 750 ppm group died during delivery.

Clinical signs

There were no treatment-related signs.

Body weight and body weight gain

In the 750 ppm group, mean body weights of F1 males at treatment weeks 0 through 10 were significantly lower than those in the control group, which was most prominent during the first weeks 0-4 (up to -12.5%). Mean body weights of F1 females were significantly lower than those in the control group at treatment weeks 0 through 10 (up to -11.1%), on gestation days 0, 7, 14 and 20 (up to -8.3%), and on lactation days 0 and 7 (up to-8.8%) in the 750 ppm group. This was partly due to lower body weights of the animals at selection especially in F1 females because their body weight gains were comparable with the corresponding controls with the exception of treatment week 0-10 (-8.5%).

Mean body weight gains of F1 males and females in the 750 ppm group were significantly lower than those in the control group at treatment weeks 1 through 4 and at treatment week 10, respectively.

Food consumption

Mean food consumption of F1 males in the 750 ppm group was significantly lower than that in the control group at treatment weeks 1 through 4 and 10, with the strongest effect at week 1 (-15.1%).

Mean food consumption of F1 females in the 750 ppm group was significantly lower than that in the control group at treatment week 2 (-5.2%).

Achieved test material intake

Mean achieved test material intakes are presented in Table 5.6.1-7.

Table 5.6.1-7: F1 generation mean test material intakes (mg/kg bw/day)

Dose level (ppm)	Males		Females	
	Pre-mating Weeks 1-10	Post-mating Weeks 12-17	Pre-mating Weeks 1-10	Gestation Days 0-20 and Lactation Days 0-21
Mean				
30	2.13	1.29	2.20	3.04
150	10.63	6.41	11.21	15.10
750	53.0	33.5	55.5	72.9
Range				
30	1.42-3.02	1.22-1.35	1.65-2.85	1.57-5.86
150	7.12-15.22	6.01-6.62	8.60-14.62	8.01-28.58
750	35.9-72.0	31.6-35.1	42.5-71.6	40.1-133.6

Sexual maturation

No significant differences were noted in the parameters of sexual development, preputial separation in F1 parental males and vaginal opening in F1 parental females, between the control group and any of the treated groups and both parameters were well within the range of the historical control data of the four studies conducted between 2002 and 2012 that comprise a range of 40.8 - 42.0 days for preputial separation and of 30.4 - 31.1 days for vaginal opening *[see KCA 5.6.1/4 2016/1025262]*

Therefore, measurement of anogenital distance in F2 pups was not conducted.

Oestrous cycles

In contrast to the P generation, oestrous cycles of F1 females in the 750 ppm group were unaffected by treatment.

Mating performance and fertility

Mating performance and fertility were unaffected by treatment. Although a statistically significant difference was observed in the mean number of days until mating in the 750 ppm group (1.2 d), this change was not due to a lower value in the 750 ppm group but due to slightly higher values in the control (2.0 d), 30 ppm (1.7 d), and 150 ppm (1.4) groups.

Gestation index and duration of gestation

Gestation index in the 750 ppm group was significantly lower than that in the control group (75% vs. 100%) due to reduced number of live litters (18/24 compared to 22/22). The reduction of the number of live litters at 750 ppm is based on increased number of dams that died during delivery.

Duration of gestation in the 750 ppm group (22.7 days) showed no statistically significant difference but a tendency to be prolonged as compared to control (22.4 days). However, historical control data means for duration of gestation ranged from 22.1 to 22.4 days (9 studies from 1997-2012; *[see KCA 5.6.1/3 2015/1276507]*

Sperm assessment
There were no treatment-related effects.

Organ weights
At 750 ppm, the relative weight of seminal vesicles of F1 males was significantly higher than that in the control group. In addition, significantly lower values were observed in the absolute brain, pituitary, and kidney weights of F1 males in the 750 ppm group, but no significant differences were noted in the relative weights of these organs.

F1 females in the 750 ppm group showed a statistically significant increase in the relative liver weight when compared with the control (+11.5%). The absolute and relative ovary weights of F1 females in the 750 ppm group were increased significantly when compared with the controls (a: 66.6g compared with 53.3g; r: 0.02071% compared with 0.01549%). The values were well within historical control range (9 studies conducted from 1997-2012) for F1 absolute ovary weights (52.9g-68.4g for individual weights; 128.3g-132.3 g for weight of bilateral ovaries) and for F1 relative ovary weights (0.01657%-0.218% for individual weights; 0.0378%-0.0389% for weight of bilateral ovaries).
Besides, significantly lower values were observed in the absolute brain and kidney weights of F1 females in the 750 ppm group; however, no significant differences were noted in the relative weights of these organs. Relative spleen weights of F1 females in the 750 ppm group showed a tendency to increase, as compared to controls.

Macroscopic findings
There were no macroscopic findings attributable to treatment with metconazole. Four females found dead during delivery also had no treatment-related gross abnormalities.

Microscopic findings
In histopathological examination of 10 randomly selected pairs of F1 parental males and females in the control and 750 ppm groups, no treatment-related abnormalities were noted in the reproductive organs, pituitary, and adrenals. Examination of animals that did not yield successful reproduction results also showed no treatment-related abnormalities.

Livers of F1 males and females in the 750 ppm group showed significantly increased incidences of centrilobular fatty change of hepatocytes and centrilobular hypertrophy of hepatocytes, respectively, when compared with the corresponding controls. Examination of spleens revealed a significant increase in the incidence of congestion in the F1 females in the 750 ppm group. No treatment-related histopathological changes were noted in seminal vesicles of F1 males and in ovaries of F1 females in any of the treated groups.

Ovarian follicle counts
No statistically significant difference was noted in the mean number of primordial follicles in the ovaries from F1 parental females between the control group and the 750 ppm group.

Litter data: F2 litters (see Table 5.6.1-10**)**

Clinical signs
There were no treatment-related signs.

Litter size and offspring survival
Implantation sites and litter size were unaffected by treatment. Offspring survival (viability index) in the 750 ppm group was significantly decreased on day 0 when compared with that in the control group. The reduced viability index in the 750 ppm group on day 4 was due to a total litter loss in two litters during lactation days 1-4.

Sex ratio
Sex ratios were unaffected by treatment.

Offspring body weight
In the 750 ppm group, mean body weights of both F2 male and female pups were significantly lower on lactation days 0, 14 and 21 than those in the control group, which resulted in a– 10.4% for F2 males and -11.6% for F2 females at weaning. However, mean male and female body weights of 750 ppm group were well within historical control data.
In detail, male body weights on lactation day 21 were 68.0 and 60.9 g in the control and 750 ppm group, respectively. Females yielded body weights of 65.7 and 58.1 g in the control and 750 ppm group, respectively. Historical control data (9 studies, 1997-2012; *[see KCA 5.6.1/3 2015/1276507]* reported mean values for F2 body weights of 58.6 to 69.8 g for males and 55.8 to 66.7 g for females on day 21 of lactation.
Nevertheless, due to a clear dose-dependency and sustainability of this body weight deviation, and since the same observation, albeit not statistically significant, was already noticed in F1 animals, the effect is considered as treatment-related.

Terminal studies F2 offspring (see Table 5.6.1-10)

Organ weights
For F2 female pups in the 750 ppm group, final body weight was significantly lower, and the relative spleen weight was significantly higher than those in the control group. Absolute brain weights of F2 male and female pups in the 750 ppm group were significantly lower than those in the control group; however, no significant differences were noted in the relative weights of their brains.

Macroscopic findings
There were no macroscopic findings attributable to treatment with Metconazole.

Microscopic findings
There were no treatment-related microscopic findings in the spleen of F2 male and female weanlings.

Table 5.6.1-8: Summary of findings in the rat two-generation reproduction dietary toxicity study with metconazole – P Generation

Parameter	Dose level (ppm)							
	Males				Females			
	0	**30**	**150**	**750**	**0**	**30**	**150**	**750**
P GENERATION								
Number of animals/group	24	24	24	24	24	24	24	24
Mortality	0	0	0	0	0	0	0	5*
Clinical signs	No treatment-related effect							
Body weight (g)								
Week 0	146	146	146	146	117	117	117	117
Week 1	206	206	202	**196*** **	150	150	149	146
Week 2	264	263	259	**251** **	175	174	171	168
Week 6	430	416	421	424	243	245	236	**230***
Week 7	453	437	447	448	252	259	249	**240***
Week 8	473	457	467	468	261	267	257	**249***
Week 9	488	474	484	487	266	271	263	**255***
Week 10	500	488	500	502	272	279	267	**259***
Gain: Week 0-1	60	60	56	**50*** **	33	33	31	**29***
Gain: Week 0-2	118	117	113	**105** **	58	57	54	**51** **
Gain: Week 0-4	215	210	210	208	97	99	92	**88***
Gain: Week 0-6	284	270	275	278	126	128	119	**113** **
Gain: Week 0-7	307	291	301	302	135	142	132	**123***
Gain: Week 0-8	327	311	321	322	144	150	140	**132***
Gain: Week 0-10	354	342	354	356	155	162	150	**142***
Gestation: Day 0	-	-	-	-	275	284	274	265
Day 14	-	-	-	-	336	341	330	**321***
Day 20	-	-	-	-	410	411	400	**384***
Gain: Day 0-20	-	-	-	-	135	127	127	**118***
Lactation: Day 0	-	-	-	-	300	302	290	282
Gain: Day 0-21	-	-	-	-	12	8	17	**29** **
Food consumption								
Pre-pairing Week 1–10	No treatment-related effect							
Gestation: Day 0-7	-	-	-	-	17.9	18.3	17.9	17.7
(g/animal/day) Day 14-20	-	-	-	-	21.6	21.6	20.9	**19.9***
Lactation: Day 0-7	-	-	-	-	34.1	35.3	36.9	35.9
(g/animal/day) Day 7-14	-	-	-	-	51.8	51.7	52.6	50.2
Day 14-21[a]	-	-	-	-	64.5	65.3	65.7	62.9
Estrous cycle length (days)	-				4.1	4.1	4.1	**4.4***
Mating performance and fertility	No treatment-related effect							
Gestation								
Gestation index (%)	-				100.0	100.0	100.0	**66.7** **
Duration of gestation (days)	-				22.2	22.3	22.4	**23.0** **
Terminal observations	**P males after 18 weeks treatment**				**P females after weaning of litters (18 weeks treatment)**			
Number examined	24	24	24	24	24	24	24	19
Sperm assessment	No treatment-related effect				-			
Body weight at necropsy (g)	578	562	577	575	307	309	303	304

Parameter	Dose level (ppm)							
	Males				Females			
	0	**30**	**150**	**750**	**0**	**30**	**150**	**750**
Absolute organ weights (mg)								
Liver	17746	17295	17550	18750	10336	10426	10602	**11542***
Ovaries[b]	-				51.4	50.7	53.7	**60.5****
Body weight-relative organ weights (%)								
Liver	3.07	3.07	3.04	**3.25***	3.36	3.37	3.50	**3.79*****
Ovaries[b]	-				0.01670	0.01648	0.01780	**0.01991*****
Macroscopic findings	No treatment-related effect							
Microscopic findings								
Liver- centriolobular fatty change of hepatocyte	0	0	1	**19****	0	0	0	0
Liver- centriolobular hypertrophy of hepatocyte	**0**	0	0	0	0	0	0	**18****

[a]: Includes food consumption of offspring
[b]: Mean weight of both sides
*: p<0.05; **: p<0.01; ***: p<0.001
-: Not applicable

Table 5.6.1-9: Summary of findings in the rat two-generation reproduction dietary toxicity study with metconazole – F1 Generation

Parameters	Dose level (ppm)							
	Males				Females			
	0	**30**	**150**	**750**	**0**	**30**	**150**	**750**
LITTER DATA (F1 LITTERS)								
Clinical signs	No treatment-related effect							
Live litters born	-	-	-	-	24	23	24	14
Litter size								
Implantation sites	-	-	-	-	14.4	14.3	14.3	13.4
Day 0 (total)	-	-	-	-	13.3	13.4	13.3	11.8
Viability indices (%)[a]								
Day 0	-	-	-	-	97.3	98.9	99.7	96.3
Day 4	-	-	-	-	99.7	99.5	99.7	96.3
Day 21	-	-	-	-	94.8	97.8	97.4	98.2
Sex ratio[b]	-	-	-	-	0.497	0.516	0.443	0.491
Body weight (g)								
Day 0	7.0	6.9	7.0	6.8	6.6	6.6	6.7	6.4
Day 14	39.0	39.9	39.4	37.5	38.1	38.2	38.0	35.9
Day 21	63.3	64.8	65.2	60.5	61.4	61.7	62.2	57.4
Terminal observations	**F1 male offspring at Day 25-27**				**F1 female offspring at Day 25-27**			
Number examined	23	23	23	11	22	23	24	12
Body weight at necropsy (g)	93	93	91	85	87	85	84	80
Absolute organ weights (mg)								
Brain	1632	1616	1588	**1517*****	1587	1562	1538	**1481*****
Body weight relative organ weights (%)								
Spleen	0.376	0.380	0.395	**0.456*****	0.375	0.373	0.380	**0.430***
Macroscopic findings	No treatment-related effect							
Microscopic findings	No treatment-related effect							

Parameters	Dose level (ppm)							
	Males				Females			
	0	30	150	750	0	30	150	750
F1 GENERATION								
Number of animals/group	24	24	24	24	24	24	24	24
Mortality	0	0	0	0	0	0	0	4
Clinical signs	No treatment-related effect							
Body weight (g)								
Week 0	86	86	86	**77*** **	81	81	79	**72** **
Week 1	140	139	137	**123*** **	122	121	118	**110** **
Week 2	205	202	199	**181** **	163	160	158	**150** **
Week 3	272	267	263	**238*** **	193	191	188	**178** **
Week 4	334	329	325	**299*** **	222	215	214	**204** **
Week 5	381	379	373	**355** **	242	238	238	**224** **
Week 6	422	420	412	**397***	260	254	256	**238** **
Week 7	455	452	445	**427***	274	273	271	**252** **
Week 8	484	480	473	**452** **	288	284	283	**264** **
Week 9	508	503	494	**474** **	296	294	291	**272** **
Week 10	529	522	512	**491** **	305	301	300	**277*** **
Gain: Week 0-1	54	53	52	**47*** **	41	40	39	38
Gain: Week 0-2	119	116	114	**104*** **	81	79	79	78
Gain: Week 0-3	185	181	178	**162*** **	112	111	109	106
Gain: Week 0-4	248	243	239	**223*** **	141	135	135	132
Gain: Week 0-10	443	436	427	415	223	220	221	**204** **
Body weight (g) – continued								
Gestation: Day 0	-	-	-	-	305	304	304	**281***
Day 7	-	-	-	-	336	333	334	**308** **
Day 14	-	-	-	-	366	364	364	**338** **
Day 20	-	-	-	-	434	437	438	**404***
Gain: Day 0-20	-	-	-	-	129	132	134	124
Lactation: Day 0	-	-	-	-	337	328	327	**309***
Day 7	-	-	-	-	363	357	360	**331***
Gain: Day 0-21	-	-	-	-	1	4	11	14
Food consumption								
Pre-pairing: Week 1	13.9	14.0	13.9	**11.8*** **	11.1	11.5	11.5	10.5
(g/animal/day) Week 2	19.4	19.3	19.1	**17.2*** **	15.3	15.4	15.2	**14.5***
Week 3	23.7	23.5	23.1	**21.1*** **	16.2	16.1	16.2	15.4
Week 4	26.1	26.0	25.7	**23.6** **	17.0	16.6	17.0	16.0
Week 10	25.0	24.7	24.3	**23.5***	16.4	16.6	17.2	15.7
Gestation: Day 0-7	-	-	-	-	18.4	17.5	18.6	17.4
(g/animal/day) Day 7-14	-	-	-	-	22.4	21.3	21.5	20.8
Day 14-20	-	-	-	-	22.8	22.9	23.4	21.6
Lactation: Day 0-7	-	-	-	-	33.9	35.6	36.2	31.8
(g/animal/day) Day 7-14	-	-	-	-	50.3	54.3	53.6	47.3
Day 14-21[a]	-	-	-	-	61.1	64.8	64.4	57.2
Sexual maturation (preputial separation for males and vaginal opening for females)								
Age of days at completion	40.8	40.8	41.0	41.7	31.1	30.9	31.1	31.2
Body weight at completion	217.5	216.3	218.2	208.3	111.5	111.6	112.8	108.9
Oestrous cycles	-				4.3	4.6	4.1	4.1
Number of days until mating (days)	-				2.0	1.7	1.4	**1.2***
Mating performance and fertility	No treatment-related effect							

Parameters	Dose level (ppm)							
	Males				Females			
	0	**30**	**150**	**750**	**0**	**30**	**150**	**750**
Gestation								
Gestation index (%)	-				100.0	100.0	100.0	**75.0***
Duration of gestation (days)	-				22.4	22.3	22.3	22.7
Terminal observations	**F1 males after 18 weeks treatment**				**F1 females after weaning of litters (19 weeks treatment)**			
Number examined	24	24	24	24	24	24	24	20
Sperm assessment	No treatment-related effect				-			
Body weight at necropsy (g)	628	614	609	590	346	345	344	324
Absolute organ weights (mg)								
Brain	2221	2194	2205	**2083*****	2034	2004	2007	**1896*****
Pituitary	13.4	13.1	12.5	**12.2***	15.3	15.1	15.4	14.4
Kidneys[b]	1802	1803	1736	**1635****	1117	1083	1079	**1000****
Ovaries[b]	-				53.3	55.2	57.9	**66.6*****
Body weight-relative organ weights (%)								
Liver	3.25	3.25	3.13	3.42	3.46	3.40	3.58	**3.86****
Spleen	0.153	0.154	0.154	0.156	0.188	0.186	0.185	0.208
Seminal vesicles	0.405	0.408	0.424	**0.460****	-			
Ovaries[b]	-				0.01549	0.01605	0.01686	**0.02071*****
Macroscopic findings	No treatment-related effect							
Microscopic findings								
Liver- centriolobular fatty change of hepatocyte	0	0	1	**20****	0	0	0	0
Liver- centriolobular hypertrophy of hepatocyte	0	0	0	0	0	0	0	**20****
Spleen - congestion	0	0	0	0	0	1	1	**16****
Ovarian follicle counts	-				437	NS	NS	362

*: p<0.05; **: p<0.01; ***: p<0.001
-: Not applicable; NS: Not sampled
[a]: Viability indices are calculated for each litter from the following formulae and given as mean values for each group
Day 0 = (no. of pups alive on lactation day 0/no. of pups delivered) × 100.
Day 4 = (no. of pups alive on lactation day 4/ no. of pups alive on lactation day 0) × 100.
Day 21 = (no. of pups alive on lactation day 21/ no. of pups selected on lactation day 4) × 100.
[b]: Sex ratio = total no. of male pups/total no. of pups delivered.

Table 5.6.1-10: Summary of findings in the rat two-generation reproduction dietary toxicity study with metconazole – F2 Generation

Parameter	Dose level (ppm)							
	Males				Females			
	0	**30**	**150**	**750**	**0**	**30**	**150**	**750**
LITTER DATA (F2 LITTERS)								
Clinical signs	No treatment-related effect							
Live litters born	-	-	-	-	22	23	20	18
Litter size								
Implantation sites	-	-	-	-	13.5	14.0	14.9	13.5
Day 0 (total)	-	-	-	-	11.7	13.0	13.9	10.3
Viability indices (%)[a]								
Day 0	-	-	-	-	98.6	99.7	99.0	**89.1***
Day 4	-	-	-	-	100.0	95.4	98.5	88.5
Day 21	-	-	-	-	100.0	100.0	97.5	100.0
Sex ratio[b]	-	-	-	-	0.508	0.497	0.489	0.508
Body weight (g)								
Day 0	7.4	7.0	6.9	**6.4*****	7.1	6.7	6.5	**6.3***
Day 14	41.4	40.7	40.3	**37.7****	40.4	38.9	38.7	**36.5****
Day 21	68.0	67.1	66.6	**60.9****	65.7	63.5	63.6	**58.1*****
Terminal observations	**F2 male offspring at Day 25-27**				**F2 female offspring at Day 25-27**			
Number examined	22	22	20	16	22	22	20	16
Body weight at necropsy (g)	94	95	93	87	89	89	86	**79****
Absolute organ weights (mg)								
Brain	1607	1616	1606	**1520*****	1571	1567	1557	**1465*****
Body weight relative organ weights (%)								
Spleen	0.391	0.385	0.400	0.426	0.365	0.364	0.371	**0.418****
Macroscopic findings	No treatment-related effect							
Microscopic findings	No treatment-related effect							

*: $p<0.05$; **: $p<0.01$; ***: $p<0.001$
-: Not applicable; NS: Not sampled
[a]: Viability indices are calculated for each litter from the following formulae and given as mean values for each group.
Day 0 = (no. of pups alive on lactation day 0/no. of pups delivered) × 100.
Day 4 = (no. of pups alive on lactation day 4/ no. of pups alive on lactation day 0) × 100.
Day 21 = (no. of pups alive on lactation day 21/ no. of pups selected on lactation day 4) × 100.
[b]: Sex ratio = total no. of male pups/total no. of pups delivered.

III. CONCLUSIONS

Based on the results of this two-generation study in the rat, the NOAEL for general toxicity in the P and F1 males and females, as well as the NOAEL for reproductive performance in the P and F1 adults were 150 ppm (9.79, 10.78, 10.63, and 11.21 mg/kg bw/day for P males, P females, F1 males, and F1 females, respectively). The NOAEL for the growth of the F1 and F2 offspring was 150 ppm.

Based on the results of this two-generation study in the rat, the LOAEL for general toxicity in the P and F1 males and females, as well as the LOAEL for reproductive performance in the P and F1 adults were 750 ppm (49.4, 53.2, 53.0, and 55.5 mg/kg/day for P males, P females, F1 males, and F1 females, respectively) [Highest Dose Tested (HDT)]. The LOAEL for the growth of the F1 and F2 offspring was 750 ppm [HDT].

CA 5.6.2 Developmental toxicity studies

Rat

Studies on metconazole cis/trans (WL148271, 85:15 cis/trans)

Teratology study in the Rat, oral (gavage) following exposure to metconazole WL148271 (cis/trans) from gestations days 6-15 (1991b; BASF DocID MK-432-005)

Guidelines: Protocol in compliance with test method B.31 of directive 87/302/EEC
GLP: Yes
Acceptance: The study was accepted.

Preliminary study (0, 37.5, 75, 150 and 300 mg/kg bw/d)

Material and Methods:
Five groups of 10 mated female Sprague-Dawley rats were used in this study. Metconazole technical (Batch: 89-01, purity: 95.3%, cis/trans ratio: 83.7/16.3%, nominal 85:15) was administered by gavage in 1% carboxymethylcellulose. Animals were treated from day 6 to day 15 of pregnancy. Dosing volume was 10 mL/kg bw. On day 20 of gestation the females were sacrificed and subjected to post-mortem examination. Litter parameters were determined but foetuses were not examined in detail.

Findings:
A dose-related adverse response was observed in the dams treated at 150 and 300 mg/kg bw/d, including 2 and 4 mortalities, respectively. Increased water intake throughout the dosing period, persistent suppression of food consumption from initiation of treatment, initial bodyweight loss coinciding with the start of dosing followed by incomplete recovery, clinical signs (post-dosing salivation and brown stained fur), and total resorptions in 3/8 and 3/5 instances was observed at 150 and 300 mg/kg bw/d, respectively. At 75 mg/kg bw/d maternal effects were still apparent but much less marked and were confined to slightly increased water intake on GD6-7, slightly decreased food consumption and bodyweight gain from start of treatment through to termination. No clear effects were recorded in animals treated at 37.5 mg/kg bw/d, possible marginal disturbances of food intake and bodyweight gain could not be discounted.
At 300 mg/kg bw/d only 2 pregnancies were maintained to gestation day 20. At all dose levels investigated there was evidence of a dose-related reduction in litter size concomitant to an increasing risk of embryo-foetal death involving some or all concepti in the litter. Despite lower litter sizes, mean foetal weight was also impaired. However, at dosages where there were sufficient foetuses available for valid comparison there was no obvious increase in incidence of gross morphological defects.

Conclusion:
Severe maternal toxicity (increased mortality) and embryo-toxicity (100% intrauterine deaths) were observed at dose levels of 150 and 300 mg/kg bw/d. Significant maternal toxicity (reduced body weight gain) and embryo/foetotoxicity (increased number of resorptions and decreased fetal weight) was noted at 75 mg/kg bw/d. Therefore, from the results of this preliminary study 75 mg/kg bw/d was considered to be sufficiently high as the top dose for the main study.

Main study (0, 12, 30 and 75 mg/kg/d)

Material and Methods:
Four groups of mated female Sprague-Dawley rats (25 females/group) were used in this study. Metconazole technical (WL148271, Batch: 89-01, purity: 95.3%, cis/trans ratio: 83.7/16.3%, nominal 85:15) was administered by gavage as a suspension in 1% carboxymethylcellulose. Animals were treated on gestation days 6 through 15 at dose levels of 0, 12, 30 or 75 mg/kg bw/d. Dosing volume was 10 mL/kg bw. On day 20 of pregnancy, the females were sacrificed and subjected to post-mortem examination. Litter parameters were determined and fetus fixed prior to examination for skeletal or visceral abnormalities.

Findings:

Maternal data
No mortalities occurred. Post-dose salivation was noted in dams 20/25 dams at the high dose of 75 mg/kg bw/d during late gestation (d12-15). The late onset of this sign was associated with irritation of the gastrointestinal tract due to the repeated gavage of the compound. Clinical signs were not evident at the other dose levels.
Water consumption appeared to be slightly elevated at 75 mg/kg/day during the first 4 days of the dosing period. Food consumption was slightly reduced on the first 2 days of treatment at the top-dose (-10%). Related to the early reduction in food consumption, body weight gain was statistically reduced at 75 mg/kg/day at both the beginning of the dosing period (GD 6-8: -67%) as well as from the beginning of dosing throughout gestation (GD 6-20:-11%). GD 20 body weight appeared to be reduced at this dose (-4%), but was not statistically different from control. Body weight gain was statistically reduced at the beginning of dosing at 30 mg/kg/day as well (GD 6-8: -29%). No macroscopic changes related to treatment were observed in the dams at necropsy.

Litter data (see Table 5.6.2-1)

Treatment with metconazole technical at dose levels up to and including 75 mg/kg bw/d did not affect pregnancy status as the pregnancy rates were comparable for the control and the treated groups. There was no incidence of total litter loss or effects on mean corpora lutea or implantations. Pre-implantation loss was not affected. However, post-implantation loss and resorptions (both early and late) were significantly increased (4-fold of control), at the high dose of 75 mg/kg/day. As a result, the mean number of live foetuses per litter appeared to be reduced at this dose compared with controls, albeit the difference was not statistically significant. Mean foetal weights and total litter weights were also significantly reduced at this dose. Sex ratios were unaffected. A statistically significant reduction in mean foetal weight was also observed at 30 mg/kg/day; otherwise, probably an indirect consequence of the slightly increased litter size (+11%) and slightly increased litter weight (+6.5%). It should be noted, that total litter weights were increased at 12 and 30 mg/kg/day; these increases appear to be a function of the increased mean number of foetuses per litter at these doses compared to control.

A low incidence of malformations was observed in this study. Malformations were observed in 4/281, 1/315, 0/340, and 7/239 foetuses at 0, 12, 30 and 75 mg/kg/day with 3/22, 1/22, 0/24 and 6/22 litters affected respectively. No one foetus displayed more than a single malformation.
Two cases each of limb flexure without apparent skeletal effects were observed in the control group and at the high dose group; these findings should however be considered as variations rather than malformations.
Two incidences of hydrocephalus were observed at the top dose in separate litters. Hydrocephaly was not observed in any control foetus from studies conducted at the testing facility with Sprague-Dawley (SD) rats received from the same supplier (i.e. Charles River, France). Historical control data (HCD) from the testing facility (from 1990-1992; see DocID MK-432-013) indicated that hydrocephaly in SD was observed in three studies conducted with rats received from Charles River, UK and in one study with rats from Charles River, USA (range: 0 (0) – 1(1)). The foetal and litter incidences of hydrocephaly in this study, albeit low and not statistically significantly different from controls, are above in-house historical control data and were considered treatment-related. However, it should be noted that hydrocephaly was not observed in any of the other developmental rat studies. Other malformations at the top-dose included one brachygnathia, and one umbilical hernia.

A single case of interventricular septal defect/VSD was reported at both the low dose and the high dose. This finding was designated as a malformation in this study, whereas in the [redacted] (2002) study (see Summary [redacted] 2015a; 2015/1087909 below) both VSDs and small VSDs are classified as variations. Furthermore, two cases of rib distortions were seen in the control group. These lesions may be considered coincidental.
The significant increase of visceral variations at the top dose may be of a general nature rather than due to specific abnormalities. Slight increases in the incidence of dilated renal pelvis and ureter were observed at 75 mg/kg bw/d as compared to control. Historical controls per separate endpoint were not provided, but figures were presented for the combination (renal pelvis and/or ureter dilatation), and were in the range of 0-5.67% (foetal incidence) and 0-24% (litter incidence). In comparison with the combined incidences in this study 6/116=5.17% (foetal incidence) and 4/22=18.18% (litter incidence)) it appeared coincidental. It should be remarked that the findings were also observed in other developmental studies. However, since the foetal and litter incidences for these visceral variations are within the range of historical controls, the slight increases in the foetal and litter incidences observed at 75 mg/kg bw/d are not considered to be treatment-related. Likewise, the observed increased incidence of testes displacement at the top-dose was within historical control ranges and was therefore considered not treatment-related.
Small interventricular septal defects (small VSDs) were reported in all dose groups, including controls. The incidence of small VSDs at 75 mg/kg/day was slightly above the historical control range. Both VSDs and small VSDs are likely to be related to developmental delay (evidenced by lower foetal body weights) in the high dose group, and are assumed to resolve over time post-natally. It was attempted to evaluate further the relationship between VSDs and developmental delay by examining the foetal weights of litters with foetuses reported to have VSDs or small VSDs. Unfortunately, individual foetal weights were not reported in this study except for one very small foetus with a VSD.
Other visceral variations, such as the incidences of haemorrhage of the brain, although above control in the treated groups, were within the historical control range. Data for haemorrhage of the eyes showed a similar trend.

There was a dose-related increase in the incidence of foetuses with skeletal variations at 30 and 75 mg/kg bw/d. This was mainly due to increased incidences showing delay of ossification in the axial skeleton, especially the incidence of lumbar ribs, and to a lesser degree, the incidences of cervical ribs, extra pre-sacral vertebrae, and variant sternebrae.
The incidence of lumbar ribs in particular was statistically increased at both 30 and 75 mg/kg/day. At 12 mg/kg bw/d the overall incidence of skeletal abnormalities was similar to the control but the number of foetuses with lumbar ribs was twice the concurrent control group. The relationship of this intergroup difference to treatment was considered uncertain as the difference was not statistically significant.
Although slight, non-statistically significant increases in the fetal and litter incidences of the additional lumbar ribs were observed in the 12 mg/kg bw/d group, these increases were not considered treatment-related because the fetal incidence for lumbar ribs was within the range of historical control data and the litter incidence was only slightly exceeding that for historical controls. The fetal incidence for additional lumbar ribs in historical controls ranges from 8.4% to 19.6% and the litter incidence ranges from 40-50%. The incidences of all other ossification variations in the treated groups were comparable to controls. Furthermore, at 75 mg/kg bw/d less frequent observations included distorted (thoracic) ribs and caudal vertebra/disc irregularities.

Table 5.6.2-1: Litter data of teratogenicity main study in rats following treatment during gestation days 6-15 with metconazole cis/trans

Dose level (mg/kg bw/d)	**0**	**12**	**30**	**75**
No. of pregnant animals	22/25	22/25	24/25	22/25
Litter observations				
Corpora lutea	16.5	17.1	16.5	17.0
Implantations	13.6	15.1	15.1	14.5
Pre-implantation loss (%)	16.6	11.5	8.4	13.8
Post-implantation losses (%)	6.0	5.2	7.2	**24.5*** **
Total resorptions	0.8	0.8	1.0	**3.5*** **
Early resorptions (No. deaths/dam)	0.7	0.7	0.7	**1.6*** **
Late resorptions (No. deaths/dam)	0.1	0.1	0.3	**2.0*** **
Litter size	12.8	14.3	14.2	10.9
Fetal weight (g)	3.82	3.84	**3.65***	**3.51****
Total litter weights (g)	48.64	**54.70***	51.78	**38.49***
Placental weight (g)	nd	nd	nd	nd
Malformations [No. fetuses (litters)/ % foetuses affected]				
malformations • Fetuses (%)	4/281 (1.4%)	1/315 (0.3%)	0/0 (0.0%)	7/239 (2.9%)
• Litters (%)	3/22 (13.6%)	1/22 (4.5%)	0/0 (0.0%)	6/22 (27.3%)
Hydrocephaly • No. fetuses (litters) *(#HCD 0 (0)- 1(1), 0- 0.4%)*	-	-	-	2 (2)
• Fetal (litter) incidence	-	-	-	0.8% (9.1%)
Interventricular septal defect *(#HCD 0 (0)- 2(2); 0-0.74%)*	- -	1(1)/ 0.32%	- -	1(1)/ 0.42%
Umbilical hernia	-	-	-	1(1)
Brachygnathia	-	-	-	1(1)
Distortions affecting ribs	2(2)	-	-	-
Forelimb flexure[+] *(#HCD 0 (0)- 3(1); 0-1.19%)*	2(1)/ 0.71%	- -	- -	2(1)/ 0.84%
Visceral Variations				
% skeletal variations • Fetuses	5.8	6.9	5.6	**16.3***
• Litters	27.3	40.9	25.0	63.6
Dilatation renal pelvis • Fetuses *(#HCD 0 (0)- 8(6); 0-5.67%)*[&]	0/139 (0.0%)	1/156 (0.6%)	1/171 (0.6%)	2/116 (1.7%)
• Litters *(#HCD 0-24%)*[&]	0/22 (0.0%)	1/22 (4.5%)	1/24 (4.2%)	2/22 (9.1%)
Dilatation ureter • Fetuses	0/139 (0.0%)	0/156 (0.0%)	0/171 (0.0%)	4/116 (3.4%)
(#HCD 0 (0)- 8(6); 0-5.67%)[&] • Litters *(#HCD 0-24%)*[&]	0/22 (0.0%)	0/22 (0.0%)	0/24 (0.0%)	2/22 (9.1%)
Displaced testis • Fetuses	0/139 (0.0%)	0/156 (0.0%)	0/171 (0.0%)	2/116 (1.7%)
• Litters	0/22 (0.0%)	0/22 (0.0%)	0/24 (0.0%)	2/22 (9.1%)
Hemorrhage of the brain/spinal cord • Fetuses *(#HCD 0-5.41%)*	0/139 (0.0%)	3/156 (1.9%)	3/171 (1.8%)	3/116 (2.6%)
• Litters	0/22 (0.0%)	2/22 (9.1%)	3/24 (12.5%)	3/22 (9.1%)
Hemorrhage of the eyes • Fetuses *(#HCD 0-2.29%)*	1/139 (0.7%)	0/156 (0.0%)	0/171 (0.0%)	2/116 (1.7%)
• Litters	1/22 (4.5%)	0/22 (0.0%)	0/24 (0.0%)	2/22 (13.6%)

Table 5.6.2-1: Litter data of teratogenicity main study in rats following treatment during gestation days 6-15 with metconazole cis/trans

Dose level (mg/kg bw/d)	**0**	**12**	**30**	**75**
No. of pregnant animals	22/25	22/25	24/25	22/25
Small interventricular septal defects *(#HCD 0 (0)- 4(4); 0-3.15%)*				
• Fetuses *(#HCD 0 (0)- 4(4); 0-3.15%)*	4/139 (2.9%)	2/156 (1.3%)	3/171 (1.8%)	6/116 (5.2%)
• Litters	4/22 (18.2%)	2/22 (9.1%)	2/24 (8.3%)	6/22 (27.3%)
Skeletal Variations				
% skeletal variations				
• Fetuses	21.9	23.0	**42.2****	**72.2*****
• Litters	77.3	77.3	95.8	95.5
Additional cervical rib (s)				
• Fetal incidence *(#HCD 0-2.56%)*	1/138 (0.7%)	1/158 (0.6%)	4/169 (2.4%)	9/116 (7.8%)
• Litter incidence	1/22 (4.6%)	1/22 (4.6%)	2/24 (8.3%)	7/22 (31.8%)
Additional lumbar rib (s)				
• Fetal incidence *(#HCD 0-17.42%)*	11/138 (8%)	28/158 (18%)	48/169 (28%)	69/116 (59%)
• Litter incidence	8/22 (36%)	14/22 (64%)	18/24 (75%)	19/22 (86%)
One Extra Pre-Sacral Vertebra				
• Fetal incidence *(#HCD 0-2.56%)*[$]	0/138 (0.0%)	1/158 (0.6%)	6/169 (3.6%)	9/116 (7.8%)
• Litter incidence	0/22 (0.0%)	1/22 (4.6%)	6/24 (25.0%)	5/22 (22.7%)
Unossified sternebrae	33/138 (26.3%)	31/158 (19.7%)	**82/169 (47.3%)***	**55/116 (49.7%)****
• Fetal incidence				
• Litter incidence	15/22 (68%)	15/22 (68%)	22/24 (92%)	21/22 (95%)
Thoracic distorted ribs				
• Fetal incidence	0/138 (0.0%)	1/158 (0.6%)	0/169 (0.0%)	5/116 (4.3%)
• Litter incidence	0/22 (0.0%)	1/22 (4.5%)	0/24 (0.0%)	5/22 (22.7%)
Caudal vertebra/disc irregularity				
• Fetal incidence	0/138 (0.0%)	0/158 (0.0%)	0/169 (0.0%)	4/116 (3.4%)
• Litter incidence	0/22 (0.0%)	14/22 (0.0%)	18/24 (0.0%)	3/22 (13.6%)

Statistical evaluation (intergroup difference in comparison with control): *p≤0.05, **p≤0.01, ***p≤0.001

 # Historical control range (HCD) reported from 1990-1992 (see DocID MK-432-013): number of fetuses (litters); affected; fetal incidence in %

[+]Forelimb flexures were noted in fetuses allocated for skeletal examinations. No skeletal changes were noted. Thus, these changes should be considered variations rather than malformations.

[$] Based on historical control range reported for 'One additional thoracolumbar vertebra'.

[&] Based on historical control data reported for combined 'dilation renal pelvis/ureter'

Conclusion:
Metconazole cis/trans (cis:trans 80:15, WL148271) administered to pregnant rats from GD 6-15 induced maternal toxicity (reductions in body weight gain) and developmental toxicity (increases in skeletal ossification variations) at 30 and 75 mg/kg bw/d. At the top-dose embryo/foeto-toxicity (post implantation loss, reductions in foetal/litter weights) was evident. A slight but significant increase in hydrocephaly incidence was considered potentially treatment-related, but this teratogenic finding could be subsequent to the high maternal toxicity. The presence of skeletal anomalies (variations) at 12 mg/kg bw/d was ambiguous but a relationship to treatment could not be dismissed. Nevertheless, globally, the lowest dose could be considered a no-adverse effect level.
Therefore, the NOAEL for maternal and developmental toxicity was established at 12 mg/kg bw/d, the NOAEL for foetal toxicity was set at 30 mg/kg bw/d.

Executive summary of the analytical method used within the analytical phase (MK-432-003 and MK-432-005)

Principle of the method
The analytical method used in studies MK-432-003 and MK-432-005 was validated for the determination of *cis*-metconazole (BAS 555 F) in 1% methylcellulose. The study was performed by HUNTINGDON RESEARCH CENTRE LTD., UK.
Residues were extracted from 1% aqueous methylcellulose by dissolving a representative sub-sample in acetonitrile. The extract was filtered and further diluted, as necessary, using acetonitrile. Residues were analyzed by high-performance liquid chromatography with ultra-violet (UV) detection. Analysis was performed on a BDH Lichrosorb Diol column equipped with guard column (BDH Lichrospher 100 RP-18e), using an acetonitrile/water (95/5, v/v) as mobile phase.

Recovery findings
The mean recovery values were between 70% and 110% for *cis*-metconazole. The detailed results are given in the table below.

Table 5.6.2-2: Validation results of the analytical method applied in studies MK-432-003 and MK-432-005 using HPLC-UV: *cis*-metconazole (BAS 555 F) in 1% aqueous methylcellulose

Matrix	Analyte	Detector wavelength (nm)	Fortification level (mg/mL)	No of replicates	Mean recovery (%)	RSD (%)
Aqueous methylcellulose	*Cis*-metconazole	221	1	9	100	1.0
			60	10	99	1.2
			Overall	19	99	1.3

Linearity
The linearity was tested using standards at concentrations between of 1 to 5 µg/mL. Calibration solutions were prepared in acetonitrile.

Specificity
Final detection has been applied by UV detection. As only known analytes of known concentration were analyzed for dose verification purposes, no additional confirmatory technique is necessary.

Interference
No interferences from the reagent blank at the relevant retention times have been reported.

Limit of Quantitation
The limit of quantitation (LOQ) is defined as the lowest fortification level successfully tested. During this study, a limit of quantitation of 1 mg/mL for *cis*-BAS 555 F was obtained.

Limit of Detection
For BAS 555 F, the limit of detection (LOD) was 0.08 mg/mL.

Repeatability
The relative standard deviations (RSD, %) for all fortification levels were below 20%.

Stability of solutions
The chemical and physical stability of the analyte in freshly prepared specimen solutions was tested within this study. Results indicated that the analyte was chemically stable in solution for at least 24 hours when stored at ambient temperature during the day and + 4°C overnight. Furthermore, results revealed that metconazole produces a homogeneous suspension in 1% aqueous methylcellulose, which can be maintained for up to 1 hour while magnetically stirred and successfully resuspended following storage in the dark (ambient temperature during the day, + 4°C overnight) for 4 and 24 hours.

Conclusion
The analytical method used in studies MK-432-003 and MK-432-005 for the analysis of *cis*-metconazole in 1% aqueous methylcellulose uses HPLC-UV for final determination, with an LOQ of 1 mg/mL.
It could be demonstrated that the method generally fulfils the requirements with regard to repeatability, specificity, LOQ and recoveries and is therefore considered suitable for the analysis of *cis*-metconazole in 1% aqueous methylcellulose.

Teratology study in the Rat, oral (gavage) following exposure to metconazole KNF-474m (cis/trans) from gestations days 6-19 (2002)

A teratology study in rats (study author: 2002; BASF DocID 2006/8000263 and 2008/8000121) with metconazole cis/trans (KNF-474m) was conducted for non-EU authorities by another metconazole-supporting company. A detailed study summary was made available to BASF (prepared by 2015a; BASF DocID 2015/1087909) and is part of this submission.

Report:	CA 5.6.2/1 2015a Summary of the Laboratory report KRA/069 022919 - KNF-474m: Teratology study by oral gavage administration to CD rats 2015/1087909
Guidelines:	(EC) B.31, OECD 414 (2001)
GLP:	yes UK, OECD and EC

Report:	CA 5.6.2/2 2006 a KNF-474m: Teratology study by oral gavage administration to CD rats 2006/8000263
Guidelines:	EPA 870.3700, OECD 414, JMAFF No 12 Nosan No 8147
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Report:	CA 5.6.2/3 2008 a Amendment Number 1 - KNF-474m: Teratology study by oral gavage administration to CD rats 2008/8000121
Guidelines:	EPA 870.3700, OECD 414, JMAFF No 12 Nosan No 8147
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Executive Summary

Groups of 22 mated CD female rats received KNF-474m in 1% aqueous methylcellulose, administered by gavage at 0, 1, 4, 16 or 64 mg/kg bw/day on Days 6 to 19 of gestation. Controls received the vehicle alone. Termination and necropsy on Day 20 of gestation was followed by processing and detailed visceral and skeletal examination of foetuses.

There were no post-dosing or other clinical signs of response to treatment. One female in the highest dosage group (64 mg/kg bw/day) was killed on Day 18 for animal welfare reasons but this isolated occurrence was considered likely to be coincidental, unrelated to treatment. At the highest dosage (64 mg/kg bw/day) there was clear adverse maternal effect, seen as initial bodyweight stasis or slight loss (mean loss -6 g in the period GD 6 to 8), followed by bodyweight gain at a reduced rate (approx. -26% for GD 8-15, as compared to controls) together with reduced food consumption throughout treatment (approx. -20% for GD 6-19, as compared to controls).

Gravid uterus weight was decreased (-10%), and maternal bodyweight gain to termination, adjusted by subtracting gravid uterus weight, was markedly reduced (11g vs. 38g in Controls). In the 1, 4, and 16 mg/kg bw/day dose groups there was no clear adverse effect of treatment upon bodyweight or food consumption.
Numbers of corpora lutea and implantations were similar in all groups. However, at 64 mg/kg/day there was a clear increase in post implantation loss (17.7% vs. 4.4% for Controls) with increased group mean values of early resorptions/dam (1.6 vs. 0.7) and late resorptions/dam (1.0 vs. 0.0). This increased post-implantation loss resulted in a significantly lower live litter size (12.8 vs.15.0 for Control), but sex ratio was unaffected.
At 64 mg/kg/day there was also a reduction in mean foetal weight and increased mean placental weight. All placentae were noted to be swollen at necropsy and many were pale or had a mottled appearance.
There were 0, 2, 1, 2 and 3 foetuses showing malformations (0, 2, 1, 2 and 2 litters affected) in Groups 1 to 5 respectively. However, neither type nor incidence of these changes indicated any obvious adverse effect of treatment with KNF-474m.

At 64 mg/kg/day a notable increase in the incidence of minor skeletal and visceral anomalies was observed including (small) ventricular septal defects, which may both associate with delayed development (8 of the 11 affected foetuses were much smaller than the average). Furthermore, cervico-thoracic artery anomalies (variation in origin of subclavian and rudimentary/absent innominate arteries) were observed at this high dosage in 10/21 litters at 64 mg/kg bw/day as compared to 2/22 litters for both the control and the 4 mg/kg bw/day groups. These findings also occur sporadically in the historical control data.
Minor skeletal bone and/or cartilage anomalies in the high dose group included a marked increase in the incidence of cervical ribs and a marked increase in the incidence of lumbar ribs. There were also a few occurrences of 20 thoracolumbar vertebrae and of offset alignment of the pelvic girdle. This array of minor skeletal findings was considered indicative of increased foetal and/or maternal stress. The incidence of incomplete ossification of the sternebrae was also higher than control, in accord with low foetal weight.
Visceral examination also revealed at 64 mg/kg bw/day a high incidence of foetuses (20/21 litters affected) with green “deposits” in the renal papilla/renal pelvis and, to a lesser extent, ureters. This finding was not observed in the control or any other treatment group and has not been seen previously within this laboratory. Subsequent microscopic examination of selected foetuses indicated that these deposits were lipofuscin (breakdown products resulting from the oxidation of lipids and lipoproteins). The architecture of the foetal kidney appeared unaffected.
With the possible exception of swollen placentae, which occurred in two females at 16 mg/kg bw/day, there was no evidence of any adverse effect at the lower dosages on the pregnant female, and there were no effects on survival, development or morphology of the foetus.
It was concluded from the results of this study that the No Observed Adverse Effect Level (NOAEL) for KNF-474m in the rat was 16 mg/kg/day for both the pregnant female and the developing foetus.

(DocID 2015/1087909)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: Metconazole

Description:	solid (crystalline powder) / white to pale yellow
Lot/Batch #:	9Z521
Purity:	98.99%
Stability of test compound:	The test substance stability and homogeneity was proven by analytical analysis.

2. Vehicle and/or positive control: 1% w/v aqueous methylcellulose

3. Test animals:

Species:	Rat
Strain:	CD (Crl:CD(SD)IGS BR)
Sex:	Female
Age (at mating):	9 to 10 weeks
Weight (day 0 of gestation):	232 - 289 g
Source:	Charles River (UK) Ltd., Margate, Kent, England
Acclimation period:	at least 5 days
Time of dosing:	Days 6 to 19 of gestation
Diet:	pelleted rodent diet (UAR VRF1) manufactured by Usine d´Alimentation Rationnelle, France
Water:	tap water, ad libitum
Housing:	During acclimatisation: in groups of up to four females in suspended stainless steel cages with grid floors and lids. During mating (one pair per cage) and gestation (one female): in high density polypropylene cages with stainless steel grid floors and lids.
Environmental conditions:	
Temperature:	19 - 23°C
Humidity:	40 - 70%
Air changes:	15 per hour
Photo period:	12 h light / 12 h dark

B. STUDY DESIGN AND METHODS

1. Dates of experimental work: 30-Jan-2002 - 24-May-2002

2. Animal assignment and treatment:
Groups of 22 mated CD female rats were given daily oral doses by gavage, of KNF-474m suspended in 1% aqueous methylcellulose, during Days 6 to 19 of gestation at a dose volume of 4 mL/kg bodyweight. Controls received the vehicle alone. Dosages of 0, 1, 4, 16 and 64 mg/kg/day were based on a contemporary preliminary study where lower maternal bodyweight gain and slightly lower food intake were seen at 64 mg/kg/day; litter data was not obviously affected but increased placental weight and decreased fetal weight were observed at this dosage. Individual doses were calculated based on bodyweights on the nearest day of dosing; the final dose volume adjustment was based on bodyweight on Day 17 after mating.

3. Test substance preparation and analysis:
An appropriate amount of KNF-474m was ground using a pestle and mortar. An appropriate amount of the vehicle (1% methylcellulose) was placed on top of the test substance, which was then ground again, ensuring a thorough mixing of the test material and vehicle. The mixture was then transferred to an appropriate measuring cylinder (rinsing the mortar with vehicle) and made to volume with vehicle. The formulation was then mixed using a high shear homogenizer to further break down any agglomerates of test article and to produce a homogenous suspension. For dosing formulations where the concentration was less than 10 mg/mL, a 10 mg/ml stock formulation initially prepared as above and measured amounts were diluted to the appropriate required concentrations of 0.25, 1.0 and 4.0 mg/mL and mixed using a magnetic stirrer. Samples (4 x 1 ml) were taken for analysis of achieved concentration from all formulations prepared for dosing during the first and last weeks of the dosing period of this study. On both occasions, two samples from each concentration were initially analysed, the remainder were retained frozen for future analysis should any results have required confirmation.

4. Statistics:
The following parameters were analysed:
Bodyweight, bodyweight change, gravid uterine weight and bodyweight adjusted for the contribution of the gravid uterus, food consumption, litter data, placental, litter and fetal weight and the percentage incidence of skeletal variants.

Statistical analysis was performed using the following criteria:
Where 75 % of the data (across all groups) were the same value, a frequency analysis was applied. Treatment groups were compared using a pairwise Fisher's Exact tests (Fisher, 1973) for each dose group against the control.

Where Bartlett's test for variance homogeneity (Bartlett, 1937) was not significant at the 1% level, or if it was not significant after first a logarithmic or second a square root transformation, then parametric analysis was applied. When the F1 test for monotonicity of dose-response (Healey, 1999) was not significant at the 1% level, William's test was performed for a monotonic trend (Williams 1971, 1972). William's test uses the error mean square from a one-way analysis of variance.

Where Bartlett's test was significant at the 1% level, even after transformation, then nonparametric analysis was applied to the untransformed data. When the H1 test for monotonicity of dose-response (Healey, 1999) was not significant at the 1% level, then Shirley's test was performed for a monotonic trend (Shirley, 1977). Significant (i.e. $p<0.05$) inter-group differences from the control are reported.

C. METHODS

1. Observations:
Animals were observed at least twice daily and any visible signs of reaction to treatment were recorded, with details of type, severity, time of onset and duration. A full physical examination was performed on Days 0, 5, 12, 18 and 20.

2. Body weight:
Maternal bodyweight was recorded on Days 0, 3 and 6 to 20 after mating.

3. Food consumption and compound intake:
Food consumption was determined for the periods Days 0-2, 3-5, 6-9, 10-13, 14-17 and 18-19 inclusive.

4. Sacrifice and pathology:
All surviving females were sacrificed on Day 20 of gestation and subjected to a detailed necropsy and any gross pathological findings were recorded.
The reproductive tract, complete with ovaries, was dissected out and the following recorded:

- Gravid uterine weight.
- Number of corpora lutea in each ovary (assessed before removal).
- Number of implantation sites.
- Number of resorption sites (resorptions classified as early or late).
- Number and distribution of fetuses in each uterine horn.

Live fetuses were sexed, weighed and examined for external and orifice abnormalities. Individual placental weights and placental abnormalities were also recorded. Fetuses were then killed by chilling on a cool plate (approx. 4°C).
Approximately half of each litter was allocated to fresh visceral examination at necropsy and were prepared for a double staining process. For this, fetuses selected for fresh visceral examination and subsequent skeletal processing were eviscerated, identified with uterine position and dam number and then placed for approximately 5 minutes in water heated to approximately 50°C. Following immersion, each fetus was skinned, and as much fat as possible removed, prior to the fetus being placed in Industrial Methylated Spirit (IMS 99%).
This procedure was performed to allow full penetration of the stains to the skeletal (alizarin red) and cartilaginous (alcian blue) tissues.
The remaining fetuses were fixed in Bouin's fluid before examination by the Wilson (Wilson, 1965) free-hand serial sectioning technique.

During examination of the Wilson sections, a high incidence of fetuses with green “deposits” in the renal papilla/renal pelvis/ureters were observed at 64 mg/kg/day. Selected fetal tissue from this dosage and corresponding fetal tissues from the control group were subjected to histopathological processing and microscopic examination in an attempt to determine the nature of this material. The initial set of fetal tissues (from 2 litters) was stained with haematoxylin and eosin; then more specialised staining was used for fetal tissues from 2 other litters, as follows: Fouchet’s stain (to identify bile pigments), Schmorl’s stain (to identify lipofuscin) and Perls’ stain (to identify haemosiderin).

Treatment of data
Data processing for fetuses at skeletal and visceral examination following free-hand serial sectioning was performed as follows:
Structural changes are presented as malformations, minor visceral and skeletal anomalies and skeletal variants and were classified according to severity and incidence as follows:

Malformations: are rare and probably lethal, e.g. exencephaly.

Anomalies minor: differences from “normal” that are detected relatively frequently either by skeletal examination (e.g. bipartite centrum) or following free-hand serial sectioning (e.g. dilated ureter).

Skeletal Variants: alternative structures occurring commonly in the control population e.g. lumbar ribs and incomplete 5th and 6th sternebrae.

Structural changes are presented as Numeric Fetal and Litter Incidences.

II. RESULTS AND DISCUSSION

A. TEST SUBSTANCE ANALYSES
The mean concentrations of formulations analysed during the study were within ±6% of nominal concentration with the exception of two analyses. Samples analysed from formulations prepared for use during the first week of the study for Group 2 (1 mg/kg/day) were 140% of target and for Group 3 (4 mg/kg/day) were 79% of target.
These lower concentrations were prepared by dilution of a 10 mg/mL stock formulation and it is considered that the discrepancies observed resulted from a loss of homogeneity for this stock formulation during production of the lower concentrations. Analyses of the stock formulation for Week 1 demonstrated accuracy of the initial formulation. Further analyses were taken from 0.25 and 1.0 mg/mL formulations during Week 2 and 3 of the study and these analyses confirmed acceptable achieved concentration on these occasions.
Within this study 16 mg/kg/day (the dose level for Group 4) had no adverse effects upon maternal or fetal parameters, although clear adverse effects were seen at 64 mg/kg/day. It is therefore considered that the formulation discrepancies at dosages of 1 and 4 mg/kg/day, which affected up to half of the group for periods of up to 7 days, had no effect upon the interpretation of the data or conclusions reached in this study. KNF-474m was shown to be homogeneous in aqueous 1% methylcellulose suspensions and stable for at least 14 days at ambient temperature in a previous study.

B. OBSERVATIONS

1. Clinical signs of toxicity

The report text indicated no post-dosing or other clinical signs attributable to treatment. However, in the high-dosage (64 mg/kg bw/day) group, blood discharge or red staining at the vagina was recorded in one female in late gestation (Days 16-17) and in two females at terminal necropsy.

2. Mortality

One female receiving 64 mg/kg bw/day was killed on Day 18 of gestation, after showing bodyweight loss (14 g) from Day 16 of gestation, hunched posture, pallor, pale eyes and piloerection. Necropsy revealed firm and reduced caecal contents; there were 14 implantations, 2 of which were early resorptions and another 4 were late deaths. The remaining 8 live fetuses had a pale swollen placenta. The relationship of this isolated death to treatment was uncertain but it was considered likely to be coincidental.

C. BODY WEIGHT AND BODY WEIGHT GAIN

Treatment at 64 mg/kg/day was associated with bodyweight stasis (see

Table 5.6.2-3) or slight mean bodyweight loss (-6 g) from Day 6 to Day 8 of gestation. Thereafter, some recovery was evident to termination although bodyweight gain remained lower than control. This lower gain was most noticeable between Day 8 to 15 of gestation (-25.6% compared with control; $p<0.01$). Overall mean bodyweight gain, after adjustment by subtracting gravid uterus weight, was very markedly lower than control (mean of 11g vs. 38g for the control; $p<0.01$), indicating that this was a maternal effect.

Bodyweight and bodyweight change mean values at dosages of 1, 4 and 16 mg/kg/day were essentially similar to control. Slightly low overall mean adjusted bodyweight change at 16 mg/kg/day was associated with slightly higher, non-statistically significant gravid uterus weight, which was not considered to be treatment related.

D. FOOD CONSUMPTION

At 64 mg/kg/day, group mean food consumption was significantly lower (-20% for GD 6-19, as compared to control) than concurrent controls, consistent with the lower bodyweight gain. Group mean food consumption at 1, 4 and 16 mg/kg/day was unaffected by treatment.

E. NECROPSY

1. Macroscopic findings

Two females receiving 64 mg/kg bw/day had blood discharge or red staining at the vagina at termination. With this exception, maternal necropsy findings showed no adverse effect of treatment with KNF-474m.
All females surviving until scheduled termination had live young on Day 20 of gestation. All females at 64 mg/kg bw/day had swollen placentae, many also being pale and/or mottled. At 16 mg/kg bw/day, all placentae of one female were swollen and mottled, and 7 of 15 placentae were swollen in a second female. Occasional swollen placentae were also recorded in both the control and 4 mg/kg bw/day groups, indicating a low background incidence.

2. Litter data

Numbers of corpora lutea and implantations were similar in all groups. However, at 64 mg/kg/day there was a clear increase in post implantation loss (17.7% vs. 4.4% for controls; $p<0.01$) with increased group mean values of early resorptions/dam (1.6 vs. 0.7; $p<0.05$) and late resorptions/dam (1.0 vs. 0.0; $p<0.001$). This increased post implantation loss resulted in a significantly lower live litter size (12.8 vs.15.0 for control; $p<0.05$), but sex ratio was unaffected. At dosages of 1, 4 and 16 mg/kg/day, the number of in utero deaths, live young and sex ratio were unaffected by treatment (see Table 5.6.2-4)

3. Placental, litter and fetal weights

At 64 mg/kg/day there was a reduction in mean fetal weight (males and females combined were -17.6% compared with control; $p<0.01$) and increased mean placental weight (+45.5% compared with control; $p<0.01$). All placentae in this group were noted to be swollen at necropsy and many were pale or had a mottled appearance. Mean litter weight and gravid uterus weight in this group were markedly lower than control.
There was a minimal increase (+5.5% compared with control) in mean placental weight at 16 mg/kg/day; in the two females with swollen placentae, litter mean placental weights were slightly high (0.66g and 0.69g) but litter mean fetal weights (3.40g and 3.55g) and other fetal developmental parameters were unaffected. With this exception, placental, litter and fetal weight at dosages of 1, 4 or 16 mg/kg/day were essentially similar to control (see Table 5.6.2-4). Overall, the etiology of enlarged placenta and increased placental weight is unclear. No further investigations were performed on the placenta. Enlarged placenta had however, no effect on the survival of the fetuses at the dose levels of the absence of resorptions.

4. Fetal examinations

There were 0, 2, 1, 2 and 3 fetuses showing malformations (0, 2, 1, 2 and 2 litters affected) in Groups 1 to 5 respectively. However, neither type nor incidence of these changes indicated any obvious adverse effect of treatment with KNF-474m (see Table 5.6.2-5)
At 64 mg/kg/day, a dosage where fetal weight was significantly low, there was a notable increase in the incidence of minor skeletal and visceral anomalies compared with the control, as follows:

There was an increased incidence of small ventricular septal defect and of ventricular septal defect (which may both associate with delayed development (8 of the 11 affected fetuses were much smaller than the average)), and of cervicothoracic artery anomalies (variation in origin of subclavian and rudimentary/absent innominate arteries) at this high dosage (i.e., one or more of these minor anomalies occurred in 10/21 litters at 64 mg/kg bw/day, as compared to 2/22 litters for both the control and the 4 mg/kg bw/day groups). Such findings occur sporadically in the historical control data.

With regard to classification of ventricular septal defects or small VSD these are defects seen at the junction of the membranous and muscular ventricular septum of the heart (Ref. Human Embryology by W. J. Larsen, Churchill Livingston mc, 1993, pp 157-158). The "small ventricular septal defects" are extremely narrow perforations through the septum junction, which are considered unlikely to persist as the fetus/neonate develops further and are therefore not considered to be major abnormalities (or "malformations" in JMAFF's classification). In this study there is no consistent relationship with fetal weight - only 2 out of the 8 affected fetuses are the smallest in the litter.
The "ventricular septal defects" in this study are larger, but still narrow, perforations at the membranous/muscular junction; they affect the membranous region of the connection between the ventricles as a result of the failure of the muscular and membranous ventricular septa to fuse. 2 of the 3 affected fetuses are the smallest in the litter. These occurrences could be considered to be related to a delay in growth and low bodyweight and were not severe enough to classify them as malformations.
In the study summary presented by [redacted] 2015a it is referred to an expert opinion issued in 2005 by [redacted] (Head of Reproductive Studies Group at the laboratory where the KNF-474m study was conducted). In these extracts from his expert opinion it is stated that "none of the ventricular septal defects or small ventricular septal defects recorded in this study were considered large enough to be incompatible with postnatal life, and as such have been classified as visceral anomalies rather than as malformations. In man, simple ventricular septal defects (VSD) occur in approximately 12/10,000 births but it is thought that approximately 10-25% of these undergo spontaneous closure after birth (Warkany J., 1971, Congenital Malformations: notes and comments: Chapter 53, pages 489-494: Ventricular septal defects, ISBN 0-8151-9098-0). The incidence of VSD is much higher among children with a birth weight of less than 2500 g. In this rat strain at these laboratories, the incidence of VSD is very low in control animals but it does occur sporadically in association with other malformations and in cases of low fetal weight, suggestive of growth retardation. In the rat, the ventricular septum is normally closed on the 17th day of gestation (Warkany 1971). In the study with KNF-474m, 8 of the 11 affected fetuses were much smaller than the average, falling below the 95th percentile for fetal weight on day 20 of gestation. The three most affected fetuses averaged only 53% of expected bodyweight. These findings suggest that failure of the ventricular septum to be fully closed on day 20 of gestation may be associated with delays in fetal development. It seems likely that the developmental delay was established before the VSD would be apparent as this anomaly is generated at a late stage of development."
VSD levels in pups were not assessed with metconazole, so that it is difficult to establish if levels of VSD were lower at weaning than would have been present prior to birth. However, the clinical evidence from man appears to suggest that closure of the smaller defects may occur.

At this high dose (64 mg/kg bw/day), minor skeletal bone and/or cartilage anomalies included a marked increase in the incidence of cervical ribs, a variation in false/floating rib/cartilage configuration compared with control, and a marked increase in the incidence of lumbar ribs (13/14 or 14/14 ribs) compared with control. There were also a few occurrences of 20 thoracolumbar vertebrae and of offset alignment of the pelvic girdle. This array of minor skeletal findings was considered indicative of increased fetal and/or maternal stress. The incidence of incomplete ossification of the sternebrae at 64 mg/kg bw/day was also higher than control, in accordance with low fetal weight.

Visceral examination also revealed at 64 mg/kg bw/day a high incidence of foetuses (20/21 litters affected) with green 'deposits' in the renal papilla/renal pelvis and, to a lesser extent, ureters. The slightly higher incidence of hydroureter at the top dose was well within the historical control range. The finding of green 'deposits' was not observed in the control or any other treatment group and has not been seen previously within this laboratory. Subsequent microscopic examination of selected fetuses indicated that these deposits were lipofuscin (breakdown products resulting from the oxidation of lipids and lipoproteins). The architecture of the fetal kidney appeared unaffected. It was considered possible that the enlarged placentae were less effective at removing waste products, resulting in accumulation of debris in the renal pelvis, or that the material was placental in origin. At lower dosages (1, 4, and 16 mg/kg bw/day) there was no apparent adverse effect of treatment with KNF-474m on fetal development.

Table 5.6.2-3: Summary of maternal data in the teratology study with KNF-474m

Parameter	Dose (mg/kg bw/day)				
	0	**1**	**4**	**16**	**64**
Disposition of females					
Number mated	22	22	22	22	22
Number pregnant	22	22	22	22	22
Killed prematurely	0	0	0	0	1
Number with live young at termination	22	22	22	22	21
Number of litters examined	22	22	22	22	21
Clinical signs					
Vagina: blood discharge or red staining at termination					2
Skin: pallor Days 17-19					1
Bodyweight (g)					
Days 0	256	259	256	255	256
Days 6	292	292	291	290	288
Days 20	420	422	424	418	**380****
At necropsy, minus gravid uterus	329	333	334	323	**299****
Bodyweight (% difference from control)					
Day 20	0				-9.52
At necropsy, minus gravid uterus	0				-9.12

Parameter	Dose (mg/kg bw/day)				
	0	**1**	**4**	**16**	**64**
Bodyweight change (g)					
Days 6-8	8	9	8	7	**-6****
Days 8-15	43	42	44	41	**32****
Days 6-20	128	130	133	128	**92****
Day 6 to necropsy, minus gravid uterus	38	41	42	33	**11****
Bodyweight change (% difference from control)					
Days 8-15	0				-25.6
Days 6-20	0				-28.1
Day 6 to necropsy, minus gravid uterus	0				-71.1
Food consumption (g/rat/day)					
Days 0-2	29	29	29	29	29
Days 3-5	30	29	29	30	29
Days 6-9	30	30	30	30	**23****
Days 10-13	31	31	31	30	**26****
Days 14-17	33	32	34	32	**26****
Days 18-19	32	33	32	31	**25****
Approx. total consumption (g/rat) Days 6-19	410				327
Food consumption (% difference from control)					
Approx. total consumption Days 6-19	0				-20.2
Gravid uterine weight and terminal bodyweight					
Gravid uterine weight (g)	85.7	85.6	85.5	91.6	**77.0***
Adjusted Day 20 bodyweight (g)	329	333	334	323	**299****
Gravid uterine weight and terminal bodyweight (% difference from control)					
Gravid uterine weight (g)	0				-10.2
Adjusted Day 20 bodyweight (g)	0				-9.1

Statistical significance: * $p \leq 0.05$, ** $p \leq 0.01$

Table 5.6.2-4: Summary of litter data in the teratology study with KNF-474m

Parameter	Dose (mg/kg bw/day)				
	0	**1**	**4**	**16**	**64**
Number of litters examined	**22**	**22**	**22**	**22**	**21**
Litter mean values:					
Corpora lutea	15.9	16.0	15.7	16.6	15.5
Implantations	15.7	15.5	15.5	15.9	15.4
Pre-implantation losses (%) *(#HCD: 1.9-9.6%)*	2.9	4.7	3.3	4.2	2.0
Post-implantation losses (%) *(#HCD: 2.9-10.1%)*	4.4	6.1	3.3	3.6	**17.7****
Total resorptions	0.7	1.0	0.5	06.	**2.6****
Early *(#HCD: 0.4-1.4)*	0.7	1.0	0.5	0.5	**1.6***
Late *(#HCD: 0.0-0.1)*	0.0	0.0	0.0	0.0	**1.0*****
Number of live fetuses *(#HCD: 13.2-16.2)*	15.0	14.5	15.0	15.3	**12.8***
Sex ratio (male fetuses/live fetuses %)	48.1	51.1	49.7	49.3	50.8
Litter weight (g) *(#HCD: 50.17-59.83g)*	54.20	54.64	54.66	56.39	**38.55****
Fetal weight (g)					
- Males *(#HCD: 3.66-4.05g)*	3.73	3.87	3.75	3.79	**2.99****
- Females *(#HCD: 3.45-3.86g)*	3.55	3.63	3.58	3.58	**2.95****
- Combined sexes *(#HCD: 3.56-3.96g)*	3.63	3.75	3.66	3.68	**2.99****
Placental weight (g) *(#HCD: 0.51-0.57g)*	0.55	0.55	0.55	**0.58***	**0.80****
% difference from control					
Number of live fetuses	0				-14.7
Fetal weight – combined sexes	0				-17.6
Placental weight	0			+5.5	+45.5
Placental findings					
- Number examined: placentae (litters)	329 (22)	320 (22)	329 (22	337 (22)	268 (21)
- Swollen placenta	3 (2)	-	5 (3)	23 (2)	260 (21)
- Placenta pale and/or mottled	1 (1)	-	1 (1)	16 (1)	106 (9)
- Placenta with punctate foci and pale rim	-	-	-	-	5 (1)

Statistical significance: * $p \leq 0.05$, ** $p \leq 0.01$

 # Historical control data (HCD) from 28 studies (May 2000 to July 2004) as given in the study summary (2015/1087909)

Table 5.6.2-5: Summary of fetal evaluation data in the teratology study with KNF-474m

Fetal observations	Dose (mg/kg bw/day)				
	0	**1**	**4**	**16**	**64**
Malformations:					
Number examined: fetuses (litters)	329 (22)	320 (22)	329 (22)	337 (22)	268 (21)
Number affected:	-	2 (2)	2 (1)	2 (2)	3 (2)
External malformations:					
Agenesis of tail, imperforate anus				1[a](1)	
Rudimentary/threadlike tail, imperforate anus					2[bc](2)
Visceral malformations:					
Transposition of ascending aorta and dorsally displaced pulmonary trunk; ventricular septal defect				1(1)	
Retro-oesophageal aortic arch		1(1)			
Duplicated inferior vena cava		1(1)			
Diaphragmatic hernia					1[d](1)
Skeletal malformations:					
Exencephaly; brachygnathia with fused mandibles;misshapen basisphenoid; split/partially split basioccipital, cervical to 4th thoracic vertebra/cartilage			1(1)		
Termination of normal vertebral column lumbar region, malrotated hind limbs					1[b](1)
Termination of normal vertebral column lumbar region				1[a](1)	1[c](1)
Anomalies and variants					
Visceral examination:					
Number examined: fetuses (litters)	163 (22)	159 (22)	163 (22)	168 (22)	136 (21)
Visceral anomalies fetuses (litters) [%fetal incidence]					
Innominate artery: absent / rudimentary *(#HCD: 0 (0)-2(1), 0-1.22%)*	3 (2) [1.84%]	-	2 (2) [1.23%]	-	5 (3) [3.68%]
Subclavian artery: variation in origin *(#HCD: 0 (0)-2(2), 0-1.22%)* [X]	-	-	-	-	2 (2) [1.47%]
Heart: ventricular septal defect *(#HCD: 0 (0)-3(2), 0-1.83%)* [Y]	-	-	-	-	3 (3) [2.21%]
Heart: ventricular septal defect (small) *(#HCD: 0 (0)-1(1), 0-0.68%)*	-	-	-	-	8 (6) [5.88%]
Ureter(s): dilated *(#HCD: 0 (0)-6(4), 0-3.90%)*	1 (1) [0.61%]	1 (1) [0.63%]	-	-	4 (2) [2.94%]
Kidney(s)/ureter(s): green deposits	-	-	-	-	61 (20) [44.85%]

Fetal observations	Dose (mg/kg bw/day)				
	0	**1**	**4**	**16**	**64**
Skeletal examination:					
Number examined: fetuses (litters)	166 (22)	159 (22)	165 (22)	167 (22)	129 (21)
Skeletal anomalies					
Cervical rib *(#HCD: 0 (0)-4(3), 0-2.41%)*	2 (2) [1.20%]	- 	- 	1 (1) [0.60%]	25 (12) [19.38%]
Complete 14th rib with associated costal cartilage *(#HCD: 0 (0)-4(2), 0-2.45%)*	- 	- 	- 	- 	2 (2) [1.55%]
1 extra false/1 less floating rib/costal cartilage	7 (5) [4.22%]	5 (4) [3.14%]	4 (4) [2.42%]	4 (4) [2.40%]	2 (1) [1.55%]
1 less false/1 extra floating rib/costal cartilage	1 (1) [0.60%]	- 	2 (2) [1.21%]	1 (1) [0.60%]	7 (5) [5.43%]
20 thoracolumbar vertebrae	- 	- 	- 	- 	3 (2) [2.33%]
Offset alignment of pelvic girdle	- 	- 	- 	- 	2 (2) [1.55%]
Skeletal variants:					
Ribs: Number with 13	144	146	136	139	67
Supernumerary lumbar ribs (number with 13/14 or 14/14) *(#HCD: 5 (3)-35(12), 3.25-21.41%)*	22 (10) [13.25%]	13 (8) [8.18%]	29 (15) [17.58%]	28 (13) [16.77%]	62 (18) [48.06%]
Sternebrae: incompletely ossified/unossified					
5th and/or 6th *(#HCD: 56 (20)-123(22), 36.36-77.12%)*	109 (21) [65.66%]	100 (22) [62.90%]	105 (21) [63.64%]	93 (21) [55.69%]	109 (21) [84.50%]
Other *(#HCD: 1 (1)-16(9), 0.65-9.85%)*	8 (6) [4.82%]	2 (1) [1.26%]	3 (2) [1.82%]	1 (1) [0.60%]	16 (11) [12.40%]
Total *(#HCD: 56 (20)-123(22), 36.36-77.12%)*	110 (21) [66.27%]	100 (22) [62.86%]	105 (21) [63.64%]	93 (21) [55.69%]	109 (21) [84.50%]
% total (mean of means)	65.1	63.4	64.1	54.4	**84.9***

Statistical significance: * $p \leq 0.05$, ** $p \leq 0.01$

 # Historical control data (HCD) from 30 studies (May 2000 to July 2004) as given in the study summary (2015/1087909): presented as range of affected fetuses (litters), % fetal incidence

[a,b,c] Superscripts indicate observations relating to the same fetus

[c,d] Superscripts indicate fetuses from the same litter

[X] Based on historical control range reported for "systemic/pulmonary vessel abnormality/retroesophageal subclavian artery" under major abnormalities

[Y] Based on historical control range reported for "atrial/ventricular septal defects" under major abnormalities.

III. CONCLUSIONS

Maternal toxicity was evident at 64 mg/kg/day, manifested as mean bodyweight loss (-6 g) in the period GD 6 to 8, then bodyweight gain at a reduced rate (approx. -26% for GD 8-15, as compared to controls) and lower food intake (approx. -20% for GD 6-19, as compared to controls). At the high-dose (64 mg/kg/day), there was an increased incidence of post-implantation loss, reduced live litter size, increased placental weight, reduction in mean foetal weight and increased incidence of minor foetal variations. At 16 mg/kg bw/day, increased incidences of swollen placentae occurred in two females, but foetal weight was unaffected. Apart from that, at the lower dosages there was no evidence of any adverse effect on the pregnant female, and there were no effects on survival, development or morphology of the foetus.
It was concluded from the results of this study that the No Observed Adverse Effect Level (NOAEL) for KNF-474m in the rat was 16 mg/kg/day both for the pregnant female and the developing foetus.

Studies on metconazole cis (WL136184, 95% cis)

Report:	CA 5.6.2/4 [redacted] 1992c WL 136184: Preliminary teratology study in the rat MK-432-008
Guidelines:	Preliminary study
GLP:	yes (certified by Department of Health and Social Security of the Government of the United Kingdom, United Kingdom)

Report:	CA 5.6.2/5 [redacted] 1992d WL 136184: Second preliminary teratology study in the rat MK-432-006
Guidelines:	Preliminary study
GLP:	yes (certified by Department of Health and Social Security of the Government of the United Kingdom, United Kingdom)

Material and Methods:
Two preliminary studies where performed in order to select the dose levels for the main study. In these preliminary studies 6 pregnant rats (CD strain of Sprague Dawley origin) were treated with the test substance metconazole WL136184 (95.2% purity, 95% cis isomer, batch 12) in 1% methylcellulose by gavage from day 6 to day 15 of gestation. Investigations comprised daily observation for clinical signs, regular determination of maternal body weight, and food and water consumption. All females were sacrificed on day 20 of gestation for examination of their uterine contents. The second preliminary study was conducted in order to establish a suitable upper dosage level, in the absence of effects at the top-dose of the first pilot study.

First Preliminary study (*0, 6, 12, 24 and 48 mg/kg bw/d)*:

Findings:
The general condition of the females was unaffected by treatment and no deaths occurred. Bodyweight gain, feed and water consumption of females during gestation were unaffected by treatment. No treatment-related macroscopic abnormalities were observed at necropsy. Placental weight was increased at 48 mg/kg bw/d (21.2% of control) and there was an increased incidence of large placenta. Fetal weight was unaffected by treatment in all groups. No treatment-related abnormalities were observed at examination of fetuses.

Conclusion:
Oral administration of metconazole 95% cis to pregnant rats during organogenesis produced no clear maternal response at doses up to 48 mg/kg bw/d, although at this dose there was an increase in placental weight. The highest dose level for use in a main teratology study in the rat should be higher than 48 mg/kg bw/d.

Second Preliminary study (0, 6, 60 and 80 mg/kg bw/d):

Findings:
The general condition of females was unaffected by treatment and no deaths occurred. Bodyweight gain was depressed during the treatment period at 60 mg/kg (-10%) and above (-32%); at both dose-levels, the effect was visible until termination (14-17% decrease). Both feed and water consumption were unaffected by treatment. No treatment-related macroscopic abnormalities were observed at necropsy. The number of implantations was similar in all groups, but there were increased incidences of early and late resorptions at 60 and 80 mg/kg bw/d (3.17 and 2.67, respectively vs 0.50 in control for early resorptions and 0.83 and 1.00, respectively vs 0.0 in control for late resorptions). The incidence of post-implantation loss was increased at 60 and 80 mg/kg bw/d (25.5% and 23.4%, respectively as compared to 3.3% in control). Consequently, the numbers of viable young was reduced (11.7 and 12.0, respectively compared to 14.8 in control). Fetal weight was slightly lower than that of the controls at 60 mg/kg bw/d (-4.6%) and a more marked effect was observed at 80 mg/kg bw/d (-15.2%). The incidence of small fetuses (less than 2.80 g) was increased at 60 and 80 mg/kg bw/d and a small number also showed shiny skin and/or domed head. These observations mainly occurred in association with reduced fetal weight and were probably signs of fetal immaturity at these doses. Placental weight was increased at 60 (9.6%) and 80 mg/kg bw/d (11.5%) and there was an increased incidence of thickened placentae. There was no clear indication of an adverse effect of treatment upon fetal morphology.

Conclusion:
Maternal toxicity (reduced body weight gain) and embryotoxicity (increased number of resorptions and decreased fetal weight) were evident at dose levels of 60 and 80 mg/kg bw/d. Based on the results of this range finding study a dose level of 60 mg/kg bw/d was selected as a suitable high dose level in the main teratology study in the rat.

Teratology study in the Rat, oral (gavage) after exposure to metconazole WL136184 (95% cis) from gestations days 6-15 ([redacted] 1992 b; BASF DocID MK-432-009)

Guidelines: Protocol in compliance with test method B.31 of directive 87/302/EEC
GLP: Yes
Acceptance: The study was accepted.

In the main study 22 pregnant rats (CD strain of Sprague Dawley origin)/dose received metconazole WL136184 (95.2% purity, 95% cis isomer, batch 12) in 1% methylcellulose by gavage at dose levels of 0, 6, 24 and 60 mg/kg bw/d from day 6 to day 15 of gestation. Dosing volume was 10 mL/kg bw. On day 20 of pregnancy the females were sacrificed and subjected to post-mortem examination. Litter parameters were determined and fetuses fixed prior to examination for skeletal or visceral abnormalities.

Maternal data
All animals survived to scheduled sacrifice and no treatment-related clinical signs were observed during the study. A number of animals in all treated groups developed brown staining of the fur, particularly in the head region. The distribution of this finding between the groups did not suggest any toxicologically significant dose-related response.
Food consumption was slightly but statistically significantly lower during the treatment period (-7% during gestation days (GD) 6-8 and -13% GD 12-15) for females in the 60 mg/kg bw/d group. During the post-treatment period (gestation days 16-20) food consumption was again comparable to controls in this group. Top-dose females showed slightly but significantly increased water consumption immediately after completion of treatment. Bodyweight gain of top-dose females was significantly reduced during the treatment period by 21% (GD 6-16), particularly during the second half of the treatment period (-23%). This lead to an overall statistically significantly body weight gain reduction of -12% during gestation days 6-20. No treatment-related macroscopic changes were noticed in maternal animals at necropsy.

Litter data (see Table 5.6.2-6)

Treatment with metconazole cis did not affect pregnancy status as the pregnancy rate was 100% for the control and treated groups. The mean numbers of corpora lutea and total implantation sites for the treated groups were comparable to the controls and there was no treatment-related increase in pre-implantation loss. No female aborted prior to scheduled sacrifice and no female exhibited total litter loss (100% resorptions).
The number of early and late resorptions was increased at 60 mg/kg bw/d with a consequent increase in post-implantation loss and a decrease in viable litter size. Fetal weight was significantly lower at 60 mg/kg bw/d as compared to the control group. In agreement with the fetal weight effect, the placental weights were: slightly increased at the top dose.
There were no treatment-related increases in the incidence of fetal external, visceral or skeletal malformations. At the top-dose, one incidence of upturned snout and posterior cleft palate was found. Despite the fact that the latter malformation incidence (0.3%) was outside the historical control range (0-0.2%), the singularity of the event does not a priori suggest a relation with compound exposure. External fetal examinations indicated one fetus (from litter no. 68) in the 60 mg/kg bw/d group with a domed head. However, neither dilated lateral ventricles nor unossified cranial bones were observed in any fetus from this litter. As such, there was no confirmed case of hydrocephaly in this study.

In agreement with the effects on fetal and placental weight, the incidences of small fetuses (below 2.8g) and of large, thickened placentae were increased at the top dose.
Skeletal ossification data indicated that the fetal and litter incidences of the following ossification variations were increased at 60 mg/kg bw/d as compared to controls, most of which were above the historical control range: large anterior fontanelle, fetuses with an additional 14th thoracic rib or ribs, incomplete ossification of 3 or 4 sternebrae, and the presence of cervical ribs. The slight delay in ossification was possibly secondary to the reduction in pup weight.
Slight increases (compared to study control incidence) of large anterior fontanelle (fetal incidence) was also detected at 24 mg/kg bw/d. Fetal incidences were slightly outside the historical ranges and displayed dose-dependency. However, litter incidences were comparable to controls. It was therefore agreed that these events were merely indicative of general retardation of skeletal development (showing a broad variability), and not necessarily of localized bony defects, and were thus not taken into account for the determination of the NOAEL.
At necropsy slight developmental effects (bilateral dilatation of ureter at 24 mg/kg bw/d and above and bilateral dilatation kidney at the top-dose) were observed. However, these effects were not confirmed during microscopical examination and were therefore, not considered treatment-related.

Table 5.6.2-6: Litter data of teratogenicity main study in rats following treatment during gestation days 6-15 with metconazole cis

Dose level	**0**	**6**	**24**	**60**
No. of pregnant animals	*22*	*22*	*22*	*22*
Litter observation				
Corpora lutea	17.0	18.0	17.2	17.7
Implantations	16.0	16.9	16.1	16.2
Pre-implantation loss (%)	6.6	6.5	6.8	8.5
Post-implantation losses (%)	6.0	3.8	6.5	**17.1****
Total resorptions	0.95	0.64	1.05	**2.77****
Early resorptions	0.95	0.55	1.00	**2.09****
Late resorptions	0.0	0.09	0.05	0.68
Litter size	15.0	16.2	15.0	13.5*
Fetal weight (g)	3.74	3.71	3.68	**3.36*****
Small fetus (<2.80 g)	1/330 (0.3%)	2/357 (0.6%)	2/331 (0.6%)	33/296 (11.1%)
Placental weight (g)	0.51	0.51	0.53	**0.58** (+13.7%)**
Skeletal Variation				
Large anterior fontanelle				
• Fetal incidence	14/223 (6.3%)	11/241 (4.6%)	23/226 (10.2%)	35/201 (17.4%)
• Litter incidence	10/22 (45.5%)	9/22 (40.9%)	9/22 (40.9%)	12/22 (54.6%)
Incomplete Ossification of 3rd Sternebrae				
• Fetal incidence	30/223 (13.5%)	32/241 (13.3%)	20/226 (8.8%)	43/201 (21.4%)
• Litter incidence	15/22 (68.1%)	17/22 (77.3%)	12/22 (54.6%)	16/22 (72.7%)
Incomplete ossification of 4th sternebrae				
• Fetal incidence	3/223 (1.3%)		6/226 (2.7%)	16/201 (8.0%)
• Litter incidence	3/22 (13.6%)	4/22 (18.2%	5/22 (22.75)	11/22 (50.0%)
Additional Cervical Rib (s)				
• Fetal incidence	0/223 (0.0%)	0/241 (0.0%)	1/226 (0.4%)	5/201 (2.5%)
• Litter incidence	0/22 (0.0%)	0/22 (0.0%)	1/22 (4.6%	2/22 (9.1%)
Additional 14th Rib (Unilateral)				
• Fetal incidence	22/223 (9.9%)	28/241 (11.6%)	21/226 (9.3%)	35/201 (17.4%)
• Litter incidence	12/22 (54.6%)	13/22 (59.1%)	12/22 (54.6%)	18/22 (81.8%)
Additional 14th Rib (Bilateral)				
• Fetal incidence	14/223 (6.3%)	16/241 (6.6%)	25/226 (11.1%)	42/201 (20.9%)
• Litter incidence	7/22 (31.8%)	9/22 (40.9%)	10/22 (45.4%)	12/22 (54.6%)
Necropsy findings				
Hydronephrosis –unilateral				
• Fetal incidence	2/223 (0.9%)	4/241 (1.7%)	3/226 (1.3%)	1/201 (0.5%)
• Litter incidence	2/22 (9.1%)	4/22 (18.2%)	1/22 (4.5%)	1/22 (4.5%)
Hydronephrosis –bilateral				
• Fetal incidence	0/223 (0.0%)	1/241 (0.4%)	1/226 (0.4%)	5/201 (2.5%)
• Litter incidence	0/22 (0.0%)	1/22 (4.5%)	1/22 (4.5%)	4/22 (18.2%)
Hydroureter - unilateral				
• Fetal incidence	7/223 (3.1%)	5/241 (2.1%)	10/226 (4.4%)	2/201 (1.0%)
• Litter incidence	5/22 (22.7%)	4/22 (18.2%)	9/22 (40.9%)	2/22 (9.1%)

Dose level	**0**	**6**	**24**	**60**
No. of pregnant animals	*22*	*22*	*22*	*22*
Litter observation				
Hydroureter - bilateral • Fetal incidence	3/223 (1.3%)	4/241 (1.7%)	12/226 (5.3%)	13/201 (6.5%)
• Litter incidence	2/22 (9.1%)	4/22 (18.2%)	6/22 (27.3%)	9/22 (40.9%)

Statistical evaluation: *p ≤ 0.05,**p ≤ 0.01, *** p ≤ 0.001

#Historical control data (HCD) from 43 studies as provided in the study report:

- *Pre-implantation loss (%):4.5 – 27.2*
- *Post-implantation losses (%):1.7 – 10.9*
- *Total resorptions: 0.26 – 1.54*
- *Early resorption:0.26-1.46*
- *Late resorptions:0.00-0.30*
- *Fetal weight (g): 3.19 – 3.84*
- *Placental weight (g): 0.48 – 0.62*
- *Large anterior fontanelle (% fetal incidence): 0.0 – 8.4*
- *Incomplete ossification of 3rd sternebrae (% fetal incidence): 7.5-29*
- *Incomplete ossification of 4th sternebrae (% fetal incidence): 0.4 – 6.3*
- *Cervical ribs (% fetal incidence): 0-2.2*
- *Additional 14th Rib-Unilateral (% fetal incidence): 0-11.5*
- *Additional 14th Rib-Bilateral (% fetal incidence): 0-14.0*
- *Hydronephrosis –unilateral (% fetal incidence): 0-1.3*
- *Hydronephrosis –bilateral (% fetal incidence):0-2.5*
- *Hydroureter - unilateral (% fetal incidence): 0-4.2*
- *Hydroureter - bilateral (% fetal incidence): 0-2.5*

Conclusion:

Metconazole cis (WL136184, 95% cis) induced maternal and developmental toxicity at the top-dose (60 mg/kg bw/d). The NOAEL for maternal toxicity was set at 24 mg/kg bw/d (increased water consumption, decreased food consumption, decreased bodyweight gain). The NOAEL for developmental/fetal toxicity was set at 24 mg/kg bw/d (increased placental weight, decreased fetal weight and decreased litter size/viability, increased post-implantation loss (early and late resorptions)).

Executive summary of the analytical method used within the analytical phase (MK-432-009)

Principle of the method
The analytical method used in study MK-432-009 was validated for the determination of metconazole (BAS 555 F) in 1% methylcellulose. The study was performed by LIFE SCIENCE RESEARCH LIMITED, UK.
Residues were extracted from 1% aqueous methylcellulose by dissolving the total sample in acetone. After further dilution with acetone, residues were analyzed by gas chromatography (GC) with nitrogen thermionic detection. Analysis was performed on an Ultrabond 20 M column.

Recovery findings
The mean recovery values were between 70% and 110% for metconazole. The detailed results are given in the table below.

Table 5.6.2-7: Validation results of the analytical method applied in study MK-432-003 using GC-TID: metconazole (BAS 555 F) isomers in 1% aqueous methylcellulose

Matrix	Analyte	Detector settings	Fortification level (mg/mL)	No of replicates	Mean recovery (%)	RSD (%)
Aqueous methylcellulose	Metconazole	Nitrogen thermionic detector, 300°C	0.6	6	94	2.8
			8.0	6	95	2.7
			Overall	12	94	2.5

Linearity
The linearity was tested using five standards at concentrations between of 6 to 30 µg/mL. For BAS 555 F, linear correlations were obtained. No coefficients of determination (R^2) were stated. Calibration solutions were prepared in acetone.

Specificity
Final detection has been applied by nitrogen thermionic detection (TID). As only known analytes of known concentration were analyzed for dose verification purposes, no additional confirmatory technique is necessary.

Interference
No interferences from the reagent blank at the relevant retention times have been reported.

Limit of Quantitation
The limit of quantitation (LOQ) is defined as the lowest fortification level successfully tested. During this study, a limit of quantitation of 0.6 mg/mL for BAS 555 F was obtained.

Repeatability
The relative standard deviations (RSD, %) for all fortification levels were below 20%.

Stability of solutions
The chemical stability of the analyte in 1% aqueous methylcellulose was tested within this study. Results indicated that the analyte was chemically stable in 1% aqueous methylcellulose for at least 6 hours when stored at 21°C.

Conclusion
The analytical method used in study MK-432-009 for the analysis of metconazole in 1% aqueous methylcellulose uses HPLC-TID for final determination, with an LOQ of 0.6 mg/mL.
It could be demonstrated that the method generally fulfils the requirements with regard to repeatability, specificity, LOQ and recoveries and is therefore considered suitable for the analysis of metconazole in 1% aqueous methylcellulose.

Rabbit

Teratology study in the Rabbit, oral (gavage) following exposure to metconazole WL148271 (cis/trans) from gestations days 6-28 (1997a; BASF DocID MK-432-015)

Guidelines: Protocol in compliance with TG 414 from OECD (2001)
GLP: Yes
Acceptance: The study was accepted.

Preliminary study (0, 1, 5, 10, 20 and 40 mg/kg/d)

Material and Methods:
10 presumed pregnant rabbits (Hra: (NZW) SPF)/dose received metconazole cis trans (WL148271, 98.3% purity, batch AC 10575-61 and AC 9339-114; cis/trans: 80:15) in 0.5% carboxymethylcellulose by gavage at dose levels of 0, 1, 5, 10, 20 and 40 mg/kg bw/d from d6 to d28 of gestation inclusive. Dosing volume was 10 mL/kg bw. On d29 of pregnancy the females were sacrificed and subjected to post-mortem examination. Litter parameters were determined and fetuses fixed prior to examination for skeletal or visceral abnormalities.

Findings:
Maternal data:
There were three non-pregnant females, one each in the 0, 20, and 40 mg/kg bw/d groups. No mortalities, abortions or premature deliveries were noted. No relevant clinical findings were observed. Absolute food consumption values (g/day) tended to be reduced at the top-dose on d12-15, and d19-24 (by 5-6%), as well as for the entire dosage period (d6-29: -3%), but relative values (g/kg bw/d) were comparable in all dosage groups. At the top-dose, maternal bodyweights tended to be reduced at 40 mg/kg bw/d after d9 of gestation; when calculated for d6-29, difference with controls amounted to about 10%. Gravid uterine weights were slightly reduced at the top dose. Placenta appeared normal for all animals in control and treated groups. During necropsy 2/10 animals showed red areas in the stomach at the top dose. (see Table 5.6.2-8)

Litter data:
When compared to controls (control values in parentheses) top-dose animals showed altered litter averages for late resorptions: 1.1 (0.0), percentage of resorbed conceptuses/litter: 14.3% (1.2%), and an increased number of does with any resorptions: 55.6% (11.1%). A reduction of live litter size was noted at the top-dose (6.9) compared to controls (8.1). No relevant effect on fetal weight was observed (see Table 5.6.2-8)
The litter incidence of fetuses with any alterations was elevated compared to controls, but dose-responsiveness was lacking. Analyses of the litter averages for ossification sites per fetus did not identify any dose-related or statistically significant differences. With regard to variations one fetus in the 1mg/kg bw/d group had an absent spleen, and absent intermediate lobe of the lungs occurred in 2, 1 and 1 fetuses from 1, 1 and 1 litters in the 1, 5 and 10 mg/kg/day dosage groups, respectively. These variations are a common variation in this strain of rabbit. The incidences of malformed fetuses in this study were 0/73, 1/82 (1.2%), 0/85, 0/96, 0/81, and 2/62 (3.2%) in the control, 1, 5, 10, 20, and 40 mg/kg bw/d dose groups, respectively. At the top-dose one fetus was found with a domed head associated with extreme dilation of lateral ventricles and one fetus with absent hindlimb digits associated with reduced metatarsal bones during gross and soft tissue examination (or skeletal examination). Moderate to extreme dilation of the cerebral lateral ventricles is usually equated with hydrocephaly
Missing digits were report in the available control data of the laboratory (available for 1994-1996, presented in the study report) at a low rate with a maximum of one per study, and dilation of the lateral ventricles (moderate to extreme) ranged as high as three fetuses per study.

Table 5.6.2-8: Maternal and litter data of teratogenicity preliminary study in rabbits following treatment during gestation days 6-28 with metconazole cis/trans

Dose level (mg/kg bw/d)	**0**	**1**	**5**	**10**	**20**	**40**
Maternal data						
No. pregnant	9	10	10	10	9	9
Bodyweight GD 29 (kg)	3.94 ± 0.36	3.93 ± 0.28	3.98 ±0.35	3.94 ±0.32	4.02 ± 0.44	3.84 ± 0.22
Gravid uterine weight (g)	474 ± 110	494 ± 120	500 ± 148	537 ± 83	534 ± 88	449 ± 81
Body weight change GD 0-29 (kg)	0.69	0.66	0.72	0.66	0.73	0.61
Food consumption GD 6-29 (g/day)	157 ± 20	168 ± 6	157 ± 26	159 ± 9	164 ± 16	152 ± 19
Placenta appeared normal	9 (100%)	10 (100%)	10 (100%)	10 (100%)	9 (100%)	9 (100%)
Litter observation						
Litter mean values Corpora lutea	9.0 ± 1.7	9.4 ± 2.1	9.4 ± 2.3	10.1 ± 1.5	10.4 ± 1.7	9.1 ± 1.2
Implantations	8.2 ± 1.9	8.5 ± 2.0	8.9 ± 2.5	9.7 ± 1.5	9.6 ± 1.4	8.1 ± 1.6
Total resorptions	0.1 ± 0.3	0.3 ± 0.7	0.4 ± 1.3	0.1 ± 0.3	0.6 ± 1.0	1.2 ± 1.4
Early resorptions	0.1 ± 0.3	0.1 ± 0.3	0.4 ± 1.3	0.1 ± 0.3	0.4 ± 1.0	0.1 ± 0.3
Late resorptions	0	0.2 ± 0.6	0	0	0.1 ± 0.3	1.1 ± 1.3
Litter size	8.1 ± 1.9	8.2 ±2	8.5 ± 3.2	9.6 ± 1.6	9.0 ± 1.5	6.9 ± 1.7
Fetal weight (g)	42.13 ± 6.25	43.15 ±4.35	43.84 ± 9.15	40.15 ± 2.95	42.92 ± 6.54	41.65 ± 5.27

Conclusion:

The top dose of 40 mg/kg bw/d was minimally toxic to the dams (small reductions in body weight gain and food consumptions) and showed slight embryo/fetotoxicity (increased incidences of late (but not early) resorptions with a corresponding decrease in live litter size). Only one craniofacial malformation was seen in this study, i.e. one case of hydrocephaly at 40 mg/kg bw/d. Overall, all fetal morphological changes were considered unrelated to treatment. Based on the results of this range-finding study, doses of 5, 10, 20 and 40 mg/kg bw/d were recommended for the main study.

Main study (0, 5, 10, 20 and 40 mg/kg/d)

Material and Methods:
Four groups (25 females/group) of mated female New Zealand White rabbits (Hra: (NZW) SPF) received metconazole (98.3% purity, batch AC 10575-61, cis/trans: 80/15) in 0.5% carboxymethylcellulose by gavage at dose levels of 0, 5, 10, 20 and 40 mg/kg bw/d from d6 to d28 of gestation. Dosing volume was 10 mL/kg bw. On d29 of pregnancy, the females were sacrificed and subjected to post-mortem examination. Litter parameters were determined and fetuses fixed prior to examination for skeletal or visceral abnormalities. In addition to standard examinations, blood samples have been obtained before sacrifice, in order to perform both hematological and clinical chemistry analysis.

Findings:
Maternal data:
Mortality observed in this study (1 case in the control and 40 mg/kg bw/d group) was not related to treatment, but attributed to intubation accidents. Single incidences of abortions (1 in the control and one at 20 mg/kg bw/d) were not related to treatment. Regarding clinical signs localized alopecia on the back or limbs, a red substance in the cage pan and ungroomed coat were considered unrelated to treatment, as incidences were not dose-related and the observation occurred only in one or two does in any dosage group.
Dams in the 40 mg/kg bw/d group lost weight on gestation days 24-29. As a result of this bodyweight loss, bodyweight gain for the entire dosage period (d6-29) was reduced (-15%). Slight, but non-statistically significant reductions in gravid uterine weights were noted for the 40 mg/kg bw/d group. This observation was associated with a slightly smaller live litter size that, in turn, reflected a small but statistically significant increase in post-implantation loss (early and late resorptions) and a small reduction in fetal bodyweight. After correction of maternal bodyweight gains for gravid uterine weights, maternal bodyweight gains for the entire dosing period and maternal bodyweight on d29 were unaffected at any dose. Food consumption values were reduced at 40 mg/kg bw/d on d24-29, resulting in small reductions in these values on during the treatment period until termination (d6-29).
Animals in the 40 mg/kg bw/d group exhibited slight, but statistically significant reductions in hemoglobin, hematocrit and mean corpuscular volume values, as compared to controls. Additionally, the values for platelet counts and alkaline phosphatase activity were significantly increased at 40 mg/kg bw/d when compared to control values. These findings were consistent with effects observed in short-term toxicity studies in other species and a relationship with metconazole administration is thus probable.
Top-dose females showed slight, but not statistically significant increases in both absolute (+13%) and relative (+15%) liver weight. This effect was considered test substance-related.
Necropsy observations included pale liver, but association with treatment is uncertain in the absence of dose-effect relationship. There were no treatment-related microscopic changes in the maternal livers from the control and 40 mg/kg bw/d groups.

Litter data:
The mean numbers of corpora lutea and total implantation sites for the treated groups were comparable to the controls and no increase in pre-implantation loss was observed in any treated group when compared to controls. No doe exhibited total litter loss (100% resorptions).
The top-dose was associated with a small increase in post implantation loss as evidenced by statistically significant increases in the number of does with any resorptions and the rate of dead/resorbed conceptus per litter. A total of 6 resorptions (3 early and 3 late) in 5 litters were observed in the control group and a total of 22 resorptions (11 early and 11 late) from 13 litters were observed in the 40 mg/kg bw/d group. Corresponding to the slight increase in post-implantation loss, a slight, but non-statistically significant reduction in mean litter size was observed at the top-dose. Fetal weights were slightly but non-statistically significantly reduced (-5 to -7%). Placenta appeared normal throughout all groups, placenta weights were not determined in this study.
All values for the litters and conceptuses were within the ranges observed historically at the testing facility (historical control data from June 1994 - June1996 as provided in the study report). Both fetus and litter incidences of total observed alterations were equal in any dosage group.
The incidence of malformed fetuses in this study was low and consisted 0/202, 1/219 (0.5%), 2/212 (0.9%), 3/183 (1.6%), and 3/177 (1.7%) fetuses in the control, 5, 10, 20 and 40 mg/kg/day dose groups, respectively. The malformations included none in the control, one fetus with fused ribs at 5 mg/kg/day, one fetus with extreme dilatation of the lateral ventricles (hydrocephaly) and one fetus with vertebral and rib malformations at 10 mg/kg/day, two fetuses with fused ribs as well as one fetus with spina bifida and fused and malformed lumbar vertebrae at 20 mg/kg/day, and at the top dose one fetus with a short tail; one fetus with vertebral and rib malformations, and one fetus with marked dilatation of the lateral ventricles/hydrocephaly. A variety of malformations was reported, all of which were also seen in the historical control studies.
There were in total two craniofacial malformations observed in this study: One hydrocephaly at 10 mg/kg/day and one hydrocephaly at 40 mg/kg/day. Since this malformation occurs spontaneously in control animals (up to 3 fetuses were recorded with marked/extreme dilated lateral brain ventricles in the historical control data from 1994-1996), and there was no clear dose-response relationship it was not considered related to treatment in this study. (see Table 5.6.2-9)

Table 5.6.2-9: Maternal and litter data of teratogenicity main study in rabbits following treatment during gestation days 6-28 with metconazole cis/trans

Dose level (mg/kg bw/d)	**0**	**5**	**10**	**20**	**40**
Maternal data					
No. pregnant	25/25	24/25	24/25	24/25	24/25
Mortality	1 (intubation error)	-	-	-	1 (intubation error)
Abortions	1	0	0	1	0
Food consumption (g/day) • GD 6-29	159 ± *14*	158 ± *19*	160 ± *15*	154 ± *19*	151 ± *19* *(-5%)*
• GD 24-29	113 ± 31	112 ± 45	117± 63	105 ± 47 (-7%)	95 ± 55 (-16%)
Bodyweight GD 29 (kg)	4.16 ± 0.33	4.16 ± 0.35	4.20 ±0.29	4.04 ±0.35	4.09 ± 0.36
Gravid uterine weight (g)	523 ± 104	530 ± 97	518 ± 94	477 ± 132 (-9%)	462 ± 141 (-12%)
Corrected maternal body weight	3.64 ± 0.28	3.63 ± 0.32	3.68 ±0.30	3.58 ±0.32	3.63 ± 0.30
Bw gain (kg) • GD 0-29	0.49 ± 0.14	0.50 ± 0.15	0.54 ± 0.22	**0.36 ± 0.15***	0.42 ± 0.20
• GD 24-29	0.03 ± 0.13	0.05 ± 0.12	0.01 ± 0.09	0.01 ± 0.15	-0.03 ± 0.15
• GD 6-29	-0.41 ± 0.12	-0.41 ± 0.20	-0.42 ± 0.14	-0.37 ± 0.16	-0.35 ± 0.21
Liver weights • absolute	96.6 ± 13.0	96.7 ± 18.9	96.3 ± 15.8	98.4 ± 21.5	108.8 ± 24.8 (+13%)
• relative					+15%
Hematology (% of control)					**↓Hb -6%* ↓Hct -6%* ↓MCV -4%* ↑platelet +24%***
Clinical chemistry					↑AP +114%
Litter observations					
Litter mean corpora lutea *#HCD: 7.8-11.8; Mean: 9.6*	*9.3* ± 2.2	*9.4* ± 1.7	*9.2* ± 2.1	*9.1* ± 2.1	8.9 ± 2.3
Implantations *#HCD:5.0-10.5; Mean: 8.6*	9.0 ± 2.4	9.2 ± 1.8	9.2 ± 2.2	8.5 ± 2.7	8.7 ± 2.4
Total resorptions *#HCD: 0-3.2; Mean: 0.4*	0.3 ± 0.5	0.1 ± 0.3	0.3 ± 0.6	0.5 ± 0.8	1.0 ± 1.4
Early resorptions No. fetuses (litter) Mean ± SD *#HCD: 0-2.8; Mean: 0.2*	3 (3) 0.1 ± 0.3	3 (3) 0.1 ± 0.3	5 (4) 0.2 ± 0.5	6 (4) 0.3 ± 0.6	11 (6) 0.5 ± 1.1
Late resorptions No. fetuses (litter) Mean ± SD *#HCD: 0-0.8; Mean: 0.2*	3 (3) 0.1 ± 0.3	0 (0) 0.0 ± 0.0	3 (3) 0.1 ± 0.3	6 (5) 0.3 ± 0.5	11 (9) 0.5 ± 0.7
Does with any resorptions *#HCD: 0-60%; Mean: 27.2%*	5 (21.7%)	3 (12.5%)	7 (29.2%)	8 (34.8%)	**13 (56.5%)****
Litter size/live foetuses *#HCD: 4.8-10.4; Mean: 8.2*	8.8 ± 2.2	9.1 ±1.9	8.8 ±2.3	8.0 ±2.7	7.7 ±2.9
Fetal weight (g) *#HCD: 31.85-50.27; Mean:38.65*	42.72 ±4.52	41.73 ±4.10	42.08 ±3.79	42.64 ±5.95	40.41 ±5.77
Litter weights (g)	368.6	372.6	367.34	327.39	303.14
Placenta appeared normal	100%	100%	100%	100%	100%

Dose level (mg/kg bw/d)	0	5	10	20	40
Maternal data					
Fetal alterations					
Fetuses with any alteration					
• % Fetuses	6.4	9.6	8.0	8.2	7.3
• % Litters	47.8	45.8	50.0	39.1	34.8
Brain: dilation of lateral ventricles (extreme or marked)/hydrocephaly *#HCD:* *Marked dilated lateral ventricles: 0-3 fetuses (0-1%), 0-1 litter (0-5.3%);* *Extreme dilated lateral ventricle: 0-1 fetuses (0-0.8%), 0-1 litter (0-6.2%);*	0	0	1	0	1

#Historical control data (HCD) from June 1994 to June 1996 (38 studies)
*p≤0.05, **p≤0.01

Conclusion:
Maternal toxicity was evident at the top dose (reductions in food consumption, body weight loss during GD 24-29, reduced hemoglobin, hematocrit and corpuscular volume, increases in platelet counts and alkaline phosphatase activity, and increased absolute and relative liver weights). Therefore, the NOAEL for maternal toxicity is established at 20 mg/kg bw/d. Based on minimal embryotoxicity/fetotoxicity (small increases in postimplantation loss (early and late), slightly decreased fetal weights and litter size) at the top dose the NOAEL for fetal toxicity was also set at 20 mg/kg bw/d. This is contrary to the previous evaluation in the monograph (2004), where a NOAEL for fetotoxicity was established at 10 mg/kg bw/d. No malformations or variations in the fetuses were attributable to treatment with the test substance. In the absence of any treatment-related developmental toxicity up to the highest dose tested, the NOAEL for developmental toxicity was found at 40 mg/kg bw (top dose).

Executive summary of the analytical method used within the analytical phase (MK-432-015)

Principle of the method
The analytical method used in study MK-432-015 was validated for the determination of metconazole (BAS 555 F) in 0.5% aqueous carboxymethylcellulose. The study was performed by LANCASTER LABORATORIES, PA, USA.
Residues were extracted from 0.5% aqueous carboxymethylcellulose by dissolving the suspension in acetonitrile, diluting aliquots to the appropriate concentrations in the solutions, and analyzing by high-performance liquid chromatography (HPLC) using ultra-violet (UV) detection. Analysis was performed on an INERTSIL 5U ODS column using acetonitrile/water (50/50, v/v) with 0.1% phosphoric acid as mobile phase.

Recovery findings
The mean recovery values were between 70% and 110% for metconazole. The detailed results are given in the table below.

Table 5.6.2-10: Validation results of the analytical method used in study MK-432-015 using HPLC-UV: metconazole (BAS 555 F) in 0.5% aqueous carboxymethylcellulose

Matrix	Analyte	Detector wavelength (nm)	Fortification level (mg/mL)	No of replicates	Mean recovery (%)	RSD (%)
Aqueous carboxymethyl-cellulose	Metconazole	220	0.1	5	104	0.4
			4.0	5	104	1.5
			Overall	10	104	1.1

Linearity
The linearity was tested using six standards at concentrations between of 0.0017 to 0.04 µg/mL. For BAS 555 F, linear correlations with coefficients of determination (R^2) greater than 0.99 were obtained. Calibration solutions were prepared in acetonitrile.

Specificity
Final detection has been applied by ultra-violet (UV) detection. As only known analytes of known concentration were analyzed for dose verification purposes, no additional confirmatory technique is necessary.

Interference
No interferences from the reagent blank at the relevant retention times have been reported.

Limit of Quantitation
The limit of quantitation (LOQ) is defined as the lowest fortification level successfully tested. During this study, a limit of quantitation of 0.1 mg/mL for BAS 555 F was obtained.

Limit of Detection
For BAS 555 F, the limit of detection (LOD) was 0.01 mg/mL.

Repeatability
The relative standard deviations (RSD, %) for all fortification levels were below 20%.

Stability of solutions
The stability of the analytes in standard solutions was investigated within this study. Test item solutions were stored at ambient temperatures for 12 days and 11 hours and analyzed against freshly prepared standard solutions, revealing stability of the test item at least within this time period.

Conclusion
The analytical method used in study MK-432-015 for the analysis of metconazole in 0.5% aqueous carboxymethylcellulose uses HPLC-UV for final determination, with an LOQ of 0.1 mg/mL.
It could be demonstrated that the method generally fulfils the requirements with regard to repeatability, specificity, LOQ and recoveries and is therefore considered suitable for the analysis of metconazole in 0.5% aqueous carboxymethylcellulose.

Additional/complementary developmental studies in the rabbit.
The following teratogenesis studies on the rabbit were performed on both metconazole cis and cis/trans. As stated in the monograph (2004) it was “the position of the rapporteur that some aspects of these studies were questionable, in view of the interpretation difficulties which emerged when comparing data between studies on the same compound, and at the same doses. However, despite the variability encountered, some pertinent effects were detected. In the evaluation, endpoints either emerging at the top-dose, showing a dose-responsive trend, or effects showing replication between different studies were highlighted and discussed.”

Preliminary teratology study in the Rabbit, oral (gavage) following exposure to 3 isomers (metconazole cis, (-) cis, and trans) from gestations days 7-19 (1990a; BASF DocID MK-432-002)

Guidelines:	Protocol not in compliance with test method B.31 of directive 87/302/EEC.
Deviations from protocol:	Only 6 females/group. Limited examination of the fetuses: no observation of the skeletal anomalies and no indeep examination of visceral ones.
GLP:	No
Acceptance:	The study was accepted.

Materials and Methods:
6 presumed pregnant NZW rabbits (Interfauna, UK Ltd.)/dose received either metconazole cis (WL136184, 96.9% purity, batch 3454/078 and ST89/324), metconazole (-)cis (WL161053, 91 ± 1% purity, batch 3454/081 ST89/323) or metconazole trans (WL153996, 97.0% purity, batch 88-08 ST89/312) in 1% methylcellulose by gavage from d7 to d19 of gestation inclusive. Selected dose levels were 10, 28 and 80 mg/kg bw/d for cis and (-) cis materials, and 10, 20 and 40 mg/kg bw/d for the trans material. A single control group was treated with the vehicle. On d29 of pregnancy, the females were sacrificed and subjected to post-mortem examination. Litter parameters were determined and fetuses examined for gross changes. The study is considered to provide additional information.

Findings:

For tabulation of results please refer to the results table in the monograph (2004).

Maternal data:

- cis metconazole:

At 80 mg/kg bw/d all animals had cold ears (d7-11), occasional signs of anorexia and reduced or altered fecal output in 4/6 animals during the treatment period. Mean food consumption was reduced during the initial treatment period (d7-10); however, recovery occurred during the remaining dosing period and following cessation of treatment values were either superior or similar to control. There was a noticeable loss of mean bodyweight (d7-9). Although there was some overall recovery, a further marked loss of bodyweight occurred on days 24-29 of gestation, probably due to 4 dams aborting in this period and mean bodyweights at termination were lower than pre-dose values. At 28 mg/kg bw/d all animals had cold ears (d7-9). There was a slight initial bodyweight loss (d7-9) observed. At 10 mg/kg bw/d 2 dams had anorexia, abnormal feces and clinical signs similar to those observed at higher doses.

- (-)cis metconazole:

At 80 mg/kg bw/d 5/6 animals had cold ears (d7-11), anorexia and reduced or altered fecal output in 4/6 animals during the dosing period. There was a reduction in mean food consumption noted during the initial treatment period (d 7-8) with recovery to higher consumption by the end of the study. There was no significant effect on body weight or body weight gain at this exposure level. For dams with live young there was a slight loss of bodyweight (d7-9); recovery occurred thereafter with gains essentially similar to control. At 28 mg/kg bw/d cold ears were noted in 5/6 animals (d7-8 for 4 days). Although food consumption showed a marginal reduction on days 7 to 8, both pre-dose values and those subsequent to day 8 were consistently lower than control. There was no apparent effect on food consumption, body weight or body weight gain in the 10 mg/kg/day group.

- trans metconazole:

No consistent or clear signs of reaction to treatment were observed. There were no effects on food consumption, body weight, body weight gain or any of the litter parameters at any dose of the trans isomer.

Litter data:

- cis metconazole:

At 80 mg/kg bw/d, 4/5 pregnant animals aborted. Two abortions occured on day 28, the other two were presumed to have occurred between days 24 and 29 due to the marked loss of bodyweights observed for this period (210 g and 350 g). The combination of reduction in the number of implants and an increase in embryo/fetal death and post-implantation loss (late deaths and abortions) at this exposure levels resulted in only one dam with a live litter. For the 1 dam with live young on day 29, there was a significant reduction in number of live young, litter weight and mean fetal weight noted.
At 28 mg/kg bw/d, there was an apparent increase in post-implantation loss, which was reflected in a lower mean litter size. Mean fetal weights were similar to control. A higher value might have been expected due to the lower litter size but litter weight was also lower than the respective control value. At 10 mg/kg bw/d, litter values were superior to controls for nearly all parameters.
The incidence of probable malformed fetuses was 2/33 (2/4 litters), 0/40 (0/4 litters), 3/41 (2/6 litters) and 0/3 (only 1 litter) at 0, 10, 28 and 80 mg/kg bw/d, respectively. One control fetus had microphthalmia; and one fetus had great vessel malformations. In the 28 mg/kg dose group, one fetus had a domed cranium (suspected hydrocephaly); one fetus had missing hindpaws and digits; and one fetus had a retroesophageal subclavian artery.

- (-)cis metconazole:

At 80 mg/kg bw/d 1 dam totally resorbed her litter and at 28 mg/kg bw/d 1 rabbit aborted. Because the incidence was the same in each group and because 1 control dam also lost her litter, the losses at 28 and 80 mg/kg bw/d could not conclusively be related to treatment.
At 80 mg/kg bw/d a reduction in the number of implantations and an increase in embryo/fetal death and post-implantation loss (late embryonic deaths) was observed. This resulted in a reduced number of live young. Although mean fetal weight was similar to control, the low litter size resulted in a reduced litter weight.

The incidence of probable malformations in the (-)cis isomer groups was 2/33 (2/4 litters), 1/56 (1/6 litters), 2/32 (2/4 litters) and 2/9 (2/3 litters) at 0, 10, 28 and 80 mg/kg bw/d, respectively. One control fetus had microphthalmia; and one fetus had great vessel malformations. At 10 mg/kg/day, one fetus had retroesophageal subclavian artery. At 28 mg/kg/day one fetus showed retroesophageal subclavian artery, and the other fetus showed spina bifida and malrotated hindlimbs. At 80 mg/kg, one fetus had great vessel malformations, and one fetus had a misshapen cranium with enlarged fontanelles. In terms of craniofacial malformations, there were defects seen only in 2 fetuses in the 80 mg/kg/day dose group compared with 1 in the control group (see the cis isomer study above).

- trans metconazole:

There were no incidences of total litter loss at any dose, i.e., all pregnant dams had live young on day 29. Litter data at all doses showed no relationship to treatment with values similar or superior to control.
The incidence of malformations in the trans isomer groups was 2/33 (2/4 litters), 0/49 (0/5 litters), 3/39 (3/5 litters) and 0/55 (0/6 litters) at 0, 10, 20 and 40 mg/kg bw/d, respectively. Compared to controls (see above cis isomer study) there was no increase in the incidence of malformations with treatment. Three fetuses at 20 mg/kg bw/d had retroesophageal subclavian artery.

Conclusion:
At the dose rates used in this comparative study, the cis and (-)cis components of metconazole have been shown to be toxic to the dams and to possess embryotoxic potential, whereas, there were no clear treatment-related effects with the trans material. None of the isomers used in these studies showed an increase in malformations above control levels. The small number of of animals (1-6 litters with live pups) and the restricted investigations performed in this study, necessarily limited the confidence with which conclusions could be drawn from the previous data.

Teratology study in the Rabbit, oral (gavage) following exposure to metconazole cis/trans (WL148271) from gestations days 7-19 ([redacted] 1991c; MK-432-003)

Guidelines: Protocol in compliance with test method B31 of directive 87/302/EEC
GLP: Yes
Acceptance: The study was accepted.

Materials and Methods:

Preliminary study (0, 10, 30, and 90mg/kg bw/d)

6 presumed pregnant NZW rabbits (Interfauna, UK Ltd.)/dose received metconazole cis/trans (WL148271, 95.3% purity, cis/trans: 83.7/16.3, batch 89-01 Ref. ST 89/088) in 1% methylcellulose by gavage at dose levels of 0, 10, 30 and 90 mg/kg bw/d from day 7 to day 19 of gestation. Dosing volume was 2 mL/kg bw. On day 29 of pregnancy, the females were sacrificed and subjected to post-mortem examination. Litter parameters were determined and fetuses examined for gross changes. In a pilot study comprising 3 pairs of non-pregnant females, marked toxicity (anorexia, cold ears and marked bodyweight loss) was observed at 100 and 400 mg/kg bw/d, whereas at 25 mg/kg bw/d the findings were equivocal, with 1 animal showing an adverse response, whilst the other was unaffected.

Main study (0, 4, 10, 25, and 62.5 mg/kg bw/d)

16/17 presumed pregnant NZW rabbits (Interfauna, UK Ltd.)/dose received metconazole cis/trans (WL148271, 95.3% purity, cis/trans: 83.7/16.3, batch 89-01 Ref. ST 89/088) in 1% methylcellulose by gavage at dose levels of 0, 4, 10, 25 and 62.5 mg/kg bw/d from day 7 to day 19 of gestation. Dosing volume was 2 mL/kg bw. On day 29 of pregnancy, the females were sacrificed and subjected to post-mortem examination. Litter parameters were determined and fetuses fixed prior to examination for skeletal or visceral abnormalities.

Additional study (0, 2, 4 and 10 mg/kg bw/d)

According to the test facility, the singular incidence of some cranial malformations at the lowest dose of the main study (4mg/kg bw/d) justified the conduct of an additional test, in order to better characterize the potential developmental toxicity between 2-10 mg/kg bw/d.
18/19 presumed pregnant NZW rabbits (Interfauna, UK Ltd.)/dose received metconazole cis/trans (WL148271, 95.3% purity, cis/trans: 83.7/16.3, batch 89-01 Ref. ST 89/088) in 1% methylcellulose by gavage at dose levels of 0, 2, 4 and 10 mg/kg bw/d from day 7 to day 19 of gestation. Doses were selected on the basis of the previous main teratogenicity study. Dosing volume was 2 mL/kg bw. On day 29 of pregnancy, the females were sacrificed and subjected to post-mortem examination. Litter parameters were determined and fetuses examined for gross changes. Fetuses were subsequently examined for skeletal abnormalities.

Findings:

Preliminary study (0, 10, 30, and 90mg/kg bw/d)

Maternal data:

Considerable maternal toxicity was observed at 90 mg/kg bw/d. At this top dose clinical signs of a generally non-specific nature associated with initial inappetence and marked bodyweight loss were noted. Five of six pregnancies were terminated by total resorption (4) or abortion (1). There was reduced food intake throughout treatment, although this recovered and was higher than controls after treatment ended. At 30 mg/kg bw/d, minimal effects on maternal parameters (transient inappetence and weight loss d7-9) were observed. At 10 mg/kg bw/d, marginal decreases compared to control values in respect of maternal food consumption (d7-10) and weight gains (d7-11) were noted.

Litter data:

At 90 mg/kg bw/d, the single litter with live young was severely compromised in terms of high incidence of in utero death and post-implantation loss, and the number of live young (3 surviving fetuses), litter weight, and fetal weight were reduced.

At 30 mg/kg bw/d, a possible increased incidence of post-implantation loss and slightly lower mean fetal weight were noted. There was also a possible increase in post-implantation loss in the 10 mg/kg/day group. At 10 mg/kg bw/d, there was also possible association between treatment and the incidence of embryofetal deaths and fetal defects (3/48 fetuses in 2/6 litters). However, the small number of animals in each group limits the power of the study to detect such effects.

The incidence of fetuses malformed in this preliminary study was 1/51, 3/48, 6/49 and 1/3 fetuses in the control, 10, 30, and 90 mg/kg/day groups, respectively. One fetus in the control group had forelimb flexure. In the 10 mg/kg/day group, two fetuses had fused parietal and frontal bones, and one fetus showed forelimb flexure. In the 30 mg/kg/day group, one fetus had short tail, two fetuses had great vessel malformations, two fetuses had alterations in the frontal bones, and one fetus had a missing forelimb. Due to the variety of defects with no clear dose-response relationship, and since no skeletal evaluations were done, and the number of litters in each group is small, no conclusion can be drawn from these data.

Main study (0, 4, 10, 25, and 62.5 mg/kg bw/d)

Selected maternal and litter observations are presented in Table 5.6.2-11.

Maternal data:

There were no treatment-related mortalities observed. At 62.5 mg/kg bw/d, there were 3 abortions (days 28/29) and total resorption of two litters. The abortion at 10 mg/kg bw/d (d19/21) was considered unlikely to be related to treatment.

The majority of the top-dose animals responded to the start of treatment by a period of anorexia/reduced or altered fecal output. This was mainly confined to the first week of dosing although it persisted for longer in some animals. Cold ears were occasionally noted at this dose.

Mean food intake at 62.5 mg/kg bw/d was markedly reduced on d7-8 of pregnancy. However, steady recovery occurred during the remaining dosing period, and after cessation of treatment, values were superior to control.

There was also an initial slight decrease in food consumption at 25 mg/kg bw/d. At the top-dose, mean bodyweight gain on d7-9 was lowered significantly. This recovered over the remainder of the study, although further loss of bodyweight was observed over gestation days 24-29 due to 3 dams aborting and 2 others totally resorbing their litters (all late embryonic deaths). For mean values for dams with live young, bodyweight recovery was not maintained after cessation of treatment, due in part, to a high number of late resorptions (with concomitant low litter size and weight) and a clear difference from control was evident at termination. For dams with live young at 25, 10 and 4 mg/kg bw/d, although there appeared to be a slight dose-related bodyweight loss during d7-9, gains thereafter were similar or superior to control. There were a number of non-pregnant females in the 0, 4, 10 and 25 mg/kg/day groups, reducing the number of females with live young to 12, 15, 11, and 11, respectively. No treatment-related macroscopic changes were noticed in maternal animals at necropsy.

Litter data:
At 62.5 mg/kg bw/d, there was a statistically significant increase in late embryonic deaths which resulted in a markedly increased post-implantation loss and reduced mean litter size. At 25 and 10 mg/kg bw/d, values for late embryonic death were slightly higher than controls and this was reflected in the relatively higher post-implantation loss and slightly lower mean litter size.
At 62.5 mg/kg bw/d, there was a marked (and highly significant) reduction in mean litter weight. This was not only due to the lower mean litter size but was also a reflexion of a concomitantly lower mean fetal weight. At the other doses, litter weights were slightly lower than controls, and the findings were mainly related to the decreased litter sizes, since fetal weights were unaffected. Values for post-implantation loss, late resorptions, and live litter size were far outside the historical control range at the top dose, showing sever maternal toxicity (see Table 5.6.2-11).

The incidence of malformed fetuses was 2/113 (2/12 litters), 9/124 (7/15 litters), 2/88 (2/11 litters), 9/88 (7/11 litters) and 8/42 (4/10 litters) in control, 4, 10, 25 and 62.5 mg/kg bw/d, respectively. Increase of incidences of malformed fetuses were apparent at 4, 25 and 62.5 mg/kg bw/d, but not at 10 mg/kg/day. Thus, a clear dose-response relationship is lacking. There were a number of severe malformations reported in the study and one or more of these were seen in all dose groups, including controls. One control fetus was observed with forelimb flexure and mal-rotated hindlimb, and one fetus with spina bifida. The incidence of any specific malformation at 4 mg/kg bw/d was not increased (except for a single incidence of hydrocephaly/cebocephaly). Thus, the finding at the lowest dose was considered irrelevant. The increased incidence of fetal malformations was within the test facility's historical control data up to and including the dose level of 10 mg/kg bw/d (see Table 5.6.2-11).
Specifically, at 4 mg/kg/day one fetus had forelimb flexure, transposition of the great vessels, retroesophageal aorta and subclavian artery, duplicated inferior vena cava, and interventricular septal defect; two further fetuses showed microphthalmia; another fetus had retroesophageal subclavian artery; two fetuses shoed dilated ascending aorta/arch (one with marked narrowing of the pulmonary trunk); one fetus had cranial schisis with microphthalmia, cleft lip and palate, great vessel malformations, and interventricular septal defect; one fetus had cebocephaly, anophthalmia, hydrocephaly, and malformed facial bones; and one fetus had lumbar scoliosis due to vertebral malformations.

At 10 mg/kg/day one fetus showed forelimb flexure and the second malformed fetus had transposition of the great vessels, inter ventricular septal defect, small right ventricle, retroesophageal subclavian artery. Four fetuses with hydrocephaly were identified in the 25 mg/kg bw/d group in addition to two fetuses with peromelia (one with limb oligodactyly), one fetus had retroesophageal subclavian artery and cystic testes; one fetus had reduced stomach and small intestine and apparent absence of large intestine; and one fetus showed cebocephaly, facial bone malformations, umbilical hernia and absent kidney and ureter. At the top dose four fetuses had retroesophageal subclavian artery (one also with forelimb flexure); one fetus had dilated ascending aorta/aortic arch, narrow pulmonary trunk, and left ventricle; another fetus showed sacral scoliosis due to vertebral malformations, brachyury and caudal vertebral malformations; one fetus showed brachyury and caudal vertebral malformations, and another fetus had microphthalmia, reduced pinna, malformed facial bones, scoliosis and associated vertebral malformations, amelia and peromelia.
The presence of 4 hydrocephalies at 25 mg/kg bw/d was considered treatment-related. The presence of a single incidence of hydrocephaly at 4 mg/kg bw/d was considered to be an isolated finding without a dose-response relationship and in addition the incidence was covered by the hystorical control data provided.
The incidence of visceral anomalies was essentially comparable in all groups. At 62.5 mg/kg bw/d, the percentage of skeletal variations was significantly higher than control and other test groups, and was principally due to the incidence of cranial anomalies and cervical ribs. However, this conclusion may be questionable, since only about 30% of the fetuses survived at the top-dose. Although at 25 mg/kg bw/d the percentage of skeletal anomalies was similar to controls, it was noted that the incidences of sutural bones and cervical ribs were also higher than control, with no instances of either at 10 or 4 mg/kg bw/d. Slight intergroup variations in mean incidence of fetuses with extra (13) ribs or variants sternebrae did not suggest any adverse effect of treatment.

Table 5.6.2-11: Selected maternal and litter data of the main teratology study in rabbits with metconazole cis/trans (treatment during GD 7-19)

Dose level (mg/kg bw/d)	**0**	**4**	**10**	**25**	**62.5**
Maternal data					
Dams with live young/mated	12/16	15/16	11/17	11/16	10/16
Mortality	0	0	2 (1x found dead, probable dosing error, 1x sacrificed prior to treatment)	0	0
Abortions	1	0	1	0	3
Non-pregnant	3	1	3	5	1
Dams with total resorptions	0	0	0	0	2
Food consumption (% of control)					
• GD 7-8	-	-	-	-19%	-60%
• GD 7-19	-	-	-	-	-27%
Bodyweight gain (g)					
• GD 7-9	3	-10	-15	-37	**-142****
• GD 7-20	120	169	90	181	103
• GD 7-29	302	391	275	382	**125***
Litter observations					
Litter mean corpora lutea	11.3	10.3	11.1	10.6	11.4
Implantations	9.6	8.9	9.3	9.5	9.1
Pre-implantation loss (%)	8.0	13.7	14.2	11.2	19.5
Post-implantation loss (%)	15.5	7.5	21.4	15.0	**73.8*****
Early Resorptions	0.3	0.3	0.3	0.3	0.4
Late Resorptions	0.5	0.3	1.0	1.2	**4.3****
Live litter size	8.7	8.3	7.3	8.0	**2.8*****
Fetal weight [g (% of control)]	42.8	43.5	44.6	42.9	38.4 (-10%)
Litter weight [g (% of control)]	398.2	357.6 (-10%)	354.4 (-11%)	341.5 **(**-14%)	**156.2 (-61%)*****
Fetal malformations (% fetuses)	2/113 (1.8%)	9/124 (7.3%)	2/88 (2.3%)	9/88 (10.2%)	8/42 (19.0%)
Gross/visceral anomalies (% fetuses)	6/111 (5.4%)	7/115 (6.1%)	4/86 (4.7%)	3/79 (3.8%)	3/34 (8.8%)
Skeletal anomalies (% fetuses)	17/111 (15.3%)	20/115 (17.4%)	7/86 (8.1%)	13/79 (16.5%)	18/34 (**52.9****)
Hydrocephaly [foetuses affected (litter)]	0	1 (1)	0	4 (4)	0
Amelia/peromelia	0	0	0	2 (2)	1
Sutural bones	2 (2)	0	0	5 (3)	4 (3)
Connected jugal to maxilla	3 (3)	9 (4)	2 (2)	2 (2)	7 (3)
Cervical ribs	2 (1)	0	0	5 (3)	4 (3)

*p ≤ 0.05,**p ≤ 0.01, *** p ≤ 0.001

Historical control data (Interfauna, UK) of the testing facility from 1989-1994 (see DocID 2015/1228507):

- *Post-implantation loss (%): 3.6-17.5%, mean 10.8*
- *Early resorptions: 0.2-1.1, mean 0.5:*
- *Late resorptions: 0.1-1.3, mean 0.5*
- *Mean number of live young : 6.1-10.1, mean 8.4*
- *% malformed foetuses: 0.9%-7.5%, mean 3.0%*
- *Hydrocephaly: max. 1 fetus (1 litter)*

Conclusion:
The results obtained from this main study showed marked signs of maternal toxicity (reduced food consumption and reduced body weight gain, abortions) at the top dose of 62 mg/kg bw/d.
At this clear maternotoxic dose level, strong fetotoxic effects were observed (increased post-implantation loss (late resorptions), and associated decreased live litter size and reduced fetal/litter weights). Developmental toxicity at this top dose was evidenced by an increased incidence of structural defects (skeletal variations).
Maternotoxic effects were already observed to a smaller extent at 25 mg/kg bw/d as indicated by an initial slight decrease in food consumption and body weight gain. Thus, the maternal NOAEL was set at 10 mg/kg bw/d.
Slightly increased embryo/fetotoxic effects (post-implantation loss and late resorptions and reduced litter weight observed at 10 and 25 mg/kg bw/d, but live litter size and fetal weights was not affected. At 4 mg/kg bw/d litter weights were reduced to a similar extent than at the next two higher doses without any effect on post-implantation loss. Also, the values for post-implantation loss, late resorptions, and live litter size were well within the historical control range at all dose levels except for the high dose.

At 25 mg/kg bw/d developmental effects were evidenced by an increased incidence of hydrocephaly, which was clearly outside historical control ranges.
Since a single incidence of hydro/cebocephaly was also observed at 4 mg/kg bw/d in this main study, the study lab concluded that the possibility of a low incidence, but probably treatment-related effect in the lower dose-ranges could not be ruled out.
Nevertheless, the apparently increased incidence of the malformations and anomalies at the 4 mg/kg bw/d was not clearly associated with specific findings and these values as well as the single incidence of hydrocephaly is well within the historical control data of the test facility in the same source of animals (Interfauna). Thus, developmental NOAEL is set at 10 mg/kg bw/d in this study.
However, due to the uncertainty of a possibly treatment-related developmental effect at lower doses, an additional study was conducted (see additional study for further information/data).

Additional study (0, 2, 4 and 10 mg/kg bw/d):

Selected maternal and litter observations are presented in Table 5.6.2-12

Maternal data:
There were no obvious signs of reaction to treatment or clear dose-related differences in food consumption or bodyweight change with values for treated groups either similar or superior to control. At 10 mg/kg bw/d, dams had reduced weight gain on days 15 to 20. It is unclear whether this observation was attributable to effects of the test substance, since no dose-related decrease was observed. There were 3 mortalities, all considered to be unrelated to treatment; 2 occurred at 10 mg/kg bw/d and were due to probable intubation errors and the third rabbit died prior to the start of treatment. Incidences of occasional macroscopic findings did not suggest any association with treatment.

Litter data:
There were 3 incidences of abortions: 1 in the control group, another at 4 mg/kg bw/d and a third at 10 mg/kg bw/d. The incidence was the same in each group and is therefore not related to treatment. A high pre-implantation loss was observed in the control group with subsequent lower litter size.

The incidence of malformed fetuses was increased above control levels, i.e. 3/98, 5/116, 5/132 and 9/110 in control, 2, 4 and 10 mg/kg bw/d dose groups, respectively. Only at 10 mg/kg bw/d the incidence of malformed foetuses was above the historical control range. Severe types of malformations were seen in control animals as well as in the treated groups.
The malformations reported in the control group were one fetus with severe craniofacial defects including rhinencephaly, microcephaly, anophthalmia and associated skull defects, as well as aortic arch defects, interventricular septal defect, absent spleen and left adrenal, and complete situs inversus; one fetus with cranioschisis and ablepharia (open eye);, and one fetus with great vessel malformations and interventricular septal defect.

At 2 mg/kg/day one fetus showed cranioschisis with open eye, cleft palate, great vessel malformations, umbilical hernia, forelimb flexure, hind limb talipes and brachydactyly; one fetus showed termination of the vertebral column at sacral 2 (including anury); one fetus had brachyury; two other fetuses had great vessel malformations (one with interventricular septal defect and altered size of ventricles).
In the 4 mg/kg/day group two fetuses were reported to have lumbar scoliosis and associated vertebral changes; one fetus showed thoracic scoliosis and associated vertebral and rib changes; one fetus had and interventricular septal defect with a slightly dilated aortic arch, and another fetus showed diaphragmatic hernia.
One fetus in the 10 mg/kg/day group had a small eye and retinal dysplasia; one fetus showed lenticular degeneration; two fetuses were found with hydrocephaly, cebocephaly and associated skull defects (one with interventricular septal defect and retroesophageal subclavian artery); another fetus also showed retroesophageal subclavian artery; two fetuses had great vessel malformations and interventricular septal defects; one fetus showed umbilical hernia, cervical and thoracic scoliosis and associated axial skeletal malformations, and another fetus had forelimb flexure.

The slightly increased incidence of hydrocephaly at the top dose was just outside the reported historical control range of the test facility, and thus a treatment-related effect cannot be excluded. Although the incidence of visceral anomalies at 10 mg/kg bw/d was higher than control, principally due to the occurrence of corneal or lenticular opacities, no such findings have been observed in any other rabbit studies, and were therefore considered of doubtful toxicological relevance. The percentage incidence of skeletal anomalies was unrelated to treatment.
Slight intergroup variations in mean incidence of fetuses with extra (13) ribs or variants sternebrae did not suggest any adverse effect of treatment.

Table 5.6.2-12: Selected maternal and litter data of the additional teratology study in rabbits with metconazole cis/trans (treatment during GD 7-19)

Dose level (mg/kg bw/d)	**0**	**2**	**4**	**10**
Maternal data				
Dams with live young/mated	16/18	17/19	16/18	16/19
Mortality	0	1(died prior to treatment)	0	2 (intubation errors)
Abortions	1	0	1	1
Non-pregnant	1	1	1	0
Litter observations				
Litter mean corpora lutea	9.4	10.8	10.4	10.4
Implantations	7.0	8.8	9.4	8.3
Pre-implantation loss (%)	26.0	18.5	11.6	18.8
Post-implantation loss (%)	14.1	19.3	11.8	16.6
Early Resorptions	0.4	1.2	0.3	0.8
Late Resorptions	0.4	0.7	0.9	0.6
Live litter size	6.1	6.8	8.3	6.9
Fetal weight (g)	47.6	45.3	44.6	44.5
Litter weight (g)	281.9	300.5	356.7	298.0
Fetal malformations (% fetuses)	3/98 (4.5%)	5/116 (6.6%)	5/132 (4.0%)	9/110 (9.5%)
Gross/visceral anomalies (% fetuses)	2/95 (4.2%)	9/111 (9.8%)	6/127 (4.7%)	14/101 (12.9%)
Skeletal anomalies (% fetuses)	10/95 (12.9%)	18/111 (15.3%)	15/127 (10.5%)	13/101 (14.0%)
Hydrocephaly [foetuses affected (litter)]	0	0	0	2 (2)

Historical control data (Interfauna, UK) of the testing facility from 1989-1994 (see DocID 2015/1228507):

- *Post-implantation loss (%): 3.6-17.5%, mean 10.8*
- *Early resorptions: 0.2-1.1, mean 0.5:*
- *Late resorptions: 0.1-1.3, mean 0.5*
- *Mean number of live young : 6.1-10.1, mean 8.4*
- *% malformed foetuses: 0.9%-7.5%, mean 3.0%*
- *Hydrocephaly: max. 1 fetus (1 litter)*

Conclusion:

Based on the results of the additional study, the NOAEL for maternal and fetal toxicity is suggested at 10 mg/kg bw/d (top dose), the NOAEL for developmental toxicity was set at 4 mg/kg bw/d due to a slightly increased incidence of hydrocephaly at the top dose.

General conclusion of main and additional studies:

The findings in the additional study (Part II) were inconsistent with the previous study (Part I), although both similarities and differences from the earlier main study were observed at 4 and 10 mg/kg bw/d.

Overall, incidence of malformed fetuses at 10 mg/kg bw/d was higher than seen at the same dose in the first study (9.5% fetuses/ 37.5% litters compared to 3.8% fetuses/ 18.2% litters). Conversely, the number of malformations at 4 mg/kg bw/d was lower than in the previous study (4.0% fetuses/ 31.3% litters compared to 7.5% fetuses/ 46.7% litters). Control rates were similar, although no craniofacial malformations were noted in the previous study (Part I) or the preliminary study, whereas several severe craniofacial defects were seen in two control fetuses in the additional study. Craniofacial defects were clearly not increased above controls except perhaps at the top dose. Two fetuses at 10 mg/kg/day in the additional study and one fetus at 4 mg/kg/day in the earlier main study showed hydrocephaly associated with cebocephaly.

Considering both the main and the additional study, clear fetotoxic/developmental effects of metconazole cis/trans were observed at maternotoxic doses. Maternal toxicity was obvious at 25 mg/kg bw/d and above (decreased feed consumption and bw gain). At 25 mg/kg, an increased incidence of hydrocephaly and amelia/peromelia was found, whereas no such findings were recorded at the top dose or next-lower dose. At 62 mg/kg bw/d (top-dose) post-implantation loss associated with drastically reduced litter size was observed, which may have precluded a proper interpretation of occurring malformations. In the additional study a slightly increased incidence of hydrocephaly was detected at 10 mg/kg bw/d (but not in the main study at the same dose level). At the lower doses, (0, 2 and 4 mg/kg bw/d), no relevant increase of any malformation was detected.

Taken together, the dose of 10 mg/kg bw/d was considered a NOAEL for maternal toxicity and fetal toxicity. The NOAEL for developmental toxicity was established 4 mg/kg bw/d based upon a slightly increased incidence of post-implantation loss (late resorptions) in the main study and a slightly increased incidence of hydrocephaly in the additional study at 10 mg/kg bw/d.

For both studies (the main study Part I as well as the additional study Part II) the same batch 89-01 was used. When comparing the impurity profile of this batch to the batch used in the most recent developmental toxicity, where no relevant developmental effects were observed up to 40 mg/kg bw/d, one major significant difference in the amount of one impurity was observed. Impurity No. [redacted] (for details please refer to confidential document JCA) was present at about 96-fold higher levels in batch 89-01 as compared to batch AC105575-61. This impurity was even contained at 3-fold higher amounts above the specified impurity limit in batch 89-01. Thus, it cannot be excluded that the presence of this impurity at high amounts may be responsible for the effects seen in these two older main studies (but not in the newer developmental toxicity study in rabbits) and may also account for the non-reproducible results at the same dose levels in the two studies. Therefore, overall, the toxicological relevance of the effects seen at these two studies are considered questionable.

Teratology study in the Rabbit, oral (gavage) following exposure to metconazole cis (WL136148) from gestations days 7-19 ([redacted] 1992a;MK-432-007)

Material and Methods:

Preliminary study (0, 2, 4, 10, 25, and 40 mg/kg bw/d)

In a preliminary study 8 presumed pregnant NZW rabbits (Interfauna, UK Ltd.) per dose received metconazole cis (WL136248 , 95.2% purity, batch 12) in 1% methylcellulose by gavage at dose levels of 0, 2, 4, 10, 25 and 40 mg/kg bw/d from d7 to d19 of gestation inclusive. Dosing volume was 2 mL/kg bw. On d29 of pregnancy, the females were sacrificed and subjected to post-mortem examination. Litter parameters were determined and fetuses were examined for visceral and subsequently for skeletal abnormalities.

Main study (0, 2, 4, 10, and 40 mg/kg bw/d)

In the main study 16 presumed pregnant NZW rabbits (Interfauna, UK Ltd.) per dose received metconazole cis (WL136248 , 94.2% purity, batch 12) in 1% methylcellulose by gavage at dose levels of 0, 2, 4, 10 and 40 mg/kg bw/d from d7 to d19 of gestation inclusive. Dosing volume was 2 mL/kg bw. On d29 of pregnancy, the females were sacrificed and subjected to post-mortem examination. Litter parameters were determined and fetuses fixed prior to examination for skeletal or visceral abnormalities.

Findings:

Preliminary study (0, 2, 4, 10, 25 and 40 mg/kg bw/d):

Maternal data:
There were no abortions or total litter resorptions reported for any of the dose groups. With regard to clinical signs an increased incidence of cold ears post-dosing, occurring intermittently, was observed at the top dose. At 25 mg/kg bw/d this effect was mainly observed during the first week of treatment.
The 40 mg/kg/day dose was associated with clear maternal toxicity. There was significantly reduced food consumption during the initial treatment period, with recovery to levels higher than control over the remainder of the study. Body weight and body weight gain were slightly reduced during the initial treatment period and generally recovered over the remainder of the study, although there was a reduced body weight gain during the last days of the study (GDs 24-29). At 25 mg/kg/day, there was similar response pattern for food consumption and a slight initial bodyweight loss over the first 3 days of dosing, with an essentially similar gain to the control group thereafter.

Litter data:
Embryo/fetal death and post-implantation loss were significantly increased, and live young and litter weight were significantly reduced at 40 mg/kg/day. Mean fetal weight was slightly lower than control at this dose, but it did not show statistical significance. At 25 mg/kg/day, post-implantation loss appeared to be increased and live young and litter weight appeared to be reduced, but without any statistical significance.
Although this was a preliminary study fetuses in this study were examined for external, visceral and skeletal defects. The incidence of malformed fetuses was 1/71, 4/71, 2/50, 3/55, 3/47, and 3/31 (1/8, 2/7, 1/5, 3/6, 2/7 and 3/8 litters affected) in the control, 2, 4, 10, 25, and 40 mg/kg/day dose groups, respectively. The incidence of malformations was somewhat higher than control at all dose levels
Malformations reported in fetuses were one fetus with retroesophageal subclavian artery in the control group. At 2 mg/kg/day one fetus had retroesophageal subclavian artery; one fetus showed great vessel malformations; two fetuses were found with forelimb flexure (one with umbilical hernia). In the 4 mg/kg/day group one fetus showed retroesophageal subclavian artery and another fetus thoracic scoliosis and associated vertebral and rib defects. At 10 mg/kg/day one fetus had hindlimb brachydactyly; one fetus showed microphthalmia, and one fetus had hydrocephaly, facial bone malformations and great vessel malformation. In the 25 mg/kg/day group three fetuses were reported with hydrocephaly. At the top dose of 40 mg/kg/day three fetuses showed limb defects, one with peromelia, one with forelimb flexure and brachydactyly, and one with hindlimb brachydactyly.
A higher incidence of fetuses with peromelia/brachydactyly, liver anomalies and skeletal changes was noted at 40 mg/kg bw/d. At 25 mg/kg bw/d, there was a higher number of fetuses with hydrocephaly, which was outside the historical control range. At 10 mg/kg bw/d, 1 fetus had hydrocephaly (within historical control data), and 1 fetus of a separate litter had brachydactyly, but again the relevance of single excursions was questioned.
At 25 mg/kg bw/d and above slightly higher numbers of liver variations (including abnormal lobation, subcapsular cysts, pale subcapsular areas, and necrotic lobe) and skeletal variations were observed.

Table 5.6.2-13: Selected maternal and litter data of the preliminary teratology study in rabbits with metconazole cis (treatment during GD 7-19)

Dose level (mg/kg bw/d)	**0**	**2**	**4**	**10**	**25**	**40**
Maternal data						
Dams with live young/mated	8/8	7/8	5/8	6/8	7/8	8/8
Non-pregnant	0	1	3	2	1	0
Food consumption (% of control)						
• GD 7-8	-	-	-	-	-11%	**-29%***
• GD7-19	-	-	-	-	-7%	-12%
Body weight gain [g]						
• GD 7-9	-20	10	1	49	-27	-72
• GD7-19	171	228	221	337	178	230
Litter observations						
Litter mean corpora lutea	11.1	12.3	11.2	11.3	9.7	11.1
Implantations	9.5	11.4	10.8	9.8	8.7	9.0
Pre-implantation loss (%)	14.8	7.1	3.7	10.1	10.5	18.7
Post-implantation loss (%)	6.5	10.7	8.1	6.6	21.0	**58.0****
Early Resorptions	0.4	0.6	0.5	0.3	0.9	0.5
Late Resorptions	0.3	0.7	0.4	0.3	1.1	**4.6****
Live litter size	8.9	10.1	10.0	9.2	6.7	**3.9****
Fetal weight [g(% of control)]	44.7	42.4	43.9	43.7	**46.4**	41.1 (-8%)
Litter weight [g (% of control]	392.4	422.3	432.5	396.7	306.7 (-22%)	**155.8 (-60%)****
Fetal malformations (% fetuses)	1/71 (1.4%)	4/71 (5.6%)	2/50 (4.0%)	3/55 (5.5%)	3/47 /6.4%)	3/31 (9.7%)
Gross/visceral anomalies (% fetuses)	1/70 (1.4%)	4/67 (6.0%)	4/48 (8.3%)	2/52 (3.8%)	3/44 (6.8%)	**6/28 (21.4%)***
Skeletal anomalies (% fetuses)	11/70 (*15.7%*)	14/67 (*20.9%*)	5/48 (*10.4%*)	8/52 (*15.4%*)	13/44 (*29.5%*)	12/28 (*42.9%*)
Visceral and skeletal malformations						
Hydrocephaly	0	0	0	1 (1)	3 (2)	0
Forelimb flexures	0	2 (2)	0	0	0	1 (1)
Peromelia	0	0	0	0	0	1 (1)
Brachydactyly	0	0	0	1 (1)	0	2 (2)
Visceral anomalies:						
Liver: Total affected	0	0	1 (1)	0	3 (2)	5 (3)
Skeletal anomalies:						
Sutural bones	1	2	0	1	1	1
Connected jugal to maxilla	6	4	3	0	3	6
Cervical ribs	0	9	0	0	4	3

Historical control data (Interfauna, UK) of the testing facility from 1989-1994 (see DocID 2015/1228507):

- *Post-implantation loss (%): 3.6-17.5%, mean 10.8*
- *Early resorptions: 0.2-1.1, mean 0.5:*
- *Late resorptions: 0.1-1.3, mean 0.5*
- *Mean number of live young : 6.1-10.1, mean 8.4*
- *% malformed foetuses: 0.9%-7.5%, mean 3.0%*
- *Hydrocephaly: max. 1 fetus (1 litter)*

Conclusion:
At 25 and 40 mg/kg bw/d after treatment with metconazole 95% cis, maternal toxicity was observed as well as an increased post implantation loss (late resorptions) resulting in reduced litter size/weight. The incidence of fetuses with hydrocephaly and/or limbs malformations was increased at maternotoxic doses. However, due to the relatively few litters examined and the inconsistency of the pattern of malformations (the most severely malformed fetus was in the 10 mg/kg bw/d group), it is inappropriate to draw a causal relationship for these malformations.

Main study (0, 2, 4, 10, 40 mg/kg bw/d)

Maternal data:
There were no abortions or total litter resorptions reported for any of the dose groups.
With regard to clinical sings 7/12 pregnant animals had cold ears post dosing on at least 3 days during the treatment period at the top dose compared to 2/14 control animals. This finding was considered to be a non-specific sign.
The 40 mg/kg/day dose was associated with clear maternal toxicity. There was significantly reduced food consumption during the initial treatment period that recovered to levels equal to control over the remainder of the study. Food consumption at 10 mg/kg bw/d was slightly reduced from the onset of treatment, attaining statistical significance during days 11-14. Thereafter, intake at this dose was essentially similar to the control group.
At 10 mg/kg bw/d and above, there was an initial dose-related bodyweight loss (d7-9) which attained statistical significance .At 40 mg/kg bw/d body weight gain did not recover to the end of the study. The lower body weight gain during gestation days 24-29 was probably due to a general reduction of litter weight observed at termination. No relevant findings were observed at necropsy.

Litter data:
There were no total litter losses at any dose.
At the top-dose embryo/fetal death and post-implantation loss were significantly increased. Number of live young, litter weights and mean fetal weight were lower than control at this dose, but it did not show statistical significance.
The incidence of malformed fetuses was 1/109, 3/133, 2/135, 5/139 and 9/69 (1/14, 3/16, 2/16, 4/14 and 6/12 litters affected) in control, 2, 4, 10 and 40 mg/kg bw/d, respectively. The incidence of malformations in the 40 mg/kg/day dose group was increased above controls, but there was lack of a clear dose-response relationship at lower doses. Furthermore, percent of malformed foetuses was within the historical control range only at the top dose.
The detailed types of malformations observed in the fetuses were observed as follows:
One fetus one fetus with cervical meningocoele was found in the control group. At 2 mg/kg/d one fetus had umbilical hernia and forelimb flexure; one fetus showed acephaly, transposition of the great vessels, mal-rotated heart, interventricular septal defect, retroesophageal subclavian artery, split sternum, spina bifida, forelimb flexure, oligodactyly and brachydactyly and another fetus showed interventricular septal defect, split sternum with umbilical hernia, forelimb flexure, oligodactyly, absent humerus and radius, reduced scapula. In the 4 mg/kg/d group one fetus was reported with brachyury and one fetus with great vessel malformations and altered size of ventricles.

One fetus with brachydactyly; two fetuses with forelimb flexure; one fetus with hydranencephaly with domed cranium and one fetus with absent gonads were found at 10 mg/kg/d. At the top dose of 40 mg/kg/d three fetuses had hydrocephaly, one with partially fused parietals, one with misshapen frontals and parietals; one fetus shoed absent spleen; two fetuses had thoracic scoliosis and associated vertebral and rib malformations; two fetuses showed retroesophageal subclavian artery, and one fetus had forelimb flexure.

The types of defects at 2 mg/kg/day were some of the most severe findings in this study as evidenced by two fetuses with multiple severe malformations. Affected malformed fetuses in the 40 mg/kg/day group had individual malformations. Thus, the severity of defects did not seem to follow a dose-related pattern in this main study.
Of note were the incidences of hydrocephaly at 40 mg/kg bw/d and shortened/absent limbs/digits at 2 and 10 mg/kg bw/d, but the latter finding was obviously without dose-effect relationship. There was also an increased proportion of fetuses with both visceral and skeletal anomalies at 40 mg/kg bw/d, mainly due to abnormal liver lobulation and various non-specific skeletal anomalies. The observed liver abnormalities at the intermediate doses were of questionable relevance in the absence of any dose-related appearance. However, the incidence of fused liver lobes at the top-dose was not neglected as it was also present in the preliminary study. Although liver morphology is quite variable, the effect of treatment was considered pertinent. The slight intergroup variations in mean incidence of fetuses with extra (13) ribs or variants sternebrae did not suggest any adverse effect of treatment.

Table 5.6.2-14: Selected maternal and litter data of the 1st main teratology study in rabbits with metconazole cis (treatment GD 7-19)

Dose level (mg/kg bw/d)	**0**	**2**	**4**	**10**	**40**
Maternal data					
Dams with live young/mated	14/16	16/16	16/16	14/16	12/16
Non-pregnant	2	0	0	2	4
Food consumption (% of control)					
• GD 7-8	163	158	153	151 (-7%)	**93 (-43%)****
• GD11-14	164	164	156	**143 (-13%)****	**143 (-13%)****
• GD 24-28	132	138	123	**117**	**126**
Body weight [g]					
• GD 9	3824	3832	3742	**3746 (-2%)****	**3641**(-5%)**
• GD11	3846	3855	3763	**3778 (-2%)***	**3707 (-4%)****
• GD 29	4198	4283	4156	4165	**4026 (-4%)***
Body weight gain [g]					
• GD 7-9	43	14	29	-15	-116
• GD 7-20	211	247	226	174	117
• GD 24-29	416	464	442	404	269
Litter observations					
Litter mean corpora lutea	10.3	11.6	11.8	12.6	11.8
Implantations	8.7	9.5	9.4	11.1	9.8
Pre-implantation loss (%)	15.3	16.4	19.8	10.5	15.6
Post-implantation loss (%)	9.9	11.0	10.7	10.3	**40.7****
Early Resorptions	0.4	0.6	0.6	0.4	0.4
Late Resorptions	0.5	0.6	0.4	0.8	**3.7****
Live litter size	7.8	8.3	8.4	9.9	5.8
Fetal weight (g)	45.7	45.0	44.7	42.0 (-8%)	42.1 (-8%)
Litter weight (g)	349.7	368.4	368.5	415.8	237.6 (-32%)

Dose level (mg/kg bw/d)	0	2	4	10	40
Maternal data					
Fetal malformations (% fetuses)	1/109 (0.9%)	3/133 (2.3%)	2/135 (1.5%)	5/139 (3.6%)	**9/69 (13.0%)****
Gross/visceral anomalies (% fetuses)	8/108 (7.4%)	15/130 (11.5%)	12/133 (9.0%)	8/134 (6.0%)	12/60 (20.0%)
Skeletal anomalies (% fetuses)	23/108 (21.3%)	20/130 (15.4%	18/133 (13.5%)	23/134 (17.2%)	21/60 (35.0%)
Visceral and skeletal malformations					
Hydrocephaly	0	0	0	0	3 (3)
Hydranencephaly	0	0	0	1 (1)	0
Forelimb flexures	0	3 (3)	0	2 (2)	1 (1)
Oligodactyly	0	2 (2)	0	0	0
Brachydactyly	0	1 (1)	0	1 (1)	0
Visceral anomalies:					
Liver: Total affected	2 (2)	3 (3)	3 (2)	2 (2)	7 (5)
Skeletal anomalies:					
Sutural bones	2 (2)	2 (2)	6 (4)	3 (3)	3 (2)
Connected jugal to maxilla	4 (4)	12 (7)	5 (3)	11 (4)	7 (4)
Irregular ossification cranial bones	1 (1)	0	1 (1)	0	5 (5)
Cervical ribs	7 (4)	1 (1)	1 (1)	3 (2)	0

,**$p \leq 0.01$

Historical control data (Interfauna, UK) of the testing facility from 1989-1994 (see DocID 2015/1228507):

- *Post-implantation loss (%): 3.6-17.5%, mean 10.8*
- *Early resorptions: 0.2-1.1, mean 0.5:*
- *Late resorptions: 0.1-1.3, mean 0.5*
- *Mean number of live young : 6.1-10.1, mean 8.4*
- *% malformed foetuses: 0.9%-7.5%, mean 3.0%*
- *Hydrocephaly: max. 1 fetus (1 litter)*

Conclusion:

Maternal toxicity (decreased food consumption, bw and bw gain) was evidenced dose related at 10 mg/kg bw/d and above after administration of metconazole 95% cis to pregnant rabbits during GD 7-19, leading to a maternal toxicity NOAEL at 4 mg/kg bw/d. Fetal toxicity was evidenced by a significant increase in post-implantation loss (late resorptions) and associated decreased littzer size, reduced litter and fetal weight at the top dose. The fetal toxicity NOAEL was established at 10 mg/kg bw/d. Contrary to the previous evaluation in the monograph (NOAEL was set at 4 mg/kg bw/d), the developmental NOAEL is set at 10 mg/kg bw/d due to an increased incidence of hydrocephaly at the top dose. The percent of malformed fetuses was outside the historical control range only at the top dose, and the single incidence of one hydranencephaly with domed head at 10 mg/kg bw/d was not considered treatment related.

Executive summary of the analytical method used within the analytical phase (MK-432-007)

Principle of the method
The analytical method used in study MK-432-007 was validated for the determination of metconazole (BAS 555 F) in 1% methylcellulose. The study was performed by HUNTINGDON RESEARCH CENTRE LTD., UK.
Residues were extracted from 1% aqueous methylcellulose by dissolving a representative sub-sample in acetone. The extract was further diluted using acetone and a suitable volume was evaporated (T = 40°C) to dryness. The residue was redissolved in toluene. Residues were analyzed by gas-liquid chromatography (GLC) with alkali-flame ionization (FID). Analysis was performed on a DB-1701 GC column.

Recovery findings
The mean recovery values were between 70% and 110% for metconazole. The detailed results are given in the table below.

Table 5.6.2-15: Validation results of the analytical method applied in study MK-432-007using GLC-FID: metconazole (BAS 555 F) in 1% aqueous methylcellulose

Matrix	Analyte	Detector settings	Fortification level (mg/mL)	No of replicates	Mean recovery (%)	RSD (%)
Aqueous methylcellulose	Metconazole	Alkali-flame ionization, 300°C	1.0	10	101	1.6
			20.0	10	100	3.5
			Overall	20	101	2.7

Linearity
The linearity was tested using four standards at concentrations between of 4 to 20 µg/mL. For BAS 555 F, linear correlations were obtained. Calibration solutions were prepared in toluene.

Specificity
Final detection has been applied by alkali-flame ionization detection (FID). As only known analytes of known concentration were analyzed for dose verification purposes, no additional confirmatory technique is necessary.

Interference
No relevant interferences from the reagent blank at the relevant retention times have been reported.

Limit of Quantitation
The limit of quantitation (LOQ) is defined as the lowest fortification level successfully tested. During this study, a limit of quantitation of 1.0 mg/mL for BAS 555 F was obtained.

Limit of Detection
For BAS 555 F, the limit of detection (LOD) was 0.04 mg/mL.

Repeatability
The relative standard deviations (RSD, %) for all fortification levels were below 20%.

Stability of solutions
The chemical and physical stability of the analyte in freshly prepared specimen solutions was tested within this study. Results indicated that the analyte was chemically stable in solution for at least 24 hours when stored at ambient temperature during the day and + 4°C overnight. Furthermore, results revealed that metconazole produces a homogeneous suspension in 1% aqueous methylcellulose, which can be maintained for up to 1 hour while magnetically stirred and successfully resuspended following storage in the dark at ambient temperature for 4 hours.

Conclusion:
The analytical method used in study MK-432-007 for the analysis of metconazole in 1% aqueous methylcellulose uses GLC-FID for final determination, with an LOQ of 1.0 mg/mL.
It could be demonstrated that the method generally fulfils the requirements with regard to repeatability, specificity, LOQ and recoveries and is therefore considered suitable for the analysis of metconazole in 1% aqueous methylcellulose.

Second Teratology study in the Rabbit, oral (gavage) following exposure to metconazole cis (WL136148) from gestations days 7-19 ([redacted] 1992b; MK-432-010)

Material and Methods:
According to the test facility, the conduct of the present study was justified because incidences of brachydactily/oligodactily have been observed in the previous developmental study at 2 mg/kg bw/d (but not at higher doses).
18/19 presumed pregnant NZW rabbits (Interfauna, UK Ltd.) per dose received metconazole cis (95.2% puritiy, batch 12) in 1% methylcellulose by gavage at dose levels of 0, 0.5, 1, 2, 10 and 40 mg/kg bw/d from d7 to d19 of gestation inclusive. Dosing volume was 2 x 2 mL/kg bw, separated as two equal doses. On day 29 of pregnancy, the females were sacrificed and subjected to post-mortem examination. Litter parameters were determined and fetuses fixed prior to examination for skeletal or visceral abnormalities. The study is considered to provide additional information.

Findings:
<u>Maternal data:</u>
There were no relevant findings on mortality and abortions. No treatment-associated clinical signs were observed.
At the top dose of 40 mg/kg bw/d there was a marked and significantly reduced food consumption during the initial treatment period that recovered to levels greater than control by the end of the study. Following initiation of treatment at 40 mg/kg bw/d, there was a bodyweight loss to day 9, which affected all animals and attained statistical significance. Thereafter, good recovery occurred and weight gain throughout the remaining period of treatment was generally superior to that of the controls. Between days 24 to 29, bodyweight gain was again slightly reduced at this dose, however, this was probably due to a general reduction of litter weight observed at termination. Necropsy revealed no findings related to treatment.

Litter data:
Embryo/fetal death and post-implantation loss were increased and litter weight and mean fetal weight were significantly decreased at 40 mg/kg/day. The number of live young was lower than control at this dose, but without statistical significance.
The incidence of malformed fetuses was 7/152, 5/160, 1/156, 3/139, 9/165 and 18/126 (7/18, 5/17, 1/17, 3/17, 6/18 and 11/16 litters affected) in control, 0.5, 1, 2, 10 and 40 mg/kg bw/d, respectively. The control incidence of malformed fetuses was high so that only the 40 mg/kg/day dose group was clearly increased above control levels. All percent of malformed fetuses were within the historical control range, except for the top dose.
The types of defects reported for the individual foetuses were as follows:
In the control group two fetuses were found with retroesophageal subclavian artery; two fetuses with great vessel malformations and interventricular septal defects; one fetus with interventricular septal defect and lumbar scoliosis and associated vertebral malformation; one fetus with hydrocephaly and cebocephaly with associated facial bone malformations, and one fetus with misshapen naso-frontal region of the cranium, cleft lip and palate. In the 0.5 mg/kg/d group two fetuses had great vessel malformations, one with an interventricular septal defect; two other fetuses showed retroesophageal subclavian artery, and one fetus had spina bifida and malrotated hindlimbs. One fetus with hydrocephaly and cebocephaly with associated facial bone malformations was reported at 1 mg/kg/day. At 2 mg/kg/day two fetuses showed great vessel malformations, one with interventricular septal defect, brachyury, and rib malformations, and another fetus had forelimb flexure. Five fetuses at 10 mg/kg/d were reported with great vessel malformations, two with interventricular septal defect, one with retroesophageal subclavian artery and interventricular septal defect and altered size of ventricles and malrotated heart, and one with interventricular septal defect and forelimb flexure; one fetus showed multiple malformations including termination of the vertebral column at T6, umbilical hernia, absent adrenal, absent gonads, and malrotated hindlimbs; one fetuses had an retroesophageal aortic arch; two fetuses showed small eye and retinal dysplasia, one with hydrocephaly.
At the top dose of 40 mg/kg/d two fetuses were found with limb defects, one with ectrodactyly, one with brachydactyly; two fetuses showed retroesophageal subclavian artery; one fetus had multiple malformations including acephaly, cervical scoliosis with associated skeletal defects, great vessel malformations, gastroschisis, partially split sternum, and forelimb flexure; one fetus had hydrocephaly; two fetuses showed umbilical hernia; three other fetuses had forelimb flexure; one fetus showed small pinna; one fetus had vertebral and rib malformations and brachyury; two fetuses had great vessel malformations, retroesophageal subclavian artery and interventricular septal defect, one of which also had kidney defects and forelimb flexure; another fetus had spina bifida, scoliosis, kidney defects, and brachyury; one fetus showed microphthalmia and retinal dysplasia; and onother fetus displayed the intestine adhered to the liver and gall bladder.

Overall, the incidence of malformed fetuses at 40 mg/kg bw/d was more than twice that seen in the control group and apart from 5 fetuses in separate litters with forelimb flexure, there was no consistent pattern in the type of changes observed. In this study, there were 4 instances of hydrocephaly, 1 each at 40, 10 and 1 mg/kg bw/d and 1 in the control group. These single cases of hydrocephaly were within the historical control data. Hydrocephaly at 1 mg/kg bw/d and the control group was associated with cebocephaly, a combination occasionally seen in historic control data. However, as the incidences were the same as that of the control, a relationship to treatment in the context of this study is not conclusive.

Further 2 fetuses showed marked digit reductions, both at 40 mg/kg bw/d; similar observations have been made previously at this dose. There were no limb/digit reductions seen at 10 or at either of the lower doses.
Although the percentage incidence of fetuses with visceral anomalies at 40 mg/kg bw/d was slightly (non-statistically) higher than the control value, there is no clear evidence of any specific visceral change that could be considered a response to treatment. The previously reported increase in liver variations was not replicated. At 40 mg/kg bw/d and, to a lesser extent, 10 mg/kg bw/d there was a higher number of fetuses with skeletal anomalies, attaining statistical significance at the highest dose, primarily reflecting an increased incidence of connected jugal to maxilla, a change previously present, but not specifically associated with treatment.

Table 5.6.2-16: Selected maternal and litter data of the 2nd main teratology study in rabbits with metconazole cis (treatment GD 7-19)

Dose level (mg/kg bw/d)	**0**	**0.5**	**1**	**2**	**10**	**40**
Maternal data						
Dams with live young/mated	18/18	17/18	17/18	17/19	18/18	16/19
Non-pregnant	0	0	1	1	0	2
Food consumption [g (% of control)]						
• GD 7-8	134	144	131	144	143	**96 (-29%)****
• GD7-19	1867	1836	1763	1852	1956	**1648 (-12%)***
Body weight gain [g]						
• GD 7-9	-12	-6	4	-10	8	-109
• GD7-20	222	165	181	216	201	141
Litter observations						
Litter mean corpora lutea	10.8	11.8	11.8	11.6	11.9	11.6
Implantations	9.1	10.1	10.1	10.0	10.3	10.1
Pre-implantation loss (%)	15.9	13.4	14.2	12.1	13.0	12.2
Post-implantation loss (%)	7.1	6.4	8.6	17.1	11.5	21.1
Early Resorptions	0.3	0.2	0.5	1.5	0.7	0.4
Late Resorptions	0.4	0.5	0.4	0.3	0.5	1.8
Live litter size	8.4	9.4	9.2	8.2	9.2	7.9
Fetal weight (g)	45.7	44.1	42.9	43.9	44.0	**39.8 (-13%)****
Litter weight (g)	380.0	412.9	388.9	356.6	399.1	**306.3 (-19%)***
Fetal malformations (% fetuses)	7/152 (4.6%)	5/160 (3.1%)	1/156 (0.6%)	3/139 (2.2%)	9/165 (5.5%)	18/126 (14.3%)
Gross/visceral anomalies (% fetuses)	18/145 (12.4%)	5/155 (3.2%)	11/155 (7.1%)	6/136 (4.4%)	12/156 (7.7%)	13/108 (12.0%)
Skeletal anomalies (% fetuses)	22/145 (15.2%)	17/155 (11.0%)	21/155 (13.5%)	17/136 (12.5%)	36/156 (23.1%)	**36/108 (33.3%)***
Visceral and skeletal malformations						
Hydrocephaly	1	0	1	0	1	1
cebocephaly	1	0	1	0	0	0
Forelimb flexure/malrotated hindlimb	0	1	0	1	2 (2)	5 (5)

Visceral anomalies:						
Liver: Total affected	4(4)	2(2)	2(2)	1	1	3(3)
Skeletal anomalies:						
Sutural bones	4(2)	2(2)	5(3)	3(3)	6(5)	3(3)
Connected jugal to maxilla	7(4)	2(2)	2(2)	6(4)	11(7)	24(11)
Cervical ribs	2(2)	9(6)	4(2)	3(2)	2(2)	6(4)
Irregular ossification vertebrae	2(2)	3(3)	8(5)	2(2)	5(5)	9(6)

*p ≤ 0.05,**p ≤ 0.01

Historical control data (Interfauna, UK) of the testing facility from 1989-1994 (see DocID 2015/1228507):

- *Post-implantation loss (%): 3.6-17.5%, mean 10.8*
- *Early resorptions: 0.2-1.1, mean 0.5:*
- *Late resorptions: 0.1-1.3, mean 0.5*
- *Mean number of live young : 6.1-10.1, mean 8.4*
- *% malformed foetuses: 0.9%-7.5%, mean 3.0%*
- *Hydrocephaly: max. 1 fetus (1 litter)*

Conclusion:

Administration of 40 mg/kg bw/d metconazole 95% cis to pregnant rabbits during GD 7-19 was associated with maternal toxicity (markedly reduced food consumption and bodyweight gain), leading to a NOAEL for maternal toxicity at 10 mg/kg bw/d.

Fetal toxicity was observed at the maternotoxic top dose as evidenced by increased post-implantation loss (late resorptions), decreased litter and fetal weight. The NOAEL for fetal toxicity was thus set at 10 mg/kg bw/d. With regard to developmental toxicity at 10 and 2 mg/kg bw/d, 1 fetus in each of these two groups showed a similar structural defect as those seen at 40 mg/kg bw/d in this and previous studies. However, the findings are ambiguous and a relationship to treatment is not conclusive. The apparent increase of any skeletal anomaly at 10 mg/kg bw/d was not associated with specific changes. The only consistent malformation at the highest dose-level in this study, are the forelimb flexures/malrotated hindlimbs setting the NOAEL for developmental toxicity at 10 mg/kg bw/d.

General conclusions on the first and second experiment

In the first study, metconazole cis provoked maternal toxicity at 10 mg/kg bw and above, as illustrated by the decreased feed consumption, and decreased body weights. It was noted that, in the second experiment, these modifications were not observed at 10 mg/kg bw/d, but only at the top-dose. At the top-dose, both studies revealed an effect on fetal toxicity, with increased incidences of late embryonic deaths and post-implantation losses, and decreased litter size/weight and fetus weight. When comparing both maternal and fetal endpoints in the two experiments at 10 and 40 mg/kg bw/d, it appeared that the effect was more important in the first one. Accordingly, the incidence of hydrocephaly was increased at 10 and 40 mg/kg bw/d in the first experiment. Single incidences of hydrocephaly were observed at the lower doses in the second experiment. These single findings, as well as the appearance of 2 litters with oligodactyly at 2 mg/kg bw/d, were hardly interpreted as a result of treatment, in the absence of dose-responsiveness, or else replication in the concurrent study. It was also of note that the split-dose administration in the second study precluded a full comparison with the first experiment. In contrast, the appearance of hydrocephaly at higher doses was more consistent, as in the preliminary study (0, 2, 4, 10, 25 and 40 mg/kg bw/d), where increased incidences were observed from 10 mg/kg bw/d upwards (the absence at the top-dose in the preliminary study was explained by the highly reduced litter size).

Therefore, when taking into account the data of the first experiment, the maternal NOAEL was considered 4 mg/kg bw/d, based upon decreased feed consumption, bw, and bw gain. The fetal NOAEL was 10 mg/kg bw/d, based upon the embryonic deaths, increased post-implantation loss, and decreased litter size/weight and fetal weight. The developmental NOAEL is set at 10 mg/kg bw/d based upon the increased hydrocephaly incidence at the higher doses. During the last evaluation the NOAEL was set as low as 4 mg/kg bw/d in the monograph (2004). However, the single incidences of hydrocephaly seen at 10 mg/kg bw or lower doses were not considered treatment-related and are well within the historical control range of the test facility

Executive summary of the analytical method used within the analytical phase (MK-432-010)

Principle of the method

The analytical method used in study MK-432-010 was validated for the determination of metconazole (BAS 555 F) in 1% methylcellulose. The study was performed by HUNTINGDON RESEARCH CENTRE LTD., UK.

Residues were extracted from 1% aqueous methylcellulose by dissolving a representative sub-sample in acetone. The extract was further diluted using acetone and a suitable volume was evaporated (T = 40°C) to dryness. The residue was redissolved in toluene. Residues were analyzed by gas-liquid chromatography (GLC) with alkali-flame ionization (FID). Analysis was performed on a DB-1 GC column.

Recovery findings

The mean recovery values were between 70% and 110% for metconazole. The detailed results are given in the table below.

Table 5.6.2-17: Validation results of the analytical method applied in study MK-432-010 using GLC-FID: metconazole (BAS 555 F) isomers in 1% aqueous methylcellulose

Matrix	Analyte	Detector settings	Fortification level (mg/mL)	No of replicates	Mean recovery (%)	RSD (%)
Aqueous methylcellulose	Metconazole	Alkali-flame ionization detector, 300°C	0.125	10	98	4.2
			10	6	99	4.7
			Overall	16	98	4.3

Linearity

The linearity was tested using four standards at concentrations between of 4 to 20 µg/mL. For BAS 555 F, linear correlations were obtained. Calibration solutions were prepared in toluene.

Specificity

Final detection has been applied by alkali-flame ionization detection (FID). As only known analytes of known concentration were analyzed for dose verification purposes, no additional confirmatory technique is necessary.

Interference

No interferences from the reagent blank at the relevant retention times have been reported.

Limit of Quantitation
The limit of quantitation (LOQ) is defined as the lowest fortification level successfully tested. During this study, a limit of quantitation of 0.125 mg/mL for BAS 555 F was obtained.

Limit of Detection
For BAS 555 F, the limit of detection (LOD) was 0.005 mg/mL.

Repeatability
The relative standard deviations (RSD, %) for all fortification levels were below 20%.

Stability of solutions
The chemical and physical stability of the analyte in freshly prepared specimen solutions was tested within this study. Results indicated that the analyte was chemically stable in solution for at least 24 hours when stored at ambient temperature during the day and + 4°C overnight. Furthermore, results revealed that metconazole produces a homogeneous suspension in 1% aqueous methylcellulose, which can be maintained for up to 1 hour while magnetically stirred and successfully resuspended following storage in the dark (ambient temperature during the day, + 4°C overnight) for 6 and 24 hours.

Conclusion
The analytical method used in study MK-432-010 for the analysis of metconazole in 1% aqueous methylcellulose uses GLC-FID for final determination, with an LOQ of 0.125 mg/mL.
It could be demonstrated that the method generally fulfils the requirements with regard to repeatability, specificity, LOQ and recoveries and is therefore considered suitable for the analysis of metconazole in 1% aqueous methylcellulose.

Dermal developmental toxicity study in rabbits with metconazole cis/trans

A developmental study in rabbits via the dermal route (study author: [redacted] 2012; BASF DocID 2012/8000573) with metconazole cis/trans was conducted for non-EU authorities by another metconazole-supporting company (for the rationale please *[see KCA 5.6.2/9 2016/1025284]*. A detailed study summary was made available to BASF (prepared by [redacted] D.G, 2014a; BASF DocID 2015/1087908) and is part of this submission.

Report:	CA 5.6.2/6 [redacted] D.G., 2014a Summary of the Study Report WIL-870002: A dermal prenatal developmental toxicity study of Metconazole in rabbits 2015/1087908
Guidelines:	OECD 414 (2001)
GLP:	yes

Report:	CA 5.6.2/7 [redacted] D.G., 2012 a A dermal prenatal developmental toxicity study of Metconazole in rabbits 2012/8000573
Guidelines:	EPA 870.3700, OECD 414
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary

Groups of 25 time-mated New Zealand White rabbits [Hra: (NZW)SPF] were exposed dermally to metconazole (99.7% purity, cis/trans: 84.2% cis/15.5% trans, batch AS2122b) at doses of 30, 90, and 270 mg/kg bw/day, 6 hours per day from gestation days 6 through 28. Controls received the vehicle (water) alone. Termination and laparohysterectomy on Day 29 of gestation was followed by processing and detailed necropsy of dams and caesarean section as well as detailed external, visceral and skeletal examination of fetuses.

One of 25 does each in the 90 and 270 mg/kg/day groups aborted on gestation day 23 or 26 and 1 of 25 does in the 270 mg/kg/day group delivered prior to the scheduled necropsy on gestation day 29 following body weight losses (up to 20.5%) with corresponding reductions in food consumption and increased incidence of decreased defecation. The abortion and early delivery in the 270 mg/kg/day group were considered to be test substance-related but secondary to the effects on body weight and food consumption noted for surviving females at this exposure level. However, because similar effects on body weight and food consumption were not observed for other females at 90 mg/kg/day, the single abortion at this exposure level was not considered to be test substance-related, but was considered to be a random occurrence. All other females survived to the scheduled necropsy on gestation day 29; there were no test substance-related macroscopic findings noted at any exposure level. Clinical signs observed included decreased defecation in the 270 mg/kg bw/day group, corresponding to reduced mean food consumption. Test substance-related, adverse dermal observations, consisted of very slight to slight erythema, desquamation, and eschar for females in the 270 mg/kg/day group during the treatment period. In the 90 mg/kg/day group, test substance-related minimal dermal irritation (primarily very slight erythema) was noted during the treatment period.

However, due to the limited incidence and minimal severity of these findings, they were not considered to be adverse. No remarkable dermal observations were noted in the 30 mg/kg/day group. A test substance-related mean body weight loss following the initiation of treatment (gestation days 6-10) and a test substance-related lower mean body weight gain during the later portion of the treatment period (gestation days 20-29) were noted in the 270 mg/kg/day group, resulting in a lower mean body weight gain when the entire treatment period (gestation days 6-29) was evaluated. However, the body weight changes noted in this group during the whole treatment period were not of sufficient magnitude to produce a notable effect on mean body weights.
Mean food consumption in the 270 mg/kg/day group was lower than the control group throughout the treatment period. Mean net body weight, net body weight change, and gravid uterine weight in the 270 mg/kg/day group were similar to control group values. No test substance-related effects on maternal body weights, body weight gains, net body weights, net body weight gains, gravid uterine weights, or food consumption were noted in the 30 and 90 mg/kg/day groups. Intrauterine growth and survival and fetal morphology in the 30, 90, and 270 mg/kg/day groups were unaffected by test substance exposure.
In conclusion, 90 mg/kg bw/day was considered to be the NOAEL for maternal toxicity and 270 mg/kg bw/day was considered to be the NOAEL for embryo/fetal developmental toxicity after dermal application of metconazole to time-mated New Zealand White rabbits.

(DocID 2015/1087908)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: Metconazole

Description:	solid (powder) / white
Lot/Batch #:	AS 2122b
Purity:	99.7% (84.2% cis-isomer, 15.5% trans-isomer)

2. Vehicle and/or positive control: water (reverse osmosis-purified)

3. Test animals:

Species:	Rabbit
Strain:	New Zealand White [Hra:(NZW)SPF]
Sex:	Female
Age (at study initiation):	5.5 months
Weight (at study initiation):	2.915 – 4.036 kg
Source:	Covance Research Products, Inc., Denver, PA
Time of dosing:	Days 6 to 28 of gestation
Diet:	ad libitum
Water:	ad libitum
Housing:	Individually in clean, stainless steel cages

Environmental conditions:

Temperature:	18.7 – 18.9°C
Humidity:	42 - 58%
Air changes:	10 per hour
Photo period:	12 h light / 12 h dark

B. STUDY DESIGN AND METHODS

1. Dates of experimental work: 01-Nov-2011 to 02-Dec-2011 (in life dates)

2. Animal assignment and treatment:
Groups of 25 time-mated animals were selected by computerized randomization procedure based on body weight data. The vehicle or test substance was applied topically with a metal spatula to the approximate center of the clipped, unabraded skin of each animal and distributed to avoid running once daily (for a period of approximately 6 hours) during gestation days 6 - 28. The total percentage of body surface to which the test substance was applied was to be approximately 10% of the total body surface area. The applied doses included 0, 30, 90, and 270 mg/kg bw/day. The doses were selected based on a previous dose range-finding study with 3 groups of 5 bred female rabbits receiving 0, 250, 500, and 1000 mg/kg bw/day from gestation day 6 through 28. Body weight loss, reduced food consumption and a high incidence of early resorptions were observed in the 1000 mg/kg bw/day group. In addition, one rabbit was euthanized *in extremis* because of a large body weight loss. In the 500 mg/kg/day group, 1 rabbit was euthanized *in extremis*, 1 rabbit aborted, and 1 rabbit delivered on gestation day 29. All rabbits of the 250 mg/kg bw/day group survived to the scheduled laparohysterectomy. Lower body weight gains and food consumption relative to the control group were observed in the 250 and 500 mg/kg bw/day groups. One of the 2 rabbits in the 500 mg/kg bw/day group that survived to the gestation day 29 laparohysterectomy had a high resorption rate, while no effects on intrauterine survival were observed in the 250 mg/kg bw/day group.
The high dose level for the current study was selected to be 270 mg/kg/day. This dose level was expected to produce effects on maternal body weight gain and food consumption without causing excessive maternal toxicity that could result in death or morbidity. In addition, this dose level is expected to avoid excessive embryo-fetal lethality. The mid-dose of 90 mg/kg/day was expected to produce minimal or no maternal and prenatal developmental toxicity. The low dose of 30 mg/kg/day was expected to be a no-effect level for both maternal and prenatal developmental toxicity.

3. Test substance preparation and analysis:
The test substance (the individual doses for each animal) was weighed daily (on the afternoon prior to dose administration) for each exposure level and stored at room temperature, protected from light until application. The test substance was administered as received and moistened with a sufficient amount of reverse osmosis-purified water to form a spreadable paste that would ensure good contact with the skin. The test substance paste was prepared immediately prior to application to each animal. The test substance was applied by the Sponsor; therefore, no additional analyses were conducted.

4. Statistics:
All statistical tests were performed using WTDMS™ unless otherwise noted. Analyses were conducted using two-tailed tests (except as noted otherwise) for minimum significance levels of 1% and 5%, comparing each test substance-treated group to the control group.
Each mean was presented with the standard deviation (S.D.), standard error (S.E.), and the number of animals (N) used to calculate the mean. Data obtained from nongravid animals were excluded from statistical analyses.
Where applicable, the litter was used as the experimental unit. Mean maternal body weights (absolute and net), body weight changes (absolute and net), and food consumption, gravid uterine weights, numbers of corpora lutea, implantation sites, and viable fetuses, and fetal body weights (separately by sex and combined) were subjected to a parametric one-way ANOVA (Snedecor and Cochran, 1980) to determine intergroup differences. If the ANOVA revealed significant ($p<0.05$) intergroup variance, Dunnett's test (Dunnett, 1964) was used to compare the test substance-treated groups to the control group. Mean litter proportions (percent per litter) of prenatal data (viable and nonviable fetuses, early and late resorptions, total resorptions, pre- and postimplantation loss, and fetal sex distribution), total fetal malformations and developmental variations (external, visceral, skeletal, and combined), and each particular external, visceral, and skeletal malformation or variation were subjected to the Kruskal-Wallis nonparametric ANOVA test (Kruskal and Wallis, 1952) to determine intergroup differences. If the ANOVA revealed significant ($p<0.05$) intergroup variance, Dunn's test (Dunn, 1964) was used to compare the test substance-treated groups to the control group.

C. METHODS

1. Maternal observations and evaluations:
All rabbits were observed twice daily, once in the morning and once in the afternoon, for moribundity and mortality. Individual clinical observations were recorded from the day of receipt through gestation day 29. Animals were also observed for signs of toxicity approximately 3 hours following the initiation of test substance exposure (at the mid-point of each daily 6-hour exposure period). In addition, the presence of findings at the time of dose administration was recorded for individual animals. The application site was scored daily (prior to administration of the first daily dose) during the treatment period for erythema and edema in accordance with a 4-step grading system of very slight, slight, moderate, and severe. Individual maternal body weights were recorded on gestation days 0 and 4-29 (daily) and individual food consumption was recorded on gestation days 4-29 (daily). All surviving rabbits (including the female that delivered) were euthanized on gestation day 29 by an intravenous injection of sodium pentobarbital via a marginal ear vein.
The thoracic, abdominal, and pelvic cavities were opened by a ventral mid-line incision, and the contents were examined. The uterus and ovaries were then exposed and excised. The number of corpora lutea on each ovary was recorded. The trimmed uterus was weighed and opened, and the number and location of all fetuses, early and late resorptions, and the total number of implantation sites were recorded. The placentae were also examined. The individual uterine distribution of implantation sites was documented. Uteri with no macroscopic evidence of implantation were opened and subsequently placed in 10% ammonium sulfide solution for detection of early implantation loss. Maternal tissues were preserved in 10% neutral-buffered formalin for possible future histopathologic examination only as indicated by the gross findings. Representative sections of corresponding organs from a sufficient number of control animals were retained for comparison. The carcass of each female was then discarded.

2. Fetal evaluations:
Each viable fetus was examined externally, individually weighed, euthanized by a subcutaneous injection of sodium pentobarbital in the scapular region, and tagged for identification. The detailed external examination of each fetus included, but was not limited to, an examination of the eyes, palate, and external orifices, and each finding was recorded. Nonviable fetuses (if the degree of autolysis was minimal or absent) were examined, the crown-rump length measured, weighed, sexed, and tagged individually. Crown-rump measurements, degrees of autolysis and gross examinations, if possible, were recorded for late resorptions, and the tissues were discarded. Each viable fetus was subjected to a visceral examination. The sex of each fetus was determined by internal examination. Fetal kidneys were examined and graded for renal papillae development. Heads from all fetuses were examined by a mid-coronal slice. All carcasses were eviscerated, skinned, and fixed in 100% ethyl alcohol.
Following fixation in alcohol, each fetus was macerated in potassium hydroxide and stained with Alizarin Red S and Alcian Blue. Fetuses were then examined for skeletal malformations and developmental variations.

3. Indices:

The following indices were calculated from caesarean section records of animals in the study.

- Group Mean Litter Basis

$$Postimplantation\ loss/Litter = \frac{No.\ Dead\ Fetuses, Resorptions\ (Early/Late)/Group}{\text{No. Gravid Females/Group}}$$

- Proportional Litter Basis

$$Summation\ per\ group\ (\%) = \frac{Sum\ of\ Postimplantation\ loss/Litter\ (\%)}{\text{No. Litters/Group}}$$

Where:

$$Postimplantation\ loss/Litter\ (\%) = \frac{No.Dead\ Fetuses,Resorptions\ (Early/Late)/Litter}{\text{No. Implantation sites/Litter}} \text{ x } 100$$

II. RESULTS AND DISCUSSION

A. MATERNAL TOXICITY

1. Mortality and clinical observations

Two animals of the 270 mg/kg bw/day group were sacrificed as a consequence of abortion and early delivery, which were considered to be secondary to the body weight losses and reductions in food consumption and increased incidences of defecation. One of these two female in this group aborted 2 dead fetuses, 2 cannibalized fetuses, and 1 late resorption on gestation day 26, which was related to a body weight loss of 701 g during gestation days 6-26 (bw reduction of 20.5%) and reduced food consumption (≤ 63g/day) during gestation days 7-26 with corresponding incidences of decreased defecation (GD 10-26). Additionally, red material around the base of the tail was noted for this female on the day of abortion. Since it has been shown that rabbits with body weight loss and feed restriction during gestation are prone to abortion (Cappon et al., 2005), this abortion was considered to be secondary to the test-substance related body weight losses. The other female in the 270 mg/kg/d group that was euthanized delivered 7 live kits, 1 dead kit, and 3 cannibalized kit on gestation day 29 (prior to scheduled necropsy). This female was noted with a body weight loss of 299 g and reduced mean food consumption (≤ 75g/day) during late portion of gestation (GD 22-28) with corresponding incidences of decreased defecation during gestation days 24-28. Early delivery often occurs in rabbits that have large body weight losses late in gestation.
One animal of the 90 mg/kg bw/day group aborted 1 late resorption on gestation day 23 and was subsequently euthanized. This female showed a body weight reduction of 13.9% and a reduced mean food consumption during gestation days 18-23 with corresponding incidences of decreased defecation and/or soft stool noted during gestation days 21-23. This abortion was considered as a secondary effect to the body weight loss, often observed in rabbits (Cappon et al., 2005). However, because of the severity of body weight loss and reduced food consumption was not observed for other females at this exposure level, this single abortion at 90 mg/kg bw/day was not considered to be test substance-related, but was considered to be a random occurrence. All remaining females in the control, 30, 90, and 270 mg/kg/day groups survived to the scheduled necropsy on gestation day 29.

Decreased defecation, which corresponded to reduced mean food consumption, was noted at an increased incidence for surviving females in the 270 mg/kg/day group (15 affected animals) compared to the control group (3 affected animals) at the daily examinations. This finding was noted as early as gestation day 8 and continued sporadically through gestation day 29, and was attributed to test substance exposure.

No other test substance-related clinical findings were noted during the daily examinations at any exposure level. Other findings noted in the test substance-exposed groups during the daily examinations, including hair loss on various body surfaces, soft stool, brown material around the right hindlimb and urogenital area, and clear material around the nose, occurred infrequently, at similar frequencies in the control group, and/or in a manner that was not exposure-related. No clinical findings were noted approximately 3 hours following the initiation of test substance exposure at any exposure level. Rapid circling and vocalization, which were noted for a single female in the 90 mg/kg/day group at the time of dose administration, were not considered to be test substance-related as they were not observed in an exposure-related manner.

2. Local effects

Very slight and slight erythema were noted at an increased incidence for females in the 270 mg/kg bw/day group during the treatment period. These findings were noted as early as gestation day 11 and continued (with increased persistence and duration) through gestation day 29. Desquamation (noted for 11 females [1 to 12 occasions each] during gestation days 11-29) and a single occurrence of eschar were also noted for females in the 270 mg/kg bw/day group. The dermal irritation noted in the 270 mg/kg/day group was considered to be test substance-related and adverse.

In the 90 mg/kg/day group, evidence of minimal dermal irritation (primarily consisting of very slight erythema) was noted sporadically for 15 females during gestation days 17-29. Due to the absence of these findings in the control group, the occurrences of minimal dermal irritation were considered to be test substance-related; however, because of the transient nature, limited incidence, and minimal severity of these findings, they were not considered to be adverse.

No test substance-related dermal findings were noted for females in the 30 mg/kg/day group. Very slight erythema (5 total occurrences) was noted for 2 females at this exposure level. However, because this finding was graded very slight, was seen in only 2 animals, and did not persist throughout the treatment period, it was not considered to be test substance-related.

3. Body weight and body weight change

Test substance-related significant ($p<0.01$) mean body weight losses (19 g and 40 g) were noted in the 270 mg/kg/day group following the first 2 days of treatment (gestation days 6-7 and 7-8, respectively) compared to mean body weight gains in the control group. Consequently, a significant ($p<0.01$) mean body weight loss was noted in this group during gestation days 6-10. Mean body weight gains in the 270 mg/kg/day group were similar to the control group during gestation days 10-13 and 13-20. However, non-statistically significant lower mean body weight gains, body weight losses, or absence of body weight gain noted in the 270 mg/kg/day group during the latter portion of the treatment period (gestation days 23-28) resulted in a slightly lower mean body weight gain in this group during the cumulative gestation days 20-29 interval. As a result of the effects on body weight change noted during the early and late portions of the treatment period, a significantly ($p<0.05$) lower mean body weight gain (-31%) was noted in the 270 mg/kg/day group when the entire treatment period (gestation days 6-29) was evaluated. However, these body weight changes failed to produce a noteworthy effect on mean body weights in the 270 mg/kg/day group. Mean net body weight, net body weight change, and gravid uterine weight in the 270 mg/kg/day group were unaffected by test substance administration. Mean maternal body weights, body weight gains, net body weights, net body weight gains, and gravid uterine weights in the 30 and 90 mg/kg bw/day groups were unaffected by test substance administration. Differences from the control group were slight and not statistically significant (see Table 5.6.2-18).

Table 5.6.2-18: Maternal body weight and body weight change [g]

	Dose (mg/kg bw/day)			
	0	**30**	**90**	**270**
Mean body weight change at gestation days				
6-10	66	88	85	**-19****
10-13	43	58	55	34
13-20	162	173	141	149
20-29	106	21	111	70
6-29	377	340	400	**261***
Mean body weight in g (% of control)				
Gestation day 6	3398	3435 (1.1)	3422 (0.7)	3412 (0.4)
Gestation day 29	3775	3775 (0.0)	3810 (0.9)	3656 (-3.2)

* = p≤0.05; ** = p≤0.01

4. Food consumption

Test substance-related lower mean food consumption, evaluated as g/animal/day and g/kg bw/day, was noted in the 270 mg/kg bw/day group throughout the treatment period and during the entire treatment period (gestation days 6-29) the differences from the control group were occasionally significant (-15% during GD 6-29). The lower mean food consumption in this group generally corresponded to the mean body weight losses/lower mean body weight gains noted during the treatment period. Maternal food consumption in the 30 and 90 mg/kg bw/day groups was unaffected by test substance administration. Differences from the control group were slight and not statistically significant (see Table 5.6.2-19).

Table 5.6.2-19: Maternal food consumption [g/animal/day]

	Dose (mg/kg bw/day)			
	0	**30**	**90**	**270**
Mean food consumption [g/animal/day] on gestation days				
6-10	168	182	178	**133****
10-13	152	172	162	128
13-20	158	173	156	**131***
20-29	124	125	131	106
6-29	146	157	151	**124***

* = p≤0.05; ** = p≤0.01

5. Gross pathology

One of the two female in the 270 m g/kg bw/d group, that was euthanized subsequent to abortion had cystic ovidcuctt at necropsy. In the other female at the top dose, which was sacrificed because of early delivery, dark red contents were noted in the urinary bladder. The female which was sacrificed in the 90 mg/kg bw/d group showed no abnormalities at necropsy. At the scheduled necropsy on gestation day 29, not test substance-related abnormalities were observed at any exposure level.

6. Cesarean section data

Intrauterine growth and survival were unaffected by test substance administration at exposure levels of 30, 90, and 270 mg/kg bw/day. Parameters evaluated included post-implantation loss, live litter size, mean fetal body weights, and fetal sex ratios. Mean numbers of corpora lutea and implantation sites and the mean litter proportions of pre-implantation loss were similar across all groups (see Table 5.6.2-20).

A significantly ($p<0.01$) lower mean male fetal weight was noted in the 270 mg/kg/day group (39.7 g) compared to the concurrent control group (44.0 g). However, the value was within the historical control data range (36.6 g - 45.2 g) and no statistically significant differences were noted when mean female or combined fetal weights were compared to the concurrent control group. Therefore, the lower mean male fetal weight noted in the 270 mg/kg/day group was not considered to be test substance-related. In addition, the mean litter proportion of post-implantation loss (primarily late resorptions) in the 270 mg/kg/day group (10.6% per litter) was higher than the concurrent control group value (3.6% per litter). This resulted in a corresponding lower mean litter proportion of viable fetuses (89.4% per litter) compared to the concurrent control group value (96.4% per litter). However, these differences were not statistically significant when compared to the concurrent control group, the values were within the respective historical control data ranges, and the mean number of viable fetuses in this group was similar to the concurrent control group. These differences could also be attributed to 2 females (nos. 65481 and 65385) in the 270 mg/kg/day group with 55.6% and 60.0% percent per litter post-implantation loss (late resorptions), respectively. Therefore, the higher mean litter proportion of post-implantation loss and lower mean litter proportion of viable fetuses were not considered to be test substance-related (see Table 5.6.2-20).

Table 5.6.2-20: Maternal toxicity – Cesarean section observations

Observation	Dose (mg/kg bw/day)			
	0	**30**	**90**	**270**
# Animals assigned (mated)	25	25	25	25
# Animals pregnant	25	22	23	25
Pregnancy rate (%)	100	88	92	100
Maternal Wastage				
# Died	0	0	0	0
# Died pregnant	NA	NA	NA	NA
# Nonpregnant	NA	NA	NA	NA
# Aborted	0	0	1	1
# Premature delivery	0	0	0	1
Total # Corpora lutea (Corpora lutea/dam)	242 (9.7)	215 (9.8)	218 (9.9)	239 (10.4)
Total # Implantations (Implantations/dam)	222 (8.6)	182 (8.3)	187 (8.5)	211 (9.2)
Total # Litters	25	22	22	23
Total # Live fetuses (Live fetuses/Dam)	214 (8.6)	177 (8.0)	177 (8.0)	190 (8.3)
Total # Resorptions	8	4	10	21
Early	7	3	8	2
Late	1	1	2	19
Resorptions/Dam	0.3	0.1	0.5	0.9
Early	0.3	0.1	0.4	0.1
Late	0.0	0.0	0.1	0.8
Litters with total resorption	0	0	0	0
Mean fetal weight (g)	43.2	41.8	43.8	40.2
Males	44.0	42.7	44.0	**39.7****
Females	42.6	40.5	43.7	40.1
Sex ratio (% male)	49.1	52.5	47.8	47.2
Pre-implantation loss (%)	7.9	15.3	12.9	11.7
Post-implantation loss (%)	3.6	2.3	5.9	10.6

**: $p<0.01$

B. DEVELOPMENTAL TOXICITY

The numbers of fetuses (litters) available for morphological evaluation were 214 (25), 177 (22), 177 (22), and 190 (23) in the control, 30, 90, and 270 mg/kg bw/day groups, respectively. Malformations were observed in 5 (4), 3 (2), 6 (4), and 6 (5) fetuses (litters) in these same respective exposure groups during external, visceral, and skeletal examinations and they were considered spontaneous in origin (see Table 5.6.2-21, Table 5.6.2-22, and Table 5.6.2-23).

In the 270 mg/kg/day group, 1 fetus was noted with hydrocephaly with a domed head (increased cavitation of both lateral ventricles and the third ventricle). Carpal and/or tarsal flexure (with no apparent skeletal origin) were noted for another fetus in this group and for 2 fetuses from the same litter in the 90 mg/kg/day group; both fetuses in the 90 mg/kg/day group also exhibited paw hyperflexion (with no apparent skeletal origin) and hydrocephaly (increased cavitation of both lateral ventricles and the third ventricle). In addition, bilateral microphthalmia was noted for 2 fetuses from the same litter in the 90 mg/kg/day group (this was not the same litter with the carpal/tarsal flexure and hydrocephaly); no skeletal origins were apparent for the microphthalmia.

Because the aforementioned malformations were observed in single fetuses or litters, were not observed in an exposure-related manner, and were within the ranges observed in the historical control data, they were not considered to be related to test substance exposure.
No external developmental variations were observed in this study. Visceral and skeletal developmental variations observed in the test substance-exposed groups consisted primarily of major blood vessel variation, extra papillary muscle present in the heart, retrocaval ureter, accessory spleen(s), hemorrhagic ring around the iris, absent or small gallbladder, small heart, pale and misshapen liver, 13th full rib(s), 27 presacral vertebrae, 13th rudimentary rib(s), and sternebra(e) nos. 5 and/or 6 unossified, and hyoid arch(es) bent. These findings were not considered to be test substance-related as they did not occur in a dose-related manner, were noted similarly in the concurrent control group, and/or the mean litter proportions were within the ranges of the developmental historical control data.

Table 5.6.2-21: Developmental toxicity – External Examinations

	Dose (mg/kg bw/day)			
	0	**30**	**90**	**270**
#Fetuses (litters) examined	214 (25)	177 (22)	177 (22)	190 (23)
#Fetuses (litters) with malformations	0 (0)	0 (0)	4 (2)	2 (2)
Mean % affected per litter	0.0	0.0	2.3	1.0
Hydrocephaly with or without dome head[$] [[#]Hist. Control: 0 (0) – 5 (1)]	0 (0)	0 (0)	0 (0)	1 (1)
Carpal and/or tarsal flexure[$] [[#]Hist. Control: 0 (0) – 3 (2)]	0 (0)	0 (0)	2 (1)	1 (1)
Paw hyperflexion[$] [[#]Hist. Control: 0 (0) – 2 (1)]	0 (0)	0 (0)	2 (1)	0 (0)
Microphthalmia and/or anophthalmia[$] [Hist. Control: 0 (0) – 2 (1)]	0 (0)	0 (0)	2 (1)	0 (0)

[$] Fetal (litter incidence)
[#] *Historical control data of rabbit studies from the laboratory as indicated in the study summary*

Table 5.6.2-22: Developmental toxicity – Visceral Examinations

	Dose (mg/kg bw/day)			
	0	**30**	**90**	**270**
#Fetuses (litters) examined	214 (25)	177 (22)	177 (22)	190 (23)
#Fetuses (litters) with malformations	1 (1)	0 (0)	2 (1)	2 (2)
Mean % affected per litter	0.5	0.0	1.1	0.8
Malpositioned kidney[$]	0 (0)	0 (0)	0 (0)	1 (1)
Lungs – lobular agenesis[$]	1 (1)	0 (0)	0 (0)	0 (0)
Hydrocephaly[$]	0 (0)	0 (0)	2 (1)	0 (0)
Stenotic pulmonary trunk[$]	0 (0)	0 (0)	0 (0)	1 (1)
Bulbous aorta[$]	0 (0)	0 (0)	2 (1)	1 (1)

[$] Fetal (litter incidence)

Table 5.6.2-23: Developmental toxicity – Skeletal Examinations

	Dose (mg/kg bw/day)			
	0	**30**	**90**	**270**
#Fetuses (litters) examined	214 (25)	177 (22)	177 (22)	190 (23)
#Fetuses (litters) with malformations	4 (3)	3 (2)	2 (2)	2 (2)
Mean % affected per litter	2.0	1.5	1.6	1.0
Vertebral anomaly with or without associated rib anomaly[$]	0 (0)	0 (0)	1 (1)	0 (0)
Sternebrae fused[$]	0 (0)	0 (0)	1 (1)	0 (0)
Costal cartilage animaly[$]	0 (0)	0 (0)	1 (1)	1 (1)
Rib anomaly[$]	0 (0)	1 (1)	0 (0)	1 (1)
Vertebral centra anomaly[$]	1 (1)	0 (0)	0 (0)	0 (0)
Bent rib(s) [$]	1 (1)	0 (0)	0 (0)	0 (0)

[$] Fetal (litter incidence)

Use of historical control data

Of the 99 studies in the current WIL Research historical control data for rabbits, only 2 studies were performed via the dermal route other than the metconazole study. No malformations were observed in the 343 control group fetuses in these 2 studies (20 litters/study). With only 2 other dermal exposure studies conducted in the laboratory, dermal HCD are insufficient for comparison. Although the dermal route of exposure is used very infrequently for studies of developmental toxicity in rabbits, the route of administration should have no effect on the incidence of malformations. Malformations that are the result of test substance administration are the result of systemic exposure to the developing embryo/fetus. This is true whether the compound is administered via oral, i.v., dermal, subcutaneous, inhalation or any other route. If the dermal route used in the metconazole study were a confounder, we would expect other intrauterine parameters to be affected compared to the mean historical control data. The control animals in the metconazole study compare very favorably to the overall historical control data (see Table 5.6.2-24). Therefore, although there is little historical control data via the dermal route, comparison to historical data by all routes of administration is appropriate.

Table 5.6.2-24: Comparison of mean control parameters between the dermal study with metconazole vs historical control data from all routes of administration

Endpoint	**Study control mean**	**Historical control mean**
Pregnancy Rate	100%	96%
Mean No. Viable Fetuses	8.5	8.7
Mean % Viable Fetuses	96.4%	95.4%
Mean Fetal Weight	43.2g	40.7g
External Malformation Incidence	0%	0.4%
Visceral Malformation Incidence	0.5%	0.9%
Skeletal Malformation Incidence	2.0%	0.9%
Total Malformation Incidence	2.5%	2.0%

As mentioned above, 2 fetuses from the same litter in the mid dose group (90 mg/kg/day) and 1 fetus in the high dose group (270 mg/kg/day) had hydrocephaly. One historical control data set had a mean litter proportion incidence of 1.2% (the maximum observed) for hydrocephaly; however, there was only 1 occurrence of hydrocephaly in that study. Three other control data sets had 2 fetuses with hydrocephaly as was seen in the metconazole study. In addition, 7 control data sets had 1 fetus each with hydrocephaly. A recently conducted oral gavage study demonstrated that 5 control group fetuses with hydrocephaly originated from 1 control group litter. The resulting incidence is much greater than seen in the 90 and 270 mg/kg/day metconazole groups. While hydrocephaly is not observed very often in control fetuses, previous control groups have been observed with a similar incidence to that observed in the present study, including one study in which fetuses from two different control group litters were affected (see Table 5.6.2-25).

Table 5.6.2-25: Historical control data on Hydrocephaly

Hydrocephaly				
Study No.	**Number of Affected Fetuses**	**Number of Affected Litters**	**Percent of Affected Litters**	**Mean Litter Proportion Affected**
6	2	1	5.0	0.9%
9	1	1	6.3	0.9%
35	1	1	4.5	0.5%
43	1	1	5.0	0.6%
49	1	1	5.9	1.2%
55	2	2	9.1	1.1%
58	2	1	4.8	0.9%
84	1	1	4.0	0.4%
105	1	1	4.5	0.4%
115	1	1	5.0	0.7%
Conducted 4/2013 (gavage)	5	1	5.0	2.5%
Metconazole (90 mg/kg/day)	2	1	4.5	1.1%
Metconazole (270 mg/kg/day)	1	1	4.3	0.5%

In the metconazole study, the same 2 fetuses in the 90 mg/kg/day group that had hydrocephaly also had carpal and/or tarsal flexure and paw hyperflexion. In addition, 1 fetus in the 270 mg/kg/day group had carpal and/or tarsal flexure. For the carpal and/or tarsal flexure, one control study had 3 fetuses affected. Two of these fetuses also had paw hyperflexion. It is not uncommon to see carpal and/or tarsal flexure and paw hyperflexion occurring in the same fetuses. In addition, 2 recently conducted studies had 2 control group fetuses each with carpal and/or tarsal flexure.

All of the aforementioned findings were classified as malformations (structural anomalies that alter general body conformity, disrupt or interfere with normal body function, or may be incompatible with life) in the original metconazole study report. However, based on a review of the literature (Fratta et al., 1965) and previous experience with this malformation, it would be more appropriate to refer to carpal and/or tarsal flexure and paw hyperflexion as developmental variations (alterations in anatomic structure that are considered to have no significant biological effect on animal health or body conformity and/or occur at high incidence, representing slight deviations from normal) that are most likely to be reversible when there is no underlying skeletal origin for these conditions.

Table 5.6.2-26: Historical control data on carpal/and or tarsal Flexure

Carpal and/or Tarsal Flexure				
Study	**Number of Affected Fetuses**	**Number of Affected Litters**	**Percent of Affected Litters**	**Mean Litter Proportion Affected**
11	1	1	4.8	0.5%
19	3	2	9.5	1.4%
25	1	1	5.3	0.9%
28	1	1	4.3	0.5%
34	2	1	4.0	0.8%
38	1	1	4.5	0.5%
72	1	1	4.2	0.4%
Conducted 4/2013 (gavage)	2	2	10.0	1.1%
Conducted 5/2013 (i.v.)	2	1	5.3	1.5%
Metconazole (90 mg/kg/day)	2	1	4.5	1.1%
Metconazole (270 mg/kg/day)	1	1	4.3	0.5%

Microphthalmia was noted in 2 fetuses from the same litter in the 90 mg/kg/day group. However, no microphthalmia was seen in the 270 mg/kg/day group. Therefore, because there was no dose-response relationship, the microphthalmia noted in the 90 mg/kg/day group should not be considered a test substance-related effect.

III. CONCLUSIONS

Maternal toxicity (mean body weight losses and lower mean body weights gains with corresponding reduced mean food consumption and decreased defecation) was noted at 270 mg/kg/day. The severity of these effects resulted in 1 abortion and 1 early delivery at this exposure level. Evidence of dermal irritation was observed in the 270 mg/kg bw/day group. No test substance-related maternal toxicity was observed at exposure levels of 30 and 90 mg/kg/day. Intrauterine growth and survival and fetal morphology were unaffected by test substance exposure at all exposure levels.
In conclusion, 90 mg/kg bw/day was considered to be the NOAEL for maternal toxicity and 270 mg/kg bw/day was considered to be the NOAEL for embryo/fetal developmental toxicity after dermal application of metconazole to time-mated New Zealand White rabbits.

REFERENCES

Fratta, I.D.; Sigg, E.B.; Maiorana, K. Teratogenic effects of Thalidomide in Rabbits, Rats, Hamsters, and Mice. *Toxicol and Appl Pharmacol* 1965, *7*, 268-286.

CA 5.7 Neurotoxicity Studies

Data submitted in this AIR3 process previously not peer-reviewed in the EU:
Metconazole is neither an organophosphate compound nor a carbamate, and no signs indicative of neurotoxic effects were observed in any of the toxicity studies.
Thus, no further studies to evaluate the neurotoxic potential of metconazole were considered necessary. Based on information from public available evaluations of non-EU authorities two subchronic neurotoxicity studies were performed on metconazole cis/trans.
A 2-week and 4-week neurotoxicity study with metconazole cis/trans (KNF-474m) were conducted for non-EU authorities by another metconazole-supporting company. Detailed study summaries were made available to BASF ([redacted] 2015a, b) and are part of this submission.

The results of these studies are listed in Table 5.7-1; comprehensive summaries are provided further below (see CA 5.7.1/1 and CA 5.7.1/2).

Table 5.7-1: Summary of neurotoxicity studies with metconazole

Study, Species, dose levels	Purity (%), cis/trans content, batch no.	NOAEL (mg/kg bw/day)	Critical effects	Reference (BASF DocID)
2-week Neurotoxicity, Sprague-Dawley rat, 0, 11.0, 59.6 and 216.8 mg/kg bw (males); 0, 10.6, 52.8 and 206.1 mg/kg bw (females); via diet	98.99% cis/trans: 85/15 batch: 9Z521	Neurotoxicity: 216.8 (m) / 206.1 (f) (3000 ppm, HDT) Systemic toxicity: 11.0 (m) / 10.6 (f) (100 ppm)	No neurotoxic effects were observed Reduced: food consumption and body weight as well as body weight gain	Summary: [redacted] 2015a ; (2015/1087911) Study author : [redacted] 2002a (2002/8000062)
4-week Neurotoxicity, Sprague-Dawley rat, 0, 4.8, 15.69, 47.08 mg/kg bw (males); 0, 5.1, 17.62, 49.82 mg/kg bw (females); via diet	98.99% cis/trans: 85/15 batch: 9Z521	Neurotoxicity: ≥ 47.08 (m) / 49.82 (f) (500 ppm, HDT) Systemic toxicity: 4.8 (m) / 5.1 (f) (50 ppm)	No neurotoxic effects were observed Reduced: food consumption and body weight gain	Summary: [redacted] 2015b (2015/1087912) Study author : [redacted] 2002b (2002/8000063)

HDT: highest dose tested

In the 2-week range finder sub-chronic neurotoxicity study, metconazole cis/trans administration did not result in any neurotoxicity up to 3000 ppm, the highest concentration tested (equivalent to 216.8 mg/kg/day for males and 206.1 mg/kg/day for females). The NOAEL for the general toxicity was set at 100 ppm in this 2-week study based on reduced food consumption resulting in body weight loss and body weight gain reductions (at 540 ppm and above) with marked effects on food intake and weight gain at 3000 ppm, that resulted in a number of secondary changes in many organs, indicating that this top dose was too high. Additionally, at 3000 ppm reduced relative liver and kidney weights were observed in both sexes with corroborating hepatic macroscopic findings (pale/enlarged).

In the 4-week sub-chronic neurotoxicity study, administration to metconazole cis/trans did not result in any neurotoxicity up to 500 ppm, the highest dose tested (equivalent to 47.08 mg/kg/day for males and 49.82 mg/kg/day for females). Neuro-behavioural screening and macroscopic and histopathological examination of the associated tissues (including anatomical measurements of the brain) revealed no treatment-related findings. In addition, no unusual signs or patterns of behaviour were observed at any of the routine observations. The NOAEL for the general toxicity was 50 ppm (equivalent to 4.84 mg/kg bw/day in males and 5.10 mg/kg bw/day in females), based on reduced food consumption and body weight gain reduction.

Based on the available data on neurotoxicity with metconazole cis/trans the conclusion for relevant endpoints for the current re-registration is as follow:

Neurotoxicity (Regulation (EU) N° 283/2013, Annex Part A, point 5.7)

Acute neurotoxicity	No findings indicative of neurotoxic potential reported. Testing not necessary.	
Repeated neurotoxicity	No neurotoxicity reported in 2-week and 4-week neurotoxicity studies: $NOAEL_{neurotoxicity}$: 47.08 mg/kg bw (rat, male) 49.82 mg/kg bw (rat, female) (highest dose tested) $NOAEL_{systemic\ toxicity}$: 4.8 / 5.1 mg/kg bw/day	
Additional studies (e.g. delayed neurotoxicity, developmental neurotoxicity)	No data submitted for delayed neurotoxicity or developmental neurotoxicity - not required	

CA 5.7.1 Neurotoxicity studies in rodents

A 2-week range finder sub-chronic neurotoxicity study in rats (study author: [redacted] 2002 a; BASF DocID 2002/8000062) with metconazole cis/trans (KNF-474m) was conducted for non-EU authorities by another metconazole-supporting company. A detailed study summary was made available to BASF (prepared by [redacted] 2015a; BASF DocID 2015/1087911) and is part of this submission.

Report:	CA 5.7.1/1 [redacted] 2015a Summary of the Laboratory report KRA065/020005 - KNF-474m: Preliminary neurotoxicity study by dietary administration to CD rats for 2 weeks 2015/1087911
Guidelines:	None applicable for the design
GLP:	no

Report:	CA 5.7.1/2 [redacted] 2002 a KNF-474m - Preliminary neurotoxicity study by dietary administration to CD rats for 2 weeks 2002/8000062
Guidelines:	<none>
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Executive Summary

The sub-chronic neurotoxic potential of KNF-474m to Sprague-Dawley (Crl:CD®(SD)) rats by dietary administration was assessed over a period of 2 weeks in order to aid selection of dietary concentrations to be used in a main four week neurotoxicity study. Three groups each of five males and five females received metconazole cis/trans (KNF-474m) in the diet at concentrations of 100, 540 or 3000 ppm and a similarly constituted control group received untreated diet throughout the period. Achieved dosages over the 2-week period were 11.0, 59.6 and 216.8 mg/kg/day for males and 10.6, 52.8 and 206.1 mg/kg/day for females receiving 100, 540 and 3000 ppm, respectively. During the study clinical condition, bodyweight, food consumption, neuro-behavioural investigations, organ weights and macro-pathology investigations were undertaken. Neuro-behavioural investigations (assessment in-the-hand and in a standard arena) were performed on all animals before treatment commenced and in week 2. In addition, sensory reactivity and grip strength observations and motor activity measurements were performed in week 2.

The continuous administration of KNF-474m to CD rats for two weeks at dietary concentrations of up to 3000 ppm did not result in any neurotoxicity. Neuro-behavioural screening revealed no clear treatment-related findings and no unusual signs or patterns of behaviour were observed at routine observations.

Initial bodyweight losses followed by low bodyweight gains together with low food intake were observed at 3000 ppm and all animals were recorded as "thin", both in-life and at necropsy. Similar but much less marked effects were seen in males receiving 540 ppm; in females receiving this dietary concentration there was only low food consumption observed in week 1. The marked effects upon food intake and weight gain at 3000 ppm resulted in a number of secondary changes in many organs. These findings are indicative of a non-specific toxic effect and also demonstrate that 3000 ppm exceeded the maximum tolerated dosage (MTD).

High food scatters occurred in week 1 in males and females receiving 540 or 3000 ppm suggesting that at these concentrations the diet was initially unpalatable to the rats.

Increased relative kidney weights were reported in males given 3000 ppm and increased relative liver weights were reported in males and females given 3000 ppm. The macro-pathological examination revealed pale/enlarged livers and also livers in which the lobular pattern was accentuated. The nature of the change in the liver and kidneys was not established since no histopathological examination was performed.

The findings from this study indicated that the highest dietary concentration for the main 4-week neurotoxicity study should be in the region of 540 ppm and that the lowest concentration should be below 100 ppm.

(DocID 2015/1087911)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: Metconazole

Description:	solid (crystalline powder) / white to pale yellow
Lot/Batch #:	9Z521
Purity:	98.99% (85:15, *cis:trans*)
Stability of test compound:	The test substance was stable in the diet as determined in an earlier experiment (HLS No. KRA 067/014561)

2. Vehicle: Diet

3. Test animals:

Species:	Rats
Strain:	Crl:CD(SD)
Sex:	Male and female
Age (at start of dosing):	40-43 days (males); 41-43 (females)
Weight (at start of dosing):	173 – 209 g (males); 137 – 160 g (females)
Source:	Charles River (UK) Ltd.
Acclimation period:	12 days
Diet:	Rat and Mouse No. 1 Maintenance Diet, ad libitum
Water:	Tap water, ad libitum

Housing:	In groups (5 animals per cage, same sex) in stainless steel cages with stainless steel mesh lids and floor
Environmental conditions:	
Temperature:	19 - 23°C
Humidity:	45 - 70%
Air changes:	at least 15 air changes per hour
Photo period:	12 h light / 12 h dark (06:00 - 18:00 / 18:00 - 06:00)

B. STUDY DESIGN AND METHODS

1. In-life dates: 12-Nov-2001 to 26-Nov-2001

2. Animal assignment and treatment:
There were 5 animals per sex and dose level, allocated randomly to the cages at the time of arrival. The test item was applied at dietary concentrations of 0, 100, 540 and 3000 ppm for two weeks. The actual doses were 0, 11, 59.6, and 216.8 mg/kg bw/day for males and 0, 10.6, 52.8, and 206.1 mg/kg bw/day for females, respectively.

3. Test substance preparation and analysis:
Test diets were prepared in weekly batches by an initial preparation of a premix where the required amount of KNF-474m was mixed with an approximately equal amount of sieved diet and hand-stirred. A further amount of sieved diet, equal to the weight of the mixture, was added and stirred until it appeared visibly homogenous. This doubling-up process was repeated until half the final weight of premix was reached and the mixture was ground using a mechanical grinder. The weight of the mixture was then made up to the final required weight of the premix, using coarse diet, and was then mixed in a Turbula mixer for 100 cycles. Aliquots of the premix were diluted further with coarse diet to produce either further premixes or the test diets; each mix was performed for a further 100 cycles in a Turbula mixer.

4. Statistics:
All statistical analyses were carried out separately for males and females, using the individual animal as the basic experimental unit. The following data types were analysed at each time-point separately: bodyweight (using gains over appropriate study periods), and neuro-behavioural data (arena rearing and activity counts, grip strength and motor activity). For organ weights and bodyweight changes, homogeneity of variance was tested using Bartlett's test. Whenever this was found to be statistically significant a Behrens-Fisher test was used to perform pairwise comparisons, otherwise a Dunnett's test was used. Inter-group differences in macroscopic pathology were assessed using Fisher's Exact test. The functional observation battery numerical data (activity and rearing in the standard arena, grip strength and motor activity) were subjected to statistical analysis. The following statistical analyses were performed:

If the data consisted predominantly of one particular value (relative frequency of the mode exceeds 75%), the proportion of animals with different values from the mode was analysed using Fisher's Exact test. Otherwise, Bartlett's test was performed to test for variance heterogeneity between groups. Where significant (1% level) heterogeneity was found, the data were logarithmically transformed and re-tested for heterogeneity. If no statistically significant heterogeneity of variance was detected (with or without logarithmic transformation), a one way analysis of variance was carried out. If the analysis of variance showed evidence (at the 5% level) of differences between the groups, Student's t-test was used to test for differences between treatment groups and the control group. If heterogeneity was significant and could not be stabilised by logarithmic transformation, the Kruskal-Wallis test on ranks was performed on the untransformed data. If the Kruskal-Wallis test showed evidence (5% level) of differences between the groups, the Wilcoxon Rank-Sum test was used to test for differences between the treatment groups and the control group.
Unless stated in the text table, group mean values or incidences for the treated groups were not significantly different from those of the controls ($p>0.05$).

C. METHODS

1. Observations:
Animals were inspected visually at least twice daily for evidence of ill-health or reaction to treatment and cages were inspected daily for evidence of ill-health in the occupants. Detailed observations, including palpation, were performed weekly. Observations made during the neuro-behavioural screening also served as clinical signs. During the acclimatisation period, observations of the animals and their cages were recorded at least once daily.

2. Body weight:
The weight of each animal was recorded one week before treatment commenced (Day -7), on the day that treatment commenced (Day 0), twice weekly throughout the treatment period and before necropsy. The bodyweights of all animals were checked on Day 1 before feeding to ensure that the bodyweight variations did not exceed ±20% of the mean for the appropriate sex.

3. Food consumption and compound intake:
The weight of food supplied to each cage, that remaining and an estimate of any spilled was recorded for each week throughout the treatment period. From these records the mean weekly consumption per animal (g/rat/week) was calculated for each cage. Food conversion efficiencies were calculated for each sex/group for each week of treatment.

4. Clinical assessment of neurotoxicity:
Neuro-behavioural investigations (assessment in-the-hand and in a standard arena) were performed on all animals before treatment commenced and in week 2. In addition, sensory reactivity and grip strength observations and motor activity measurements were performed in week 2.
All animals were subjected to the procedures detailed below on the specified occasions. The functional observational battery was performed at approximately the same time of day on each occasion and the observer was unaware of the experimental group to which the animal belonged. The animals were not necessarily all tested on the same day but the number of animals was balanced across the groups on each day of testing.

During and after removal from the home cage, the following parameters were assessed (in-the-hand observations): ease of removal from the cage, exophthalmos, fur condition, piloerection, reactivity to handling, salivation, lacrimation and vocalisation. Each animal was then placed in a standard arena (approximately 653 mm × 500 mm) with the floor marked into six sections of equal size for scoring activity counts and the following assessed during a two-minute period: activity count, arousal, convulsions, tremor, twitches, defecation count, gait, grooming, palpebral closure, posture, rearing count and urination. The following sensory reactivity investigations were performed: approach response, body temperature†, bodyweight†, grip strength, pupil reflex†, righting reflex†, auditory startle reflex, tail pinch response and touch response (†- not performed in week 2 in error). In addition, motor activity was also performed in week 2.

5. Sacrifice and pathology:
All animals were killed by carbon dioxide asphyxiation and were subject to a detailed necropsy. After a review of the history of each animal, a full macroscopic examination of the tissues was performed. All external features and orifices were examined visually. Any abnormality in the appearance or size of any organ and tissue (external and cut surface) was recorded and the required tissue samples preserved in appropriate fixative. The adrenals, brain, epididymides, heart, kidneys, liver, lungs (including bronchi), ovaries, spleen, testes, thymus, thyroid with parathyroids, and uterus with cervix, taken from each animal, were dissected free of adjacent fat and other contiguous tissue and the weights recorded. Bilateral organs were weighed together. The weight of each organ was expressed as a percentage of the bodyweight recorded immediately before necropsy. Selected tissues were preserved but no microscopic examination was performed.

II. RESULTS AND DISCUSSION

A. OBSERVATIONS

1. Clinical signs of toxicity

At 3000 ppm thin build was recorded from day 4 for all animals, and brown staining on the head was seen during the second week of treatment in two males receiving this dietary concentration. There were no treatment related signs in animals receiving 100 or 540 ppm.

2. Mortality

No animals died prematurely.

B. BODY WEIGHT AND BODY WEIGHT GAIN

Bodyweight losses were seen in week 1 in all animals receiving 3000 ppm; the losses were greater in the first part of the week (day 0 to 3) than in the second part (day 3 to 7). Small gains were seen in these animals in week 2. Over the two-week period animals receiving 3000 ppm did not regain their initial bodyweight and overall losses were recorded (-9% for the males and -7% for the females). In addition, overall bodyweight gains were also low for males receiving 540 ppm (78% of the control value).

There was no effect of treatment on the bodyweight gains of animals receiving 100 ppm or of females receiving 540 ppm (see Table 5.7.1-1).

Table 5.7.1-1: Mean body weight gain (g) (selected periods)

Level (ppm)	Day 0-3		Day 3-7		Day 0-14	
	M	F	M	F	M	F
Control	27 (3.8)	9 (3.3)	31 (4.8)	12 (3.5)	111 (12.4)	35 (8.0)
100	24 (2.0)	10 (2.5)	32 (4.2)	11 (1.6)	106 (7.2)	38 (6.2)
540	**19** (2.5)**	7 (4.0)	25 (2.2)	13 (2.3)	**87* (7.1)**	39 (8.3)
3000	**-23** (3.7)**	**-17** (1.0)**	**-4** (2.7)**	**-2** (2.2)**	**-17** (2.1)**	**-11** (4.0)**

Significant when compared with control; * - $p<0.05$, ** $p<0.01$
Parenthesis – standard deviation

C. FOOD CONSUMPTION AND FOOD EFFICIENCY

Food consumption was markedly low during the first week of treatment, when compared with the controls, for animals receiving 3000 ppm; marginally low consumption was seen in males receiving 100 ppm and in males and females receiving 540 ppm. Low food intake continued during week 2 for animals receiving 3000 ppm, though the magnitude of the effect was less severe.

The animals receiving 540 or 3000 ppm scattered more food than the controls in week 1 whilst in week 2 large amounts of scattered diet were only recorded for males receiving 3000 ppm. This indicated that there was an initial palatability problem with the treated diets at the higher concentrations.

The amount of food eaten by females receiving 100 ppm was similar to that of the controls.

Overall food conversion efficiency was not calculable for animals receiving 3000 ppm due to the bodyweight losses in these animals. When compared with the controls, low food conversion efficiency was recorded during both treatment weeks for males receiving 540 ppm.

Food conversion efficiencies of animals receiving 100 ppm and of females receiving 540 ppm were not affected by treatment.

D. NEUROBEHAVIOURAL SCREENING

1. In-the-hand observations

In-the-hand observations were unaffected by treatment.

2. Arena observations

Elevated gait (slight) was seen in week 2 in three males receiving 3000 ppm. This finding is occasionally reported in control animals and is thought unlikely to be related to treatment.

3. Sensory reactivity and grip strength

Sensory reactivity findings and grip strength values were unaffected by treatment.

4. Motor activity

Motor activity scores showed considerable intra- and inter-group variation during week 2 of treatment; the differences could not, unequivocally, be attributed to treatment. High and low beam scores in all treated female groups were low, when compared with the controls, at 6 and 12 minutes (low beam scores) and 6, 12 and 18 minutes (high beam scores).
However, in the absence of any pre-treatment data and given the flat response across all groups, the small number of animals in each sex/group and the absence of a similar effect in the males, it is difficult to confidently state that there is an effect on motor activity in these animals (see Table 5.7.1-2).

Table 5.7.1-2: Mean motor activity (high and low beam scores) for females during week 2

Level (ppm)	Beam Level	Time (minutes)										
		6	**12**	**18**	**24**	**30**	**36**	**42**	**48**	**54**	**60**	**Total**
Control	High	57.4	39.6	25.6	9.0	7.0	17.2	6.2	4.4	4.6	2.0	173.0
100	High	**29.2***	23.4	15.2	16.0	5.8	4.6	8.4	3.6	0.8	2.4	109.4
540	High	**20.6****	28.6	19.0	15.6	2.0	0.6	2.4	3.0	3.4	5.0	100.2
3000	High	**19.2****	26.8	18.8	7.4	6.2	1.4	3.8	2.0	3.8	4.4	93.8
Control	Low	259.4	199.0	125.0	83.0	68.8	94.0	47.8	32.8	30.6	23.6	964.0
100	Low	164.8	120.6	107.4	123.2	54.0	48.8	40.8	46.4	16.8	12.6	735.4
540	Low	**141.0***	143.2	129.8	109.8	28.2	4.6	20.8	24.8	33.8	27.8	663.8
3000	Low	**137.6****	132.0	132.0	47.8	52.8	6.8	7.8	57.8	67.8	39.6	682.0

Significant when compared with control group; * - $p<0.05$, ** $p<0.01$

E. TERMINAL PROCEDURES

1. Organ weights

Evaluation of the organ weight data revealed statistically significant changes in many of the absolute organ weights of animals which had received 3000 ppm and, to a lesser extent, in males which had received 540 ppm. These changes were attributed to the marked effect of treatment on bodyweight and included variations seen in brain, adrenal, epididymides, heart, lungs, testes, thymus and thyroid weight. In addition, uterus weights were low in several females treated at 3000 ppm, though statistical significance was not attained, and this change was also attributed to the effect of treatment on bodyweight (see Table 5.7.1-3).

Slightly higher bodyweight-relative kidney weights were recorded for males given 3000 ppm and bodyweight-relative liver and spleen weights were high in both sexes given 3000 ppm. The organ weights of animals given 100 or 540 ppm were considered to be unaffected by treatment (see Table 5.7.1-4).

Table 5.7.1-3: Mean absolute organ weights (g) after 2 weeks of treatment

Group/sex	**1M**	**2M**	**3M**	**4M**	**1F**	**2F**	**3F**	**4F**
Level (ppm)	**0**	**100**	**540**	**3000**	**0**	**100**	**540**	**3000**
Number of animals	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**
Tissue								
Brain	1.99	1.98	1.98	**1.86***	1.80	1.80	1.84	**1.66***
Adrenals	0.047	**0.039***	**0.038****	**0.034****	0.054	0.49	0.054	**0.045****
Epididymides	0.536	0.525	**0.441****	**0.345****				
Heart	1.211	1.132	**1.037***	**0.703****	0.748	0.789	0.776	**0.619****
Lungs & bronchi	1.605	1.511	1.451	**1.055****	1.104	1.140	1.098	**0.875****
Testes	3.22	2.99	3.00	**2.41***				
Thymus	0.489	0.546	0.570	**0.345***	0.470	0.505	0.430	**0.315***
Thyroids (+ paras)	0.013	0.014	0.012	**0.009***	-	-	-	-
Uterus and cervix					0.431	0.501	0.375	0.163
HCD†	**M**				**F**			
	n	**Mean**	**5%**	**95%**	**n**	**Mean**	**5%**	**95%**
Brain	346	2.006	1.87	2.15	356	1.874	1.74	2.01
Adrenals	346	0.0526	0.040	0.070	356	0.0647	0.049	0.084
Epididymides	331	0.8490	0.627	1.121				
Heart	316	1.2827	1.050	1.527	326	0.914	0.760	1.085
Lungs & bronchi	158	1.5603	1.278	2.040	167	1.2985	1.025	1.735
Testes	346	3.282	2.80	3.69				
Thymus	316	0.4627	0.301	0.685	326	0.4393	0.296	0.613
Thyroids (+ paras)	196	0.0142	0.010	0.020	204	0.0114	0.007	0.017
Uterus and cervix					191	0.5471	0.320	1.035

† HCD – Historical control data as presented in the study summary document
- No noteworthy findings
Significant when compared with control; * - p<0.05, ** p<0.01

Table 5.7.1-4: Mean relative organ weights (g) after 2 weeks of treatment

Group/sex	**1M**	**2M**	**3M**	**4M**	**1F**	**2F**	**3F**	**4F**
Level (ppm)	**0**	**100**	**540**	**3000**	**0**	**100**	**540**	**3000**
Number of animals	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**
Tissue								
Kidneys	0.856	0.858	0.851	**0.929***	0.938	**0.841***	0.870	0.892
Liver	4.889	4.822	4.760	**6.501***	4.162	4.068	4.718	**6.605****
Spleen	0.2549	0.2341	0.2283	**0.4006****	0.2462	0.2749	0.2428	**0.3387****

Significant when compared with control; * - p<0.05, ** p<0.01

2. Macroscopic findings at necropsy

Macroscopic examination at necropsy revealed treatment-related findings in the livers of animals given 3000 ppm. These findings included pale liver, lobular pattern accentuated and enlarged liver. Thin build observed in-life for animals receiving 3000 ppm was confirmed at necropsy. Small prostate and seminal vesicles were recorded for 4/5 males given 3000 ppm and for 1/5 males given 540 ppm. Thin uterus was recorded in 4/5 females given 3000 ppm and this finding correlates with the low uterus and cervix weights reported for these animals (these low weights were attributed to the effect seen on bodyweight). The macroscopic changes seen in the male reproductive organs were also considered to be related to the reduction in bodyweight seen in these animals (see Table 5.7.1-5).

Table 5.7.1-5: Summary of macroscopic findings after 2 weeks of treatment

Group/sex	**1M**	**2M**	**3M**	**4M**	**1F**	**2F**	**3F**	**4F**
Level (ppm)	**0**	**100**	**540**	**3000**	**0**	**100**	**540**	**3000**
Number of animals	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**
Tissue								
Liver								
Pale	0	0	0	3	0	0	0	0
Lobular pattern accentuated	0	0	0	2	0	0	0	2
Enlarged	0	0	0	**5****	0	0	0	**4***
Prostate								
Small	0	0	1	**4***	-	-	-	-
Seminal Vesicles								
Small	0	0	1	**4***	-	-	-	-
Uterus								
Thin	-	-	-	-	0	0	0	**4****

- No noteworthy findings

Significant when compared with control; * - $p<0.05$, ** $p<0.01$

All other macroscopic changes were of the type normally encountered in young CD rats at these laboratories.

III. CONCLUSIONS

It was concluded that the administration of metconazole cis/trans (KNF-474m) to rats for two weeks via the diet at concentrations of up to 3000 ppm (equivalent to 216.8 mg/kg/day in males and 206.1 mg/kg/day in females) did not result in any neurotoxicity, though at 3000 ppm the maximum tolerated dosage was exceeded. The no-observed-adverse-effect level (NOAEL) in this study was considered to be 100 ppm (equivalent to 11.0 mg/kg/day in males and 10.6 mg/kg/day in females). The findings from this study demonstrated that the highest dietary concentration in the main four week study should be in the region of 540 ppm and the lowest concentration should be below 100 ppm.

A 4-week sub-chronic neurotoxicity study in rats (study author: [redacted] 2002 b; BASF DocID 2002/8000063) with metconazole cis/trans (KNF-474m) was conducted for non-EU authorities by another metconazole-supporting company. A detailed study summary was made available to BASF (prepared by [redacted] 2015b; BASF DocID 2015/1087912) and is part of this submission.

Report:	CA 5.7.1/3 [redacted] 2015b Summary of the Laboratory report KRA068/022386 - KNF-474m: Neurotoxicity study by dietary administration to CD rats for 4 weeks 2015/1087912
Guidelines:	OECD 424 (1997)
GLP:	yes (certified by UK Department of Health)

Report:	CA 5.7.1/4 [redacted] 2002 b KNF-474m - Neurotoxicity study by dietary administration to CD rats for 4 weeks 2002/8000063
Guidelines:	OECD 424, JMAFF No 12 Nosan No 8147
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Executive Summary

The neurotoxic potential of the test substance metconazole cis/trans (KNF-474m) to Sprague-Dawley (Crl:CD® (SD)) rats by dietary administration was assessed over a period of four weeks. Three groups of ten male and ten female rats received KNF-474m in the diet at concentrations of 50, 170 or 500 ppm for four weeks and a similarly constituted control group received untreated diet throughout the treatment period. Achieved dosages at dietary concentrations of 50, 170 and 500 ppm were 4.84, 15.69 and 47.08 mg/kg/day for males and 5.10, 17.62, and 49.82 mg/kg/day for females receiving 50, 170 or 500 ppm, respectively. During the study clinical condition, body weight, food consumption, food conversion efficiency, neuro-behavioural investigations, brain anatomical measurements and weight, macro-pathology and neuro-histopathology investigations were undertaken. Neuro-behavioural investigations (assessment in the hand, in a standard arena, manipulations and motor activity measurements) were performed on all animals before treatment commenced and in week 4.

Continuous administration of KNF-474m to CD rats for four weeks at dietary concentrations up to 500 ppm did not result in any neurotoxicity. Neuro-behavioural screening and macroscopic and histopathological examination of the associated tissues (including anatomical measurements of the brain) revealed no treatment-related findings. In addition, no unusual signs or patterns of behaviour were observed at any of the routine observations. Consequently, there was no evidence that KNF-474m was neurotoxic to CD rats at dietary levels up to 500 ppm (47.08 mg/kg/day in males and 49.82 mg/kg/day in females).

The growth performance of animals receiving 170 or 500 ppm was inferior to that of the controls, particularly for females receiving 500 ppm with food conversion efficiency being low in females receiving this dietary concentration. This was associated with a sustained effect on food intake and was, considered to represent a non-specific indicator of general toxicity.

It was concluded that the administration of KNF-474m in the diet to CD rats, at concentrations up to 500 ppm (equivalent to 47.08 mg/kg/day in males and 49.82 mg/kg/day in females) produced no evidence of neurotoxicity. Non-specific toxicity was evident at 170 and 500 ppm in both sexes. The no-observed-adverse-effect level (NOAEL) for neurotoxicity in this study was considered to be 500 ppm (equivalent to 47.08 mg/kg/day in males and 49.82 mg/kg/day in females).

(DocID 2015/1087912)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: Metconazole (Code No. KNF-474m)

Description:	solid (crystalline powder) / white to pale yellow
Lot/Batch #:	9Z521
Purity:	98.99% (85:15, *cis:trans*)
Stability of test compound:	The test substance was stable in the diet as determined in an earlier experiment (HLS No. KRA 067/014561)

2. Vehicle: Diet

3. Test animals:

Species:	Rats
Strain:	Crl:CD(SD)
Sex:	Male and female
Age (at start of dosing):	42 days
Weight (at start of dosing):	198 – 248 g (males); 147 – 188 g (females)
Source:	Charles River (UK) Ltd.
Acclimation period:	11 days
Diet:	Rat and Mouse No. 1 Maintenance Diet, ad libitum
Water:	Tap water, ad libitum
Housing:	In groups (5 animals per cage, same sex) in stainless steel cages with stainless steel mesh lids and floor

Environmental conditions:

Temperature:	19 - 23°C
Humidity:	45 - 70%
Air changes:	at least 15 air changes per hour
Photo period:	12 h light / 12 h dark (06:00 - 18:00 / 18:00 - 06:00)

B. STUDY DESIGN AND METHODS

1. In-life dates: 14-Jan-2002 to 14-Feb-2002

2. Animal assignment and treatment:
There were 10 animals per sex and dose level, allocated randomly to the cages at the time of arrival. The test item was applied at dietary concentrations of 0, 50, 170 and 500 ppm for four weeks. The actual doses were 0, 4.84, 15.69, and 47.08 mg/kg bw/day for males and 0, 5.1, 17.62, and 49.82 mg/kg bw/day for females, respectively.

3. Test substance preparation and analysis:
Test diets were prepared in weekly batches by an initial preparation of a premix where the required amount of KNF-474m was mixed with an approximately equal amount of sieved diet and hand-stirred. A further amount of sieved diet, equal to the weight of the mixture, was added and stirred until it appeared visibly homogenous. This doubling-up process was repeated until half the final weight of premix was reached and the mixture was ground using a mechanical grinder. The weight of the mixture was then made up to the final required weight of the premix, using coarse diet, and was then mixed in a Turbula mixer for 100 cycles. Aliquots of the premix were diluted further with coarse diet to produce either further premixes or the test diets; each mix was performed for a further 100 cycles in a Turbula mixer.

Homogeneity and stability of the test material in the test diets were confirmed in an associated study (Huntingdon Life Sciences Report No. KRA 067/014561) at nominal concentrations of 30 and 3000 ppm during refrigerated storage for 8 days and for 10 days ambient storage following 8 days refrigerated storage.
Achieved concentrations were confirmed as acceptable in this study (see Table 5.7.1-6).

Table 5.7.1-6: Achieved concentrations

Test Group	Concentration in diet (ppm feed)	Mean concentration by analysis (ppm)		Actual concentration as a % of the nominal concentration	
		Week 1	Week 3	Week 1	Week 3
2	50	47.7	48.7	95	97
3	170	169	172	99	101
4	500	468	501	94	100

4. Statistics:
All statistical analyses were carried out separately for males and females. The following data types were analysed at each time point separately: bodyweight (using gains over appropriate study periods), neuro-behavioural data (rearing and activity counts, grip strength, hind-limb foot splay, bodyweight, temperature and motor activity), brain weights, pathological findings: for the number of animals with and without each finding.
For categorical data, including pathological findings, the proportion of animals was analysed using Fisher's Exact test (Fisher, 1973) for each treated group versus the control.

For continuous data, Bartlett's test (Bartlett, 1937) was first applied to test the homogeneity of variance between the groups. Using tests dependent on the outcome of Bartlett's test, treated groups were then compared with the control group, incorporating adjustment for multiple comparisons where necessary. For bodyweight gains and brain weights, whenever Bartlett's test was found to be statistically significant, a Behrens-Fisher test was used to perform pairwise comparisons, otherwise a Dunnett's test was used. The following sequence of statistical tests was used for the functional observational battery:
If 75% of the data (across all groups) were the same value, for example c, then a frequency analysis was applied. Treatment groups were compared using pairwise Fisher's Exact tests (Fisher, 1973) for each dose group against the control both for i) values <c versus values ≥c, and for ii) values ≤c versus values >c, as applicable. If Bartlett's test for variance homogeneity (Bartlett, 1937) was not significant at the 1% level, or if it was not significant after first a logarithmic or second a square-root transformation then parametric analysis was applied (a one way analysis of variance). If the analysis of variance showed evidence (at the 5% level) of differences between the groups, Student's t-test was used to test for differences between treatment groups and the control group. If Bartlett's test was significant at the 1% level, then logarithmic and square-root transformations were tried. If Bartlett's test was still significant, then non-parametric (Kruskal-Wallis) tests were applied (Kruskal and Wallis 1952 and 1953). If the Kruskal-Wallis test showed evidence (5% level) of differences between the groups, the Wilcoxon Rank-Sum test was used to test for differences between the treatment groups and the control group.
Significant differences between control and treated groups were expressed at the 5% ($p<0.05$) and 1% ($p<0.01$) levels.

C. METHODS

1. Observations:
Animals were inspected visually at least twice daily for evidence of ill-health or reaction to treatment. Cages and cage-trays were inspected daily for evidence of ill-health amongst the occupants, such as loose faeces. Any deviation from normal was recorded at the time in respect of nature and severity, date and time of onset, duration and progress of the observed condition, as appropriate. In addition, a more detailed weekly physical examination was performed on each animal to monitor general health. Further observations were made as part of the neuro-behavioural screening examinations. During the acclimatisation period, observations of the animals and their cages were recorded at least once per day.

2. Body weight:
The weight of each rat was recorded one week before treatment commenced (week -1), on the day that treatment commenced (week 0), weekly throughout the treatment period and before necropsy. The bodyweights of all animals were checked on Day 1 before feeding to ensure that the bodyweight variations did not exceed ± 20% of the mean for the appropriate sex.

3. Food consumption and food conversion efficiency:
The weight of food supplied to each cage, that remaining and an estimate of any spilled was recorded for each week throughout the treatment period. From these records the mean weekly consumption per animal (g/rat/week) was calculated for each cage. Food conversion efficiencies were calculated for each group/sex for each week of treatment.

4. Neuro-behavioural screening:
Neuro-behavioural investigations (assessment in the hand, in a standard arena, manipulations and motor activity measurements) were performed on all animals before treatment commenced and in week 4. The functional observational battery and motor activity recordings were performed at approximately the same time of day on each occasion and the observer was unaware of the experimental group to which the animal belonged. The animals were not necessarily all tested on the same day but the number of animals was balanced across the groups on each day of testing. After removal from the home cage, the following parameters were assessed (in-the-hand observations): ease of removal from the cage, exophthalmos, fur condition, piloerection, reactivity to handling, salivation, lacrimation and vocalisation. Each animal was then placed in a standard arena (approximately 653 mm × 500 mm) with the floor marked into six sections of equal size for scoring activity counts and the following assessed during a three-minute period: activity count, arousal, convulsions, tremor, twitches, defecation, gait, grooming, palpebral closure, posture, rearing count and urination. After completion of arena observations, the following manipulation measurements were made: approach response, body temperature, body weight, grip strength, pupil reflex, righting reflex, auditory startle reflex, tail pinch response, landing foot-splay, pupil closure reflex and touch response. Finally, motor activity was assessed over a one hour period (observed in ten six-minute periods) in which each animals was placed on a clear polycarbonate cage with four high and four low infra-red beams (to assess cage floor and rearing activity levels).

5. Sacrifice and pathology:
Animals were killed by an overdose of barbiturate by intra-peritoneal injection and exposure of the heart to permit perfusion with glutaraldehyde/paraformaldehyde fixative via the left ventricle. The sequence in which the animals were killed after completion of treatment was selected to allow satisfactory inter-group comparison. After a review of the history of each animal, a full macroscopic examination of the tissues was performed. All external features and orifices were examined visually. The cranial roof was removed to allow observation of the brain, pituitary gland and cranial nerves. After ventral mid-line incision, the neck and associated tissues and the thoracic, abdominal and pelvic cavities and their viscera were exposed and examined in situ. Any abnormal position, morphology or interaction was recorded. The tissues were checked and the carcass retained.
Samples (or the whole) of the following tissues from all animals were preserved in glutaraldehyde/paraformaldehyde fixative by in situ perfusion followed by immersion; brain, spinal cord, dorsal root ganglia and fibres, ventral root fibres, eyes, optic nerves, skeletal muscle, sciatic and tibial nerves. The brain from each animal was weighed and then transected from the spinal cord above the first cervical spinal nerve and the olfactory lobes removed. Measurements were taken of the length between the rostral part of the cerebral hemispheres to the most caudal part of the cerebellum and also the width between the widest parts of the cerebral hemispheres. The tissues submitted were examined [five male and five female rats with the lowest animal numbers of Groups 1 (control) and 4 (500 ppm)] for histopathological examination.

II. RESULTS AND DISCUSSION

A. OBSERVATIONS

1. Clinical signs of toxicity

The appearance and behaviour of the animals at routine examinations were unaffected by treatment.
One male receiving 500 ppm (No. 33) had a palpable swelling on the right lower dorsal area. In-life signs were restricted to the functional observational battery results. The animal was limping from week 1 and had low activity scores throughout. From week 3 onwards the animal was hunched and it had an elevated gait during week 4. This swelling affected the landing foot-splay results; the week 4 score was low when compared with the pre-treatment result. Macroscopic and microscopic findings for this animal indicted that it had an abscess of the muscle adjacent to the right hip joint with minimal dermal haemorrhage and slight subcutaneous inflammation. In the absence of any similar finding in any other animals, these findings are considered incidental and not related to treatment, but rather related to the individual animal's condition involving a swollen, subcutaneous/intramuscular lesion identified as an abscess.

2. Mortality

No animals died prematurely.

B. BODY WEIGHT AND BODY WEIGHT GAIN

When compared with the controls, low body weight gains were observed in week 1 in males receiving 170 ppm and in animals receiving 500 ppm. At the highest concentration the effect was more marked in females than in the males. Subsequent body weight gain was considered unaffected by treatment. As a result of the effect in week 1, the overall weight gain of females receiving 500 ppm was lower than that of the controls.
Body weight gains of animals receiving 50 ppm and of females receiving 170 ppm were considered unaffected by treatment. (see Table 5.7.1-7)

Table 5.7.1-7: Mean body weight gain (g) (selected periods)

	Week 0-1		Week 1-4		Week 0-4	
Level (ppm)	**M**	**F**	**M**	**F**	**M**	**F**
Control	51	22	96	54	147	77
50	53	22	90	53	144	75
170	46	20	**78***	48	124	68
500	**41***	**13***	100	49	141	62

Significant when compared with control; * - $p<0.05$
Parenthesis – standard deviation

C. FOOD CONSUMPTION AND FOOD EFFICIENCY

Animals receiving 170 or 500 ppm consumed less food than the controls in week 1; in females receiving 500 ppm this effect persisted throughout the treatment period, and consequently, the total food intake of these animals was low when compared with the controls. Food scatter values were generally higher than the controls in treated male groups but the effect across the groups was not always dosage-related. In females the treated groups scattered more food than the controls in week 1 but thereafter scatter values were variable throughout the groups and similar to the controls.

When compared with the controls food conversion efficiency was low in week 1 for males receiving 170 ppm and for animals receiving 500 ppm. It was also low in week 2 for females receiving 170 or 500 ppm. Slightly low overall food conversion efficiencies were recorded for females receiving 170 or 500 ppm (see Table 5.7.1-8).

Table 5.7.1-8: Mean food consumption/food conversion efficiency (selected periods)

	Week 1		Week 2	Week 1-4	
Level (ppm)	**M**	**F**	**F**	**M**	**F**
Control (g)	196	141	146	-	579
Control (%)	26.1	15.9	16.0	-	13.3
50 (g)	199	140	141	-	570
50 (%)	26.7	15.9	15.0	-	13.2
170 (g)	189	130	144	-	565
170 (%)	24.2	15.3	12.6	-	12.0
500 (g)	184	126	137	-	531
500 (%)	22.1	10.2	12.5	-	11.7

Parenthesis – units; g = food consumption; % = food conversion efficiency
- No noteworthy findings

D. NEUROBEHAVIOURAL SCREENING

1. In-the-hand observations

In-the-hand observations were unaffected by treatment.

2. Arena observations

Arena observations showed some inter-group variation but there were no-treatment-related changes.

Rearing counts during week 1 and 2 in treated males were lower than those of the controls but this reflected a trend that was apparent before treatment commenced and was not present at the motor activity assessments. Consequently, this was not attributed to treatment. (see Table 5.7.1-9)

Table 5.7.1-9: Summary of rearing count

Group/sex Level (ppm) Number of animals	1M 0 10	2M 50 10	3M 170 10	4M 500 10	1F 0 10	2F 50 10	3F 170 10	4F 500 10
Pre-treatment	11.3	9.9	9.7	8.5	-	-	-	-
Week 1	5.9	**2.5****	**2.5****	**2.5****	-	-	-	-
Week 2	5.6	2.6	2.7	2.5	-	-	-	-

- No noteworthy findings

3. Manipulations

Manipulations were unaffected by treatment.

4. Motor activity

Motor activity was unaffected by treatment.

E. TERMINAL PROCEDURES

1. Macroscopic findings at necropsy

Macroscopic examination of the animals killed on completion of the treatment period revealed no treatment-related findings.

2. Brain parameters

Absolute brain weights were unaffected by treatment. The anatomical measurements made of the brain did not indicate any treatment-related differences between the control and treated animals.

3. Microscopic findings

There was no microscopic pathology findings considered to be related to treatment with KNF-474m.

III. CONCLUSIONS

It was concluded that the administration of KNF-474m to Sprague-Dawley rats, via the diet, at concentrations up to 500 ppm (equivalent to 47.08 mg/kg/day in males and 49.82 mg/kg/day in females) produced no evidence of neurotoxicity. Non-specific toxicity was evident at 170 and 500 ppm in both sexes. The no-observed-adverse-effect level (NOAEL) for neurotoxicity in this study was considered to be at least 500 ppm (equivalent to at least 47.08 mg/kg/day in males and 49.82 mg/kg/day in females).

CA 5.7.2 Delayed polyneuropathy studies

Based on the structure of metconazole (it is not an organophosphate), the active substance would not be expected to produce delayed neuropathy. Also none of the impurities in the proposed technical specification for which approval is sought are organophosphates**.** Thus, metconazole does not belong to a chemical family for which testing for delayed neurotoxicity is required.

CA 5.8 Other Toxicological Studies

CA 5.8.1 Toxicity studies of metabolites

Assessment of metabolites in food of plant and animal origin
With regard to the metabolites in food of plant and animal origin a relevance assessment has been performed according to the TTC (Threshold of Toxicological Concern) concept, which is the approach as described in the 'Scientific Opinion on Evaluation of the Toxicological Relevance of Pesticide Metabolites for Dietary Risk Assessment' (EFSA Journal 2012; 10(07): 2799). It is laid down in this EFSA Scientific Opinion that 'the TTC approach is the most appropriate tool to evaluate the toxicological relevance of metabolites associated with chronic dietary exposure. The TTC values, established for genotoxic and toxic compounds based on the Cramer et al., (1978;Food Cosmet. Toxicol. 16**,** 255–276) scheme, were considered sufficiently conservative'.
This approach assesses whether chemical structures are of concern for which no or only limited information on the toxicological profile is available, and considers whether the predicted exposure is above or below a threshold of toxicological concern (Cramer et al., 1978; BASF DocID 1978/1001324; Kroes et al., 2004; BASF DocID 2004/1036074; Munro et al., 1996; BASF DocID 1996/1005180). The proposed threshold levels are 0.0025 µg/kg bw/day for potentially genotoxic compounds and 1.5 µg/kg bw/day for non-genotoxic Cramer Class III compounds.
According to the presented assessments (see chapter MCA 6.9.) exposure of the metconazole metabolites remains well below the acceptable long-term exposure threshold value of 1.5 µg/kg bw/day for Cramer Class III compounds.
However, five metabolites M555F001 cis (Reg. No. 4111795), M555F011 cis (Reg.No. 4111112), M555FF021 cis (Reg.No. 4558878), M555F030 cis (Reg.No. 4110625), and M555F031 cis (Reg.No. 5968488) exceeded the TTC genotoxicity threshold value of 0.0025 µg/kg bw/day (see chapter MCA 6.9.)
M555F001 cis was detected in rat urine (5% and 14% of the administered dose in males and females, respectively) and thus, it is already covered by assessment of already performed rat studies with metconazole.
Metconazole metabolites in food (plant or animal origin) are M555F011 cis (Reg.No. 4111112), M555F021 cis (Reg.No. 4558878), M555F030 cis (Reg.No. 4110625), and M555F031 cis (Reg.No. 5968488). M555F021 cis has been demonstrated to be formed in rats after administration of metconazole, but only in minor amounts (see also M-CA 5.1). Therefore all of these metabolites are not regarded as covered by toxicological testing of the parent compound.
Thus, in agreement with the RMS an Ames test was conducted with each of these four metabolites (see Table 5.8.1-1).

With regard to the triazole-derived metabolites, no toxicological assessment has been conducted, as that would be beyond the scope of the metconazole assessment and will be reconsidered based on the outcome of the on-going UK assessment.

Studies evaluated in the original monograph (2004) and the addendum of the monograph (2006):
Studies submitted for the last Annex I listing consisted of an acute oral toxicity study with plant metabolite 555FM011.

Table 5.8.1-1: Summary of toxicity studies with Metconazole metabolites

Metabolite	Study	Species (strain)/Test System	Findings (Dose levels tested)	References* (BASF DocID)
M555F011 cis (Reg.No. 4111112)	Acute oral LD50	Rat (Sprague-Dawley)	>5000 mg/kg bw (males and females)	[redacted] **1997; (MK-470-025)**
M555F011 cis (Reg.No. 4111112)	Microbial Mutagenicity Assay (Ames)	*S. typhimurium :* TA98, TA100, TA1535, TA1537 and TA1538; *E. coli :* WP2 *uvrA*-	± S9: Negative (3.3 - 5200 µg/plate)	Woitkowiak C., 2015a*; (2014/1035851)
M555F021 cis (Reg.No. 4558878)	Microbial Mutagenicity Assay (Ames)	*S. typhimurium :* TA98, TA100, TA1535, TA1537 and TA1538; *E. coli :* WP2 *uvrA*	± S9: Negative (3.3 - 5300 µg/plate)	Woitkowiak C., 2015b*; (2014/1035852)
M555F030 cis (Reg.No. 4110625)	Microbial Mutagenicity Assay (Ames)	*S. typhimurium :* TA98, TA100, TA1535, TA1537 and TA1538; *E. coli :* WP2 *uvrA*	± S9: Negative (3.3 - 5000 µg/plate)	Woitkowiak C., 2015c*; (2014/1035853)
M555F031 cis (Reg.No. 5968488)	Microbial Mutagenicity Assay (Ames)	*S. typhimurium :* TA98, TA100, TA1535, TA1537 and TA1538; *E. coli :* WP2 *uvrA*	± S9: Negative (10 - 5000 µg/plate)	Woitkowiak C., 2015d*; (2014/1035854)

*new studies
already peer-reviewed study is indicated in bold

Presence of Structural alerts – <u>QSAR evaluation of metabolites</u>

For all plant metabolites the presence for potential structural alerts was evaluated with different QSAR models. Models used were the OASIS TIMES and VEGA (Caesar, ISS and SarPy).

OASIS TIMES

OASIS TIMES is a hybrid statistical and knowledge-based model for toxicity prediction. The Tissue Metabolism Simulator (TIMES), developed by LMC (Bourgas University, Bulgaria; http://oasis-lmc.org/) integrates on the same platform a metabolic simulator and QSAR models for predicting toxicity of selected metabolites. The metabolic simulator generates plausible metabolic maps from a comprehensive library of biotransformations and abiotic reactions. It allows prioritization of chemicals according to toxicity of their metabolites. Of OASIS TIMES the prediction models for Ames test (DocID 2015/1198619) and in vitro chromosome aberration (DocID 2015/1198620) were considered and therefore predictivity is limited to these test systems only. Q(SAR) Model Reporting Formats (QMRF) for both endpoints are provided in DocIDs 2015/1198609 and 2015/1198616.

The reactivity model describing interactions of chemicals with DNA is based on an alerting group approach. Only those toxicophores extracted from the training set that have clear interpretation for the molecular mechanism causing the ultimate effect are included in the model. The mechanistic interrelation between alerts and related parametric ranges generalising the effect of the rest of the molecules on the alert is also considered. The structural component of the model is based on the structural similarity between chemicals in the training set which were correctly predicted by the model. The structural neighbourhood of atom-centered fragments is used to determine this similarity. The training set consists of 1514 chemicals for Ames and 808 chemicals for chromosomal aberration.

The derived model is combined with metabolic simulator TIMES used for predicting metabolic activation of chemicals with the S9 mix. The metabolic simulator is trained to reproduce documented maps for mammalian liver metabolism for 261 chemicals. Parent chemicals and each of the generated metabolites are submitted to a battery of models to screen for a general effect and mutagenicity mechanisms. Thus, chemicals are predicted to be mutagenic as parents only, parents and metabolites, and metabolites only. Mutagenicity could be due to the parent chemical only or as a result of its metabolic activation (i.e., the parent is inactive but it is transformed to a mutagenic metabolite), or both parent structure and metabolites could be mutagenic.

VEGA
Using the VEGA platform, access to a series of QSAR (quantitative structure-activity relationship) models for regulatory purposes was obtained. Of the models offered by VEGA [http://www.vega-qsar.eu/] only the three independent statistical prediction models for mutagenicity (Ames) were selected.
The data obtained for the metabolites of metconazole can be found in DocIDs 2015/1198621, 2015/1198622 and 2015/1198623 for the various models applied (CEASAR, ISS, SarPy, respectively.

CAVEAT on reliability of QSAR modules implied
With regard to the QSAR evaluations as implied in OECD TIMES and in VEGA it should be noted that for nearly all analysis the algorithm reported are out of structural domain error. As a consequence the predictivity is solely based on the proposed DNA-interaction via the structural alert, not (VEGA) or not appropriately (OASIS TIMES) taking into account possible functional group interaction and stereochemical hindrance. It is well acknowledged that these structural activity predictions are therefore of limited validity. If suitable the analysis was conducted in comparison to the parent compound metconazole or related structures with available toxicological data, in order to assess whether same or other predictions than for the compared compound were made.

Chemical Similarity
With regard to evaluation of chemical similarity the general proposals given by e.g. by the EFSA Scientific Opinion on Evaluation of the Toxicological Relevance of Pesticide Metabolites for Dietary Risk Assessment [EFSA Journal 2012;10(07):2799] or the were followed. The following general molecular modifications were considered to probably not cause higher toxicity of the metabolites:

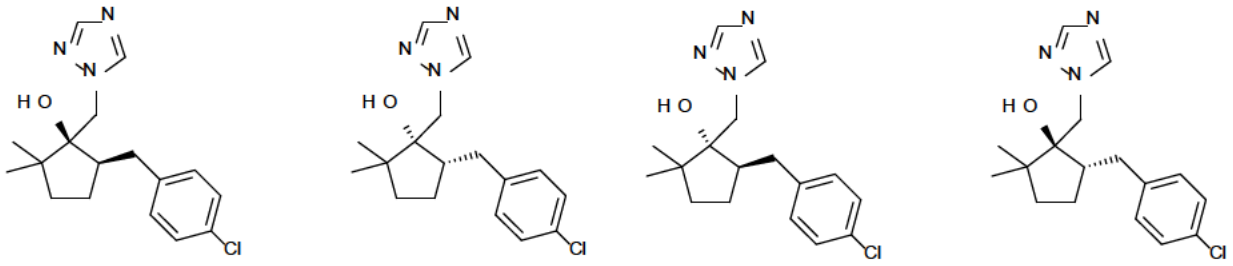
- Simple demethylation of the ring or side chain
- Simple hydroxylation of the ring system without any cleavage of the ring
- Hydroxylation of another ring position
- Conjugation of molecules with amino acids

Comparison was made to parent as well as to other similar compounds where toxicological data were available that allowed a further evaluation of the compound under consideration. In addition increased hydrophilicity and thus considered faster excretion was taken into account.

Toxicological Assessment of the Active Ingredient

For the convenience of the reviewer the assessment for metconazole is shortly summarized below, previous to the assessments of the metabolites.

TGAI Metconazole (BAS 555 F; Reg. No. 4056343 - sum of cis- and trans-isomers):



cis-isomers
Reg.no. 4079468

trans-isomers
Reg.no. 4079654

a) QSAR predictions on cis- and trans-metconazole (Reg. No. 4079468; CL. No. 354801):

QSAR evaluation

Since the QSAR prediction was exactly the same for the both isomers in all modules applied, the results for cis- and trans-metconazole are summarized as follow:

Active Substance	QSAR tools applied	Structural alerts for genotoxicity	Reliability / Total Domain	Evaluation
cis-isomer: Reg. No. 4079468 **trans-isomer: Reg. No. 4079654**	OA	Ames parent: negative Ames metabolites: negative	Low / Out of Domain	**Not genotoxic**
		CA parent: negative CA metabolites: 2/16 positive	Low / Out of Domain	
	VE	CAESAR: not mutagenic	Moderate / could be out of Domain	
		ISS: not mutagenic	Low / Out of Domain	
		SarPy: not mutagenic	Moderate / could be out of Domain	

OA = OASIS-TIMES
VE = Vega
CA = Chromosomal aberration

OASIS TIMES [see molecules 1 and 2 of report DocID 2015/1198402 for cis- and trans-metconazole, respectively] predicted cis-metconazole and in silico generated metabolites to be not mutagenic in the Ames test, with the limitation that the molecule was out of the prediction domain.
OASIS TIMES CA [see molecule 1 and 2 of report DocID 2015/1198403 for cis- and trans-metconazole, respectively] prediction was positive for 2/16 in silico generated metabolites with the alert "Substituted Phenols", with the limitation that the molecule was out of the prediction domain.

The VEGA prediction in all modules (CAESAR, ISS and SarPy) was not mutagenic and the reliability of this prediction was low to moderate [see molecule 0 and 1 for cis- and trans-metconazole in reports DocID 2015/1198404; DocID 2015/1198405, and DocID 2015/1198406].

b) Toxicity studies with Metconazole (BAS 555 F):

Cis-metconazole (BAS 505 F; Reg. No. 4079468; CL. No. 354801)

The genotoxic potential of cis-metconazole was assessed based on toxicity studies with batch N12, containing Reg. No. 4079468 at 952 g/kg (95.2%).

Bacterial mutagenicity (Ames test; batch N12); DocID MK-435-003: **negative**

In vitro mammalian gene mutation (MLTK, batch N12); DocID MK-435-007: **negative**

In vitro chromosome aberration (CA, batch N12); DocID MK-435-005: **negative**

In vivo unscheduled DNA synthesis (UDS, batch N12); DocID MK-435-004: **negative**

In vivo chromosome aberration (MNT, batch N12); DocID MK-435-006: **negative**

Based on experimental data that were clearly negative, cis-metconazole is assessed to have no genotoxic potential.

Trans-metconazole (BAS 505 F; Reg. No. 4079654; CL. No. 354802)

The genotoxic potential of trans-metconazole was assessed based on toxicity studies with batch 89-01 (cis/trans content: 79.8%/15.5%) and with batch AC 9339-114 (cis/trans content: 83.7%/13.7%) in in vitro and in vivo genotoxicity studies, respectively.

Bacterial mutagenicity (Ames test; batch 89-01); DocID MK-435-001: **negative**

In vitro chromosome aberration (CA, batch 89-01); DocID MK-435-002:

-S9 negative;
+S9 (24h) positive at 50 µg/mL
+S9 (48 h) negative

In vivo unscheduled DNA synthesis (UDS, batch AC 9339-114); DocID MK-435-008: **negative**

In vivo chromosome aberration (MNT, batch AC 9339-114); DocID MK-435-009: **negative**

c) Conclusion on metconazole technical (BAS 555 F; Reg. No. 4056343; CL. No. 900768, cis/trans mixture):

Individual QSAR analysis was performed for the cis- and the trans-metconazole structure. However, the results are identical irrespective of the isomer.

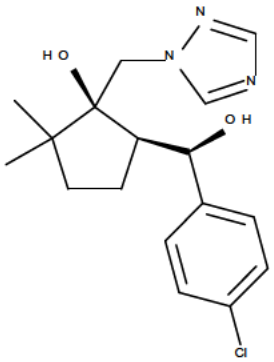
For cis-metconazole (BAS 505 F; Reg. No. 4079468; CL. No. 354801) no structural alert was identified regarding mutagenicity. The prediction was “non-mutagen” in all models used and is in concordance with available in vitro data (see MCA 5.4).
Regarding in vitro chromosome aberration, the structural alert “Substituted Phenols” was received for some hydroxylated metabolites. The structural alert is not reflected by experimental data (see MCA 5.4). In vitro chromosome aberration in human lymphocytes (DocID MK-435-005) and in vivo micronucleus test in mice (DocID MK-435-006) were negative. This alert is not predictive and is therefore rejected.

For trans metconazole (BAS 505 F; Reg. No. 4079654; CL. No. 354802) no structural alert was identified regarding mutagenicity. The prediction was “non-mutagen” in all models used and is in concordance with available in vitro data conducted with the cis/trans mixture (see MCA 5.4).
Regarding in vitro chromosome aberration the structural alert “Substituted Phenols” was received for some hydroxylated metabolites. The structural alert is partly reflected by experimental data (see MCA 5.4). In vitro chromosome aberration test in CHO cells with the cis/trans metconazole (DocID MK-435-002) was negative without addition of S9 mix (parent compound), and positive after 24 but not 48 hours after addition of S9 mix (metabolites). However, the *in vitro* testing for chromosome aberrations using the rodent CHO cells is known to produce more false positive results compared to usage of p53 unimpaired and DNA repair competent human lymphocytes (Kirkland et al., 2007; DocID 2007/1071065). Testing of 19 genotoxins that are known to produce false positive results in vitro, revealed a false positive response of 53% using the CHO cells comparted to the false positive rate of 17% in human lymphocytes for evaluation of chromosomal aberrations by in vitro micronucleus assay (Fowler et al., 2012; DocID 2012/13690).
Furthermore, metconazole batches N12 (cis-metconazole) and AC 9339-114 (cis/trans metaconazole) were tested for chromosome aberration by an in vitro micronucleus test with mice (DocID MK-435-006 and DocID MK-435-009) and was clearly negative. Thus, this structural alert that was received for cis-metconazole and trans-metconazole did not reflect the data available from in vitro and in vivo studies.
By weight of evidence, the QSAR alert for the in vitro CA endpoint, simulating the rodent CHL and CHO cells (DocID 2015/1198616), is assessed to be not predictive and is therefore rejected.

It is concluded that metconazole TGAI (BAS 555 F; Reg. No. 4056343) has no genotoxic properties based on the results from several in vitro and in vivo genotoxicity studies.

Toxicological Assessment of Metabolites

M555F011 cis - Reg. No. 4111112



M555F011 cis ((1RS,5SR)5-[(SR)-(4-chlorophenyl)(hydroxy)methyl]-2,2-dimethyl-1-(1H-1,2,4-triazol-1-ylmethyl)cyclopentanol; CAS-No. 155413-23-3) was identified in fish, plant (rape, banana and wheat straw) and in the water.

QSAR evaluation

Specified metabolite	QSAR tools applied	Structural alerts for genotoxicity	Reliability / Total Domain	Evaluation
M555F011 cis Reg. No. 4111112	OA	Ames parent: negative Ames metabolites: negative	Low / Out of Domain	**Not genotoxic**
		CA parent: negative CA metabolites: 2/24 positive	Low / Out of Domain	
	VE	CAESAR: not mutagenic	Moderate / could be out of Domain	
		ISS: not mutagenic	Low / Out of Domain	
		SarPy: not mutagenic	Moderate / could be out of Domain	

OA = OASIS-TIMES
VE = Vega
CA = Chromosomal aberration

OASIS TIMES [see molecule 2 of report DocID 2015/1198619] predicted M555F011 and in silico generated metabolites to be not mutagenic in the Ames test, with the limitation that the molecule was out of the prediction domain.
OASIS TIMES CA [see molecule 2 of report DocID 2015/1198620] prediction was positive for 2/24 in silico generated metabolites with the alert "Substituted Phenols", with the limitation that the molecule was out of the prediction domain.

The VEGA prediction in all modules (CAESAR, ISS and SarPy) was not mutagenic and the reliability of this prediction was low to moderate [see molecule 1 of reports DocID 2015/1198621; DocID 2015/1198622, and DocID 2015/1198623].

Available experimental data:

M555F011 cis was of low acute toxicity based on LD_{50} that was greater than 5000 mg/kg bw derived from an acute oral study with rats (DocID MK-470-025), that was already submitted and peer-reviewed in the Annex I registration process.

Since M555F011 cis was not detectable the rat (see CA 5.1), its toxicological properties are not covered by the assessment of already performed rat studies. However, this metabolite represent a simply hydroxylation of the parent compound without any molecule cleavage and therefore higher toxicity compared to the parent is not expected. Therefore, a genotoxicity assessment based on an Ames assay is considered to be acceptable.

Mutagenicity of M555F011 cis was investigated in the Ames test with no indication of a mutagenic effect (DocID 2014/1035851).

Conclusion:

No conclusive structural alert was identified for M555F011 regarding mutagenicity. The prediction was “non-mutagen” in all models used and is in concordance with available in vitro data.

Regarding in vitro chromosome aberration the structural alert “Substituted Phenols” was received for some hydroxylated metabolites. Exactly the same structural alert was also received for cis-metconazole and did not reflect the data available from in vitro and in vivo studies, which were clearly negative and was rejected. Based on weight of evidence, this structural alert for M555F011 is assessed to be not predictive for this structure class and is therefore rejected.

Thus, M555F011 is considered to be of no toxicological relevance and the ADI derived for metconazole can be applied to this metabolite as well.

Available toxicological studies with M555F011

Oral LD50 study in albino rats with AC 382390 ([redacted] 1997; BASF DocID MK-470-025)

Guidelines: OECD 401; EEC 92/69 B 1
GLP: Yes (laboratory certified by United States Environmental Protection Agency)
Acceptance: The study was accepted.

Material and methods:

AC 382390 (= M555F011= metconazole metabolite M11 = M36; Batch No. AC11021-26A; purity: 91.5%)

Five male and 5 female Sprague-Dawley rats were fasted overnight and thereafter dosed by oral intubation with AC 382390 at a limit dose of 5000 mg/kg bw. For this purpose the test substance was ground with a mortar and pestle and then mixed with 0.5% carboxymethyl cellulose in water to achieve a uniform weight/volume dispersion of the test material in the diluent. The test dispersion was prepared fresh on the day of dosing at a nominal concentration of 50% and administered at a constant volume of 10 mL/kg bw. The animals were observed for mortality and clinical signs of toxicity daily during the 14-day study period. Body weights were recorded on the day of dosing (day 0), day 7 and at termination (day 14). Necropsies were performed on all survivors at the end of the 14-day observation period.

Findings:

All animals survived the 14-day study period. There were no clinical signs observed and all animals gained weight throughout the 14-day observation period. There were no gross pathological findings observed in animals which were sacrificed at the termination of the study.

Conclusion:

For metabolite M555F011 (former AC 382390), the oral LD_{50} value was found to be greater than 5000 mg/kg bw for male and female rats.

Report:	CA 5.8.1/1 Woitkowiak C., 2015a Reg.No. 4111112 (metabolite of BAS 555 F, Metconazole) - Salmonella typhimurium / Escherichia coli - Reverse mutation assay 2014/1035851
Guidelines:	OECD 471 (1997), (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.13/B.14 No. L 142, EPA 870.5100
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

S. typhimurium (strains TA98, TA 100, TA 1535, and TA 1537) and E. coli (uvrA) were exposed to Reg.No. 4111112 (Metabolite of metconazole; batch L86-10) using DMSO as a solvent in the presence and absence of metabolic activation in standard plate and pre-incubation tests. Triplicate plates were used per dose and per test condition. Vehicle and positive controls were included in each experiment. The test substance was used at concentrations in the range of 3.3 to 5200 µg/plate in the presence and absence of S9-mix. Bacteriotoxic effects were observed depending on the strain and test condition from about 100 (standard plate test) or 333 (pre-incubation test) µg/plate onward. A biologically relevant increase in the number of revertant colonies was not noticed in any of the strains tested in presence or absence of metabolic activation in any of the experiments. The positive controls induced the appropriate response in the corresponding strains in the absence or presence of metabolic activation. According to the results of the study the test substance the test substance was not mutagenic under the experimental conditions of the study.

(Doc ID 2014/1035851)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material	Reg.No. 4111112 (Metabolite of metconazole)
Description:	Solid / white
Lot/Batch #:	L86-10
Purity:	97.8% (tolerance ±1.0%)
Stability of test compound:	The stability of the test substance under storage conditions is guaranteed until 01 Aug 2016 as indicated by the sponsor, and the sponsor holds this responsibility.
Homogeneity:	The homogeneity of the test substance was ensured by mixing before preparation of the test substance solutions.
Vehicle:	DMSO

2. Control Materials:

Vehicle control: The vehicle control with and without S-9 mix only contained the vehicle used for the test substance at the same concentration and volume for all tester strains.

Sterility control: Additional plates were treated with soft agar, S9 mix, buffer, vehicle or the test substance but without the addition of tester strains.

Positive control compounds tested without addition of metabolic activation system:

Strain	Mutagen	Solvent	Concentration
TA 100	MNNG	DMSO	5 µg/plate
TA 1535	MNNG	DMSO	5 µg/plate
TA 1537	9-Aminoacridine	DMSO	100 µg/plate
TA 98	NOPD	DMSO	10 µg/plate
E. coli	4-NQO	DMSO	5 µg/plate

Positive control compounds tested with addition of metabolic activation system:

Strain	Mutagen	Solvent	Concentration
TA 100	2-Aminoanthracene	DMSO	2.5 µg/plate
TA 1535	2-Aminoanthracene	DMSO	2.5 µg/plate
TA 1537	2-Aminoanthracene	DMSO	2.5 µg/plate
TA 98	2-Aminoanthracene	DMSO	2.5 µg/plate
E. coli	2-Aminoanthracene	DMSO	60 µg/plate

3. Activation:

S9 was produced from the livers of male Wistar rats treated with 80 mg/kg bw phenobarbital (i.p.) and β-naphthoflavone (orally) for three consecutive days. The S9-mix was prepared freshly prior to each experiment. For this purpose, a sufficient amount of S9-fraction is thawed at room temperature and 1 volume of S9-fraction is mixed with 9 volumes of S9-supplement (cofactors). This preparation, the so-called S9-mix, was kept on ice until used.
The rat liver S9-mix was prepared immediately before use and had the following composition:

Component	Concentration
Phosphate buffer (pH 7.4)	15 mM
Glucose 6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM
S9	10 %

To demonstrate the efficacy of the S9 mix, the S9 batch was characterized with benzo(a)pyrene.

4. Test organisms: S. typhimurium strains: TA98, TA100, TA1535, TA1537
E. coli : WP2 uvrA

5. Test concentrations:
Standard plate test (1st experiment):
Triplicate plates in one experiment were prepared for each concentration (neg. control, 33, 100, 333, 1000, 2600, and 5200 µg/plate) and condition (i.e. with and without S9) for all tester strains.

Standard plate test (2nd experiment):
Triplicate plates in one experiment were prepared for each concentration (neg. control, 10, 33, 100, 333, 1000, and 2600 µg/plate) and condition (i.e. with and without S9) for TA98, TA100, TA1535, and TA1537. Based on bacteriotoxicity that was observed in the 1st standard plate test with the TA tester strains this second experiment was performed.

Standard plate test (3rd experiment):
Triplicate plates in one experiment were prepared for each concentration (neg. control, 10, 33, 100, 333, 1000, and 2600 µg/plate) and condition (i.e. with and without S9) for TA100. Furthermore, triplicate plates in one experiment were prepared for each concentration (neg. control, 3.3, 10, 33, 100, 333, and 1000 µg/plate) in the presence of S9 mix for TA1537. Due to a bacteriotoxicity shift observed in the second standard plate test in tester strain TA 1537 with S9 mix and due to technical reason with TA 100, the experimental parts were repeated.

Pre-incubation test (4th experiment):
Triplicate plates in one experiment were prepared for each concentration (neg. control, 3.3, 10, 33, 100, 333, and 1000 µg/plate) and condition (i.e. with and without S9) for all tester strains.

B. TEST PERFORMANCE:

1. Dates of study period: 21 October 2014 – 07 November 2014

2. Standard plate test:
Test tubes containing 2-mL portions of soft agar (overlay agar), which consists of 100 mL agar (0.8% [w/v] agar + 0.6% [w/v] NaCl) and 10 mL amino acid solution (minimal amino acid solution for the determination of mutants: 0.5 mM histidine + 0.5 mM biotin for Salmonella typhimurium strains or 0.5 mM tryptophan for E. coli) were kept in a water bath at about 42 - 45°C, and the remaining components were added in the following order:

0.1 mL test solution or vehicle (negative control)
0.1 mL fresh bacterial culture
0.5 mL S9 mix (**with metabolic activation**) **or** 0.5 mL phosphate buffer (**without metabolic activation**)

After mixing, the samples were be poured onto Minimal glucose agar plates within approximately 30 seconds. After incubation at 37°C for 48 – 72 hours in the dark, the bacterial colonies (his^+ or trp^+ revertants) were counted. The colonies were counted using the Sorcerer Image Analysis System with the software program Ames Study Manager (Perceptive Instruments Ltd., Haverhill, UK). Colonies were counted manually, if precipitation of the test substance hinders the counting using the Image Analysis System.

3. Preincubation test:
0.1 mL test solution or vehicle, 0.1 mL bacterial suspension and 0.5 mL S9 mix (**with metabolic activation**) or phosphate buffer (**without metabolic activation**) were incubated at 37°C for the duration of about 20 minutes using a shaker. Subsequently, 2 mL of soft agar was added and, after mixing, the samples were poured onto the agar plates within approximately 30 seconds. After incubation at 37°C for 48 – 72 hours in the dark, the bacterial colonies were counted. The colonies were counted using the Sorcerer Image Analysis System with the software program Ames Study Manager (Perceptive Instruments Ltd., Haverhill, UK). Colonies were counted manually, if precipitation of the test substance hindered the counting using the Image Analysis System.

4. Statistics:
No special statistical tests were performed.

5. Evaluation criteria:
Toxicity
Toxicity detected by a

- decrease in the number of revertants (factor ≤ 0.6)
- clearing or diminution of the background lawn (= reduced his^- or trp^- background growth)

was recorded for all test groups both with and without S9 mix in all experiments and indicated in the tables. Single values with a factor ≤ 0.6 were not detected as toxicity in low dose groups.

Acceptance criteria
Generally, the experiment was considered valid if the following criteria were met:

- The number of revertant colonies in the negative controls was within the range of the historical negative control data for each tester strain.
- The sterility controls revealed no indication of bacterial contamination.
- The positive control substances both with and without S9 mix induced a distinct increase in the number of revertant colonies within the range of the historical positive control data or above.
- Fresh bacterial culture containing approximately 10^9 cells per mL were used.

Assessment criteria
The test substance was considered positive in this assay if the following criteria were met:

- A dose-related and reproducible increase in the number of revertant colonies, i.e. at least doubling (bacteria strains with high spontaneous mutation rate, like TA 98, TA 100 and E.coli WP2 uvrA) or tripling (bacteria strains with low spontaneous mutation rate, like TA 1535 and TA 1537) of the spontaneous mutation rate in at least one tester strain either without S9 mix or after adding a metabolizing system.

A test substance was generally considered non-mutagenic in this test if:

- The numbers of revertants for all tester strains were within the historical negative control data range under all experimental conditions in at least two experiments carried out independently of each other.

II. RESULTS AND DISCUSSION

A. STANDARD PLATE TEST

Toxicity
A bacteriotoxic effect (reduced his^- or trp^- background growth, decrease in the number of his^+ or trp^+ revertants) was observed in the standard plate test depending on the strain and test conditions from about 100 µg/plate onward.

Solubility
No test substance precipitation was found with and without S9 mix.

Mutagenicity
No relevant increase in the number of his^+ or trp^+ revertants was observed in the experiments with or without S9 mix.

Table 5.8.1-2: Bacterial gene mutation assay with Reg. No. 4111112 – Mean number of revertants (Standard Plate Test; 1st experiment)

Bacteria	**Salmonella typhimurium**								**E. coli**	
Strain	**TA 98**		**TA 100**		**TA 1535**		**TA 1537**		**uvrA**	
Metabol. activation	**+S9**	**-S9**	**+S9**	**-S9**	**+S9**	**-S9**	**+S9**	**-S9**	**+S9**	**-S9**
Neg. control (DMSO)#	28 ± 6	19 ± 5	47 ± 8	51 ± 12	11 ± 3	13 ± 3	9 ± 1	8 ± 2	73 ± 8	62 ± 7
Test item (µg/plate)										
33	27 ± 7	21 ± 5	45 ± 1	51 ± 7	14 ± 3	15 ± 1	8 ± 3	6 ± 1	62 ± 2	52 ± 14
100	24 ± 3	20 ± 9	54 ± 9	51 ± 7	17 ± 4	16 ± 5	9 ± 1	8 ± 3	67 ± 23	61 ± 7
333	25 ± 10	25 ± 2	46 ± 5	59 ± 7	10 ± 4	15 ± 3	5 ± 1	7 ± 5	79 ± 5	53 ± 3
1000	18 ± 2	20 ± 2	39 ± 14	32 ± 3	6 ± 3	10 ± 5	5 ± 3	7 ± 2	66 ± 15	51 ± 12
2600	0[B]	0	0	0	0	0	0	0	51 ± 10	30 ± 6
5200	0	0	0	0	0	0	0	0	25 ± 10	20 ± 8
Pos. control	1144 ± 172	403 ± 9	944 ± 353	2773 ± 359	154 ± 29	3899 ± 231	88 ± 15	783 ± 117	237 ± 27	1146 ± 23

#: due to rounding of numbers they may vary compared to the original report
B: reduced background growth

Table 5.8.1-3: Bacterial gene mutation assay with Reg. No. 4111112 – Mean number of revertants (Standard Plate Test; 2nd experiment)

Bacteria	**Salmonella typhimurium**								**E. coli**	
Strain	**TA 98**		**TA 100**		**TA 1535**		**TA 1537**		**uvrA**	
Metabol. activation	**+S9**	**-S9**	**+S9**	**-S9**	**+S9**	**-S9**	**+S9**	**-S9**	**+S9**	**-S9**
Neg. control (DMSO)#	25 ± 9	27 ± 2	-[T]	-	10 ± 4	18 ± 4	9 ± 3	6 ± 2	-	-
Test item (µg/plate)										
10	28 ± 2	21 ± 4	-	-	11 ± 5	16 ± 4	9 ± 3	7 ± 2	-	-
33	31 ± 2	25 ± 3	-	-	12 ± 1	13 ± 1	8 ± 4	8 ± 2	-	-
100	25 ± 4	19 ± 5	-	-	11 ± 4	11 ± 3	10 ± 3	10 ± 3	-	-
333	22 ± 2	23 ± 3	-	-	7 ± 2	9 ± 2	6 ± 3	6 ± 4	-	-
1000	20 ± 4	16 ± 5	-	-	5 ± 1[B]	3 ± 1[B]	0[B]	3 ± 2[B]	-	-
2600	0[B]	1 ± 2[B]	-[T]	-	1 ± 1[B]	1 ± 1[B]	0[B]	0[B]	-	-
Pos. control	1757 ± 169	434 ± 37	-	-	191 ± 31	4263 ± 136	176 ± 31	525 ± 204	-	-

#: due to rounding of numbers they may vary compared to the original report
B: reduced background growth; T: Technical fault

Table 5.8.1-4: Bacterial gene mutation assay with Reg. No. 4111112 – Mean number of revertants (Standard Plate Test; 3rd experiment)

Bacteria	**Salmonella typhimurium**								**E. coli**	
Strain	**TA 98**		**TA 100**		**TA 1535**		**TA 1537**		**uvrA**	
Metabol. activation	**+S9**	**-S9**	**+S9**	**-S9**	**+S9**	**-S9**	**+S9**	**-S9**	**+S9**	**-S9**
Neg. control (DMSO)#	-	-	28 ± 6	29 ± 10	-	-	6 ± 2	-	-	-
Test item (µg/plate)										
3.3	-	-	-	-	-	-	5 ± 2	-	-	-
10	-	-	32 ± 5	32 ± 10	-	-	7 ± 1	-	-	-
33	-	-	33 ± 11	29 ± 10	-	-	8 ± 2	-	-	-
100	-	-	32 ± 8	28 ± 7	-	-	6 ± 1	-	-	-
333	-	-	40 ± 7	25 ± 5	-	-	4 ± 2	-	-	-
1000	-	-	10 ± 9 B	11 ± 3 B	-	-	0 B	-	-	-
2600	-	-	0 B	0 B	-	-	-	-	-	-
Pos. control	-	-	401 ± 100	3526 ± 131	-	-	541 ± 112	-	-	-

#: due to rounding of numbers they may vary compared to the original report
B: reduced background growth; T: Technical fault

B. PREINCUBATION TEST

Toxicity
In the preincubation assay bacteriotoxicity (reduced his- or trp- background growth, decrease in the number of his^+ or trp^+ revertants) was observed depending on the strain and test conditions from about 333 µg/plate onward.

Solubility
No test substance precipitation was found with and without S9 mix.

Mutagenicity
No relevant increase in the number of his^+ or trp^+ revertants was observed in the experiments with or without S9 mix.

Table 5.8.1-5: Bacterial gene mutation assay with Reg. No. 4111112 – Mean number of revertants (Preincubation Test; 4th experiment)

Bacteria	**Salmonella typhimurium**								**E. coli**	
Strain	**TA 98**		**TA 100**		**TA 1535**		**TA 1537**		**uvrA**	
Metabol. activation	**+S9**	**-S9**	**+S9**	**-S9**	**+S9**	**-S9**	**+S9**	**-S9**	**+S9**	**-S9**
Neg. control (DMSO)#	23 ± 3	18 ± 2	30 ± 4	31 ± 1	9 ± 2	11 ± 1	8 ± 3	9 ± 4	68 ± 15	57 ± 12
Test item (µg/plate)										
3.3	21 ± 3	12 ± 4	26 ± 5	26 ± 2	10 ± 2	8 ± 2	9 ± 4	7 ± 4	59 ± 6	63 ± 11
10	24 ± 4	21 ± 2	36 ± 7	28 ± 7	10 ± 2	9 ± 4	8 ± 2	7 ± 2	69 ± 1	57 ± 7
33	27 ± 3	20 ± 5	30 ± 13	23 ± 4	9 ± 1	9 ± 1	9 ± 5	9 ± 2	69 ± 16	59 ± 8
100	17 ± 6	15 ± 2	25 ± 8	22 ± 6	9 ± 5	8 ± 3	10 ± 4	6 ± 3	64 ± 10	58 ± 13
333	23 ± 2	14 ± 4	27 ± 6	13 ± 3	10 ± 4	9 ± 2	8 ± 3	4 ± 3	72 ± 14	54 ± 11
1000	6 ± 3 B	0 B	13 ± 2 B	4 ± 1 B	5 ± 3 B	4 ± 3 B	0 B	0 B	57 ± 11 B	28 ± 7 B
Pos. control	1628 ± 75	370 ± 12	454 ± 29	2186 ± 69	177 ± 15	3375 ± 64	113 ± 14	812 ± 208	217 ± 11	428 ± 4

#: due to rounding of numbers they may vary compared to the original report
B: reduced background growth; T: Technical fault

C. DISCUSSION

According to the results of the present study, the test substance did not lead to a relevant increase in the number of revertant colonies either without S9 mix or after adding a metabolizing system in several experiments carried out independently of each other (standard plate test and preincubation assay).

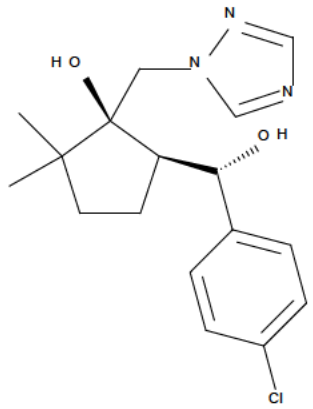
Besides, the results of the negative as well as the positive controls performed in parallel corroborated the validity of this study, since the values fulfilled the acceptance criteria of this study. In this study with and without S9 mix, the number of revertant colonies in the negative controls was within the range of the historical negative control data for each tester strain.

In addition, the positive control substances both with and without S9 mix induced a significant increase in the number of revertant colonies within the range of the historical positive control data.

III. CONCLUSION

Under the experimental conditions chosen here, it is concluded that Reg. No. 4111112 (Metabolite of metconazole) is not a mutagenic test substance in the bacterial reverse mutation test in the absence and the presence of metabolic activation.

M555F021 cis - Reg. No. 4558878



M555F021 cis ((1RS,5SR)-5-[(RS)-(4-chlorophenyl)(hydroxy)methyl]-2,2-dimethyl-1-(1H-1,2,4-triazol-1-ylmethyl)cyclopentanol; CAS-No. 153208-75-4) was identified in rat (urine: not detectable, faeces: 1 - 6% in males and ≤ 1% in females), fish, plant (wheat straw) and in the water.

QSAR evaluation

Specified metabolite	QSAR tools applied	Structural alerts for genotoxicity	Reliability / Total Domain	Evaluation
M555F021 cis Reg. No. 4558878	OA	Ames parent: negative Ames metabolites: negative	Low / Out of Domain	**Not genotoxic**
		CA parent: negative CA metabolites: 2/24 positive	Low / Out of Domain	
	VE	CAESAR: not mutagenic	Moderate / could be out of Domain	
		ISS: not mutagenic	Low / Out of Domain	
		SarPy: not mutagenic	Moderate / could be out of Domain	

OA = OASIS-TIMES
VE = Vega
CA = Chromosomal aberration

OASIS TIMES [see molecule 3 of report DocID 2015/1198619] predicted M555F021 and in silico generated metabolites to be not mutagenic in the Ames test, with the limitation that the molecule was out of the prediction domain.
OASIS TIMES CA [see molecule 3 of report DocID 2015/1198620] prediction was positive for 2/24 in silico generated metabolites with the alert "Substituted Phenols", with the limitation that the molecule was out of the prediction domain.

The VEGA prediction in all modules (CAESAR, ISS and SarPy) was not mutagenic and the reliability of this prediction was low to moderate [see molecule 2 of reports DocID 2015/1198621; DocID 2015/1198622 and DocID 2015/1198623, respectively].

Available experimental data:

Since M555F021 cis was detectable in the rat (see CA 5.1) only in faeces (bile: not examined, urine: not detected), its toxicological properties are not covered by the assessment of already performed rat studies. However, this metabolite represent a simply hydroxylation of the parent compound without any molecule cleavage and thus higher toxicity compared to the parent is not expected. Therefore, a genotoxicity assessment based on an Ames assay is considered to be acceptable.

Mutagenicity of M555F021 was investigated in the Ames test with no indication of a mutagenic effect (DocID 2014/1035852).

Conclusion:

No conclusive structural alert was identified for M555F021 regarding mutagenicity. The prediction was “non-mutagen” in all models used and is in concordance with available in vitro data.

Regarding in vitro chromosome aberration the structural alert “Substituted Phenols” was received for some hydroxylated metabolites. Exactly the same structural alert was also received for cis-metconazole and did not reflect the data available from in vitro and in vivo studies, which were clearly negative and was rejected. Based on weight of evidence, this structural alert for M555F021 is assessed to be not predictive for this structural domain and is therefore rejected.

Thus, M555F021 is considered to be of no toxicological relevance and the ADI derived for metconazole can be applied to this metabolite as well.

Available toxicological studies with M555F021

Report:	CA 5.8.1/2 Woitkowiak C., 2015b Reg.No. 4558878 (metabolite of BAS 555 F, Metconazole) - Salmonella typhimurium / Escherichia coli - Reverse mutation assay 2014/1035852
Guidelines:	OECD 471 (1997), (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.13/B.14 No. L 142, EPA 870.5100
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary
S. typhimurium (strains TA98, TA 100, TA 1535, and TA 1537) and E. coli (uvrA) were exposed to Reg. No. 4558878 (Metabolite of metconazole; batch L86-8) using DMSO as a solvent in the presence and absence of metabolic activation in standard plate and pre-incubation tests. Triplicate plates were used per dose and per test condition. Vehicle and positive controls were included in each experiment. The test substance was used at concentrations in the range of 3.3 to 5300 µg/plate in the presence and absence of S9-mix. Bacteriotoxic effects were observed depending on the strain and test condition from about 333 µg/plate onward. A biologically relevant increase in the number of revertant colonies was not noticed in any of the strains tested in presence or absence of metabolic activation in any of the experiments. The positive controls induced the appropriate response in the corresponding strains in the absence or presence of metabolic activation. According to the results of the study the test substance the test substance was not mutagenic under the experimental conditions of the study.

(Doc ID 2014/1035852)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material	Reg. No. 4558878 (Metabolite of metconazole)
Description:	Solid / white
Lot/Batch #:	L86-8
Purity:	95.6% (tolerance ±1.0%)
Stability of test compound:	The stability of the test substance under storage conditions is guaranteed until 01 Aug 2016 as indicated by the sponsor, and the sponsor holds this responsibility.
Homogeneity:	The homogeneity of the test substance was ensured by mixing before preparation of the test substance solutions.
Vehicle:	DMSO

2. Control Materials:

Vehicle control: The vehicle control with and without S-9 mix only contained the vehicle used for the test substance at the same concentration and volume for all tester strains.

Sterility control: Additional plates were treated with soft agar, S9 mix, buffer, vehicle or the test substance but without the addition of tester strains.

Positive control compounds tested without addition of metabolic activation system:

Strain	Mutagen	Solvent	Concentration
TA 100	MNNG	DMSO	5 µg/plate
TA 1535	MNNG	DMSO	5 µg/plate
TA 1537	9-Aminoacridine	DMSO	100 µg/plate
TA 98	NOPD	DMSO	10 µg/plate
E. coli	4-NQO	DMSO	5 µg/plate

Positive control compounds tested with addition of metabolic activation system:

Strain	Mutagen	Solvent	Concentration
TA 100	2-Aminoanthracene	DMSO	2.5 µg/plate
TA 1535	2-Aminoanthracene	DMSO	2.5 µg/plate
TA 1537	2-Aminoanthracene	DMSO	2.5 µg/plate
TA 98	2-Aminoanthracene	DMSO	2.5 µg/plate
E. coli	2-Aminoanthracene	DMSO	60 µg/plate

3. Activation:

S9 was produced from the livers of male Wistar rats treated with 80 mg/kg bw phenobarbital (i.p.) and β-naphthoflavone (orally) for three consecutive days. The S9-mix was prepared freshly prior to each experiment. For this purpose, a sufficient amount of S9-fraction is thawed at room temperature and 1 volume of S9-fraction is mixed with 9 volumes of S9-supplement (cofactors). This preparation, the so-called S9-mix, was kept on ice until used.
The rat liver S9-mix was prepared immediately before use and had the following composition:

Component	Concentration
Phosphate buffer (pH 7.4)	15 mM
Glucose 6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM
S9	10 %

To demonstrate the efficacy of the S9 mix, the S9 batch was characterized with benzo(a)pyrene.

4. Test organisms: S. typhimurium strains: TA98, TA100, TA1535, TA1537
E. coli : WP2 uvrA

5. Test concentrations:

Standard plate test (1st experiment):
Triplicate plates in one experiment were prepared for each concentration (neg. control, 33, 100, 333, 1000, 2650, and 5300 µg/plate) and condition (i.e. with and without S9) for all tester strains.

Standard plate test (2nd experiment):
Triplicate plates in one experiment were prepared for each concentration (neg. control, 10, 33, 100, 333, 1000, and 2650 µg/plate) and condition (i.e. with and without S9) for TA98, TA100, TA1535, and TA1537. Based on bacteriotoxicity that was observed in the 1st standard plate test with the TA tester strains this second experiment was performed.

Pre-incubation test (3rd experiment):
Triplicate plates in one experiment were prepared for each concentration (neg. control, 3.3, 10, 33, 100, 333, and 1000 µg/plate) and condition (i.e. with and without S9) for all Salmonella strains. For E. coli the same conditions were applied using concentrations of 10, 33, 100, 333, 1000, and 2650 µg/plate.

B. TEST PERFORMANCE:

1. Dates of study period: 04 Nov 2014 – 28 Nov 2014

2. Standard plate test:
Test tubes containing 2-mL portions of soft agar (overlay agar), which consists of 100 mL agar (0.8% [w/v] agar + 0.6% [w/v] NaCl) and 10 mL amino acid solution (minimal amino acid solution for the determination of mutants: 0.5 mM histidine + 0.5 mM biotin for Salmonella typhimurium strains or 0.5 mM tryptophan for E. coli) were kept in a water bath at about 42 - 45°C, and the remaining components were added in the following order:

0.1 mL test solution or vehicle (negative control)
0.1 mL fresh bacterial culture
0.5 mL S9 mix (**with metabolic activation**) **or** 0.5 mL phosphate buffer (**without metabolic activation**)

After mixing, the samples were be poured onto Minimal glucose agar plates within approximately 30 seconds. After incubation at 37°C for 48 – 72 hours in the dark, the bacterial colonies (his^+ or trp^+ revertants) were counted. The colonies were counted using the Sorcerer Image Analysis System with the software program Ames Study Manager (Perceptive Instruments Ltd., Haverhill, UK). Colonies were counted manually, if precipitation of the test substance hinders the counting using the Image Analysis System.

3. Pre-incubation test:
0.1 mL test solution or vehicle, 0.1 mL bacterial suspension and 0.5 mL S9 mix (**with metabolic activation**) or phosphate buffer (**without metabolic activation**) were incubated at 37°C for the duration of about 20 minutes using a shaker. Subsequently, 2 mL of soft agar was added and, after mixing, the samples were poured onto the agar plates within approximately 30 seconds. After incubation at 37°C for 48 – 72 hours in the dark, the bacterial colonies were counted. The colonies were counted using the Sorcerer Image Analysis System with the software program Ames Study Manager (Perceptive Instruments Ltd., Haverhill, UK). Colonies were counted manually, if precipitation of the test substance hindered the counting using the Image Analysis System.

4. Statistics:
No special statistical tests were performed.

5. Evaluation criteria:
Toxicity
Toxicity detected by a

- decrease in the number of revertants (factor ≤ 0.6)
- clearing or diminution of the background lawn (= reduced his- or trp- background growth)

was recorded for all test groups both with and without S9 mix in all experiments and indicated in the tables. Single values with a factor ≤ 0.6 were not detected as toxicity in low dose groups.

Acceptance criteria
Generally, the experiment was considered valid if the following criteria were met:

- The number of revertant colonies in the negative controls was within the range of the historical negative control data for each tester strain.
- The sterility controls revealed no indication of bacterial contamination.
- The positive control substances both with and without S9 mix induced a distinct increase in the number of revertant colonies within the range of the historical positive control data or above.
- Fresh bacterial culture containing approximately 10^9 cells per mL were used.

Assessment criteria
The test substance was considered positive in this assay if the following criteria were met:

- A dose-related and reproducible increase in the number of revertant colonies, i.e. at least doubling (bacteria strains with high spontaneous mutation rate, like TA 98, TA 100 and E.coli WP2 uvrA) or tripling (bacteria strains with low spontaneous mutation rate, like TA 1535 and TA 1537) of the spontaneous mutation rate in at least one tester strain either without S9 mix or after adding a metabolizing system.

A test substance was generally considered non-mutagenic in this test if:

- The numbers of revertants for all tester strains were within the historical negative control data range under all experimental conditions in at least two experiments carried out independently of each other.

II. RESULTS AND DISCUSSION

A. STANDARD PLATE TEST

Toxicity

A bacteriotoxic effect (reduced his^- or trp^- background growth, decrease in the number of his^+ or trp^+ revertants) was observed in the standard plate test depending on the strain and test conditions from about 333 µg/plate onward.

Solubility

No test substance precipitation was found with and without S9 mix.

Mutagenicity

No relevant increase in the number of his^+ or trp^+ revertants was observed in the experiments with or without S9 mix.

Table 5.8.1-6: Bacterial gene mutation assay with Reg. No. 4558878 – Mean number of revertants (Standard Plate Test; 1st experiment)

Bacteria	**Salmonella typhimurium**								**E. coli**	
Strain	**TA 98**		**TA 100**		**TA 1535**		**TA 1537**		**uvrA**	
Metabol. activation	**+S9**	**-S9**	**+S9**	**-S9**	**+S9**	**-S9**	**+S9**	**-S9**	**+S9**	**-S9**
Neg. control (DMSO)#	27 ± 5	19 ± 4	45 ± 7	29 ± 15	26 ± 6	10 ± 4	8 ± 1	8 ± 1	64 ± 11	52 ± 15
Test item (µg/plate)										
33	26 ± 4	23 ± 4	44 ± 8	26 ± 8	20 ± 2	12 ± 3	8 ± 2	7 ± 3	69 ± 1	65 ± 1
100	27 ± 3	22 ± 6	50 ± 12	26 ± 5	19 ± 1	13 ± 2	7 ± 2	8 ± 1	76 ± 5	65 ± 9
333	23 ± 3	21 ± 5	44 ± 12	28 ± 2	20 ± 6	12 ± 1	8 ± 1	6 ± 4	60 ± 6	56 ± 2
1000	25 ± 1	13 ± 4	14 ± 5	10 ± 2	14 ± 5	11 ± 3	4 ± 1	4 ± 2	63 ± 10	56 ± 2
2650	0 B	0 B	0 B	0 B	0 B	0 B	0 B	0 B	53 ± 4 B	27 ± 6 B
5300	0 B	0 B	0 B	0 B	0 B	0 B	0 B	0 B	11 ± 1 B	15 ± 6 B
Pos. control	1899 ± 100	369 ± 18	457 ± 6	3138 ± 177	200 ± 17	4254 ± 252	190 ± 9	1323 ± 161	269 ± 4	986 ± 67

#: due to rounding of numbers they may vary compared to the original report
B: reduced background growth

Table 5.8.1-7: Bacterial gene mutation assay with Reg. No. 4558878 – Mean number of revertants (Standard Plate Test; 2nd experiment)

Bacteria	**Salmonella typhimurium**								**E. coli**	
Strain	**TA 98**		**TA 100**		**TA 1535**		**TA 1537**		**uvrA**	
Metabol. activation	**+S9**	**-S9**	**+S9**	**-S9**	**+S9**	**-S9**	**+S9**	**-S9**	**+S9**	**-S9**
Neg. control (DMSO)#	24 ± 1	18 ± 5	29 ± 2	26 ± 3	10 ± 3	13 ± 1	8 ± 2	7 ± 2	-	-
Test item (µg/plate)									-	-
10	23 ± 3	19 ± 5	34 ± 9	25 ± 6	9 ± 4	10 ± 3	7 ± 4	25 ± 6	-	-
33	22 ± 3	19 ± 5	36 ± 6	26 ± 6	9 ± 3	8 ± 3	9 ± 2	26 ± 6	-	-
100	19 ± 4	22 ± 4	27 ± 2	29 ± 1	9 ± 3	11 ± 3	7 ± 1	29 ± 1	-	-
333	19 ± 1	16 ± 3	28 ± 6	28 ± 3	12 ± 4	9 ± 4	7 ± 2	28 ± 3	-	-
1000	22 ± 10	12 ± 3	20 ± 2	34 ± 2	11 ± 2	9 ± 0	6 ± 1	34 ± 2	-	-
2650	0[B]	0[B]	0[B]	0[B]	0[B]	0[B]	0[B]	0[B]	-	-
Pos. control	1680 ± 183	335 ± 25	731 ± 67	4046 ± 69	192 ± 34	4545 ± 205	182 ± 24	579 ± 57	-	-

#: due to rounding of numbers they may vary compared to the original report
B: reduced background growth

B. PREINCUBATION TEST

Toxicity

In the preincubation assay bacteriotoxicity (reduced his^- or trp^- background growth, decrease in the number of his^+ or trp^+ revertants) was observed depending on the strain and test conditions from about 333 µg/plate onward.

Solubility

No test substance precipitation was found with and without S9 mix.

Mutagenicity

No relevant increase in the number of his^+ or trp^+ revertants was observed in the experiments with or without S9 mix.

Table 5.8.1-8: Bacterial gene mutation assay with Reg. No. 4558878 – Mean number of revertants (Pre-incubation test; 3rd experiment)

Bacteria	**Salmonella typhimurium**								**E. coli**	
Strain	**TA 98**		**TA 100**		**TA 1535**		**TA 1537**		**uvrA**	
Metabol. activation	**+S9**	**-S9**	**+S9**	**-S9**	**+S9**	**-S9**	**+S9**	**-S9**	**+S9**	**-S9**
Neg. control (DMSO)#	24 ± 4	20 ± 5	33 ± 7	34 ± 3	9 ± 3	11 ± 1	9 ± 2	8 ± 2	53 ± 8	55 ± 8
Test item (µg/plate)										
3.3	23 ± 2	16 ± 1	30 ± 2	34 ± 2	9 ± 4	10 ± 2	10 ± 2	9 ± 3	-	-
10	23 ± 7	21 ± 6	30 ± 13	28 ± 4	8 ± 1	13 ± 2	9 ± 1	7 ± 1	63 ± 6	52 ± 6
33	25 ± 7	18 ± 1	34 ± 13	36 ± 11	9 ± 4	9 ± 3	8 ± 3	7 ± 2	62 ± 13	54 ± 3
100	27 ± 6	20 ± 2	27 ± 3	24 ± 6	9 ± 2	7 ± 4	7 ± 2	7 ± 1	62 ± 4	51 ± 10
333	11 ± 2	10 ± 1	6 ± 3	9 ± 2	5 ± 3	2 ± 1	3 ± 1	5 ± 1	60 ± 2	55 ± 8
1000	0 B	0 B	0 B	0 B	0 B	0 B	0 B	0 B	52 ± 10	26 ± 8
2650	-	-	-	-	-	-	-	-	23 ± 3 B	15 ± 4 B
Pos. control	636 ± 196	469 ± 9	595 ± 25	1793 ± 58	138 ± 10	1215 ± 158	144 ± 18	640 ± 172	209 ± 25	418 ± 66

#: due to rounding of numbers they may vary compared to the original report
B: reduced background growth

C. DISCUSSION

According to the results of the present study, the test substance did not lead to a relevant increase in the number of revertant colonies either without S9 mix or after adding a metabolizing system in several experiments carried out independently of each other (standard plate test and pre-incubation assay).

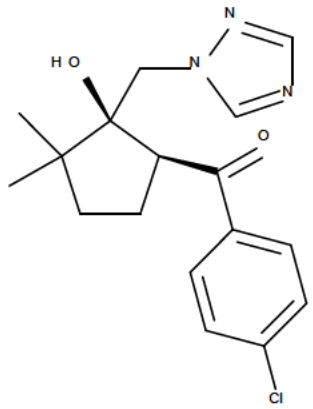
Besides, the results of the negative as well as the positive controls performed in parallel corroborated the validity of this study, since the values fulfilled the acceptance criteria of this study. In this study with and without S9 mix, the number of revertant colonies in the negative controls was within the range of the historical negative control data for each tester strain.

In addition, the positive control substances both with and without S9 mix induced a significant increase in the number of revertant colonies within the range of the historical positive control data.

III. CONCLUSION

Under the experimental conditions chosen here, it is concluded that Reg. No. 4558878 (Metabolite of metconazole) is not a mutagenic test substance in the bacterial reverse mutation test in the absence and the presence of metabolic activation.

M555F030 cis - Reg. No. 4110625



M555F030 cis ((1RS,5SR)-5-(4-chlorobenzoyl)-2,2-dimethyl-1-(1H-1,2,4-triazol-1-ylmethyl) cyclopentanol; CAS-No. 153208-73-2) was identified in soil and in the water.

QSAR evaluation

Specified metabolite	QSAR tools applied	Structural alerts for genotoxicity	Reliability / Total Domain	Evaluation
M555F030 cis Reg. No. 4110625	OA	Ames parent: negative Ames metabolites: negative	Low / Out of Domain	**Not genotoxic**
		CA parent: negative CA metabolites: negative	Low / Out of Domain	
	VE	CAESAR: not mutagenic	Moderate / could be out of Domain	
		ISS: not mutagenic	Low / Out of Domain	
		SarPy: not mutagenic	Moderate / could be out of Domain	

OA = OASIS-TIMES
VE = Vega
CA = Chromosomal aberration

OASIS TIMES [see molecule 4 of report DocID 2015/1198619] predicted M555F030 and in silico generated metabolites to be not mutagenic in the Ames test, with the limitation that the molecule was out of the prediction domain.
OASIS TIMES CA [see molecule 4 of report DocID 2015/1198620] prediction was negative for the parent compound and all in silico generated metabolites, with the limitation that the molecule was out of the prediction domain.

The VEGA prediction in all modules (CAESAR, ISS and SarPy) was not mutagenic and the reliability of this prediction was low to moderate [see molecule 3 of reports DocID 2015/1198621; DocID 2015/1198622 and DocID 2015/1198623, respectively].

Available experimental data:

Since M555F030 cis was not detectable in the rat (see CA 5.1), its toxicological properties are not covered by the assessment of already performed rat studies. However, this metabolite represent a simply oxidation of the parent compound without any molecule cleavage and thus higher toxicity compared to the parent is not expected. Therefore, a genotoxicity assessment based on an Ames assay is considered to be acceptable.
Mutagenicity of M555F030 cis was investigated in the Ames test with no indication of a mutagenic effect (DocID 2014/1035853).

Conclusion:

No structural alert was identified for M555F030 regarding mutagenicity. The prediction was "non-mutagen" in all models used and is in concordance with available in vitro data.
No structural alert was identified regarding chromosomal aberration potential of M555F030. The prediction for in vitro CA with metabolic activation was negative in the module applied.

Thus, M555F030 is considered to be of no toxicological relevance and the ADI derived for metconazole can be applied to this metabolite as well.

Available toxicological studies with M555F030

Report: CA 5.8.1/3
Woitkowiak C., 2015c
Reg.No. 4110625 (metabolite of BAS 555 F, Metconazole) - Salmonella typhimurium / Escherichia coli reverse mutation assay
2014/1035853

Guidelines: OECD 471, (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.13/B.14, EPA 870.5100

GLP: yes
(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary
S. typhimurium (strains TA98, TA 100, TA 1535, and TA 1537) and E. coli (uvrA) were exposed to Reg. No. 4110625 (Metabolite of BAS 555 F; batch L86-8) using DMSO as a solvent in the presence and absence of metabolic activation in standard plate and pre-incubation tests. Triplicate plates were used per dose and per test condition. Vehicle and positive controls were included in each experiment. The test substance was used at concentrations in the range of 3.3 to 5000 µg/plate in the presence and absence of S9-mix. Bacteriotoxic effects were observed depending on the strain and test condition from about 333 (pre-incubation test) or 2500 (standard plate test) µg/plate onward. A biologically relevant increase in the number of revertant colonies was not noticed in any of the strains tested in presence or absence of metabolic activation in any of the experiments. The positive controls induced the appropriate response in the corresponding strains in the absence or presence of metabolic activation. According to the results of the study the test substance the test substance was not mutagenic under the experimental conditions of the study.

(Doc ID 2014/1035853)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material Reg. No. 4110625 (Metabolite of metconazole)

Description:	Solid / white
Lot/Batch #:	L85-58
Purity:	98.7% (tolerance ±1.0%)
Stability of test compound:	The stability of the test substance under storage conditions is guaranteed until 01 Aug 2016 as indicated by the sponsor, and the sponsor holds this responsibility.
Homogeneity:	The homogeneity of the test substance was ensured by mixing before preparation of the test substance solutions.
Vehicle:	DMSO

2. Control Materials:

Vehicle control: The vehicle control with and without S-9 mix only contained the vehicle used for the test substance at the same concentration and volume for all tester strains.

Sterility control: Additional plates were treated with soft agar, S9 mix, buffer, vehicle or the test substance but without the addition of tester strains.

Positive control compounds tested without addition of metabolic activation system:

Strain	Mutagen	Solvent	Concentration
TA 100	MNNG	DMSO	5 µg/plate
TA 1535	MNNG	DMSO	5 µg/plate
TA 1537	9-Aminoacridine	DMSO	100 µg/plate
TA 98	NOPD	DMSO	10 µg/plate
E. coli	4-NQO	DMSO	5 µg/plate

Positive control compounds tested with addition of metabolic activation system:

Strain	Mutagen	Solvent	Concentration
TA 100	2-Aminoanthracene	DMSO	2.5 µg/plate
TA 1535	2-Aminoanthracene	DMSO	2.5 µg/plate
TA 1537	2-Aminoanthracene	DMSO	2.5 µg/plate
TA 98	2-Aminoanthracene	DMSO	2.5 µg/plate
E. coli	2-Aminoanthracene	DMSO	60 µg/plate

3. Activation:

S9 was produced from the livers of male Wistar rats treated with 80 mg/kg bw phenobarbital (i.p.) and β-naphthoflavone (orally) for three consecutive days. The S9-mix was prepared freshly prior to each experiment. For this purpose, a sufficient amount of S9-fraction is thawed at room temperature and 1 volume of S9-fraction is mixed with 9 volumes of S9-supplement (cofactors). This preparation, the so-called S9-mix, was kept on ice until used.
The rat liver S9-mix was prepared immediately before use and had the following composition:

Component	Concentration
Phosphate buffer (pH 7.4)	15 mM
Glucose 6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM
S9	10 %

To demonstrate the efficacy of the S9 mix, the S9 batch was characterized with benzo(a)pyrene.

4. Test organisms: S. typhimurium strains: TA98, TA100, TA1535, TA1537
E. coli : WP2 uvrA

5. Test concentrations:
Standard plate test (1st experiment):
Triplicate plates in one experiment were prepared for each concentration (neg. control, 33, 100, 333, 1000, 2500, and 5000 µg/plate) and condition (i.e. with and without S9) for all tester strains.

Pre-incubation test (2nd experiment):
Triplicate plates in one experiment were prepared for each concentration (neg. control, 33, 100, 333, 1000, 2500, and 5000 µg/plate) and condition (i.e. with and without S9) for all tester strains.

Pre-incubation test (3rd experiment):
Triplicate plates in one experiment were prepared for each concentration (neg. control, 3.3, 10, 33, 100, 333, and 1000 µg/plate) and condition (i.e. with and without S9) for Salmonella strains TA 100 and TA 1537, as bacteriotoxicity was observed in the second experiment for these strains.

B. TEST PERFORMANCE:

1. Dates of study period: 28 Oct 2014 – 20 Nov 2014

2. Standard plate test:
Test tubes containing 2-mL portions of soft agar (overlay agar), which consists of 100 mL agar (0.8% [w/v] agar + 0.6% [w/v] NaCl) and 10 mL amino acid solution (minimal amino acid solution for the determination of mutants: 0.5 mM histidine + 0.5 mM biotin for Salmonella typhimurium strains or 0.5 mM tryptophan for E. coli) were kept in a water bath at about 42 - 45°C, and the remaining components were added in the following order:

0.1 mL test solution or vehicle (negative control)
0.1 mL fresh bacterial culture
0.5 mL S9 mix (**with metabolic activation**) **or** 0.5 mL phosphate buffer (**without metabolic activation**)

After mixing, the samples were be poured onto Minimal glucose agar plates within approximately 30 seconds. After incubation at 37°C for 48 – 72 hours in the dark, the bacterial colonies (his^+ or trp^+ revertants) were counted. The colonies were counted using the Sorcerer Image Analysis System with the software program Ames Study Manager (Perceptive Instruments Ltd., Haverhill, UK). Colonies were counted manually, if precipitation of the test substance hinders the counting using the Image Analysis System.

3. Pre-incubation test:
0.1 mL test solution or vehicle, 0.1 mL bacterial suspension and 0.5 mL S9 mix (**with metabolic activation**) or phosphate buffer (**without metabolic activation**) were incubated at 37°C for the duration of about 20 minutes using a shaker. Subsequently, 2 mL of soft agar was added and, after mixing, the samples were poured onto the agar plates within approximately 30 seconds. After incubation at 37°C for 48 – 72 hours in the dark, the bacterial colonies were counted. The colonies were counted using the Sorcerer Image Analysis System with the software program Ames Study Manager (Perceptive Instruments Ltd., Haverhill, UK). Colonies were counted manually, if precipitation of the test substance hindered the counting using the Image Analysis System.

4. Statistics:
No special statistical tests were performed.

5. Evaluation criteria:
Toxicity
Toxicity detected by a

- decrease in the number of revertants (factor ≤ 0.6)
- clearing or diminution of the background lawn (= reduced his^- or trp^- background growth)

was recorded for all test groups both with and without S9 mix in all experiments and indicated in the tables. Single values with a factor ≤ 0.6 were not detected as toxicity in low dose groups.

Acceptance criteria
Generally, the experiment was considered valid if the following criteria were met:

- The number of revertant colonies in the negative controls was within the range of the historical negative control data for each tester strain.
- The sterility controls revealed no indication of bacterial contamination.
- The positive control substances both with and without S9 mix induced a distinct increase in the number of revertant colonies within the range of the historical positive control data or above.
- Fresh bacterial culture containing approximately 10^9 cells per mL were used.

Assessment criteria
The test substance was considered positive in this assay if the following criteria were met:

- A dose-related and reproducible increase in the number of revertant colonies, i.e. at least doubling (bacteria strains with high spontaneous mutation rate, like TA 98, TA 100 and E.coli WP2 uvrA) or tripling (bacteria strains with low spontaneous mutation rate, like TA 1535 and TA 1537) of the spontaneous mutation rate in at least one tester strain either without S9 mix or after adding a metabolizing system.

A test substance was generally considered non-mutagenic in this test if:

- The numbers of revertants for all tester strains were within the historical negative control data range under all experimental conditions in at least two experiments carried out independently of each other.

II. RESULTS AND DISCUSSION

A. STANDARD PLATE TEST

Toxicity
A bacteriotoxic effect (reduced his^- or trp^- background growth, decrease in the number of his^+ or trp^+ revertants) was observed in the standard plate test depending on the strain and test conditions from about 2500 µg/plate onward.

Solubility
No test substance precipitation was found with and without S9 mix.

Mutagenicity
No relevant increase in the number of his^+ or trp^+ revertants was observed in the experiments with or without S9 mix.

Table 5.8.1-9: Bacterial gene mutation assay with Reg. No. 4110625 – Mean number of revertants (Standard Plate Test; 1st experiment)

Bacteria	**Salmonella typhimurium**								**E. coli**	
Strain	**TA 98**		**TA 100**		**TA 1535**		**TA 1537**		**uvrA**	
Metabol. activation	**+S9**	**-S9**	**+S9**	**-S9**	**+S9**	**-S9**	**+S9**	**-S9**	**+S9**	**-S9**
Neg. control (DMSO)#	26 ± 2	27 ± 3	57 ± 7	45 ± 6	10 ± 2	15 ± 3	8 ± 4	8 ± 2	60 ± 11	49 ± 10
Test item (µg/plate)										
33	30 ± 3	24 ± 6	58 ± 4	55 ± 7	11 ± 4	10 ± 2	8 ± 3	6 ± 2	56 ± 8	56 ± 5
100	35 ± 7	24 ± 3	61 ± 11	52 ± 7	10 ± 4	11 ± 4	9 ± 5	7 ± 4	57 ± 3	52 ± 7
333	29 ± 5	22 ± 6	51 ± 4	56 ± 3	12 ± 3	12 ± 3	6 ± 2	8 ± 4	58 ± 4	43 ± 7
1000	28 ± 6	26 ± 2	60 ± 8	52 ± 4	11 ± 5	14 ± 1	7 ± 3	8 ± 1	67 ± 7	49 ± 9
2500	24 ± 4	23 ± 3	53 ± 10	50 ± 7	6 ± 2	7 ± 3	2 ± 1	6 ± 2	56 ± 5	46 ± 5
5000	19 ± 7 B	15 ± 1 B	31 ± 2 B	26 ± 4 B	6 ± 1 B	10 ± 3 B	1 ± 1 B	2 ± 1 B	52 ± 3 B	49 ± 5 B
Pos. control	1577 ± 252	360 ± 31	1815 ± 420	3018 ± 134	188 ± 21	4181 ± 121	155 ± 32	794 ± 177	228 ± 38	1030 ± 5

B. PREINCUBATION TEST

Toxicity
In the preincubation assay bacteriotoxicity (reduced his^- or trp^- background growth, decrease in the number of his^+ or trp^+ revertants) was observed depending on the strain and test conditions from about 333 µg/plate onward.
Solubility
No test substance precipitation was found with and without S9 mix.
Mutagenicity
No relevant increase in the number of his^+ or trp^+ revertants was observed in the experiments with or without S9 mix.

Table 5.8.1-10: Bacterial gene mutation assay with Reg. No. 4110625 – Mean number of revertants (Pre-incubation Test; 2nd experiment)

Bacteria	**Salmonella typhimurium**								**E. coli**	
Strain	**TA 98**		**TA 100**		**TA 1535**		**TA 1537**		**uvrA**	
Metabol. activation	**+S9**	**-S9**	**+S9**	**-S9**	**+S9**	**-S9**	**+S9**	**-S9**	**+S9**	**-S9**
Neg. control (DMSO)#	26 ± 3	20 ± 8	36 ± 2	45 ± 2	10 ± 0	11 ± 2	7 ± 2	7 ± 0	61 ± 12	61 ± 5
Test item (µg/plate)										
33	29 ± 3	21 ± 4	28 ± 5	46 ± 1	10 ± 2	9 ± 1	6 ± 4	8 ± 3	72 ± 6	58 ± 5
100	29 ± 8	19 ± 5	38 ± 9	47 ± 4	10 ± 3	9 ± 2	8 ± 2	6 ± 1	70 ± 9	52 ± 3
333	23 ± 3	23 ± 2	29 ± 6	32 ± 13	9 ± 3	9 ± 2	7 ± 3	4 ± 1	67 ± 7	52 ± 9
1000	23 ± 1	17 ± 7	20 ± 3	13 ± 1	11 ± 2	9 ± 2	4 ± 0	2 ± 1	63 ± 9	51 ± 3
2500	14 ± 5	9 ± 3	5 ± 2	0	4 ± 2	2 ± 1	0	0	60 ± 3	27 ± 10
5000	0[B]	0 [B]	0 [B]	0 [B]	0 [B]	0 [B]	0 [B]	0 [B]	55 ± 2	14 ±4 [B]
Pos. control	1750 ± 257	345 ± 10	390 ± 58	400 ± 119	125 ± 4	1537 ± 295	120 ± 24	585 ± 147	164 ± 6	465 ± 26

#: due to rounding of numbers they may vary compared to the original report
B: reduced background growth

Table 5.8.1-11: Bacterial gene mutation assay with Reg. No. 4110625 – Mean number of revertants (Pre-incubation Test; 2nd experiment)

Bacteria	**Salmonella typhimurium**								**E. coli**	
Strain	**TA 98**		**TA 100**		**TA 1535**		**TA 1537**		**uvrA**	
Metabol. activation	**+S9**	**-S9**	**+S9**	**-S9**	**+S9**	**-S9**	**+S9**	**-S9**	**+S9**	**-S9**
Neg. control (DMSO)#	-	-	-	32 ± 6	-	-	12 ± 4	11 ± 3	-	-
Test item (µg/plate)										
3.3	-	-	-	30 ± 9	-	-	11 ± 2	9 ± 1	-	-
10	-	-	-	30 ± 8	-	-	10 ± 4	12 ± 1	-	-
33	-	-	-	31 ± 3	-	-	14 ± 0	9 ± 3	-	-
100	-	-	-	32 ± 1	-	-	9 ± 2	11 ± 3	-	-
333	-	-	-	28 ± 5	-	-	12 ± 3	6 ± 2	-	-
1000	-	-	-	18 ± 2	-	-	6 ± 4	4 ± 2	-	-
Pos. control	-	-	-	2146 ± 128	-	-	126 ± 25	678 ± 165	-	-

#: due to rounding of numbers they may vary compared to the original report

C. DISCUSSION

According to the results of the present study, the test substance did not lead to a relevant increase in the number of revertant colonies either without S9 mix or after adding a metabolizing system in several experiments carried out independently of each other (standard plate test and pre-incubation assay).

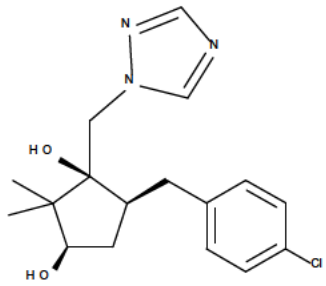
Besides, the results of the negative as well as the positive controls performed in parallel corroborated the validity of this study, since the values fulfilled the acceptance criteria of this study. In this study with and without S9 mix, the number of revertant colonies in the negative controls was within the range of the historical negative control data for each tester strain.

In addition, the positive control substances both with and without S9 mix induced a significant increase in the number of revertant colonies within the range of the historical positive control data.

III. CONCLUSION

Under the experimental conditions chosen here, it is concluded that Reg. No. 4110625 (Metabolite of metconazole) is not a mutagenic test substance in the bacterial reverse mutation test in the absence and the presence of metabolic activation.

M555F031 cis - Reg. No. 5968488



M555F031 (1RS,3SR,5RS)-5-(4-chlorobenzyl)-2,2-dimethyl-1-(1H-1,2,4-triazol-1-ylmethyl) cyclopentane-1,3-diol) was identified in goat and hen.

QSAR evaluation

Specified metabolite	QSAR tools applied	Structural alerts for genotoxicity	Reliability / Total Domain	Evaluation
M555F031 cis Reg. No. 5968488	OA	Ames parent: negative Ames metabolites: negative	Low / Out of Domain	**Not genotoxic**
		CA parent: negative CA metabolites: 2/14 positive	Low / Out of Domain	
	VE	CAESAR: not mutagenic	Moderate / could be out of Domain	
		ISS: not mutagenic	Low / Out of Domain	
		SarPy: not mutagenic	Moderate / could be out of Domain	

OA = OASIS-TIMES
VE = Vega
CA = Chromosomal aberration

OASIS TIMES [see molecule 5 of report DocID 2015/1198619] predicted M555F031 and in silico generated metabolites to be not mutagenic in the Ames test, with the limitation that the molecule was out of the prediction domain.
OASIS TIMES CA [see molecule 5 of report DocID 2015/1198620] prediction was positive for 2/14 in silico generated metabolites with the alert "Substituted Phenols", with the limitation that the molecule was out of the prediction domain.

The VEGA prediction in all modules (CAESAR, ISS and SarPy) was not mutagenic and the reliability of this prediction was low to moderate [see molecule 4 of reports DocID 2015/1198621; DocID 2015/1198622 and DocID 2015/1198623, respectively].

Available experimental data:

Since M555F030 cis was not detectable in the rat (see CA 5.1), its toxicological properties are not covered by the assessment of already performed rat studies. However, this metabolite represent a simply hydroxylation of the parent compound without any molecule cleavage and thus higher toxicity compared to the parent is not expected. Therefore, a genotoxicity assessment based on an Ames assay is considered to be acceptable.

Mutagenicity of M555F031 was investigated in the Ames test with no indication of a mutagenic effect (DocID 2014/1035854).

Conclusion:

No structural alert was identified for M555F031 regarding mutagenicity. The prediction was "non-mutagen" in all models used and is in concordance with available in vitro data.

Regarding in vitro chromosome aberration the structural alert "Substituted Phenols" was received for some hydroxylated metabolites. Exactly the same structural alert was also received for cis-metconazole and did not reflect the data available from in vitro and in vivo studies, which were clearly negative and was rejected. Based on weight of evidence, this structural alert for M555F031 is assessed to be not predictive for this compound class and is therefore rejected.

Thus, M555F031 is considered to be of no toxicological relevance and the ADI derived for metconazole can be applied to this metabolite as well.

Available toxicological studies with M555F031

Report:	CA 5.8.1/4 Woitkowiak C., 2015d Reg.No. 5968488 (metabolite of BAS 555 F, Metconazole) - Salmonella typhimurium / Escherichia coli reverse mutation assay 2014/1035854
Guidelines:	OECD 471 (1997), (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.13/B.14 No. L 142, EPA 870.5100
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

S. typhimurium (strains TA98, TA 100, TA 1535, and TA 1537) and E. coli (uvrA) were exposed to Reg.No. 5968488 (Metabolite of metconazole; batch L86-12) using DMSO as a solvent in the presence and absence of metabolic activation in standard plate and pre-incubation tests. Triplicate plates were used per dose and per test condition. Vehicle and positive controls were included in each experiment. The test substance was used at concentrations in the range of 10 to 5000 µg/plate in the presence and absence of S9-mix. Bacteriotoxic effects were observed depending on the strain and test condition from about 2500 µg/plate in the standard plate test and at 2500 µg/plate in all strains and test conditions in the pre-incubation test. A biologically relevant increase in the number of revertant colonies was not noticed in any of the strains tested in presence or absence of metabolic activation in any of the experiments. The positive controls induced the appropriate response in the corresponding strains in the absence or presence of metabolic activation. According to the results of the study the test substance the test substance was not mutagenic under the experimental conditions of the study.

(Doc ID 2014/1035854)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material Reg. No. 5968488 (Metabolite of metconazole)

Description: Solid / white

Lot/Batch #: L86-12

Purity: 98.9% (tolerance ±1.0%)

Stability of test compound: The stability of the test substance under storage conditions is guaranteed until 01 Nov 2016 as indicated by the sponsor, and the sponsor holds this responsibility.

Homogeneity: The homogeneity of the test substance was ensured by mixing before preparation of the test substance solutions.

Vehicle: DMSO

2. Control Materials:

Vehicle control: The vehicle control with and without S-9 mix only contained the vehicle used for the test substance at the same concentration and volume for all tester strains.

Sterility control: Additional plates were treated with soft agar, S9 mix, buffer, vehicle or the test substance but without the addition of tester strains.

Positive control compounds tested without addition of metabolic activation system:

Strain	Mutagen	Solvent	Concentration
TA 100	MNNG	DMSO	5 µg/plate
TA 1535	MNNG	DMSO	5 µg/plate
TA 1537	9-Aminoacridine	DMSO	100 µg/plate
TA 98	NOPD	DMSO	10 µg/plate
E. coli	4-NQO	DMSO	5 µg/plate

Positive control compounds tested with addition of metabolic activation system:

Strain	Mutagen	Solvent	Concentration
TA 100	2-Aminoanthracene	DMSO	2.5 µg/plate
TA 1535	2-Aminoanthracene	DMSO	2.5 µg/plate
TA 1537	2-Aminoanthracene	DMSO	2.5 µg/plate
TA 98	2-Aminoanthracene	DMSO	2.5 µg/plate
E. coli	2-Aminoanthracene	DMSO	60 µg/plate

3. Activation:

S9 was produced from the livers of male Wistar rats treated with 80 mg/kg bw phenobarbital (i.p.) and β-naphthoflavone (orally) for three consecutive days. The S9-mix was prepared freshly prior to each experiment. For this purpose, a sufficient amount of S9-fraction is thawed at room temperature and 1 volume of S9-fraction is mixed with 9 volumes of S9-supplement (cofactors). This preparation, the so-called S9-mix, was kept on ice until used.
The rat liver S9-mix was prepared immediately before use and had the following composition:

Component	Concentration
Phosphate buffer (pH 7.4)	15 mM
Glucose 6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM
S9	10 %

To demonstrate the efficacy of the S9 mix, the S9 batch was characterized with benzo(a)pyrene.

4. Test organisms:

S. typhimurium strains: TA98, TA100, TA1535, TA1537 (regularly checked for rfa, UV sensitivity, R factor plasmid)
E. coli: WP2 uvrA (regularly checked for UV sensitivity)

5. Test concentrations:

Standard plate test (1st experiment):
Triplicate plates in one experiment were prepared for each concentration (neg. control, 33, 100, 333, 1000, 2500, and 5000 µg/plate) and condition (i.e. with and without S9) for all tester strains.

Pre-incubation test (2nd experiment):
Triplicate plates in one experiment were prepared for each concentration (neg. control, 10, 33, 100, 333, 1000, and 2500 µg/plate) and condition (i.e. with and without S9) for all tester strains.

B. TEST PERFORMANCE:

1. Dates of study period: 14 January 2015 – 23 January 2015

2. Standard plate test:
Test tubes containing 2-mL portions of soft agar (overlay agar), which consists of 100 mL agar (0.8% [w/v] agar + 0.6% [w/v] NaCl) and 10 mL amino acid solution (minimal amino acid solution for the determination of mutants: 0.5 mM histidine + 0.5 mM biotin for Salmonella typhimurium strains or 0.5 mM tryptophan for E. coli) were kept in a water bath at about 42 - 45°C, and the remaining components were added in the following order:

0.1 mL test solution or vehicle (negative control)
0.1 mL fresh bacterial culture
0.5 mL S9 mix (**with metabolic activation**) **or** 0.5 mL phosphate buffer (**without metabolic activation**)

After mixing, the samples were be poured onto Minimal glucose agar plates within approximately 30 seconds. After incubation at 37°C for 48 – 72 hours in the dark, the bacterial colonies (his^+ or trp^+ revertants) were counted. The colonies were counted using the Sorcerer Image Analysis System with the software program Ames Study Manager (Perceptive Instruments Ltd., Haverhill, UK). Colonies were counted manually, if precipitation of the test substance hinders the counting using the Image Analysis System.

3. Pre-incubation test:
0.1 mL test solution or vehicle, 0.1 mL bacterial suspension and 0.5 mL S9 mix (**with metabolic activation**) or phosphate buffer (**without metabolic activation**) were incubated at 37°C for the duration of about 20 minutes using a shaker. Subsequently, 2 mL of soft agar was added and, after mixing, the samples were poured onto the agar plates within approximately 30 seconds. After incubation at 37°C for 48 – 72 hours in the dark, the bacterial colonies were counted. The colonies were counted using the Sorcerer Image Analysis System with the software program Ames Study Manager (Perceptive Instruments Ltd., Haverhill, UK). Colonies were counted manually, if precipitation of the test substance hindered the counting using the Image Analysis System.

4. Statistics:
No special statistical tests were performed.

5. Evaluation criteria:
Toxicity
Toxicity detected by a

- decrease in the number of revertants (factor ≤ 0.6)
- clearing or diminution of the background lawn (= reduced his^- or trp^- background growth)

was recorded for all test groups both with and without S9 mix in all experiments and indicated in the tables. Single values with a factor ≤ 0.6 were not detected as toxicity in low dose groups.

Acceptance criteria
Generally, the experiment was considered valid if the following criteria were met:

- The number of revertant colonies in the negative controls was within the range of the historical negative control data for each tester strain.
- The sterility controls revealed no indication of bacterial contamination.
- The positive control substances both with and without S9 mix induced a distinct increase in the number of revertant colonies within the range of the historical positive control data or above.
- Fresh bacterial culture containing approximately 10^9 cells per mL were used.

Assessment criteria
The test substance was considered positive in this assay if the following criteria were met:

- A dose-related and reproducible increase in the number of revertant colonies, i.e. at least doubling (bacteria strains with high spontaneous mutation rate, like TA 98, TA 100 and E.coli WP2 uvrA) or tripling (bacteria strains with low spontaneous mutation rate, like TA 1535 and TA 1537) of the spontaneous mutation rate in at least one tester strain either without S9 mix or after adding a metabolizing system.

A test substance was generally considered non-mutagenic in this test if:

- The numbers of revertants for all tester strains were within the historical negative control data range under all experimental conditions in at least two experiments carried out independently of each other.

II. RESULTS AND DISCUSSION

A. STANDARD PLATE TEST

Toxicity
A bacteriotoxic effect (reduced his^- or trp^- background growth, decrease in the number of his^+ or trp^+ revertants) was observed in the standard plate test depending on the strain and test conditions from about 2500 µg/plate onward.

Solubility
No test substance precipitation was found with and without S9 mix.

Mutagenicity
No relevant increase in the number of his^+ or trp^+ revertants was observed in the experiments with or without S9 mix.

Table 5.8.1-12: Bacterial gene mutation assay with Reg.No. 5968488 – Mean number of revertants (Standard Plate Test; 1st experiment)

Bacteria	**Salmonella typhimurium**								**E. coli**	
Strain	**TA 98**		**TA 100**		**TA 1535**		**TA 1537**		**uvrA**	
Metabol. activation	**+S9**	**-S9**	**+S9**	**-S9**	**+S9**	**-S9**	**+S9**	**-S9**	**+S9**	**-S9**
Neg. control (DMSO)#	28 ± 6	22 ± 2	40 ± 4	24 ± 2	10 ± 2	10 ± 1	11 ± 1	8 ± 3	56 ± 9	55 ± 4
Test item (µg/plate)										
33	27 ± 6	24 ± 9	35 ± 10	22 ± 2	16 ± 3	12 ± 1	10 ± 2	10 ± 1	62 ± 10	56 ± 6
100	27 ± 3	25 ± 2	33 ± 1	24 ± 5	11 ± 3	10 ± 2	12 ± 3	8 ± 2	49 ± 3	49 ± 5
333	29 ± 4	24 ± 7	33 ± 3	19 ± 2	15 ± 4	11 ± 1	12 ± 2	8 ± 2	55 ± 8	54 ± 6
1000	31 ± 6	23 ± 6	38 ± 10	21 ± 4	11 ± 2	13 ± 4	11 ± 4	7 ± 4	36 ± 5	50 ± 2
2500	28 ± 9	27 ± 1	19 ± 7	17 ± 1	14 ± 3	11 ± 2	11 ± 2	7 ± 2	59 ± 4	48 ± 7
5000	0 ± 0 B	0 ± 0 B	0 ± 0 B	0 ± 0 B	0 ± 0 B	0 ± 0 B	0 ± 0 B	0 ± 0 B	32 ± 3 B	33 ± 3 B
Pos. control*	1505 ± 198	426 ± 25	492 ± 160	3018 ± 134	248 ± 39	4574 ± 184	130 ± 22	925 ± 240	137 ± 26	932 ± 51

#: due to rounding of numbers they may vary compared to the original report
*: details see under Material and Methods
B: reduced background growth

B. PREINCUBATION TEST

Toxicity

In the preincubation assay bacteriotoxicity (reduced his^- or trp^- background growth, decrease in the number of his^+ or trp^+ revertants) was observed under all test conditions at a concentration of 2500 µg/plate.

Solubility

No test substance precipitation was found with and without S9 mix.

Mutagenicity

No relevant increase in the number of his^+ or trp^+ revertants was observed in the experiments with or without S9 mix.

Table 5.8.1-13: Bacterial gene mutation assay with Reg. No. 5968488 – Mean number of revertants (Pre-incubation Test; 2nd experiment)

Bacteria	**Salmonella typhimurium**								**E. coli**	
Strain	**TA 98**		**TA 100**		**TA 1535**		**TA 1537**		**uvrA**	
Metabol. activation	**+S9**	**-S9**	**+S9**	**-S9**	**+S9**	**-S9**	**+S9**	**-S9**	**+S9**	**-S9**
Neg. control (DMSO)#	30 ± 9	26 ± 2	42 ± 0	28 ± 2	10 ± 3	8 ± 3	8 ± 1	8 ± 3	58 ± 6	66 ± 4
Test item (µg/plate)										
33	30 ± 7	25 ± 4	42 ± 9	29 ± 7	9 ± 3	9 ± 3	9 ± 2	7 ± 2	64 ± 2	67 ± 11
100	34 ± 11	24 ± 7	39 ± 5	24 ± 4	8 ± 2	7 ± 4	12 ± 4	11 ± 4	67 ± 8	64 ± 3
333	29 ± 3	27 ± 6	36 ± 9	32 ± 6	11 ± 1	11 ± 2	8 ± 4	7 ± 2	58 ± 11	60 ± 12
1000	30 ± 3	21 ± 5	41 ± 6	24 ± 2	10 ± 3	8 ± 2	11 ± 2	11 ± 1	71 ± 4	62 ± 12
2500	29 ± 10	29 ± 12	30 ± 3	35 ± 6	8 ± 1	9 ± 4	11 ± 3	10 ± 3	61 ± 11	59 ± 5
5000	14 ± 3[B]	7 ± 3[B]	5 ± 3[B]	4 ± 3[B]	4 ± 1[B]	0[B]	0[B]	0[B]	31 ± 5	20 ± 5[B]
Pos. control*	962 ± 330	386 ± 14	535 ± 126	3007 ± 81	171 ± 51	2589 ± 123	80 ± 14	420 ± 122	148 ± 11	412 ± 92

#: due to rounding of numbers they may vary compared to the original report
*: details see under Material and Methods
B: reduced background growth

C. DISCUSSION

According to the results of the present study, the test substance did not lead to a relevant increase in the number of revertant colonies either without S9 mix or after adding a metabolizing system in two experiments carried out independently of each other (standard plate test and pre-incubation assay).

Besides, the results of the negative as well as the positive controls performed in parallel corroborated the validity of this study, since the values fulfilled the acceptance criteria of this study.

In this study with and without S9 mix, the number of revertant colonies in the negative controls was within the range of the historical negative control data for each tester strain.

In addition, the positive control substances both with and without S9 mix induced a significant increase in the number of revertant colonies within the range of the historical positive control data.

III. CONCLUSION

Under the experimental conditions chosen here, it is concluded that Reg. No. 5968488 (Metabolite of metconazole) is not a mutagenic test substance in the bacterial reverse mutation test in the absence and the presence of metabolic activation.

CA 5.8.2 Supplementary studies on the active substance

Studies presented in the original monograph (2004) and the addendum of the monograph (2006):

The cis-isomer of metconazole was tested in a subacute mechanistic study to investigate the mechanism of action of liver activation and hepatotoxic effects in male rats and male mice. In both species increased liver weights, centrilobular hypertrophy/vacuolation, increased CYP-450 protein content and induction of CYP isoenzyme activities were observed.

The effects of three isomers of metconazole (isomer mix, the cis- and the trans-isomer) were tested in the Whole Embry Culture (WEC), an in-vitro rat embryo-toxicity assay for the independent evaluation of effects on growth and differentiation. For this dossier the WEC study was re-evaluated according to the commonly accepted interpretations today. All metconazole preparations tested showed general embryo-toxicity with the potency ranking of cis/trans > cis > trans.

These data have been evaluated by European authorities and Belgium as Rapporteur member state (European Commission Peer Review Program) and were considered acceptable.

Table 5.8.2-1: Overview of already peer-reviewed supplementary studies with metconazole

Study, species	Test Substance/Dose Levels	Effect	LOAEL	Reference
28-day oral short term mechanistic study; Mouse (CD-1; male)	cis: batch: ST90/369 (purity: 94.2%; cis/trans: not mentioned) / 300 ppm (62.5 mg/kg bw/d; day 0-7) (58.3 mg/kg bw/d; day 0-28) 8 animals/ group/time point	Liver weights↑ (d7+22%, d28 +13%) CYP450↑ (~1.5-fold) EMND↑ (CYP3A, ~1.4-fold) ECOD↑ (CYP1A &CYP2B, ~1.5-fold) EROD (CYP1A) no change LA-11-H↑ (only day 28: 1.4-fold) LA-12-H no change Midzonal vacuolisation Inflammatory cell foci Centrilobular hypertrophy	300 ppm	[redacted] 1991a (MK-440-005)
28-day oral short term mechanistic study; Rat (F-344; male)	cis: batch: ST90/369 (purity: 94.2%; cis/trans: not mentioned) / 1000 ppm (102.7 mg/kg bw/d; day 0-7) (86.7 mg/kg bw/d; day 0-28) 8 animals/ group/time point	Liver weights↑ (d7+9%, d28 +4%) CYP450↑ (~1.5-fold) EMND↑ (CYP3A, ~1.5-fold) ECOD↑ (CYP1A &CYP2B, ~1.7-fold) EROD↑ (only at day 7:~1.1-fold) LA-11-H no change LA-12-H no change Midzonal vacuolisation	1000 ppm	[redacted] 1991a (MK-440-005)

Study, species	Test Substance/Dose Levels	Effect	LOAEL	Reference
In vitro rat embryo growth and development assay rat embryo (SD-CD rats), day 10 of gestation	cis/trans: batch: 89-01 (purity: 95.3%; cis/trans: 79.8/15.5) cis: batch: 12 (purity: 95.3%; cis/trans: 95.2/0.1), trans: batch: 88.08 (purity and cis/trans not mentioned) / 3, 10, 30, 100 µg/mL, 10 embryos/ concentration	Cis/trans: Embryo development dismorphogeneses	≥ 30 µg/mL ≥ 10 µg/mL	[redacted], 1991 (MK-432-004)
		Cis: No effect on embryo development dismorphogeneses	- ≥ 10 µg/mL	
		Trans: No effect on embryo development dismorphogeneses at 100 µg/mL	- 100 µg/mL	

Submission of not yet peer-reviewed studies in this AIR3-Dossier:

A two-week mechanistic study measuring enzyme induction, cell proliferation, and reactive oxygen species in mice was already submitted to the RMS, but was not yet peer-reviewed for the last Annex I inclusion. However, this mechanistic study was taken under consideration and evaluated for the classification and labelling process of metconazole and supported a mechanism of liver tumour formation not relevant to humans, which does not require a classification for carcinogenicity (see also Chapter M-CA 5.5).

An aromatase assay using recombinant CYP19 enzyme was performed to elucidate the mode of action of metconazole as an aromatase inhibitor and to compare activities of rat versus human enzyme (see also Chapter M-CA 5.8.3 discussion on endocrine disrupting properties). Metconazole cis/trans, cis, and trans showed aromatase inhibition in both rat and human enzyme, but the inhibition was considerably more pronounced in rat as compared to human.

A 28-day immunotoxicity study in rats was conducted for non-EU authorities by another metconazole-supporting company. A detailed study summary was made available to BASF and is part of this submission. Metconazole did not demonstrate any immunotoxic potential up to the highest dose tested.

In order to address the mechanism by which slightly prolonged duration of gestation and dystocia occurred at the high dose in the 2-generation study with cis/trans metconazole (see Chapter M-CA 5.6), a mechanistic 1-generation study in rats was performed investigating hormone measurements during late gestation and liver CYP protein analysis. The delayed onset of parturition and difficult delivery might be associated with the inhibition of increases in the E/P (estradiol/progesterone) ratio observed on gestation days 19 and 21 in this study, which was mainly attributed to decreased serum 17ß-estradiol concentrations. Additionally, increased hepatic CYP proteins of isozymes, which are involved in the estradiol metabolism in the liver were observed and the study author hypothesized that the inhibition of increases in the E/P ratio at this top dose may be due to an excessive acceleration of 17ß-estradiol metabolism.

A comparative metabolome analysis of cis and cis/trans metconazole in Wistar rats following administration in the diet for 28 days has been performed to investigate the toxicity profile and mode of action patterns of both test substances. Effects on the plasma metabolome in rats seem to be pronounced for metconazole cis than for metconazole cis/trans. At 1500 ppm, the metabolite profiles of metconazole cis and metconazole cis/trans showed a correlation on the level of the 97th percentile and 87th percentile for female and male animals, respectively, indicating a high similarity between the two forms.

Table 5.8.2-2: Overview of not yet peer-reviewed supplementary studies on metconazole

Study, species	Test Substance/Dose Levels	Critical effect	LOAEL	Reference (BASF DocID)
2-week hepatic enzyme induction, cell proliferation, and ROS production, CD-1 Mouse (female)	cis/trans : batch 9Z521 (purity :98.53% ; cis/trans : 82.68/15.85) 0,30,300,1000 ppm (0, 4.49, 47.6, 151 mg/kg bw/day) 18 female animals/group	ALT↑ (2.5-fold), AST↑ (1.9-fold) T.Bil.↓ /T.Chol↓ Liver weights↑ (1.5-fold) <u>Histopathology:</u> Hepatocellular hypertrophy Hepatocellular vacuolation <u>CYP enzymes (7 days):</u> CYP450 ↑ (3-fold) ECOD (CYP1A&2B) ↑ (3.1-fold), PROD (CYP2B)↑ (4.5-fold) <u>CYP proteins (7 days):</u> CYP1A (4.5-fold) CYP2B (11.5-fold) CYP3A (3.9-fold) <u>PCNA LI (liver) ↑:</u> 3 days (~8.5-fold) 7 days (~6-fold) 14 days (~3-fold, n.s.) <u>Oxidative stress markers (day 14):</u> LPO↑ (2.3-fold) 8-OHdG (~1-fold)	1000 ppm ≥ 300 ppm ≥ 300 ppm ≥ 300 ppm ≥ 300 ppm ≥ 300 ppm ≥ 300 ppm ≥ 300 ppm n.s.	[redacted], 2004a (2004/1032005)
Human and rat recombinant Aromatase (CYP 19) Assay	<u>cis/trans</u>: batch 14955000 (purity: 98%, cis/trans: 81/17); <u>cis:</u> batch 3 (purity: 100%; cis/trans: 97.2/2.8); <u>trans:</u> batch 1 (purity 100%; cis/trans: 0.8/99.2) / 10^{-4} - 10^{-12} M	inhibition of recombinant aromatase activity (human < rat): <u>IC_{50} human:</u> cis/trans: 0.721 µM cis: 0.569 µM trans: 2.47 µM <u>IC_{50} rat:</u> cis/trans: 0.157 µM cis: 0.223 µM trans: 0.579 µM	Not applicable	Mentzel, 2015a (2015/1205961)

Study, species	Test Substance/Dose Levels	Critical effect	LOAEL	Reference (BASF DocID)
Immuno-toxicity study (28 days), Wistar rat (male)	cis/trans: batch 1362353 (purity: 97%; cis/trans: 84.6/15.1) / 0, 70, 210, 630 ppm (5.4, 17, 52 mg/kg bw/day) 8 animals/group	Systemic toxicity: reduced body weight gain at top dose No immunotoxic effects.	LOAEL systemic toxicity: 630 ppm (52 mg/kg bw/day) LOAEL immunotoxicity: >630 ppm (52 mg/kg bw/day) (HDT)	Summary: [redacted] 2014a (2015/1087910) Study author: [redacted], 2010 a (2010/8000287)
Mechanistic 1-generation study: Measurement of serum steroid hormone concentrations and hepatic drug-metabolizing enzyme contents during late gestation - SD rat (female)	cis/trans: batch: 9Z521 (purity: 98.99%; cis/trans: 83.13/15.86) / 30, 150 and 750 ppm (1.8, 8.9 and 43 mg/kg bw/day) 24 females/group	Parental toxicity: reduced body weight gain Fetotoxicity: number of live fetuses↓, resorptions and fetal deaths↑ Inhibition of decrease of E/P (estradiol/progesterone) ratio on GD 19 and 21; Decreased serum estradiol on GD 19 and 21; Increased CYP protein: CYP3A4 and CYP2B	43 mg/kg bw/day (750 ppm, HDT)	Summary: [redacted], 2015(b) (2015/1087914) Study author: [redacted], 2002b (2006/8000262)
Metabolome profile analy-sis of cis and cis/trans met-conazole in Wistar rats (28-day dietary study)	cis/trans: batch COD-001163 (purity: 98.7%); 500 and 1500 ppm cis: batch AC 10925-24B (purity: 97.4%); 1500 and 5000 ppm 5 males and females/group	high level of similarity of metabolite profile between cis and cis/trans metconazole	Not applicable	[redacted], 2015a (2014/1035855)

n.s. not significant

HDT: highest dose tested

For convenience of the reviewer brief summaries of the respective studies are presented and detailed study summaries of the supplementary studies which were not yet evaluated are provided below.

Based on the available data, the conclusion for relevant endpoints for the current renewal was as follows:

Other toxicological studies

Supplementary studies on the active substance	Metconazole cis: 28-day mechanistic study (male rats and mice): hepatic CYP induction (rat, mouse) Metconazole cis/trans: 14-day mechanistic study in female mice: CYP2B induction (protein and enzyme activity) and transient hepatocellular proliferation after 3 and 7 days at 300 and 1000 ppm 28-day immunotoxicity study in rats: no immunotoxin potential Mechanistic 1-generation study: extended gestation length and dystocia may be associated with inhibition of decrease of E/P (estradiol/progesterone) ratio during late gestation, mainly due to decreases in serum estradiol. Metconazole cis/trans, cis, and trans: Recombinant aromatase assay in rat and human enzyme: inhibition of aromatase activity (human < rat; cis/trans = cis > trans): Whole Embryo culture: general embryotoxicity potential cis/trans > cis > trans. Metconazole cis and cis/trans: Comparative metabolome analysis: high level of similarity of metabolite profile between cis and cis/trans metconazole

Mechanistic study, 28 day dietary study in rats and mice ([redacted], 1991a) (MK-440-005)

Guidelines: Not available. Mechanistic study.
Deviations: Not applicable.
GLP: Yes
Acceptance: The study was accepted.

Materials and methods:
8 male rats/dose/time point (F344 Fisher) and 8 female CD-1 mice/dose/time point were fed a diet of metconazole (total purity 94.2%; enantiomeric purity not mentioned); code no. WL136184=KNF-S-474c, batch no. ST90/369) at a dose level of 0 and 300 ppm (mice) or 0 and 1000 ppm (rats) during 7 or 28 days. Achieved doses: mice (300 ppm): 62.5 mg/kg bw/d (d0-7) and 58.3 mg/kg bw/d (d0-28); rat (1000 ppm): 102.7 mg/kg bw/d (d0-7) and 86.7 mg/kg bw/d (d0-28). As a positive control, 8 male rats and mice were fed a diet of Phenobarbital, at a dose level of 0.05% during 28 days. Samples were checked analytically to ensure accuracy, homogeneity and stability of the administered diet. After treatment, 2/8 animals were anaesthetised with barbiturate, their livers perfused with glutaraldehyde, removed and processed for electron microscopical examination. The other animals were sacrificed by cervical dislocation, and livers were processed for biochemical analysis. The livers of the last group were homogenised, and whole homogenates were analysed for the activity of cyanide-insensitive palmitoyl-CoA activity, DNA- and protein content. Washed microsomal fractions were assayed for protein and Cytochtrome-P450 (CYP450) content, and for CYP450 isoenzyme subfamilies activities: Ethylmorphine N-demethylase (EMND, a marker for CYP3A activitiy), 7-Ethoxycoumarin-O-deethylase (ECOD, a marker for CYP1A and CYP2B activity), 7-Ethoxyresorufin-O-deethylase (EROD, a marker for CYP1A activity), and lauric acid 11- and 12-hydroxylase (LA-11-H and LA-12H; markers for CYP4A activity). The palmitoyl-CoA activity was analysed to monitor the potential for peroxisomal fatty acid β-oxidation induction.

Findings:
No mortality was observed. No relevant clinical signs were observed. On d7, body weight of rats were lowered compared to controls (-5%, $p<0.05$); the difference was maintained until d28 of treatment. In the mice, treatment was without effect on body weight.
Relative (r), but not absolute (a), liver weight was increased in the rat on d7 (+9%; $p<0.01$) and on d28 (+4%, $p<0.05$). In the mice, both absolute and relative liver weights were increased on d7 (a:+20%, $p<0.05$%, r:+22%, $p<0.001$), and on d28 (a:+12%, r:+13%, $p<0.01$). Phenobarbital treatment resulted in a significant increase of liver weight (a:+41%, r:+31%).

Histopathology:
In both rats and mice, treated with metconazole (cis) during 28d, an increased incidence of slight to moderate midzonal vacuolisation in was observed. Both form and distribution of them were consistent with accumulation of intracellular lipid. In contrast, 0/6 rats and 1/6 mice exhibited centrilobular hypertrophy. This was expected, since in the 28-days study, the finding was only observed at a higher dose, or with the isomer mix.

The majority of the phenobarbital-treated animals showed hepatocellular hypertrophy after 28d. Electron microscopical examination of the 2 metconazole-treated rats revealed no obvious differences in the quantity of smooth endoplasmatic reticulum (SER) when compared to controls, whereas Phenobarbital-treated rats showed some increase of SER in periportal hepatocytes. In the mice, metconazole provoked a slight increase in SER in centriclobular hepatocytes, in association with a reduction in the amount of rough endoplasmatic reticulum (RER). In Phenobarbital-treated mice, only an increase in SER in centrilobular hepatocytes was noted.

Table 5.8.2-3: Histopathological findings in the liver of Metconazole and phenobarbital treated rats and mice.

Endpoint	Rat			Mouse		
	Control	Metconazole	Phenobarbital	Control	Metconazole	Phenobarbital
		1000 ppm	0.05%		300 ppm	0.05%
Inflammatory cell foci	1	0	1	0	2	0
MZ vacuolisation	0	5	1	0	3	0
Diffuse vacuolisation	0	0	0	0	1	0
CL hypertrophy	0	0	5	0	1	6

Numbers indicate incidence/6 animals; MZ: midzonal, CL:centrilobular

Biochemical analysis:

Analyses performed on the whole liver homogenates of rats and mice, treated either by Metconazole or the positive control phenobarbital, revealed no meaningful differences in both DNA- or protein content, when compared to control animals. Likewise, neither metconazole nor phenobarbital had an effect on peroxisomal fatty acid β-oxidation (palmitoyl-CoA). In contrast, microsomal protein content was time-dependently induced in both Metconazole- or Phenobarbital treated rats and mice. In common to the effect of Phenobarbital, total CYP450-content, and enzymatic activities of EMND (CYP3A) and of ECOD (CYP1A and CYP2B) were slightly induced after 28d of treatment.

While EROD-activity was only marginally increased in the rats after 7d of treatment, and remained uninduced after 28 days, Phenobarbital-treated rats showed an increase of enzymatic activity when compared to controls.

Lauric acid-12-hydroxylase, which is a known peroxisome proliferator, was not induced by metconazole in both rat and mouse, whereas, like Phenobarbital, the compound produced an slight increase in lauric acid-11-hydroxylase in the mouse after 28d, which was however only marginally increased (up to 1.4-fold for metconazole).

Table 5.8.2-4: Biochemical analysis of liver microsomes from metconazole and phenobarbital treated rats and mice (% of control)

Endpoint	Rat			Mouse		
	Metconazole		Phenobarbital	Metconazole		Phenobarbital
	d7	d28	d28	d7	d28	d28
Protein content	117*	139***	146***	114*	124***	128**
CYP450 content	154***	140***	197***	152***	139***	146***
EMND (CYP3A)	149***	141***	172***	139***	159***	213***
ECOD (CYP2B)	173***	178***	439***	156**	143***	277***
EROD (CYP1A1)	114*	-	300***	-	-	184***
LA-11-H	-	-	189***	-	140**	165***
LA-12-H	-	-	130*	-	-	-

Statistical evaluation *: $p \leq 0.05$, **: $p \leq 0.01$, ***: $p \leq 0.001$

Conclusion:

Metconazole (cis), administered during 7d and 28d in rats and mice induced some CYP450-dependent isoenzymes, in a pattern similar, but not identical to Phenobarbital. In contrast to Phenobarbital, the compound induced some midzonal vacuolation in both rats and mice. On the contrary, unlike Phenobarbital, the test compound did not induce a centrilobular hypertrophy. This was in line with the findings in the full 28d-feeding study, where this effect was observed at a 10x higher dose (the administration of 1000 ppm of the cis/trans isomer during 28d induced such an effect predominantly in the female rats).

Report: CA 5.8.2/1
[redacted] 2015a
Metconazole (BAS 555 F) (+/- cis racemate), Metconazole (-/+ cis, -/+ trans racemate) - Metabolome analysis conducted for a screening study in Wistar rats - Administration via the diet
2014/1035855
Guidelines: <none>
GLP: no

Executive Summary

Metconazole cis (batch: AC 10925-24B, purity: 97.4%) or metconazole cis/trans (batch: COD-001163, purity: 98.7%) were administered daily to groups of 5 male and female Wistar rats for 28 days at dietary concentrations of 1500 and 5000 ppm, or 500 and 1500 ppm, respectively. Groups of 10 males and females received only the ground diet for the same time-period and served as controls. Blood samples for metabolome analysis were taken retro-orbitally on study days 7, 14, and 28 from overnight fasted animals under isoflurane anesthesia and the obtained EDTA-plasma was covered with nitrogen and frozen at -80°C.

The plasma metabolome was examined by metanomics GmbH following proprietary sample work up using GC/MS and LC/MS-MS techniques. The metabolome as investigated in this study refers to 297 endogenous metabolites in plasma. These cover a broad range such as carbohydrates, amino acids, fatty acids, hormones, etc. The analytical data were evaluated via MetaMap®Tox database for specific metabolic changes of each dose group and comparing the entire metabolome and reference compounds ("profile comparison") using Spearman and Pearson correlations.

Metconazole cis at 5000 ppm did have a strong effect on the food consumption and body weights of animals of both sexes. Due to the reduced general state of the animals, the treatment of the animals had to be discontinued at this top dose. At 1500 ppm, a significant effect on food consumption and body weights was observed. Metconazole cis/trans at 1500 ppm did have a slight effect on food consumption and body weights of animals of both sexes, whereas animals administered 500 ppm did not show any effect on these parameters.

Both compounds did induce effects on the plasma metabolome of rats of both sexes. Comparing the treatments with 1500 ppm metconazole cis and metconazole cis/trans, the effects on the metabolome of metconazole cis seem to be more pronounced than for metconazole cis/trans as it is reflected in the higher profile strength for metconazole cis (1.6 for male and 1.83 for female animals) when compared to the profile strength for metconazole cis/trans (0.99 for male and 1.04 for female animals). This is also reflected in the similarities observed with patterns in the data base, which are dominated by effects on the liver and indirect effects on thyroids. However the pattern for the indirect thyroid effect is not corroborated by the data obtained in the subacute and subchronic rat studies. At 1500 ppm, the metabolite profiles of metconazole cis and metconazole cis/trans showed a correlation on the level of the 97th percentile and 87th percentile for female and male animals, respectively, indicating a high similarity between the two forms.

(DocID 2014/1035855)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material 1:	Metconazole cis
Description:	not specified
BASF Test item No.:	01/0051-1
Lot/Batch #:	AC 10925-24B
Purity:	97.4%
Test Material 2:	Metconazole cis/trans
Description:	not specified
BASF Test item No.:	04/0609-8
Lot/Batch #:	COD-001163
Purity:	98.7%
2. Vehicle:	food
3. Test animals:	
Species:	Rat
Strain:	Wistar rats (Crl:Wi(Han)
Sex:	male and female
Age:	10 weeks (at start of administration)
Source:	Charles River, Sulzfeld, Germany
Diet:	Ground Kliba mouse/rat maintenance diet “GLP”, meal, supplied by Provimi Kliba SA, Kaiseraugst, Switzerland, ad libitum
Water:	water, ad libitum
Housing:	Group housing (5 animals per cage) in the study with metconazole cis/trans and individual housing in the study with metconazole cis.

B. STUDY DESIGN AND METHODS

1. Dates of work: In-life:

metconazole cis:	26. Aug. 2005 - 23. Sep. 2005
metconazole cis/trans:	13. Jun. 2013 - 12 Jul. 2013

2. Animal assignment and treatment:

Metconazole cis or metconazole cis/trans were administered daily to groups of 5 male and female Wistar rats for 28 days at dietary concentrations of 1500 and 5000 ppm, and 500 and 1500 ppm, respectively. Groups of 10 males and females received only the ground diet for the same time period and served as controls. Blood samples for metabolome analysis were taken retro-orbitally on study day 7, 14, and 28 from overnight fasted animals under isoflurane anesthesia and the obtained EDTA-plasma was covered with nitrogen and frozen at -80°C. In total, 40 plasma samples of metconazole cis and metconazole cis/trans treatments, respectively, were used for analysis and evaluation.

3. Metabolite profiling:

The plasma metabolome was examined by metanomics GmbH following proprietary sample work up. Proteins were removed from plasma samples by precipitation. Subsequently, polar and non-polar fractions were separated for both GC-MS and LC-MS/MS analysis by adding water and a mixture of ethanol and dichloromethane.
Absolute quantification was performed by means of stable isotope-labelled standards. For all metabolites, changes were calculated as the ratio of the mean of metabolite levels in individual rats in a treatment group relative to mean of metabolite levels in rats in a matched control group (time point, dose level, sex).

A. Broad profiling

GC/MS analysis

The non-polar fraction was treated with methanol under acidic conditions to yield the fatty acid methyl esters derived from both free fatty acids and hydrolyzed complex lipids. The non-polar and polar fractions were further derivatised with O-methyl-hydroxylamine hydrochloride and pyridine to convert oxo-groups to O-methyl-oximes and subsequently with a silylating agent before analysis.

LC/MS-MS analysis

The non-polar and fractions were reconstituted in appropriate solvent mixtures. HPLC was performed by gradient elution using methanol/water/formic acid on reversed phase separation columns. Mass spectrometric detection technology was applied which allows target and high sensitivity MRM (Multiple Reaction Monitoring) profiling in parallel to a full screen analysis.

B. Catecholamine and steroid hormone analysis

Steroids hormones, catecholamines and their metabolites were measured by online SPE-LC-MS/MS (Solid phase extraction-LC-MS/MS).

3. MetaMap®Tox evaluation:

The sex- and day-stratified heteroscedastic t-test ("Welch test") was applied to compare metabolite levels of dose groups with respective controls. A significance of $p < 0.05$ was applied. On the basis of 297 analytes, 15 significant metabolite changes can be expected on a significance level of 0.05 (“false positive” rate). Therefore, up to 15 significantly changed metabolites, the metabolome is considered as not affected by the test compound.

Test substance related changes in the metabolome were analyzed as follows:
1) Analysis of specific metabolic changes for each dose group
2) Comparison with the entire metabolome of reference compounds, called “profile comparison” using Spearman and Pearson correlations. In order to assess the size and relevance of a correlation coefficient a reference distribution of correlation coefficients was derived by calculating all pairwise coefficients of the whole data base stratified by sex (male/female) and dose (high/low). As each stratum comprises approximately 500 treatments (t-profiles) the quantiles of each reference distribution are based on approximately 130000 r-values/stratum.
Based on these analysis, a threshold value of 0.40 for male animals and 0.50 for female animals displays approximately the 95th percentile of all correlation coefficients obtained by the profile

comparison. Correlation coefficients above these values are considered as indicating a clear match between two treatments.

II. RESULTS AND DISCUSSION

A. OBSERVATIONS

Animals administered 5000 ppm metconazole cis showed reduced general state of health and thus the treatment was discontinued on day 14 and the animals were sacrificed.

B. BODY WEIGHT AND FOOD CONSUMPTION

Metconazole cis

At 5000 ppm, food consumption was significantly reduced on day 7 (-76% and -74% for males and females, respectively) and day 14 (-59% and -53% for males and females, respectively).
Body weight was decreased on day 7 (-24% and -23% for males and females, respectively) and day 14 (-35% and -29% for males and females, respectively).

At 1500 ppm, food consumption was significantly reduced on day 7 (-17% and -49% for males and females, respectively) and day 14 (-17% and -27% for males and females, respectively).
Body weight was decreased on day 7 (-10% and -13% for males and females, respectively), day 14 (-12% and -16% for males and females, respectively), and day 28 (-13% and -19% for males and females, respectively).

Metconazole cis/trans

At 1500 ppm, no test substance-related effects on food consumption were observed in males and females.
Body weight was decreased on day 7 (-11% in females), day 14 (-8% and -9% for males and females, respectively), and day 28 (-9% in females)

At 500 ppm, no test substance-related effects on food consumption and body weight were observed in males and females.

C. METAMAP®TOX EVALUATION

1. Changed metabolites - Key changes

Metconazole cis

At 5000 ppm, the interpretation of the changes in the metabolome in males and females was not possible, since the animals had to be sacrificed in a moribund state on day 14.

At 1500 ppm, strong changes in the metabolite profile of male and female animals as compared to the control group (no. of significantly changed metabolites 4-6 times above the false positive rate at $p < 0.05$ of 15 metabolites; based on 297 possible metabolites) were observed. Nineteen and 27 metabolites have been significantly and consistently changed in males and females, respectively, on at least two out of the three different time points (see Table 5.8.2-5).

Metconazole cis/trans

At 1500 ppm, on day 7, metconazole cis/trans produced slight changes in metabolite profile of males. From study day 14 onwards, clear changes in the metabolite profile of male and female animals as compared to that of the control animals (no. of significantly changed metabolites 2-3 times above the false positive rate at $p < 0.05$ of 15 metabolites; based on 297 possible metabolites) were observed. Ten and 5 metabolites have been significantly and consistently changed in males and females, respectively, on at least two out of the three different time points (see Table 5.8.2-5).

Table 5.8.2-5: Metabolite changes in rats administered 1500 ppm metconazole cis or metconazole cis/trans for 7,14, and 28 days

Day	Sex	Changes*	Metconazole	No. of changed metabolites
7	♂	↑	cis	14
			cis/trans	10
		↓	cis	55
			cis/trans	16
		Total	cis	**69**
			cis/trans	**26**
	♀	↑	cis	47
			cis/trans	29
		↓	cis	21
			cis/trans	17
		Total	cis	**68**
			cis/trans	**46**
14	♂	↑	cis	17
			cis/trans	16
		↓	cis	34
			cis/trans	15
		Total	cis	**51**
			cis/trans	**31**
	♀	↑	cis	57
			cis/trans	25
		↓	cis	42
			cis/trans	30
		Total	cis	**99**
			cis/trans	**55**
28	♂	↑	cis	31
			cis/trans	45
		↓	cis	66
			cis/trans	6
		Total	cis	**97**
			cis/trans	**51**
	♀	↑	cis	82
			cis/trans	13
		↓	cis	18
			cis/trans	18
		Total	cis	**100**
			cis/trans	**31**

*relative to control

At 500 ppm, slight changes in the metabolite profile of male and female animals as compared to that of the control group (no. of significantly changed metabolites 1-2 times above the false positive rate at $p < 0.05$ of 15 metabolites; based on 297 possible metabolites) were observed. Four and 2 metabolites have been significantly and consistently changed in males and females, respectively, on at least two out of the three different time points (see Table 5.8.2-6).

Table 5.8.2-6: Metabolite changes in rats administered 500 ppm metconazole cis/trans for 7,14, and 28 days

Day	Sex	Changes*	No. of changed metabolites
7	♂	↑	14
		↓	10
		Total	**24**
	♀	↑	10
		↓	10
		Total	**20**
14	♂	↑	6
		↓	16
		Total	**22**
	♀	↑	11
		↓	8
		Total	**19**
28	♂	↑	11
		↓	9
		Total	**20**
	♀	↑	14
		↓	4
		Total	**18**

*relative to control

2. Toxicological mode of action

Pattern ranking

Metconazole cis

At 5000 ppm, the comparison of metabolite changes in males and females against the established specific metabolite patterns present in MetaMap®Tox was not possible, since the animals had to be sacrificed in a moribund state on day 14.

At 1500 ppm, the comparison of metabolite changes against the established specific metabolite patterns present in MetaMap®Tox did show similarities with patterns for triazole aromatase inhibition and oxidative stress in the liver in males, and for liver effects (liver toxicity, enzyme induction, cholestasis), indirect effects on the thyroid (potentially due to increased excretion of thyroid hormones), and diuretic effects on the kidney for females.
The metabolome similarities to pattern for triazole aromatase inhibition were recorded in males. Because of the lack of a similar pattern for female animals, no such similarity could be observed for females.
The metabolome similarities to pattern for indirect effects on the thyroids were recorded in female animals. However, neither in subacute nor in subchronic studies with metconazole cis and metconazole cis/trans thyroid effects were observed in rats of both sexes [see Chapter M-CA 5.3].

Metconazole cis/trans
At 1500 ppm, the comparison of metabolite changes against the established specific metabolite patterns present in MetaMap®Tox did show similarities with patterns for liver enzyme induction, liver toxicity and indirect effects on the thyroid (potentially due to increased excretion of thyroid hormones) in males, but did not show any similarity for females.
The metabolome similarities to pattern for indirect effects on the thyroids were recorded in male animals. However, neither in subacute nor in subchronic studies with metconazole cis and metconazole cis/trans thyroid effects were observed in rats of both sexes [see Chapter M-CA 5.3].

At 500 ppm, the comparison of metabolite changes against the established specific metabolite patterns present in MetaMap®Tox did show weak similarities with patterns for liver enzyme induction in males, but did not show any similarity for females.

Profile comparison with reference compounds
Metconazole cis
At 5000 ppm, the total profile comparison of the metabolite profile of males and females could not be conducted, since the animals had to be sacrificed in a moribund state on day 14.

At 1500 ppm, the total profile comparison of the metabolite profile (profile strength 1.6 and 1.83 for males and females, respectively) did show matches with those of compounds present in MetaMap®Tox as compared to that for male and female animals, and was ranked on the level of the 87th and 97th percentile of all correlations in the data base for males and females, respectively.

Metconazole cis/trans
At 1500 ppm, the total profile comparison of the metabolite profile (profile strength 0.99 and 1.04 for males and females, respectively) did show matches with those of compounds present in MetaMap®Tox as compared to that for male and female animals, and was ranked on the level of the 87th and 97th percentile of all correlations in the data base for males and females, respectively.

At 500 ppm, the total profile comparison of the metabolite profile (profile strength 0.81 and 0.68 for males and females, respectively) did show matches with those of compounds present in MetaMap®Tox as compared to that for male and female animals, and was ranked on the level of the 39th and 93th percentile of all correlations in the data base for males and females, respectively.

Comparing both substances tested, the same level of similarity between the two forms in males and females receiving 1500 ppm and reasonable level of similarity female animals treated with 500 ppm metconazole cis/trans was observed. In contrary, very low similarity in male animals administered 500 ppm metconazole cis/trans was evaluated. However, this effect is based on the dose dependency of the metabolome changes observed.

III. CONCLUSION

Both compounds did induce effects on the plasma metabolome of rats of both sexes. Comparing the treatments with 1500 ppm metconazole cis and metconazole cis/trans, the effects on the metabolome of metconazole cis seem to be more pronounced than for metconazole cis/trans as it is reflected in the higher profile strength for metconazole cis (1.6 for male and 1.83 for female animals) as compared to the profile strength for metconazole cis/trans (0.99 for male and 1.04 for female animals). This is also reflected in the similarities observed with patterns in the data base, which are dominated by effects on the liver and indirect effects on thyroid. However the pattern for the indirect thyroid effect is not corroborated by the data obtained in the subacute and subchronic rat studies. At 1500 ppm, the metabolite profiles of metconazole cis and metconazole cis/trans showed a correlation on the level of the 97th percentile and 87th percentile for female and male animals, respectively, indicating a high similarity between the two forms.

Report: CA 5.8.2/2
[redacted] 2004a
Metconazole: 2-week hepatic drug-metabolizing enzyme induction, cell proliferation, and reactive oxygen species production study in mice
2004/1032005
Guidelines: none
GLP: no

Executive Summary
Metconazole was administered via the diet to groups of 18 female Wistar rats at dose levels of 30, 300, and 1000 ppm for 3,7, or 14 days. At the end of the treatment, the animals were killed and subjected to blood sampling, necropsy, measurements of liver weights, hepatocellular proliferation, histopathology, and reactive oxygen species (ROS) production in the liver (oxidative stress makers). Interim kills of 6 animals per group were performed after 3 and 7 days of treatment and subjected to necropsy, measurements of organ weights, cell proliferation, and hepatic drug-metabolizing enzymes (after 7 days only).
There were neither abnormalities in clinical signs nor deaths in any dose group during the study. Body weight and food consumption for each dose group were comparable to controls. Increased levels of transaminases AST (up to 1.9-fold), ALT (up to 2.5-fold) and cytochrome P-450 isozymes and decreased levels of total cholesterol and bilirubin were observed in the 300 and 1000 ppm dose group. The elevation in PROD (CYP 2B) activity was more pronounces than ECOD (CYP1A, 2B) activity, i.e. up to 4.5-fold in PROD and 3.1-fold in ECOD as compared to controls at 300 and 1000 ppm. Both CYP2B (up to 11-fold) and to a lesser extent CYP3A (up to 4-fold) protein levels were increased at the mid and high dose.
In addition, at the top dose cell proliferation was dose-dependently increased after 3 and 7 days at 300 and 1000 ppm.
Measurement of the oxidative stress makers indicated a significant up to 2.6-fold increase in lipid peroxide at 300 and 1000 ppm, whereas no significant change in 8-OHdG levels were observed at any dose level. Thus, metconazole is considered to lead to increased ROS levels generated secondarily during the metabolic microsomal CYP enzyme induction, that is not sufficient to also induce oxidative DNA damage.
These biochemical findings correlated with a dose-dependent increase in liver weights (absolute and relative) and histopathological alterations like hepatocellular hypertrophy and hepatocellular vacuolation in the 300 and 1000 ppm dose groups.
Taken together, increased plasma ALT and AST activities up to 2.5-fold as well as up-to 2-fold increased hepatic lipid peroxidation (LPO), increased liver weights and microscopic evidence of hypertrophy/vacuolation are indicative of a liver-enzyme inducting compound. It is suggested that metconazole is a hepatic drug-metabolizing enzyme inducer acting primarily via CYP2B induction similar to phenobarbital and has a potential to enhance cell proliferation (mitogenic activity) like a known non-genotoxic-mitogenic hepatocarcinogen. The effects were observed at the carcinogenic dose levels 300 ppm and 1000ppm (see also Chapter 5.5).
The no-observed-adverse-effect level (NOAEL) of the test substance for these effects was considered to be 30 ppm (4.5 mg/kg b.w./day for female mice), suggesting the threshold level for its effects on the non-linear non-genotoxic mode of action (MOA) for induction of mouse liver tumors.

(DocID 2004/1032005)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: Metconazole

Chemical name:	(lRS,5RS,lRS, 5RS)-5-(4-chlorobenzyl)-2,2-dimethyl-1-(1H-1,2,4-triazol-1-ylmethyl)cyclopentazole
Description:	solid (crystal) / white
Lot/Batch #:	9Z521
Purity:	98.53% (cis 82.68%, trans 15.85%)
Stability of test compound:	The stability of the test substance under storage conditions over the test period was guaranteed by the sponsor.

2. Vehicle: none

3. Test animals:

Species:	Mouse
Strain:	ICR (Crj:CD-1)
Sex:	Female
Age:	10 weeks (at start of administration)
Weight at dosing:	27.1 – 34.5 g
Source:	Charles River Japan Inc., Atsugi Breeding Center, Kanagawa, Japan
Acclimation period:	7 days
Diet:	Certified diet MF Mash (Oriental Yeast Co., Ltd., Tokyo, Japan)
Water:	filtered and sterilized well water, ad libitum
Housing:	Group housing (6 animals per cage) before grouping and single housing after grouping in aluminum cages with wire-mesh floors.

Environmental conditions:

Temperature:	20 - 26°C
Humidity:	40 - 70%
Air changes:	at least 10 per hour
Photo period:	12 h light / 12 h dark (07:00 - 19:00 / 19:00 - 07:00)

B. STUDY DESIGN AND METHODS

1. Dates of experimental work: 06/25/2004 - 07/23/2004

2. Animal assignment and treatment:
Female mice were chosen as they have shown to be more sensitive regarding hepatic tumor induction, as observed in a previously performed carcinogenicity study.
On day of arrival, the animals were subjected to an acclimatization period during which they received ground diet and drinking water ad libitum. Prior to the first detailed clinical observation, the animals were distributed according to weight among the individual test groups. The weight variation of the animals used did not exceed 20 percent of the mean weight.
The test substance was administered daily via the diet for 2 weeks. Control animals received only the ground diet without test substance addition. Animals were administered a diet with 0, 30, 300, and 1000 ppm of the test item for 2 weeks. Eighteen animals were used for each dose group. After 3 and 7 days of treatment, 6 animals from each group were killed in numerical order from the lowest animal number and subjected to necropsy, measurements of organ weights, cell proliferation, and hepatic drug-metabolizing enzymes (hepatic enzymes only after 7 days of treatment). After 14 days (2 weeks) of treatment, all the rest of animals were killed and subjected to blood sampling, necropsy, measurements of organ weights, cell proliferation and reactive oxygen species production (oxidative stress makers), and histopathology.

3. Test substance preparation and analysis:
Prior to the present study, a stability test was performed on test diet samples and it was confirmed that the test substance was stable in the diet under the following conditions:

- stored under a sealed, dark and cold (at about 5°C) condition for 4 weeks
- and then kept under a condition exposed to an ambient air in an animal room at room temperature for 2 weeks.

Based on the results of the stability test, preparation of test diets for the present study was conducted once prior to initiation of treatment. For each dose level, a required amount of the test substance was mixed with a part of the basal diet in a mortar to provide a premix. The premix was then added to the rest of the basal diet, and they were blended by a blending machine, HP-60 (Kanto Kongoki Industrial Co., Ltd., Tokyo), to obtain good homogeneity of the test substance in the diet. No adjustment for purity was made when calculating the amount of the test substance to be mixed. The basal diet was used as a control diet for the control group. Formulated diets were sealed in plastic bags and stored in a test diet storage room (at about 5°C) until provided to animals. After the sealed bags were opened in the animal room, the diets were used within 14 days to guarantee the stability of the test substance in the diet.
Chemical analyses for homogeneity and concentration of the test substance in test diets were performed for each dose level on samples (approximately 50 g each) taken from the top, middle, and bottom portions of the mixer. The diet for control animals was also analyzed to verify no contamination with the test substance. At chemical analyses, the test substance in the diet was extracted with acetone and analyzed by gas chromatography. Analytical values were divided by the purity of the test substance and expressed as the total concentrations including impurities.

4. Statistics:
Statistical significance of the difference between the control and treated groups was estimated at 5% and 1% levels of probability.
The data on body weight, food consumption, liver weights, blood biochemical parameters, hepatic microsomal enzymes, hepatic cell proliferation and hepatic oxidative stress markers were evaluated by Bartlett's test for equality of variance. When group variances were homogeneous, a parametric analysis of variance of a one way layout type was conducted to determine if any statistical differences existed among groups. When the analysis of variance was significant, Dunnett's multiple comparison test was applied. When the group variances were heterogeneous, the data were evaluated by Kruskal-Wallis non-parametric analysis of variance. When significant, Dunnett-type mean rank sum test was applied. Fisher's exact probability test (one-tail analysis) was used to analyze the data on clinical signs, mortality, necropsy and histopathology findings.

C. METHODS

1. Clinical examinations:

Mortality
All animals were checked daily for deaths and morbidity during the study. Mortality rates were expressed as ratio of cumulative number of animals found dead or killed *in extremis* to the effective number of animals per group.

Clinical observations
A cage side examination was conducted at least once daily for any signs of morbidity, pertinent behavioral changes and signs of overt toxicity. Abnormalities and changes were documented daily for each affected animal.

2. Food consumption and intake of test substance

Food consumption for each animal was measured once a week during the study. The weekly consumption was obtained by the measurement of total amount consumed for a period of 3 consecutive days. The value was divided by the number of days for measurement, and daily food consumption per animal was obtained. Group mean food consumption (g/mouse/day) was calculated from daily food consumption by each animal. An average of the group mean food consumption throughout the treatment period was also calculated as a mean of the weekly data. Group mean test substance intake (mg/kg/day) was calculated weekly according to the following formula:

$$IT_x = \frac{FCx}{BWx}\ x\ C$$

FC_x = mean daily food consumption on study day x [g]
BW_x = body weight on study day x [g]
IT_x= intake of test substance on study day x [mg/kg bw/day]
C = concentration [ppm]

3. Body weight data
Body weights of all animals were recorded weekly during the study. Group mean body weights were calculated at each measurement. In addition, all animals at each scheduled sacrifice were weighed just before necropsy and the body weight data were used for calculation of relative organ weights (ratio to body weight).

4. Clinical pathology
Blood biochemical examinations were performed on all surviving animals after 2 weeks (14 days) of treatment. Animals were laparotomized under ether anesthesia and blood samples were withdrawn from the posterior vena cava using heparinized syringes. These animals were not fasted before blood sampling. Plasma samples obtained from the collected blood were used for measurements of the following parameters: Alkaline phosphatase (ALP), Glutamic oxaloacetic transaminase (GOT), Glutamic pyruvic transaminase (GPT), y-Glutamyl transpeptidase (GGTP), Total cholesterol (T.Chol), Total bilirubin (T.Bil).

5. Sacrifice and pathology:

Weight parameters
Liver weight was measured for all animals killed on schedule after 3, 7, and 14 days of treatment. Relative liver weight was calculated from the ratio of liver weight to body weight.

Necropsy
Necropsy was performed on all animals. Animals at interim sacrifices after 3 and 7 days of treatment were euthanized by exsanguination from the abdominal aorta and posterior vena cava under deep ether anesthesia and then necropsied. Animals at terminal sacrifice after 14 days of treatment were also euthanized and necropsied in the same manner after blood sampling. Gross examinations at necropsy were limited to the organs and tissues in the thoracic and abdominal cavities. All gross findings were recorded for each animal.

Organ/tissue fixation
Following organ weight measurement at each scheduled sacrifice after 3, 7, and 14 days of treatment, tissues of the liver (left lobe) and duodenum were sampled from each animal and fixed in 10% neutral-buffered formalin or methanol. In addition to the collecting above samples, median, right and caudal lobes of the liver from each animal killed after 7 days of treatment were perfused with 1.15% KCl solution to remove blood as much as possible. After completion of perfusion, the hepatic samples were frozen in liquid nitrogen and stored in a deep freezer (lower than -70°C) until analysis of drug-metabolizing enzymes. Furthermore, a part of the liver (left lobe) was embedded in a Tissue Mount (Chiba Medical Co., Ltd., Saitama, Japan) with dry ice-alcohol and then stored in a deep freezer (lower than -70°C) for possible future analysis of gap junction protein Cx32.
At terminal sacrifice after 14 days of treatment, median lobes of the liver were sampled from each animal in addition to the sampling of hepatic left lobes and duodenum. The samples of the hepatic left lobes were frozen in liquid nitrogen and stored in a deep freezer (lower than - 70°C) until use (measurement of oxidative stress markers). The rest of liver tissues at each scheduled sacrifice were frozen or fixed in 10% neutral-buffered formalin and stored as much as possible for future analysis.

Histopathology
Paraffin sections of the liver from all animals killed on schedule after 2 weeks (14 days) of treatment were prepared by a routine method. Microscopic examinations were performed on the preparations stained with hematoxylin and eosin (H&E). In addition, frozen sections of the liver from a representative animal (Animal No. 467) in the 1000 ppm group were stained with Oil red 0 and examined microscopically.

6. Measurement of hepatic drug-metabolizing enzymes:
Preparation of hepatic microsomal fraction
The frozen sample of the liver (median lobe, right lobe and caudal lobe) were defrosted and homogenized with 1.15% KCl using a homogenizer Polytron® (Kinematica GmbH, Luzern, Switzerland). The liver homogenate was centrifuged at 9000 x g for 10 min, the pellet was discarded and the supernatant was centrifuged again at 105000 x g for 60 min. The pellet (Microsomal fraction) was suspended in 0.1 M Na/K-phosphate buffer (pH 7.4) and stored at -70°C.
Measurement of microsomal enzymes
The following parameters were measured by using the suspension of hepatic microsomal fraction: Microsomal protein content, Cytochrome P-450 content, Ethoxycoumarin O-dealkylase activity (ECOD; CYP1A1, 1A2, 2B), and Pentoxyresorufin O-dealkylase activity (PROD; CYP2B).
In addition, protein contents of P-450 isozymes (CYPlA, CYP2B, and CYP3A) were determined by Western blot analysis using goat or rabbit polyclonal antibodies (Daiichi Pure Chemicals Co., Ltd., Tokyo, Japan) against these CYPs. The concentration of each identified CYP was quantified with an imaging densitometer, Model GS-700 (Nippon Bio-Rad Laboratories, Tokyo, Japan).
Measurement of cell proliferation in liver
The liver and duodenum tissues fixed in methanol for each animal were embedded in paraffin and their paraffin sections were subjected to immunohistochemistry for proliferating cell nuclear antigen (PCNA). Numbers of PCNA-positive cells per approximately 1000 hepatocytes were counted and PCNA labeling index (%) was determined. Cell proliferation activity was evaluated by PCNA labeling index for each group. The duodenum tissue was used as positive control for PCNA staining.
Measurement of hepatic oxidative stress marker
Measurements of lipid peroxide (LPO, malondialdehyde) and 8-hydroxydeoxyguanosine (8-OHdG) were performed on the liver tissues (frozen sample of median lobe) from animals killed on schedule after 14 days of treatment.
Measurement of hepatic lipid peroxide
A part of the frozen sample (median lobe) was processed to give a liver homogenate and the absorbance of the peroxide (LPO, malondialdehyde) was determined by the TBA method using a spectrophotometer (UV-2200, Shimazdu Corporation, Kyoto, Japan). The concentration of lipid peroxide in the liver tissue was calculated as follows:

Concentration of lipid peroxide (nmol/mL) = f (absorbance of test sample) / F (absorbance of standard sample) x 10 nmol/mL liver homogenate

Measurement of hepatic 8-OHdG
DNA was extracted from a part of the same frozen sample (median lobe) mentioned in the above section. The concentration of 8-OHdG in the liver tissue was determined using an Elisa kit for 8-hydroxydeoxyguanosine (8-OHdG) and by micro plate-reader.

II. RESULTS AND DISCUSSION

A. TEST SUBSTANCE ANALYSES

Homogeneity of the test substance in test diets was checked for each dose level on samples taken from the top, middle, and bottom portions of the mixer. The coefficient of variation for each dose level was within 2.7%, indicating that good homogeneity was obtained by the preparation method used.
Concentrations of the test substance in test diets were 95-98% of the target concentrations. The test diets were considered to be appropriate for use in the study.

Table 5.8.2-7: Concentration control analysis of the test substance in the diet

Nominal concentration (ppm)	Recovery [ppm] Mean ± SD (out of 6 samples)	Recovery [%]
30	29 ± 1.2	97
300	294 ± 8.4	98
1000	953 ± 23.7	95

B. OBSERVATIONS

1. Clinical signs of toxicity
No abnormal clinical signs of toxicity were observed.

2. Mortality
No animal died prematurely during the study.

C. BODY WEIGHT AND BODY WEIGHT GAIN
There were no significant differences in body weight change between treated and control groups during the study.

D. FOOD CONSUMPTION AND COMPOUND INTAKE
There were no significant differences in food consumption between treated and control groups during the study.

The test substance intake (mg/kg/day) for each group was calculated by multiplying the group mean food consumption by the nominal concentration of the test substance in the diet and then dividing by the mean body weight.

Table 5.8.2-8: Calculated mean daily test substance intake of female rats

Concentration in the vehicle (ppm)	Mean daily test-substance intake (mg/kg/day)
	Females
30	4.49
300	47.6
1000	151

E. CLINICAL PATHOLOGY

Clinical chemistry findings

Statistically significant changes were observed for glutamic oxaloacetic transaminase (GOT)/ aspartate amino transferase (AST), glutamic pyruvic transaminase (GPT)/ alanine amino transferase (ALT), total cholesterol (T.Chol) and total bilirubin (T.Bil) for animals after 14 days of treatment (see Table 5.8.2-9)

In the 1000 ppm group, significant increases in AST (approximately 1.9-fold increase) and ALT (approximately 2.5-fold increase) and significant decreases in T.Chol (approx. - 55%) and T.Bil (approx. -30%) were observed.

In the 300 ppm group, a significant decrease in T.Bil and a decreasing trend in T.Chol were also noted. In the 30 ppm group, there were no significant changes in any parameters.

Table 5.8.2-9: Overview on blood chemistry parameters in animals after 14 days of treatment (% of control)

Dose	GOT (AST)	GPT (ALT)	T. Chol	T. Bil
0	100	100	100	100
30	115	129	102	111
300	115	129	74	**78****
1000	**194****	**254****	**44****	**67****

Statistical evaluation **: $p \leq 0.01$

F. PATHOLOGY

1. Organ weights

Dose-dependent increased absolute and relative liver weights were observed at 300 ppm and 1000 ppm. At 1000 ppm, relative liver weights were increased by approximately 50%, as compared to controls, following 3, 7, or 14 days of treatment. At 300 ppm, relative liver weights were increased up to 17%, as compared to controls, following 14 days of treatment, but the changes were of no statistical significance (see Table 5.8.2-10).

In the 30 ppm group, no abnormalities were observed in the liver weights for animals at each scheduled sacrifice.

Table 5.8.2-10: Absolute and relative liver weights

	Absolute liver weight (g)			Relative to body weight (%)		
Dose (ppm)	**3 days**	**7 days**	**14 days**	**3 days**	**7 days**	**14 days**
0	1.36 ± 0.18 (100%)	1.51 ± 0.06 (100%)	1.44 ± 0.14 (100%)	4.55 ± 0.33 (100%)	4.65 ± 0.16 (100%)	4.27 ± 0.36 (100%)
30	1.36 ± 0.31 (100%)	1.43 ± 0.19 (95%)	1.46 ± 0.11 (101%)	4.61 ± 0.65 (101%)	4.52 ± 0.38 (97%)	4.52 ± 0.43 (106%)
300	1.61 ± 0.15 (118%)	1.68 ± 0.22 (111%)	1.57 ± 0.18 (109%)	5.11 ± 0.18 (112%)	5.31 ± 0.63 (114%)	4.99 ± 0.27 (117%)
1000	**2.07 ± 0.15** (152%)**	**2.04 ± 0.21** (135%)**	2.07 ± 0.55 (144%)	**6.75 ± 0.54** (148%)**	**6.79 ± 0.59** (146%)**	**6.67 ± 0.78** (156%)**

Statistical evaluation ** : $p < 0.01$ (Dunnett's test)

2. Gross lesions

In the 1000 ppm group, enlarged livers were observed in almost all animals killed on schedule after 3, 7, and 14 days of treatment. In addition, white spots were found in 2 of 6 animals killed on schedule after 7 days of treatment. In the 300 and 30 ppm group, there were no treatment-related gross abnormalities.

3. Histopathology

Histopathological examination was performed on the liver from all animals at terminal sacrifice after 14 days of treatment. In the 1000 ppm group, slight to moderate diffuse hepatocellular hypertrophy was observed in all animals. In addition, slight hepatocellular vacuolation was noted in 5 of 6 animals. The vacuoles in hepatocytes were demonstrated to be lipid droplets by Oil Red O stain. In 300 ppm group, mild diffuse hepatocellular hypertrophy was observed in all animals and slight hepatocellular vacuolation was found in 2 of 6 animals. In the 30 ppm group, no treatment-related histological changes were observed.

Table 5.8.2-11: Treatment related necropsy and hiostpathological findings in female mice

	Liver enlargement			Hepatocellular hypertrophy	Hepatocellular vacuolation
Dose (ppm)	**3 days**	**7 days**	**14 days**	**14 days**	**14 days**
0	0 / 6	0 / 6	0 / 6	0 / 6	0 / 6
30	0 / 6	0 / 6	0 / 6	0 / 6	0 / 6
300	0 / 6	0 / 6	0 / 6	6 / 6	2 / 6
1000	6 / 6	6 / 6	5 / 6	6 / 6	5 / 6

G. HEPATIC DRUG-METABOLISING ENZYMES

Microsomal CYP enzyme activity

In the 300 and 1000 ppm group ethoxycoumarin O-dealkylase (ECOD: CYP1A1, 1A2, 2B) activity, and pentoxyresorufin O-dealkylase (PROD: CYP2B) activity were significantly increased when compared to controls. The elevation in PROD activity was more evident than that in ECOD activity (4.5-fold in PROD and 3.1-fold in ECOD as compared to controls).
In the 30 ppm group, there were no significant changes in any parameters.

Table 5.8.2-12: Hepatic microsomal cytochrome P-450 enzyme activities (after 7 days of treatment; % of control)

Dose	CYP content	ECOD	PROD
0	100	100	100
30	117	115	136
300	**207***	**206***	**379****
1000	**307***	**311****	**448****

Microsomal CYP protein (Western Blot analysis)

In the 1000 ppm group, contents of cytochrome P-450 isoenzymes CYP1A, CYP2B and CYP3A were significantly increased when compared to controls, especially with a marked elevation in CYP2B (11.5-fold when compared to controls).
In the 300 ppm group, contents of CYP2B and CYP3A were significantly increased when compared to controls. In the 30 ppm group, there were no significant changes in any cytochrome P-450 isoenzyme content.

Table 5.8.2-13: Hepatic microsomal CYP protein content (after 7 days of treatment; % of control)

Dose	CYP P-450 isozyme content		
	CYP1A	CYP2B	CYP3A
0	100	100	100
30	99	162	82
300	222	**396***	**251****
1000	**451****	**1149****	**395****

Statistical evaluation *: $p \leq 0.05$; **: $p \leq 0.01$

Hepatic cell proliferation activity

Increased PCNA labelling index (PCNA LI), a marker for cell proliferation activity, was significantly increased at 1000 ppm, compared to control values, after 3 days (850%), 7 days (600%), and to a lesser extent after 14 days (300%, not significant) (see Table 5.8.2-14). Since the PCNA labelling method is not a very sensitive marker for cell proliferation, the increase in hepatocellular proliferation observed at 300 ppm after 3 and 7 days was considered treatment-related. No significantly increased PCNA indices in the liver were seen at 30 ppm dietary concentration.

Table 5.8.2-14: PCNA LI in the liver (after 3, 7, and 14 days of treatment; % of control)

Dose	PCNA LI in the liver		
	3 days	**7 days**	**14 days**
0	100	100	100
30	100	100	[400][$]
300	**150**	**200**	100
1000	**850****	**600****	300

Statistical evaluation *: p≤0.05; **: p≤0.01
[$] [LI increase at 30 ppm / 14-days were caused by two outliers values from a total of 6 animals:
PCNA LI in %: 1.2–0.3–0.1–0.1–0.8–0.0 (30 ppm) vs. 0.1–0.3–0.1–0.0–0.1–0.1 (control)]

Hepatic oxidative stress markers

In 1000 and 300 ppm groups, LPO was significantly increased when compared to controls, but there were no significant differences in 8-OHdG between the treated and control groups.
In the 30 ppm group, there were no significant changes in any parameter.

Table 5.8.2-15: Hepatic oxidative stress markers (after 14 days of treatment; % of control)

Dose (ppm)	LPO (nmol/g tissue)	8-OHdG (ng/mg DNA)
0	100	100
30	145	94
300	**261****	91
1000	**231***	92

Statistical evaluation *: p≤0.05; **: p≤0.01

H. DISCUSSION

Metconazole was administered in feed to female ICR (Crj:CD-l) mice at dose levels of 0, 30, 300, and 1000 ppm for 3, 7 or 14 days to evaluate its effects on hepatic drug-metabolizing enzyme induction, hepatocellular proliferation, and reactive oxygen species (ROS) production in the liver.
Chemical analysis of test diets confirmed that homogeneity and concentration of the test substance in test diets were within acceptable limits.
There were neither abnormalities in clinical signs nor deaths in any dose group during the study. Body weight and food consumption for each dose group were comparable to controls.

An increase in AST and ALT up to 2.5-fold was observed at 1000 ppm. T.Chol and T.Bil were decreased in the 300 and 1000 ppm dose group. These changes were considered to be treatment-related as similar effects have been observed in former repeated dose toxicity studies. Dose-dependent related increased liver weights (absolute and relative), as well as liver hypertrophy/vacuolation were also observed in the 300 and 1000 ppm dose groups.

These findings were accompanied by increased ECOD (Major catalysts: CYP1A1, 1A2, 2B) and PROD (CYP2B) enzyme activities in these two dose groups, whereas PROD activity was more pronounced than ECOD activity. Furthermore, Western blot analysis revealed a predominant increase in CYP2B and to a lesser extent CYP3A protein contents at 300 and 1000 ppm. Based on the necropsy findings and enzyme activity/content measurements (primarily via CYP2B) a similarity to enzyme inducing effects of phenobarbital was suggested.
Cell proliferation in the liver was dose-dependently increased at 300 and 1000 ppm after 3 and 7 days but not after 14 days. These transient effects were evaluated as typical effects for a non-genotoxic-mitogenic hepatocarcinogen (similar to phenobarbital), which enhances cell proliferation at the beginning of the treatment but returns to normal thereafter although treatment may be continued.
Lipid peroxide was increased in the liver at terminal sacrifice in the 300 and 1000 ppm dose groups, whereas no change in the 8-OHdG level was observed at any dose level. Thus, metconazole is considered to lead to increased ROS levels generated secondarily during the metabolic microsomal enzyme induction, that is not sufficient to also induce oxidative DNA damage.

The increased liver weights and the observed enzyme induction were associated with the histopathological correlates as evidenced by hepatocellular hypertrophy and slight vacuolation in the 300 and 1000 ppm dose groups.
Based on the results described above, it has been suggested that metconazole is a hepatic drug-metabolizing enzyme inducer primarily via CYP2B, similar to phenobarbital and has a potential to enhance transient cell proliferation (mitogenic activity) like a known non-genotoxic-mitogenic hepatocarcinogen.
The occurrence of hepatocellular tumours observed in female mice at concentrations of 300 ppm and 1000 ppm in a long-term oral carcinogenicity study (see Chapter M-CA 5.5.) is considered to be via a phenobarbital-like mechanism. In the present study, the no-observed-adverse-effect level (NOAEL) of the test substance was found to be 30 ppm (4.49 mg/kg bw/day), suggesting the threshold of its effects on hepatic microsomal enzyme induction, cell proliferation, and ROS production (oxidative stress).

III. CONCLUSIONS

The administration of metconazole cis/trans via the diet to ICR (Crj:CD-1) female mice for 14 days revealed comparable signs of toxicity at concentrations of 300 and 1000 ppm similar to the known non-genotoxic-mitogenic hepatocarcinogen phenobarbital. A threshold for the effects on hepatic CYP enzyme induction, ROS production, and transient cell proliferation was derived at 30 ppm, corresponding to the NOAEL of this study.

Report: CA 5.8.2/3
[redacted] 1991a
Exploratory investigation into the effects of WL148271, WL136184 and WL153996 upon growth and development of rat embryos in vitro
MK-432-004
Guidelines: <none>
GLP: yes
(certified by Department of Health and Social Security of the Government of the United Kingdom, United Kingdom)

Materials and methods:
For assessing these data one has to be aware that the used approach in 1991 to combine the WEC for metabolic activation of a test substance with a S9 mix could not be established in the following years (Flick et al., 2006a; BASF DocID 2006/1051874). Its metabolic capacity is still insufficient for most pro-teratogens. However, a few successful uses had been described, like for cyclophosphamide where only a relative small metabolic transformation into highly potent metabolites is needed. But the S9 fraction as well as the co-factors have an embryo-toxic potential itself and increase the sensitivity of embryos for exposure independent of the metabolic activation of a test item. Thereby, the background incidence of effects is increased and bias the interpretation of the study. In this study this had been manifested in a control showing an unusual high incidence of six dysmorphogeneses (1x flexion abnormal, 2x pericardium abnormal, 1x hind brain abnormal, 1x mid brain abnormal, 1x forebrain open, and 2x forebrain abnormal, see Tab. 2B in the report). However, significant increased incidence above this background level are worth to be assessed.

The effects of three isomers of metconazole were tested in the Whole Embry Culture (WEC), an in-vitro rat embryo-toxicity assay for the independent evaluation of effects on growth and differentiation according to the commonly accepted interpretations today (Flick et al., 2006a; BASF DocID 2006/1051874 and Flick et al., 2009a; BASF DocID 2009/1132282).

Embryo culture:
Rat embryos were obtained from mated Sprague Dawley CD-rats (gestation day 10). Uterus from ether-anaesthetised rats were removed, deciduas released, and embryos dissected out. Only overtly healthy embryos with the visceral yolk-sac and ectoplacental sac intact, but with Reichert's membrane torn open and removed, were explanted. Embryos were incubated in roller-bottles (5/bottle) during 48h (60 rev/min, 38°C, 1 mL culture/embryo) in heat-inactivated, filter-sterilised rat serum, supplemented with an equal volume of Hepes-buffered Eagle's Minimum Essential Medium (MEM) in the presence of 5/5/90% (0-24h) and 20/5/75% (24-48h) of $O_2/CO_2/N_2$.

Treatment and evaluation.
10 rat embryos/concentration were incubated in the presence of metconazole cis/trans (WL148271; purity 95.3%, cis/trans: 79.8/15.5; batch: 89-01), metconazole cis (WL136184; purity: 95.3%; cis/trans: 95.2/0.1; batch: 12) or metconazole trans (WL153996; purity and cis/trans content not specified; batch: 88.08), diluted in stock solutions in distilled water and in the presence of metabolic activating system (S9, obtained from Aroclor-1254 pretreated rats, supplemented with 0.5 mM NADP and 5 mM G6P, final concentration 8 µL/mL). These stock solutions were used to aim final concentrations of 0, 3, 10, 30 and 100 µg/mL in the culture medium of the main experiment.

These concentrations have been selected based on a range-finding experiment (5 embryos/concentration) testing the cis/trans isomer mix at 0, 0.1, 1, 10 and 100 µg/mL before.
The effectiveness of the metabolic activating system was tested by treatment of the embryos with cyclophosphamide (CP) at 5 µg/mL.
Parameters of growth were yolk sac diameter, crown-rump and head length. A morphological score as indicated in the study report and the somite number were used to determine the differentiation of embryos. The differentiation landmarks were e.g. yolk sac, allantois, heart, caudal neural tube, brain, otic and optic system, branchial bars and limb-buds.

Findings:
In the preliminary range-finding experiment, metconazole cis/trans showed effects on growth (crown-rump and head length), and on differentiation (somite number and morphological score) at 100 µg/mL. Borderline effects might have been observed on differentiation at 10 µg/mL. However, the potential effects at 10 µg/ml could not be reproduced in the main experiment and were assessed more likely caused by chance than being treatment-related. Also dysmorphogeneses, like yolk sac abnormal, pericardium abnormal, hindbrain open, forelimb buds, abnormal observed at 10 µg/ml were considered as incidental because no concentration-dependency were observed in the range finding and/or the main experiment, or these findings were also seen in the control of the main experiment at comparable incidences. Based on this evaluation, only a part of the dysmorphogeneses listed in the report could be used for the assessments of the isomers of metconazole. Only these dysmophogeneses were listed in this summary.

In the main experiment, embryos incubated in the presence of metconazole cis/trans exhibited significant reductions in growth (crown-rump and head length) and differentiation (somite number and partially morphological score) parameters at 30 and 100 µg/mL. These parameters were essentially similar in all dose groups of embryos incubated with either the cis- or the trans-isomer of metconazole. (see Table 5.8.2-16)

Table 5.8.2-16: Effect of metconazole isomers and of cyclophosphamide on rat general embryo development (growth and differentiation) in-vitro

Endpoint	Metconazole isomer	Dose metconazole (µg/mL)					Dose CP (µg/mL)
		0	3	10	30	100	5
Yolk sac diameter (mm)	**Cis/trans**	**3.94 mm**	0%	4%	-6%	-4%	**-8%**
	Cis		2%	-2%	2%	2%	
	Trans		-1%	-1%	1%	-3%	
Crown-rump length (mm)	**Cis/trans**	**3.62 mm**	-3%	1%	**-7%**	**-11%**	**-30%**
	Cis		2%	-6%	-2%	-3%	
	Trans		0%	-1%	-1%	-4%	
Head-length (mm)	**Cis/trans**	**1.77 mm**	-5%	-1%	**-12%**	**-17%**	**-40%**
	Cis		6%	-5%	-1%	-6%	
	Trans		2%	-3%	-1%	-3%	
Somite number	**Cis/trans**	**26.1**	-1%	-2%	**-10%**	**-16%**	**-43%**
	Cis		0%	-2%	-2%	-2%	
	Trans		-1%	0%	-3%	-1%	
Morphological score	**Cis/trans**	**38.5**	-6%	1%	-1%	**-11%**	**-44%**
	Cis		1%	-2%	-1%	-2%	
	Trans		1%	-2%	0%	-2%	

Numbers indicate incidence /10 embryos evaluated; Morphological score calculated on 13 developmental landmarks (score 0 to 4 per parameter). The toxicologically relevant relative changes are given bolded.

An increased incidence of morphologic abnormalities (dysmorphogeneses) occurred in embryos treated with metconazole cis/trans in 6 out of 13 anomalies, metconazole cis in 3 out of 13 anomalies. Metconazole trans induced morphologic abnormalities only at the top-dose and manifested in only 2 out of 13 used dysmorphogeneses in the morphological scoring. Metconazole showed a common pattern of dysmorphogeneses based on abnormalities on posterior neuropore, and otic system caused by all three isomers at relative high incidences. No further concentration-dependent dysmorphogeneses were induced by the three isomers. However, the potential of the isomers to cause dysmorphogeneses was different. Metconazole cis/trans and cis showed within the common pattern of dysmorphogeneses a comparable potency causing abnormalities at ≥ 10 µg/mL whereas metconazole trans had a lower potency causing anomalies only at the highest concentration tested 100 µg/mL. However, metconazole cis/trans caused further three dysmorphogenesis in hind- and mid-brain and branchial arches in an incidence only slightly above the background incidence of spontaneous abnormalities at 100 µg/mL (see Table 5.8.2-17).

Table 5.8.2-17: Effect of metconazole isomers and of cyclophosphamide on rat embryo dysmorphology in-vitro

Detected abnormality	Metconazole isomer	Dose Metconazole (µg/mL)					Dose CP (µg/mL)
		0	3	10	30	100	5
Posterior neuropore	Cis/trans	0	3	**8**	**9**	**10**	7
	Cis		0	**9**	**10**	**9**	
	Trans		1	1	2	**9**	
Otic system	Cis/trans	0	0	0	**3**	**7**	7
	Cis		0	0	**5**	**3**	
	Trans		0	0	0	**4**	
Fore-limb buds	Cis/trans	0	1	2	0	4	9
	Trans		0	2	0	3	
	Cis		0	0	0	1	
Hind-brain	Cis/trans	1	0	0	2	4	5
	Cis		0	1	2	2	
	Trans		0	2	1	0	
Mid-brain	Cis/trans	1	0	0	2	4	5
	Cis		0	1	1	1	
	Trans		0	2	1	0	
Branchial part	Cis/trans	0	0	0	0	2	0
	Cis		0	0	0	0	
	Trans		0	0	0	0	

Numbers indicate incidence /10 embryos evaluated. The toxicologically relevant observations are given bolded.

All metconazole preparations tested interfered with posterior neuropore closure and otic system development in vitro. However, these parameters did not indicate a specific teratogenic effect in vivo, but corroborate the already noted general embryo-toxicity based on growth and differentiation parameters. (see also Chapter M-CA 5.6)

Conclusion:

Metconazole cis/trans showed effects on embryo development at ≥30 µg/mL based on retardation in growth and differentiation. Neither metconazole cis nor trans showed a comparable effect on embryo development up to 100 µg/mL. However, metconazole cis/trans and cis showed a potential to cause dysmorphogeneses at ≥10 µg/mL and above, but metconazole trans only at 100 µg/mL. The pattern of dysmorphogeneses of all three isomers was comparable at the highest concentration tested indicating that all isomers may act via the same mode of action in embryo-toxicity. However, the potencies of metconazole cis/trans and cis were higher to cause dysmorphogeneses in vitro manifested at 10 and 30 µg/mL but for metconazole trans only at 100 µg/mL.

Mechanistic 1-generation study: Measurement of serum steroid hormone concentrations and hepatic drug-metabolizing enzyme contents during late gestation in rats following exposure to metconazole KNF-474m (cis/trans) (2002)

A mechanistic 1-generation study in rats (study author: 2002 b; BASF DocID 2006/8000262) with metconazole cis/trans (KNF-474m) was conducted for non-EU authorities by another metconazole-supporting company. A detailed study summary was made available to BASF (prepared by 2015b; BASF DocID 2015/1087914) and is part of this submission.

Report:	CA 5.8.2/4 2015b Summary of the Laboratory Report IET 02-0058 - KNF-474m: A Measurement of serum steroid hormone concentrations and hepatic drug-metabolizing enzyme during late gestation in rats fed diets containing KNF-474m
BASF DocID:	2015/1087914
Guidelines:	none
GLP:	no

Report:	CA 5.8.2/5 2006 b A measurement study of serum steroid hormone concentrations and hepatic drug-metabolizing enzyme contents during late gestation in rats fed diets containing KNF-474m 2006/8000262
Guidelines:	EPA 870.3800
GLP:	yes (certified by Ministry of Agriculture, Forestry and Fisheries of Japan, Japan)

Executive Summary

This supplemental study was performed to clarify the mechanisms by which prolonged duration of gestation and maternal death during delivery occurred at the highest dose tested (750 ppm) in the 2-generation with metconazole with cis/trans in rats (see Chapter M-CA 5.6; summary DocID 2015/1087913). Groups of 24 female Crj:CD(SD)[IGS] rats were given diets containing metconazole at concentrations of 0, 30, 150, or 750 ppm for 3 weeks and mated with normal untreated males of the same strain. Treatment of females with metconazole was continued during gestation. It has been reported in rats that towards the end of the gestation period increased serum estradiol concentrations, and an increased concentration ratio of 17ß-estradiol to progesterone (E/P ratio) due to a decrease in progesterone concentrations with regression of corpora lutea from gestation day 19 onward, are required to trigger parturition. Thus, in this females were euthanized on gestation day 19 or 21, ant their serum sex steroid hormone concentrations and hepatic drug-metabolizing enzyme contents were measured.
No test substance treatment effects were noted in any parameters of clinical findings, body weights, body weight gain, food consumption, reproductive results, and organ weights in the 30 and 150 ppm groups.

In the 750 ppm group, consistent and significant decreases in mean body weight gains (- 31.5% during pre-mating weeks 1-3, and -17.5% during gestation days 0-21 compared with the controls) and food consumption throughout the study period were indicative of parental toxicity. In addition, slight significant decreases in the mean number of corpora lutea and implantation sites were observed at the top dose. These effects were of questionable toxicological significance, since the parameters were not altered in the 2-generation study with metconazole cis/trans even after a longer treatment period. Reduced number of live fetuses, a significant increase in percent resorptions and fetal deaths, and significant increases in relative liver and ovary weights were noted at the highest dose tested, as compared to control.

Serum 17ß-estradiol concentrations stayed constant on both gestation days 19 and 21 in the 30 and 150 ppm groups, and no test substance treatment effects were noted in these groups. In the 750 ppm group, 17ß-estradiol concentrations were lower than controls on gestation days 19 and 21 (significant only for gestation day 21). Progesterone value in the 750 ppm group tended to be slightly but not statistically significantly higher than that of the control group on gestation day 21 (with large standard deviations). In the 750 ppm group, the changes in hormone concentration were mainly due to the changes in estradiol and resulted in statistically significant decreases in the E/P ratios on both gestation days 19 and 21 when compared with the controls. E/P ratios in the 30 and 150 ppm groups were comparable with the corresponding controls on both gestation days 19 and 21.
CYP2B1 protein levels were significantly elevated in treated groups, with a slight but relevant increase only at the top dose on gestation days 19 and 21 (1.9-fold and 2.2-fold). CYP3A2 contents in the 30 and 150 ppm groups were comparable to those in the control group, while those in the 750 ppm group were consistently and remarkably higher than controls on both gestation days 19 and 21 (12- and 8-fold increases over the control, respectively).
Histopathological examination of the ovaries in the control group versus 750 ppm group revealed a statistically significant increase in the ratio of the number of corpora lutea with PCNA-positive lutein cells to the total number of corpora lutea in the 750 ppm group.
Progesterone concentrations in this 750 ppm group tended to be slightly but not statistically significantly higher than that of the control group on gestation day 21 with large standard deviations. It was suggested that corpora lutea may not have entered the stage of functional regression yet and may thus, still be active for progesterone production during late pregnancy, which was possibly in line with the finding of higher-than-the-control ratio of corpora lutea containing PCNA-positive lutein cells. However, the large variations of the measured serum progesterone levels make it difficult to draw a final conclusion.
Based on the results of this study, parental toxicity was demonstrated at 750 ppm by decreased mean body weight gain of approximately 31.5% and 17.5% during the pre-mating and gestation periods, respectively. Treatment-related reproductive effects were seen at 750 ppm in the main 2-generation study were prolonged duration of gestation and several cases of maternal death during delivery (see Chapter M-CA 5.6; summary DocID 2015/1087913). The delayed onset of parturition and difficult delivery might be associated with the inhibition of increases in the E/P ratio observed on gestation days 19 and 21 in this study. At the highest dose tested, 750 ppm, it is hypothesized that the inhibition of increases in the E/P ratio may be due to an excessive acceleration of 17ß-estradiol metabolism due to remarkable increase in hepatic CYP contents of isozymes, which are involved in the estradiol metabolism in the liver.

Based on these results, the NOAEL for maternal toxicity in this study is 150 ppm. No adverse effects for increases in the E/P ratio were demonstrated at this dietary dose level, although non-adverse changes like increases in the hepatic microsomal protein and cytochrome P-450 contents were observed.

(DocID 2015/1087914)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:	Metconazole (Code No. KNF-474m)
Description:	solid (crystalline) / white
Lot/Batch #:	9Z521
Purity:	98.99% (83.13:15.86, cis:trans)
Stability of test compound:	not specified
2. Vehicle:	Basal diet
3. Test animals:	
Species:	Rat
Strain:	Crl:CD(SD) [IGS]
Sex:	Female
Age (at start of dosing):	10 days
Weight (at start of dosing):	213 –244 g (females)
Source:	Charles River Japan, Inc., Japan
Acclimation period:	7 days
Diet:	Certified pulverized feed MF Mash, Oriental Yeast Co., Ltd., Japan
Water:	Well water passed through a rapid filtration unit with sand filter and an absorption unit with charcoal filter and sterilized with sodium hypochlorite, ad libitum
Housing:	Animals were housed in suspended wire-mesh stainless steel cages during acclimatization, pre-mating growth, and post-mating (males) periods. Animals were housed in aluminium cages with wire-mesh floors and fronts during the mating period. Post-mating females were continuously housed alone in aluminium cages until necropsy on gestation day 19 or 21. Animals were housed by sex, up to 5 per cage, or during mating, in pairs (1 male with 1 female). Females were housed individually after mating.

Environmental conditions:

Temperature:	22 - 26°C
Humidity:	40 - 70%
Air changes:	at least 10 per hour
Photo period:	12 h light / 12 h dark

B. STUDY DESIGN AND METHODS

1. Dates of experimental work: 17-May-2002 to 06-Jul-2002

2. Animal assignment and treatment:
A total of 109 females (age: 8 weeks) and 109 males (age: 12 weeks) were received from supplier. After the acclimatization period, 96 females were selected and assigned to 4 groups based on their body weight, and 96 males were selected and used for mating at 13 weeks of age.
Groups of 24 females were administered dietary concentrations of 0, 30, 150 or 750 ppm of metconazole for 3 weeks before mating, throughout approximately 1-week mating, and approximately 3-week gestation period until necropsy on gestation day 19 or 21. Female rats of the treatment groups received basal feed when they mated overnight with males. Females in the control group and all males received untreated diet throughout.

Mating procedure
Females were examined daily for regularity of the estrous cycle following completion of treatment week 2, and mating was initiated from day 4 after completion of treatment week 3. Female showing pro-estrus or estrus vaginal smears were placed into the male cages in the evening and cohabited overnight on a 1:1 basis. Healthy males were used for mating in ascending order of the animal number. Females were examined next morning for the presence of the vaginal plugs or sperm in vaginal smears to ascertain the occurrence of copulation. Copulated females were housed individually and the day on which evidence of mating was found was designated as gestation day 0. Females without evidence of copulation were separated from males and examined continuously for the estrous cycle until next mating. The mating period did not exceed one week.

3. Test substance preparation and analysis:
For each dose level, the specified amount of the test substance was mixed in a mortar with a small part of the basal feed. Then, the premix was stirred into the remaining part of the basal feed in a mixer to obtain a test diet of the prescribed concentration. Test diet batches were prepared once every four weeks during the pre-mating growth period and once every two weeks during the breeding period. At the first preparation, homogeneity and concentration of the test substance were analysed for all test diets. At second preparation and onward, concentrations of the test substance in the diets were confirmed for all test diets immediately after preparation.

4. Statistics:
The following tests were used to estimate significance of differences between the control group and the treated groups.
The data on the body weights, body weight gains, and food consumption, the number of corpora lutea, implantation sites, and live foetuses, organ weights, serum steroid hormone concentrations, and hepatic drug-metabolizing enzyme contents were evaluated as follows: Equality of variances was first evaluated by Bartlett's test ($\alpha = 0.05$). When group variances were homogenous, a parametric analysis of variance in one-way classifications ($\alpha = 0.05$) was used to determine if any statistical differences exist among groups. If the analysis of variance was significant, Dunnett's test ($\alpha = 0.05$ or 0.01) was performed. When Bartlett's test indicated that the variances were not homogeneous, Kruskal-Wallis test ($\alpha = 0.05$) was used for detecting any statistical differences among groups and if significant, Dunnett-type mean rank test or Scheffe-type mean rank test ($\alpha = 0.05$, 0.01, or 0.001) was performed.

The data on the number of apoptotic bodies per corpus luteum and mean numbers of PCNA-positive lutein cells per corpus luteum was evaluated between the control group and the high-dose group as follows: Equality of variances was first evaluated by F test ($\alpha = 0.05$) and if group variances were homogeneous, Student's t test ($\alpha = 0.05$, 0.01 or 0.001) was used. When group variances were not homogeneous, Aspin-Welch's test was used ($\alpha = 0.05$, 0.01 or 0.001).
Fisher's exact probability test ($\alpha = 0.05$, 0.01, or 0.001) was used for the data on the incidence of clinical findings, mating and fertility indices, incidences of gross pathological and histopathological findings, and the ratio of the number of corpora lutea with PCNA-positive lutein cells to the total number of corpora lutea.
Mann-Whitney's U-test ($\alpha = 0.05$ or 0.01) was used for the data on the E/P (estradiol / progesterone) ratio and percent resorption and foetal death.

C. METHODS

1. Observations:
Female rats were examined for mortality and clinical signs twice daily on working days and once daily on holidays by cage observation. In addition, a more detailed weekly physical examination was also performed.

2. Body weight:
Females were weighed at the start of treatment, weekly until mating, on gestation days 0, 7 and 14, and on the day of necropsy (gestation day 19 or 21)

3. Food consumption and compound intake:
Food consumption was recorded for each cage on the day of body weight measurement before mating. Individual food consumption was recorded for the periods of gestation days 0 - 7 and 7 - 14, and 14 - 19 or 14 - 21. Food consumption during the mating period was not determined.

4. Sacrifice and pathology:
Males were euthanized after completion of the mating period.
Female rats (24 per group) were divided into two equal parts, and were sacrificed on gestation day 19 or 21 by decapitation under light anesthesia with ether inhalation. All females were subjected to detailed necropsy, and all macroscopic abnormalities were recorded. The number of corpora lutea and the numbers of implantations, live fetuses, resorptions, and dead fetuses were recorded for each female. The percent resorptions and fetal deaths was calculated for each litter from the following formula:

$$\%\ \text{resorptions and fetal deaths} = \frac{\text{number of resorptions and fetal deaths}}{\text{number of implantations}} * 100$$

The liver and ovary from all pregnant females were weighed. The ovary weights are expressed as the mean values of both sides. The relative weights of the liver and ovary to the final body weights were calculated for each female. After the organ weight measurement, the median lobe of the liver was processed and frozen for drug-metabolizing enzyme measurements. Remaining part of the liver, the right ovary, and a piece of duodenum were fixed in 10% neutral-buffered formalin and preserved. The left ovary and a piece of duodenum were fixed in methanol and preserved.

The histopathology of ovaries was conducted for the 8 females selected for the examinations of steroid hormones in serum and the liver drug-metabolising enzymes in each group. Sections of right ovary in all groups were stained with hematoxylin and eosin and examined microscopically with special attention to regressing corpora lutea. Then, the number of TUNEL-positive apoptotic bodies per corpus luteum and the number of PCNA-positive lutein cells per corpus luteum (numbers of corpora lutea with or without PCNA-positive cells in a section of ovary were counted) in right and left ovaries, respectively, were estimated for control and 750 ppm females. Both of TUNEL and PCNA staining methods were validated by the results from the control staining of the duodenum.

5. Steroid hormone concentration in serum:
On gestation day 19 or 21, blood samples were collected from all females in the afternoon by decapitation. For each dose group, serums from eight females, in the ascending order of the animal number, were analysed for 17ß-estradiol and progesterone concentrations using the radioimmunoassay. The concentration ratio of 17ß-estradiol to progesterone (E/P ratio) was calculated for each females

6. Hepatic drug-metabolising enzymes content:
The frozen livers of the females selected for the serum hormone measurement were homogenized and centrifuged to form microsome pellets, and cytochrome P-450 isozymes, CYP1A1, CYP2B1, CYP3A2, and CYP4A1 were determined by Western blot assays. The chromophoric band corresponding to the isozyme was quantitated for its density using the GS-700 Imaging Densitometer (Nippon Bio-Rad Laboratories K. K.), and CYP isozyme content in total microsomal protein [pmol/mg protein] was calculated by the linear regression of the calibration curve derived from the standard molar solution of the isozyme.

II. RESULTS AND DISCUSSION

Results are presented in Table 5.8.2-18, Table 5.8.2-19, and Table 5.8.2-20.

A. TEST SUBSTANCE ANALYSES

The analyses for homogeneity demonstrated that the coefficient of variation of mean test substance concentrations in each treated group diet ranged from 0.5% to 3.3%, indicating that the test substance was homogeneously distributed in the test diets. In the analyses for concentration, the test substance was detected in the samples from the treated groups at levels ranging from 98% to 107% of the nominal concentrations, indicating that the test diets were properly prepared. No test substance was detected in any control diets.

B. OBSERVATIONS

1. Mortality

There were no deaths among females, up to 750 ppm, the highest dose tested (HDT).

2. Clinical signs of toxicity

There were no treatment-related signs of toxicity, up to 750 ppm (HDT).

C. BODY WEIGHT AND BODY WEIGHT GAIN

In the 750 ppm group, mean body weights and body weight gains of females were consistently and significantly lower than those in the control group throughout the study period. Mean body weight gain decrease of 31.5% and 17.5% were recorded during the pre-mating and gestation periods, respectively.

A statistically significant increase in body weight gain, transiently observed in the 30 ppm group at treatment week 3 was considered to be incidental.

D. FOOD CONSUMPTION AND COMPOUND INTAKE

Mean food consumption of females in the 750 ppm group was significantly lower compared with the control group at treatment week 1 and consistently during the gestation period. Statistically significant increases in the 30 ppm group at treatment weeks 2 and 3 were considered to be incidental.

The mean achieved test material intakes are summarized in the table below.

Table 5.8.2-18: Mean test material intakes [mg/kg bw/day]

Dose level [ppm]	Females	
	Pre-mating (Weeks 1-3)	Gestation Days (0-21)
Mean		
30	1.82	
150	8.89	
75	43.0	
Range		
30	1.85-2.02	1.50-1.91
150	8.91-9.84	7.29-9.55
750	44.6-47.9	36.8-43.8

E. MATING DATA

1. Reproductive performance

Mating and fertility were unaffected by treatment.
In the 750 ppm group, mean numbers of corpora lutea, implantations, and live fetuses were significantly lower, and the mean percent resorption and fetal deaths was significantly higher than the corresponding control.

F. NECROPSY

1. Organ weights

At 750 ppm, the absolute and relative (+30%) liver weights on gestation day 21 were significantly higher, compared with the control group. The relative ovary weight on gestation day 19 in this group was significantly higher (+14%), compared with the control group.

2. Gross lesions

There were no macroscopic findings attributable to the treatment with metconazole.

3. Histopathology

No significant differences were noted in the histology of corpora lutea and follicles in the ovary between the control group and any of the treated groups.
The mean number of apoptotic bodies per corpus luteum in the 750 ppm group was comparable with the control group. However, the ratio of the number of corpora lutea with PCNA-positive lutein cells to the total number of corpora lutea in the 750 ppm group was significantly higher, compared with the control group. The mean number of PCNA-positive lutein cells per corpus luteum in the 750 ppm group was also slightly higher than the control.

G. EFFECT OF METCONAZOLE ON STEROID HORMONES

In the 750 ppm group, serum 17ß-estradiol concentrations were lower than controls on both gestation days 19 and 21, and a statistically significant difference was noted in the values on gestation day 21. The serum progesterone concentration in this group was slightly but not statistically significantly higher than the control on gestation day 21 (with large standard deviations). Thus, the changes in hormone concentrations were due to decreased estradiol levels and resulted in significantly decreased E/P rations at the top dose on both gestation days 19 and 21 when compared with the control.

Serum 17ß-estradiol and progesterone concentrations and E/P ratios on gestation days 19 and 21 were unaffected by treatment in the 30 and 150 ppm group. A statistically significant decrease in the 17ß-estradiol concentration on gestation day 21 in the 30 ppm group was considered incidental because no significant difference was noted between the value in the 150 ppm group and that in the control group.

H. EFFECT OF METCONAZOLE ON HEPATIC DRUG-METABOLISING ENZYMES

Hepatic microsomal protein contents on gestation day 19 were significantly increased in the 150 and 750 ppm groups when compared with the control. Cytochrome P-450 contents were significantly increased in the 750 ppm group on gestation days 19 and 21 and in the 150 ppm group on gestation day 21.

No CYP1A1 contents were detected in all groups including the control group on either gestation day 19 or 21. CYP4A1 contents were increased significantly in treated groups, but the magnitudes of the increases were only less than twice of the corresponding controls. CYP2B1 protein levels were significantly elevated in treated groups, with a slight but relevant increase only at the top dose on gestation days 19 and 21 (1.9-fold and 2.2-fold). On the other hand, CYP3A2 contents in the 30 and 150 ppm groups were comparable to those in the control group, while those in the 750 ppm group were consistently and remarkably higher than controls on both gestation days 19 and 21 (12- and 8-fold increases over the control, respectively).

Table 5.8.2-19: Summary of findings in the late gestation rat serum steroid hormones and drug-metabolizing enzymes measurement study with metconazole

Parameter	Dose level (ppm)			
	Females			
	0	**30**	**150**	**750**
Number of animals/group	24	24	24	24
Mortality	0	0	0	0
Clinical signs	No treatment-related effect			
Body weight (g)				
Pre-Mating: Week 0	228	228	228	228
Week 1	246	248	247	**234****
Week 2	260	264	262	**246****
Week 3	264	271	266	**252***
Gain: Week 0-1	17	19	19	**6****
Gain: Week 0-2	31	35	34	**18****
Gain: Week 0-3	35	**42***	38	**24****
Gestation: Day 0	274	280	277	**260****
Day 7	309	314	311	**284****
Day 14	343	348	346	**313****
Day 19	404	413	408	**357****
Day 21	430	432	432	**393****
Gain: Day 0-7	34	35	34	**23****
Gain: Day 0-14	69	69	70	**52****
Gain: Day 0-19	129	130	130	**101****
Gain: Day 0-21	155	156	156	**128****
Food consumption (g/animal/day)				
Pre-mating: Week 1	15.8	16.7	16.2	**13.9****
Week 2	16.5	**17.6***	19.9	15.77
Week 3	15.6	**16.7***	15.8	15.0
Gestation: Day 0-7	19.7	20.0	19.8	**16.2****
Day 14	20.9	21.6	21.1	**18.3****
Day 19	21.9	22.4	21.2	**19.3***
Day 21	21.1	21.6	21.0	**19.3***
Mating performance and fertility	No treatment-related effect			
Reproductive outcome				
Mating index (%)	100.0	100.0	91.7	95.8
Fertility index (%)	95.8	100.0	100.0	100.0
No. of pregnant animals	23	24	22	23
No. of corpora lutea	16.7	16.3	17.3	**15.0***
No. of implantations	15.3	15.0	15.6	**13.9****
No. of live foetuses	14.6	14.3	15.0	**12.4****
Resorptions and fetal deaths (%)	4.3	5.1	4.5	**10.8****

*: $p<0.05$; **: $p<0.01$

Table 5.8.2-20: Summary of findings in the late gestation rat serum steroid hormones and drug-metabolizing enzymes measurement study with metconazole (continued)

Parameter	Dose level (ppm)							
	Females							
	Gestation day 19				Gestation day 21			
	0	**30**	**150**	**750**	**0**	**30**	**150**	**750**
Number of animals/group	11	12	11	11	12	12	11	12
Terminal observations								
Necropsy	No treatment-related effect							
Body weight at necropsy (g)								
	404	413	408	**357****	430	432	432	**393****
Absolute organ weight (mg)								
Liver	14142	14213	14109	13152	11367	12101	11376	**13470****
Ovaries [a]	61.1	61.4	62.9	61.2	60.7	58.5	61.1	60.8
Relative organ weight (% of body weight)								
Liver	3.50	3.44	3.46	3.69	2.64	2.80	2.64	3.43**
Ovaries [a]	0.0151	0.0150	0.0155	**0.0172****	0.0141	0.0136	0.0142	0.0155
Serum steroid hormone concentration								
No. of animals examined	7	8	8	8	8	8	8	8
17ß-estradiol (pg/mL)	22.3	24.1	23.8	16.2	23.9	**16.8***	20.9	**11.4****
SD (±)	*4.8*	*7.0*	*8.0*	*7.2*	*8.1*	*3.2*	*5.4*	*4.2*
Progesterone (pg/mL)	51.3	61.3	61.4	62.9	10.7	10.0	11.6	14.9
SD (±)	*7.8*	*10.6*	*10.1*	*14.9*	*2.4*	*3.4*	*2.5*	*10.1*
E/P ratio	0.441	0.396	0.402	**0.267***	2.352	1.913	1.860	**1.092****
Drug-metabolizing enzyme content								
No. of animals examined	8	8	8	8	8	8	8	8
Microsomal protein (mg/g liver)	45	49	**58****	**60****	31	32	33	33
CYP content (nmol/mg protein)	0.26	0.26	0.33	**0.46****	0.48	0.51	**0.64***	**0.76****
CYP isozymes (pmol/mg protein)								
CYP1A1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CYP2B1	6.2	**8.9****	**9.0****	**11.5****	11.9	15.5	14.4	**25.7****
CYP3A2	3.2	2.6	6.7	**39.4****	8.8	7.1	14.3	**72.7****
CYP4A1	5.2	4.6	**8.1****	**9.1****	10.6	10.8	**15.2****	12.7
Microscopic examination of right ovary								
No. of animals examined					8	0	0	8
No. of corpora lutea observed					7.0	-		5.3
No. of apoptotic bodies per corpus luteum					0.93	-	-	0.87
Microscopic examination of left ovary								
No. of animals examined					8	0	0	8
No. of corpora lutea observed					6.1	-	-	4.4
Ratio of no. of corpora lutea with PCNA-positive lutein cells to total no. of corpora lutea					23/49 (47%)	-	-	**28/35**** (80%)
No. of PCNA-positive lutein cells per corpus luteum					1.43	-	-	3.17

a: Mean weight of both sides
*: p<0.05; **: p<0.01
-: Not applicable

III. CONCLUSIONS

The dosages used in this study were the same as those used in the previously conducted reproductive toxicity study with metconazole cis/trans in rats [see Chapter M-CA 5.6.; summary DocID 2015/1087913]. In that main reproductive toxicity study adverse effects on reproduction such as prolonged estrous cycle length and duration of gestation and maternal deaths during delivery as well as general systemic toxic effects such as decreased body weight gains and food consumption and increased relative liver and ovary weights were noted in P females at 750 ppm. These effects were also observed in F1 females at the top dose level. It was concluded that the dose level of 150 ppm was the no observed adverse effect level in the reproductive toxicity study.

In the current supplemental study, parental toxicity was demonstrated at 750 ppm by decreased mean body weight gain during both, the pre-mating as well as gestation periods. Other apparent treatment-related effect was the inhibition of increases in the E/P rations on gestation days 19 and 21, mainly due to decreased estradiol levels. Progesterone concentrations in this 750 ppm group tended to be slightly but not statistically significantly higher than that of the control group on gestation day 21 with large standard deviations. It was suggested that corpora lutea may not have entered the stage of functional regression yet and may thus, still be active for progesterone production during late pregnancy, which was possibly in line with the finding of higher-than-the-control ratio of corpora lutea containing PCNA-positive lutein cells. However, the large variations of the measured serum progesterone levels make it difficult to draw a final conclusion. The delayed onset of parturition and difficult delivery might be associated with the inhibition of increases in the E/P ratio on gestation days 19 and 21 observed in this study. It was hypothesized by the study authors that the decrease in estradiol levels at the highest dose tested might be due to an excessive acceleration of 17ß-estradiol metabolism due to a remarkable increase in hepatic CYP contents involved in liver estradiol metabolism.

Based on these results, the dose level of 150 ppm is considered to be the NOAEL for female rats which demonstrated no adverse effects for increases in the E/P ratio, although non-adverse changes in increases in hepatic microsomal protein and cytochrome P-450 contents were observed at this dietary concentrations, as compared to the controls.

Immunotoxicity in the rat, oral (diet) following exposure to metconazole cis/trans for 4 weeks [redacted], 2010)

An immunotoxicty study in rats (study author: [redacted] 2010 a; BASF DocID 2010/8000287) with metconazole cis/trans was conducted for non-EU authorities by another metconazole-supporting company. A detailed study summary was made available to BASF (prepared by Buesen, 2014 a; BASF DocID 2015/1087910) and is part of this submission.

Report:	CA 5.8.2/6 [redacted] 2014 a Summary of the Study 42S0609/04122: Metconazole - Immunotoxicity study in male Wistar rats - Administration via the diet for 4 weeks 2015/1087910
Guidelines:	EPA OPPTS 870.7800
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Report:	CA 5.8.2/7 [redacted] et al., 2010 a Metconazole - Immunotoxicity study in male Wistar rats - Administration via the diet for 4 weeks 2010/8000287
Guidelines:	EPA 870.7800
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

The immunotoxic potential of metconazole cis/trans (Batch: 1362353; Purity: 99.7%) in male Wistar rats was analyzed using dietary dose levels of 0, 70, 210 and 630 ppm (corresponding to mean intake levels of 5.4, 17 and 52 mg/kg bw/day, respectively) for 28 days. The parameters used for detection of potential test substance related alterations in the morphology of the immune system included a) the determination of lymphoid organ weights (spleen and thymus) and b) the analysis of the primary humoral (IgM response) immune response to sheep red blood cells (SRBC).

Treatment with metconazole did not result in systemic toxicity in the test groups 70 and 210 ppm. However, in the high dose group (630 ppm) a significantly lower body weight gain (about 15-16% less as compared to the control) after 7 days and 14 days of treatment was observed. None of the immunotoxicologically relevant parameters mentioned above were affected by treatment with metconazole up to the highest dose level tested.

Concurrent treatment with positive control substance, cyclophosphamide monohydrate (CPA, 4.5 mg/kg bw/day) induced clear signs of immunotoxicity, demonstrating the reliability of the test system under the study conditions employed.

Based on the obtained results it can be concluded that metconazole does not bear an immunomodulatory/immunotoxic potential under the conditions of this study. The NOAEL for the immunotoxicologically relevant endpoints was determined to be 630 ppm corresponding to 52 mg/kg bw/day. The NOAEL for systemic toxicity was 210 ppm corresponding to 17 mg/kg bw/day in male Wistar rats.

(BASF DocID 2015/1087910)

I. MATERIAL AND METHODS

1. Test Material:	Metconazole
Description:	solid/ white
Lot/Batch #:	AS 2122a
Purity:	99.7% (84.6% cis; 15.1% trans)
Stability of test compound:	The test substance was stable over the study period (Expiry date June 30, 2010).
2. Vehicle control:	Rodent diet
3. Positive control:	Cyclophosphamide monohydrate (CPA)
Description:	Solid / white
Lot/Batch #:	1362353
Purity:	100% (according to supplier)
Stability of test compound:	According to the supplier the positive control substance was stable over the study period (Expiry date Oct. 2010).
Vehicle for CPA:	Drinking water
4. Test animals:	
Species:	Rat
Strain:	Crl:WI(HAN)
Sex:	Male (more sensitive gender based on previous studies)
Age:	33 ± 1 days at delivery; approx. 42 ± 1 days at start of administration
Reason for the selection:	The rat appears to be more sensitive than the mouse when comparing LOAELs from toxicity studies with metconazole
Weight at dosing:	178.8 ± 0.9 g
Source:	Charles River Laboratories, Research Models and Services GmbH, Sulzfeld, Germany
Acclimation period:	9 days

Diet:	Kliba maintenance diet for mouse/rats "GLP", Provimi Kliba SA, Kaiseraugst, Switzerland, ad libitum
Water:	Tap water in bottles, ad libitum
Housing:	4 animals per cage in H-Temp (PSU, floor area about 2065 cm^2) cages (TECNIPLAST, Hohenpeißenberg, Germany)

Environmental conditions:

Temperature:	20 - 24 °C
Humidity:	30 - 70 %
Air changes:	15/hour
Photo period:	12 h light / 12 h dark (06:00 - 18:00 / 18:00 - 06:00)

B. STUDY DESIGN

1. Dates of experimental work: 10-Nov-2009 - 25-Mar-2010
(In life dates: 19-Nov-2009 (start of administration) to 18-Dec-2009 (necropsy))

2. Animal assignment and treatment:
Metconazole was administered to groups of 8 male rats at dietary concentrations of 0, 70, 210 and 630 ppm for 28 days. The dose levels were selected based on findings of a range-finding study (BASF Project No. 10S0609/04121) in male Wistar rats at dose levels of 0, 200, 400, and 800 ppm for 2 weeks, resulting in approximately 20% lower cumulative mean body weight change (from day 0-14) at 400 ppm and approximately 30% lower cumulative mean body weight change at 800 ppm for male Wistar rats.
Additionally, 8 female mice were treated orally (gavage) with 4.5 mg/kg bw/day Cyclophosphamide monohydrate (CPA; positive control substance). CPA was administered as a solution in drinking water at a volume of 10 mL/kg. The administered volume was determined based on the most recently determined body weights.
The animals were assigned to the treatment groups by means of computer generated randomization list based on body weights.
On day 23 of the study all animals received a single intraperitoneal injection (0.5 mL) of a sheep red blood cell (SRBC)-suspension containing 4 x 10^8 cells/mL for immunization.

3. Test substance preparation and analysis:
The diets were prepared by mixing weighed amounts of test substance with a small amount of food. Subsequently, appropriate amounts of food were added to obtain the intended dietary concentrations and mixed for approx. 10 minutes in a laboratory mixer. Test substance preparations were mixed once before the start of administration.

The stability of the test substance metconazole in the diet over a period of up to 32 days was proven before the start of the study. Homogeneity and concentration analyses of the diet preparations were performed at the beginning of the administration period for all concentrations.

Table 5.8.2-21: Results of homogeneity and concentration control analysis of metconazole in rodent diet

Nominal Dose level [ppm]	Sampling	Concentration Mean ± SD# [ppm]	Mean of nominal concentration [%]	Relative standard deviation [%]
70	Nov. 18, 2009	70.9 ± 1.5	101.3	2.1
210	"	210.9 ± 3.8	100.4	1.8
630	"	611.8 ± 16.4	97.1	2.7

Relative standard deviations of the homogeneity of the metconazole samples were quite low, which indicates the homogenous distribution of metconazole in the diet preparations. The actual (mean) average test-substance concentrations were in the range of 97.1 to 101.3% of the nominal concentrations confirming the correctness of the concentrations.

The positive control substance preparation (CPA in drinking water) was prepared once at the beginning of the study, split in daily aliquots and deep frozen at -18°C. The mixtures were applied when reaching room temperature. The concentration control of the CPA solution was performed at the beginning of the study. Since the CPA formulation in drinking water was a solution a homogeneity analysis was redundant. The stability analysis conducted revealed the stability of the CPA solution for 31 days when stored frozen.
The actual CPA concentrations were 108.2 and 108.7% of the nominal concentration confirming the correctness of the concentration.

Table 5.8.2-22: Results of concentration control analysis of CPA in drinking water

Nominal Concentration [g/100 mL]	Sampling / Analysis	Analytical concentration [g/100 mL]	Mean of nominal concentration [%]
0.045	Nov. 17, 2009 / Jan. 29, 2010	0.0489	108.7
0.045	Dec. 18, 2009 / Jan. 29, 2010	0.0487	108.2

4. Statistics:

Means and standard deviations of each test group were calculated for several parameters. Further statistical analyses were performed according to following tables:

Table 5.8.2-23: Statistics of clinical examinations

Parameter	Statistical test
body weight and body weight change	For the test substance and the vehicle control groups: A comparison of each group with the control group was performed using DUNNETT's test (two-sided) for the hypothesis of equal means For the vehicle and positive control groups: A comparison of the dose group with the control group was performed using the t-test (two-sided) for the hypothesis of equal means

Table 5.8.2-24: Statistics of clinical pathology

Parameter	Statistical test
Clinical pathology parameters	Non-parametric one-way analysis using KRUSKAL-WALLIS test (two-sided). If the resulting p-value was equal or less than 0.05, a pair wise comparison of each dose group with the control group was performed using Wilcoxon-test (two-sided) for the equal medians

Table 5.8.2-25: Statistics of pathology

Parameter	Statistical test
Weight parameters	Non-parametric one-way analysis using KRUSKAL-WALLIS test (two-sided). If the resulting p-value was equal or less than 0.05, a pair wise comparison of each dose group with the control group was performed using the WILCOXON test for the hypothesis of equal medians

C. METHODS

1. Observations:

The animals were examined for morbidity or mortality twice daily on working days and once daily on weekends and public holidays. Observation for overt clinical signs of toxicity was performed at least once daily. Detailed clinical observations were performed in all animals prior to the start of the administration period and weekly thereafter. The animals were transferred to a standard arena (50 x 37.5 cm with sides of 25 cm high). The findings were ranked according to the degree of severity, if applicable.

1. abnormal behavior during handling	10. abnormal movements
2. fur	11. impairment of gait
3. skin	12. lacrimation
4. body posture	13. palpebral closure
5. salivation	14. exophthalmus
6. respiration	15. feces (appearance/consistency)
7. activity/arousal level	16. urine
8. tremors	17. pupil size
9. convulsions	

2. Food consumption and compound intake:

Food consumption was determined weekly for each cage. The average food consumption per cage was used to estimate the mean food consumption in grams per animal per day.

The mean daily intake of test substance (group means) was calculated based upon individual values for body weight and food consumption.

$$\text{Substance intake for day x} = \frac{FC_x \times C}{BW_x}$$

with FC_x as the mean daily food consumption (in g) on day x, C as the concentration in the food on day x (in mg/kg) and BW_x as body weight on day x of the study (in g).

3. Water consumption:

Drinking water consumption was determined weekly (as representative value over 3 days) and was calculated as mean water consumption in grams per animal and day.

4. Body weight:

The body weight of the animals was determined before the start of the administration period (in order to randomize the animals), at the start of the treatment (day 0), and once weekly thereafter.

5. Analysis of the primary immune response:

Immunization:
- Sterile, heparinized sheep blood was washed with sterile 0.9% NaCl solution and adjusted to $4x10^8$ RBC/mL.
- On study day 23, each rat was immunized with 0.5 mL of the SRBC solution injected intraperitoneally.
- On study day 29 blood was taken in the morning from non-fasted, isoflurane anesthetized animals from the retro-orbital venous plexus. The blood sampling procedure and the subsequent analysis of the serum samples were carried out in a randomized sequence.

SRBC ELISA:
- The ELISA was performed according to Temple et al. (1995).
- In deviation to the mentioned reference, a standard curve (8 standards in a two-fold dilution) was established using anti-SRBC IgM positive serum pool, for comparison to subsequent test runs (arbitrary lab units/mL; stock standard aliquots stored at -80°C.
- Each serum sample was applied to the ELISA in two dilutions, i.e. 1:128 and 1:256. Generally, the 1:128 dilution was reported.
- Result deviations (lab units/mL) in both dilutions of more than 25% were confirmed.
- OD values of the sample dilutions outside of the linear range of the standard curve were repeated with a couple of two-fold lower or higher dilutions.
- Generally, two in-house controls were measured with each test run.
- The ELISA was measured with a Sunrise MTP-reader, Tecan AG, Maennedorf, Switzerland, and evaluated with the Magellan-Software of the instrument producer.

6. Necropsy and pathology:

The animals were sacrificed by decapitation under isoflurane anesthesia. The exsanguinated animals were necropsied and assessed by gross pathology. The following weights were determined for all animals sacrificed at scheduled dates:

1. Anesthetized animals
2. Spleen
3. Thymus

No further histopathological examinations were performed.

II. RESULTS AND DISCUSSION

A. OBSERVATIONS

1. Clinical signs of toxicity

No clinical signs were observed throughout the study.

2. Mortality

No mortality was observed in this study.

B. FOOD CONSUMPTION, DRINKING WATER CONSUMPTION, AND COMPOUND INTAKE

No test substance-related findings were observed (see Table 5.8.2-26). No treatment-related effects on water consumption were noted.

Table 5.8.2-26: Mean food consumption per cage of rats administered metconazole or CPA for 28 days

Treatment	**Metconazole [ppm]**				**CPA**
Dose level	**0**	**70**	**210**	**630**	**4.5 mg/kg**
Food consumption per cage [g]					
- Day 6 to 7	19.69	19.55	19.98	21.41	21.40
- Day 13 to 14	19.77	20.09	20.95	19.79	21.11
- Day 20 to 21	20.01	20.68	21.20	20.92	20.96
- Day 27 to 28	20.94	21.30	23.29	22.62	19.74

The mean daily test substance intake in mg/kg body weight/day over the entire study period was calculated and is shown in the following table:

Table 5.8.2-27: Calculated intake of metconazole

Test group	**Concentration in the diet (ppm)**	**Mean daily test-substance intake (mg/kg bw/d)**
		Males
1	70	5.4
2	210	17
3	630	52

C. BODY WEIGHT AND BODY WEIGHT GAIN

Body weight in animals of test group 3 (630 ppm) was slightly, but not significantly lower from day 7 until day 28, with a maximum of -3.7% compared to control animals on day 14.
Body weights of animals treated with CPA was slightly, but also not significantly lower from day 7 until day 28, with a maximum of -5.5% on day 28.
Body weight change in animals of test group 3 (630 ppm) was lower from day 7 until day 28 (significantly lower on day 7 (by 15.9%, as compared to controls) and on day 14 (by 14.7%, as compared to controls)).
Body weight change of animals treated with CPA was significantly decreased from day 7 until day 28, with a maximum of -13.8% on day 21.

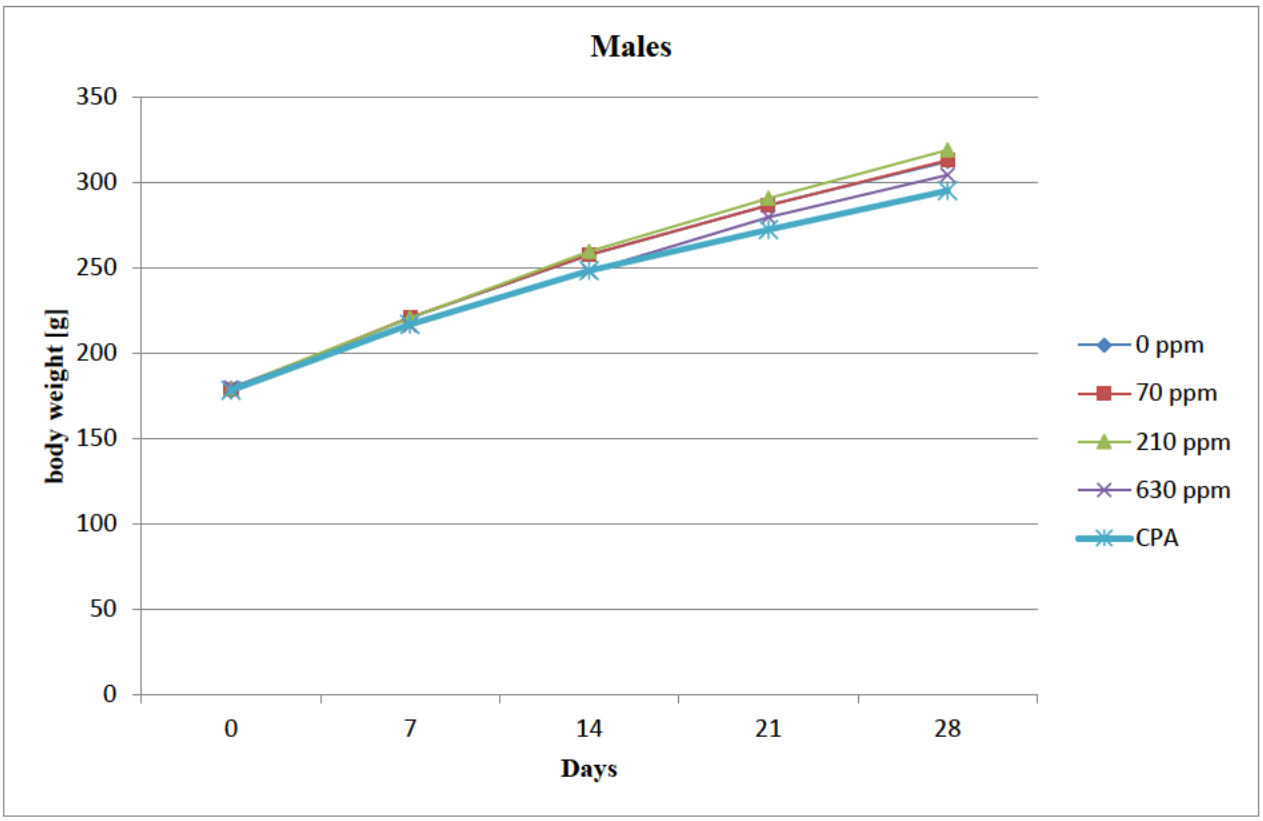


Figure 5.8.2-1: Body weight development of rats administered metconazole for 28 days

Table 5.8.2-28: Mean body weight of rats administered metconazole or CPA for 28 days

Treatment	**Metconazole [ppm]**				**CPA**
Dose level	**0**	**70**	**210**	**630**	**4.5 mg/kg**
Body weight [g]					
- Day 0	177.68	178.82	179.46	179.93	178.19
- Day 7	220.72	220.90	220.61	216.15	216.86
- Day 14	257.51	257.76	259.55	248.05	248.31
- Day 21	286.81	286.59	290.69	279.68	272.31
- Day 28	312.32	312.98	319.01	304.43	295.21
Day 28 Δ% (compared to control) #	-	0.21	2.14	-2.53	-5.48

Table 5.8.2-29: Mean body weight change of rats administered metconazole or CPA for 28 days

Treatment	**Metconazole [ppm]**				**CPA**
Dose level	**0**	**70**	**210**	**630**	**4.5 mg/kg**
Body weight [g]					
- Day 0-7	43.05	42.07	41.15	36.23**	38.68*
- Day 0-14	79.84	78.94	80.09	68.12*	70.12*
- Day 0-21	109.14	107.76	111.32	99.75	94.12*
- Day 0-28	134.65	134.15	139.55	124.50	117.02*

*: $p \leq 0.05$, **: $p \leq 0.01$

D. IMMUNOLOGICAL ANALYSES

1. Analysis of the primary T-cell dependent immune response

Six days after immunization, no changes in the SRBC IgM titres were found in male rats dosed with the test substance, whereas the SRBC titres were significantly lower in rats of test group 4 (CPA, positive control group) (see Table 5.8.2-30).

Table 5.8.2-30: Analysis of the specific primary (IgM) immune response to SRBC in rats treated with Metconazole or CPA for 28 days

	Metconazole				**CPA**
Dose [ppm]	**0**	**70**	**210**	**630**	
[mg/kg bw/day]		**5.4**	**17**	**52**	**4.5**
Specific IgM Titer (U/mL)					
- Mean ± SD	2102 ± 1722	2124 ± 1248	1581 ± 707	1917 ± 867	**584** ± 230
- Median	1206	1974	1387	1952	526

* $p \leq 0.05$; ** $p \leq 0.01$ (Kruskal-Wallis and Wilcoxon-test. two sided)

G. NECROPSY

1. Terminal body weight and organ weights

The absolute mean weights of spleen and thymus of animals in test groups 70, 210, and 630 ppm did not show relevant differences compared to the control group.
The positive control group (CPA) revealed significant decreases of spleen and thymus weights, which corresponded to the expected result.

The relative mean weights of spleen and thymus of animals in test groups 70, 210 and 630 ppm did not show relevant differences compared to the control group.
The positive control group (CPA) revealed significant decreases of spleen and thymus weights, which corresponded to the expected result.

Table 5.8.2-31: Mean absolute and relative organ weights of male rats treated with metconazole or CPA for at 28 days

	Metconazole				**CPA**
Dose [ppm]	**0**	**70**	**210**	**630**	
[mg/kg bw/day]		**5.4**	**17**	**52**	**4.5**
Terminal bodyweight [g]	291	292	296	283	273
[% of control]	100	100	102	97	94
Spleen, absolute [g]	0.551	0.579	0.561	0.586	0.393**
[% of control]	100	105	102	106	71
Spleen, relative [%]	0.19	0.198	0.189	0.207	0.144**
[% of control	100	104	99	109	76
Thymus, absolute [mg]	489	440	509	459	241**
[% of control]	100	90	104	94	49
Thymus, relative [%]	0.168	0.15	0.171	0.162	0.088**
[% of control]	100	90	102	97	52

* $p \leq 0.05$; ** $p \leq 0.01$ (Kruskal-Wallis and Wilcoxon-test, two sided)

2. Gross pathology
One animal of the positive control group (CPA) revealed a reduced organ size of thymus. No gross lesions were observed in all other test animals.

III. CONCLUSIONS

Under the conditions of the study metconazole did not reveal any signs of immunotoxicity when administered via the diet over a period of 4 weeks to male Wistar rats. The NOAEL for the immunotoxicologically relevant endpoints was determined to be 630 ppm (52 mg/kg bw/d; highest dose tested). The NOAEL for systemic toxicity was set to 210 ppm (17 mg/kg bw/d). The oral administration of the positive control substance cyclophosphamide (4.5 mg/kg bw/d) led to severe findings indicative of immunotoxicity. This was represented by significantly lower SRBC IgM antibody titres as well as reduced spleen and thymus weights. Thus, assay sensitivity was verified in the present immunotoxicity study performed in male Wistar rats.

Report: CA 5.8.2/8
Mentzel T., 2015a
Metconazole (BAS 555 F) (cis/trans isomer) - Metconazole (cis isomer) - Metconazole (trans isomer) - Human and rat recombinant Aromatase assay
2015/1205961
Guidelines: EPA 890.1200
GLP: no

Executive Summary

Metconazole cis/trans (98% pure, cis/trans ratio: 82.7/17.3, batch: 14955000), metconazole cis (100% pure, cis/trans ratio: 97.2/2.8, batch: 3) and metconazole trans (100% pure, cis/trans ratio: 0.8/99.2, batch: 1) were tested in vitro for their effect on human and rat aromatase activities (CYP 19). Human/rat CYP19 supersomes (aromatase + reductase) were exposed to the test, positive and negative control substances in concentrations ranging from 10^{-4} to 10^{-13} M as well as to the solvent DMSO. Enzyme activity was determined fluorometrically using dibenzylfluorescein as a model substrate. Resulting activity values have been fitted using the 4-parameter regression model to yield a sigmoidal inhibition curve and IC_{50} values were calculated.

For all positive controls as well as for the test substances inhibition of rat and human aromatase activities was achieved. The resulting human aromatase IC_{50} values were 0.721 µM, 0.569 µM and 2.47 µM for metconazole cis/trans, metconazole cis and metconazole trans, respectively. The resulting rat aromatase IC_{50} values were 0.157 µM, 0.223 µM and 0.579 µM for metconazole cis/trans, metconazole cis and metconazole trans, respectively. Thus, the test substance-induced aromatase inhibition was more pronounced for the rat enzyme and was 4.6-, 4.3- and 2.6-fold lower for the human enzyme by metconazole cis/trans, metconazole trans and metconazole cis, respectively.

(BASF DocID 2015/1205961)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

Test item 1	Metconazole (technical, cis/trans isomer) (Reg.No. 4056343)
Description:	Solid, powder
Lot/Batch #:	14955000
Purity:	98% (cis/trans ratio: 82.7/17.3)
Test item 2	Metconazole (cis isomer) (Reg.No. 4079468)
Description:	Solid, powder
Lot/Batch #:	3
Purity:	100% (cis/trans ratio: 97.2/2.8)
Test item 3	Metconazole (trans isomer) (Reg.No. 4079654)
Description:	Solid, powder
Lot/Batch #:	1
Purity:	100% (cis/trans ratio: 0.8/99.2)

2. Vehicle control DMSO (final concentration 1%)

3. Negative control

Test substance 1	Atrazine
CAS No.:	1912-24-9
Description:	Solid, powder
Lot/Batch #:	SZBD158XV
Purity:	99.1%
Supplier:	Sigma-Aldrich #45330
Test substance 2	Bis(2-ehtylhexyl)phthalate
CAS No.:	117-81-7
Description:	Liquid
Lot/Batch #:	SZBB167XV
Purity:	99.7%
Supplier:	Fluka #36735

4. Positive control

Test substance 1	4-OH ASDN
CAS No.:	566-48-3
Description:	Solid, powder
Lot/Batch #:	081k2133V
Purity:	99.6%
Supplier:	Sigma-Aldrich F25525

Test substance 2	Fenarimol
CAS No.:	60168-88-9
Description:	Solid, powder
Lot/Batch #:	SZBD071XV
Purity:	99.9%
Supplier:	Fluka #45484
Test substance 3	Econazol nitrate
CAS No.:	24169-02-6
Description:	Solid, powder
Lot/Batch #:	BCBL5063V
Purity:	98%
Supplier:	Sigma Aldrich #E4632
Test substance 3	Letrozole
CAS No.:	112809-51-5
Description:	Solid, powder
Lot/Batch #:	104M4759V
Purity:	98.0%
Supplier:	Sigma-Aldrich #L6545
5. Test system	Recombinant Aromatase
Species	**Human:** Corning Supersomes Human CYP19 (Aromatase) + Reductase (#456260) expressed in baculovirus/insect cells **Rat:** Corning Supersomes Rat CYP19 (Aromatase) + Reductase (#457254)

B. STUDY DESIGN AND METHODS

1. Dates of experimental work: 25-May-2015 to 20-Aug-2015

2. Test substance preparation:
The test items, negative and positive substances were weighed and topped up with the chosen vehicle (DMSO) to achieve the required concentration of the stock solution (100 mM). In order to obtain a clear solution of the test substance in the vehicle, the test substance preparation was shaken thoroughly. The further concentrations were diluted according to the planned concentrations. All test substance formulations were prepared immediately before administration. The stability of the test substance in the vehicle DMSO and in water was not determined analytically.
Final substance concentrations (except econazole nitrate and letrozole) tested were: 10^{-11}, 10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} and 10^{-4} mol/L, based on solubility limit in assay buffer of 100 µM. Econazole nitrate and letrozole were tested in concentrations ranging from 10^{-13} to 10^{-6} and 10^{-12} to 10^{-5}, respectively, their solubility limits in assay buffer were 30 µM and 1 mM, respectively.

3. Test method:
The assay was conducted in a 96-well microplate utilizing the recombinant human or rat aromatase and the fluorometric artificial substrate dibenzylfluorescein (DBF). After addition of the test compounds including dilutions and all cofactors (1.3 mM $NADP^+$, 0.4 mU Glucose-6-phosphat-dehydrogenase, 3.3 mM Glucose-6-phosphate and 3.3 mM $MgCl_2$) to each well, reaction was started with 100 µL of enzyme/substrate mix (4 pmol/mL enzyme, 0.4 µM DBF). Plates were incubated for 30 minutes at 37°C. Reaction was determined by addition of 75 µL 2 M NaOH, which results in a cleavage of the oxidized DBF to the fluorescent product fluorescein. To develop adequate signal to noise ratio, plates were incubated for another 2 hours at 37°C. Measurement was conducted at 490 nm excitation wavelength, 530 nm emission wavelength and 515 nm cut-off.
Experimental design have been adapted according to OCSPP Guideline 890.1200. The analysis described in OCSPP Guideline 890.1200 is using a radioactive substrate, while the procedure used for this study employs a non-radioactive artificial substrate.
In addition to the control substances recommended in the guideline, further additional positive controls (4-OH ASDN, fenarimol, econazol nitrate, letrozole) as well as negative controls (atrazine and bis(2-ehtylhexyl)phthalate) have been carried out not in one, but in every test run in parallel to the test compounds using a plate set up.

A serial dilution as triplicates was performed for each compound with a 50 mM potassium phosphate buffer pH 7.4. Four wells per test plate without enzyme were used as control to determine background fluorescence. Additional full enzyme activity were analysed done using DMSO only in 4 wells per plate. This analysis has been repeated in seven independent experiments.

4. Data interpretation:
The method used in this study is measuring the generation of a florescent product for the analysis of human or rat aromatase activity.
Values of background and full activity controls were determined, and ratios of full activity / background activity range were calculated. Acceptable average ratio should always be below 15% of mean full activity representing an ideal activity range for this activity measurement.

Absolute fluorescence was corrected by subtraction of mean background control of each individual plate and normalized to the full activity control to achieve % activity values.

Resulting activity values have been fitted using the 4-parameter regression model to yield a sigmoidal inhibition curve. The resulting IC_{50} values, as well as slope of the calculated curve, were used for a statistical analysis to ensure data consistency. All runs which significantly diverge from the overall analysis have been excluded from the final calculation of an average IC_{50} value.

5. Statistical analysis

Dose-response analyses were made using the log-logistic 4-parameter model. Assumptions were checked for each model calculated and a Box-Cox algorithm was used to determine the optimal lambda value using a profile likelihood approach: For each lambda value the non-linear regression model is fitted and the lambda value resulting in the largest value of the log likelihood function is picked. After transformation all results were back transformed to the original scale.
Also parameter estimates for all models were compared for each day and plate. Comparisons were made calculating the ratio and its standard error between days or plates. Significant deviations from 1 (parameters between days or plates are identical) were detected using a t-test. Significance levels were adjusted using a Bonferroni correction. In cases when parameter estimates for the ED_{50} or slope did differ significantly between days outlier days were removed. This was well possible, as in total seven independent runs have been conducted, which exceeds the minimum number of three runs by far recommended by guideline OCSPP Guideline 890.1200. This comparison over runs has been included in appendix 3: statistical analysis.

All calculations were made using R 3.2.1 (R Core Team 2015). Dose-response models including Box-Cox transformations were calculated using the "drc" package version 2.5-12.

II. RESULTS AND DISCUSSION

A. Human Aromatase Inhibition

Metconazole technical (cis/trans mixture) shows aromatase inhibition on the human enzyme with an IC_{50} of 0.721 µM and does not differ significantly from inhibition response of the pure cis isomer (IC_{50} = 0.569 µM). The trans isomer shows about 3.4 to 4.3–times less human aromatase inhibition with an IC_{50} of 2.47 µM as compared to the cis/trans mixture or the cis isomer.

Table 5.8.2-32: Mean human aromatase IC_{50} values

Test item	human aromatase IC50 [M]	
	mean	SE
Test substance		
Metconazole cis	5.69×10^{-7}	6.64×10^{-8}
Metconazole cis/trans	7.21×10^{-7}	1.18×10^{-7}
Metconazole trans	2.47×10^{-6}	3.07×10^{-7}
Positive control		
Letrozole	9.02×10^{-10}	8.03×10^{-11}
Econazole	2.30×10^{-9}	2.39×10^{-10}
4-OH ASDN	1.38×10^{-8}	2.22×10^{-8}
Fenarimol	1.26×10^{-6}	1.74×10^{-7}

In relation to the positive substances analysed within this study, metconazole cis/trans has a comparable effect on human aromatase activity as the least strong positive control fenarimol. Compared to other azole substances tested within this study the three test substances are 631 - 2738-fold less potent than letrozole, and 247 - 1074-fold less potent than econazole. Furthermore, the test items are 41 - 179-fold less potent than the positive control 4-OH ASDN for their inhibition of human CYP19 enzyme activity.

B. Rat Aromatase Inhibition

Metconazole technical (cis/trans mixture) shows aromatase inhibition on the rat enzyme with an IC_{50} of 0.157 µM and does not differ significantly from inhibition response of the pure cis isomer (IC_{50} = 0.223 µM). The trans isomer shows about 2.6 to 3.7–times less human aromatase inhibition with an IC_{50} of 0.579 µM as compared to the cis/trans mixture or the cis isomer.
In relation to the positive substances analysed within this study, metconazole cis/trans has a comparable effect on rat aromatase activity as the least strong positive control fenarimol. Compared to other azole substances tested within this study the three test substances are 103 - 378-fold less potent than letrozole, and 98 - 362-fold less potent than econazole. Furthermore, the test items are 4 - 16-fold less potent than the positive control 4-OH ASDN for their inhibition of human CYP19 enzyme activity.

Table 5.8.2-33: Mean rat aromatase IC_{50} values

Test item	rat aromatase IC50 [M]	
	mean	SE
Test substance		
Metconazole cis/trans	1.57×10^{-7}	2.28×10^{-8}
Metconazole cis	2.23×10^{-7}	2.54×10^{-8}
Metconazole trans	5.79×10^{-7}	1.21×10^{-7}
Positive control		
Letrozole	1.53×10^{-9}	1.03×10^{-10}
Econazole	1.60×10^{-9}	1.52×10^{-10}
4-OH ASDN	3.57×10^{-8}	3.48×10^{-9}
Fenarimol	1.79×10^{-7}	2.15×10^{-8}

C. Human vs. Rat Aromatase Inhibition

All three test compounds analysed within this study revealed higher inhibition of rat aromatase than of human enzyme [see Figure 5.8.2-2].
Metconazole cis/trans and metconazole trans inhibition has a 4.6- and 4.3-fold stronger response on rat than on human aromatase, respectively. Likewise metconazole cis shows a 2.6-times higher IC_{50} value on human than on rat enzyme. These differences were further analyzed using a t-test, displaying a highly significant difference between enzyme inhibition of rat and human aromatase by all compounds. Thus, all compounds are more potent towards rat aromatase than towards human aromatase.

Table 5.8.2-34: Pairwise comparison of IC_{50} values of metconazole cis/trans metconazole cis and metconazole trans for human and rat aromatase inhibition

Test substance	fold difference (human/rat)	Standard error	t-value	p-value
Metconazole cis/trans	4.58	1.00	3.57	0.0004
Metconazole trans	4.27	1.15	2.85	0.0048
Metconazole cis	2.55	0.42	3.73	0.0002

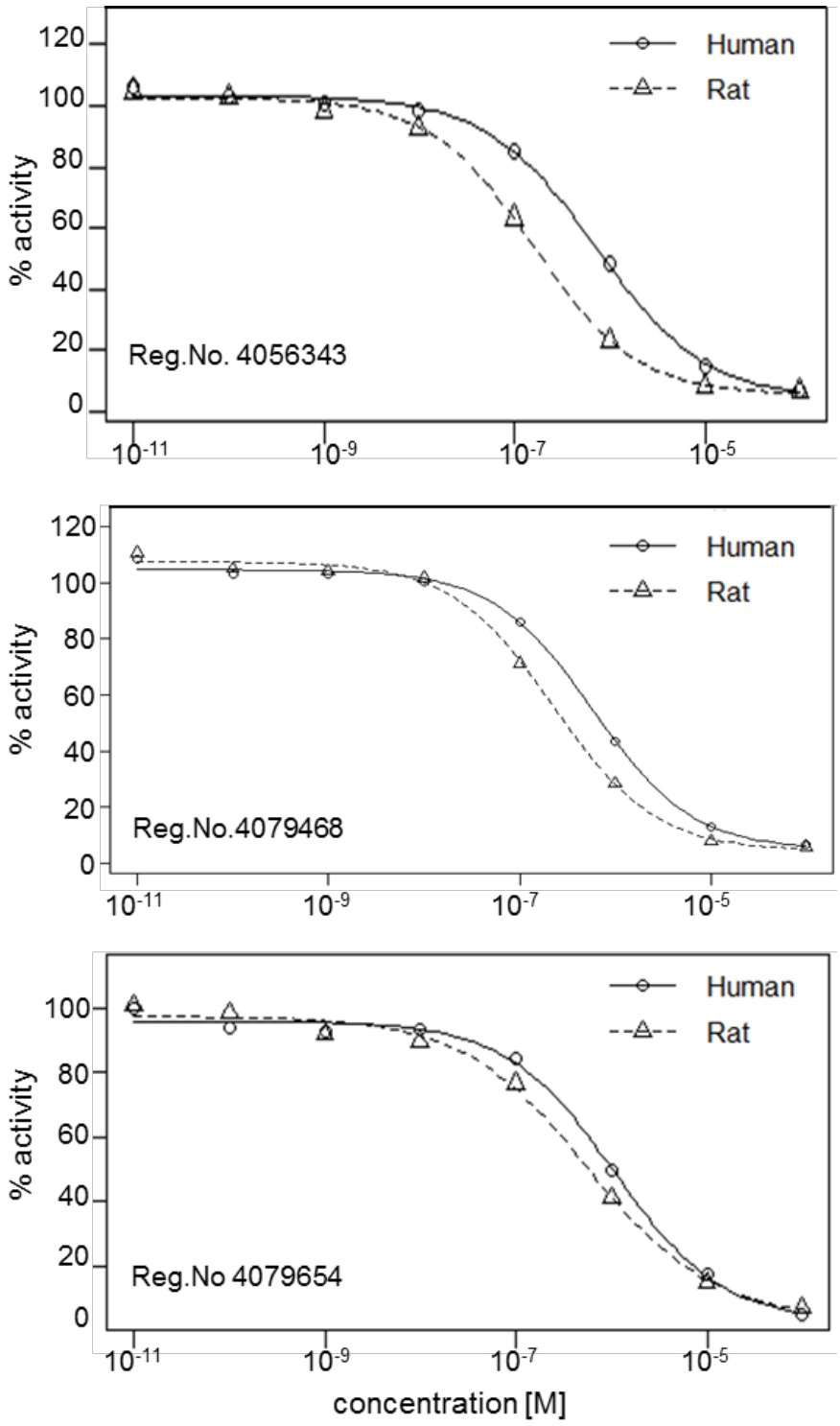


Figure 5.8.2-2: Comparison of metconazole technical (Reg.No. 4056343), metconazole cis (Reg.No. 4079468), and metconazole trans (Reg.No. 4079654) induced inhibition of rat and human aromatases

III. CONCLUSION

Under experimental conditions chosen, metconazole cis/trans, metconazole cis and metconazole trans inhibited rat and human aromatase activities. The resulting human aromatase IC_{50} values were 0.721 µM, 0.569 µM and 2.47 µM for metconazole cis/trans, metconazole cis and metconazole trans, respectively. The resulting rat aromatase IC_{50} values were 0.157 µM, 0.223 µM and 0.579 µM for metconazole cis/trans, metconazole cis and metconazole trans, respectively. The aromatase inhibition was more pronounced for the rat enzyme and is 4.6-, 4.3- and 2.6-fold lower for the human enzyme by metconazole cis/trans, metconazole trans and metconazole cis, respectively.

Literature data

As many other pesticides and chemicals, metconazole is part of the US ToxCast program. Several assays react to varying doses of metconazole, however, no conclusive picture has emerged. The following publications related to CYP-binding affinity and estrogen receptor signalling response are discussed.

Report: CA 5.8.2/9
Itokawa D.N., 2006a
Quantitative structure - Activity relationship study of binding affinity of Azole compounds with CYP2B and CYP3A
2007/1071105
Guidelines: none
GLP: no

Executive Summary: Azole compounds, such as Metconazole, are known to be metabolised by CYP isozymes, including CYP3A4 and CYP2B6. Binding affinity of azoles can be measured by UV spectral analysis, as the nitrogen atom in the azole ring induces a typical UV spectral change with a characteristic absorption at 430 (maximum) and 400 nm (minimum). In this study the binding affinities of metconazole and further azoles were determined in rat liver CYP2B and CYP3A4 using spectral analysis followed by further QSAR analysis using hydrophobicity and binding affinity as parameters.
Binding affinities observed with CYP2B and CYP3A using metconazole did not differ significantly. The pIC_{50}s and pK_D were 6.13 and 5.98 for CYP2B and 6.02 and 6.00 for CYP3A4, respectively. Good correlation with the bilinear model was observed between the binding affinities and the partition coefficient (log P). The model suggested that the optimum log P values of the azole compounds were nearly the same for these two CYPs. The sequence homology of amino acid residues around the substrate recognition site is significantly high between CYP2B and CYP3A. It was reported that sizes of the binding pocket in CYP2B and CYP3A are not much different. These explain why the optimum log P values in the correlation equations for CYP2B and CYP3A was nearly the same.

Conclusion: Supplementary information

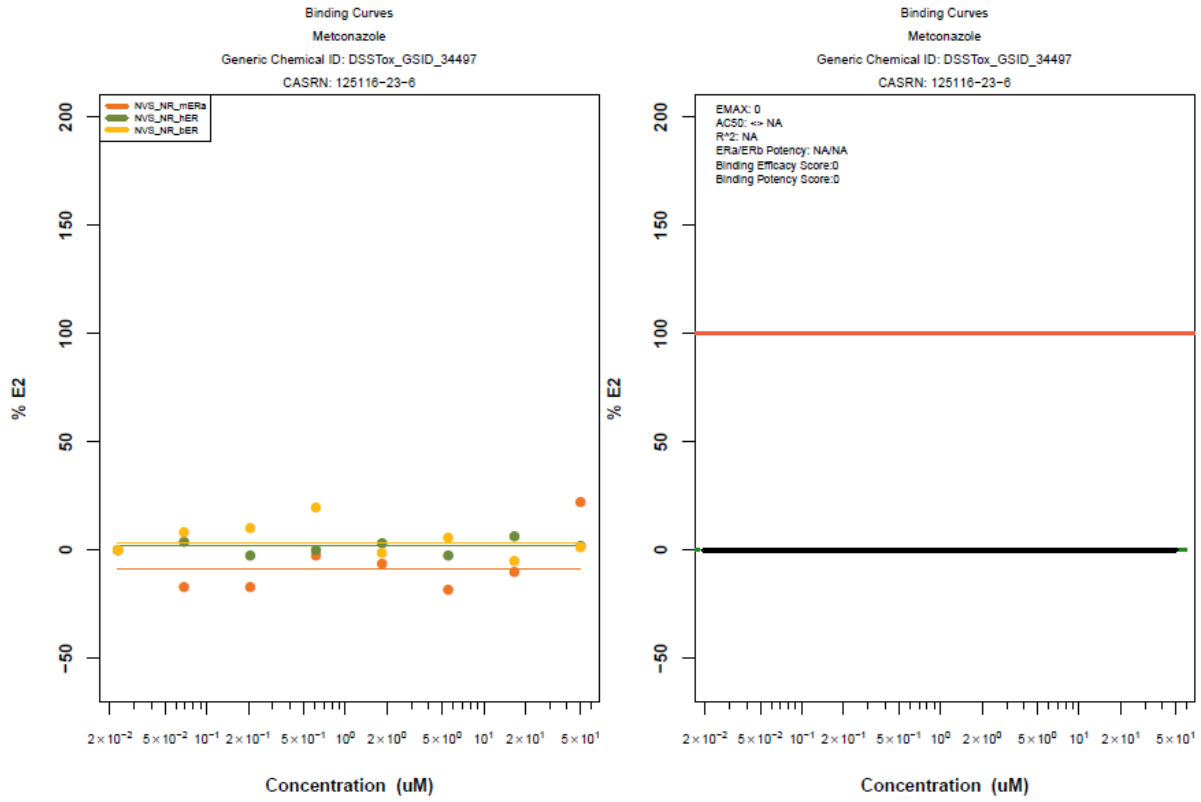
Report: CA 5.8.2/10
Itokawa D., 2007a
Quantitative structure - Activity relationship for inhibition of CYP2B6 and CYP3A4 by Azole compounds - Comparison with their binding affinity
2009/1132242
Guidelines: none
GLP: no

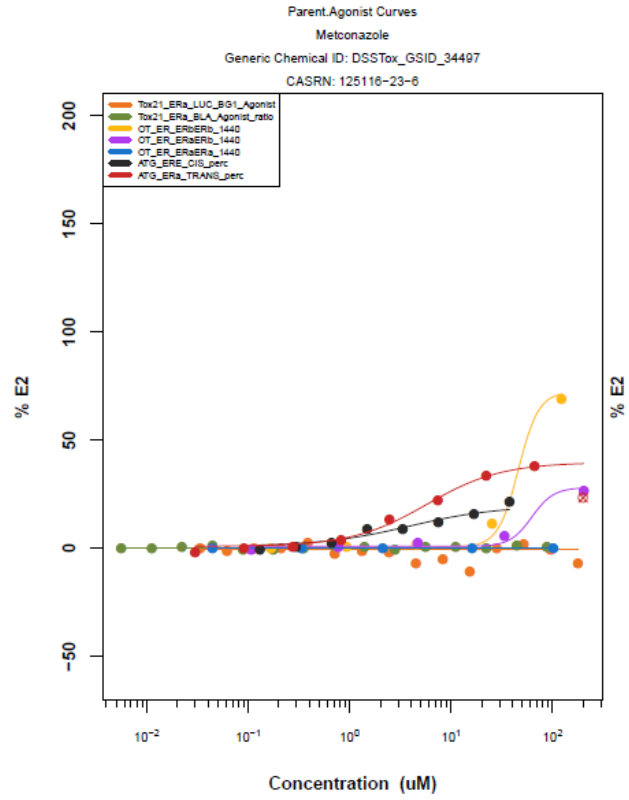
Executive Summary: Azole compounds, such as Metconazole, are known to be metabolised by CYP isozymes, including CYP3A4 and CYP2B6. In the previous study from Itokawa (DocID 2007/1071105), it was shown that the binding affinity of 18 azole compounds for rat CYP2B and CYP3A was nicely expressed by the bilinear model of log P. In this study, the same azole compounds were examined as to their inhibitory effect on the substrates for human CYP2B6 and CYP3A4. Enzyme inhibition assays using 7-Ethoxy-4-trifluoromethylcoumarin (EFC) and 7-benzyloxy-4-trifluoromethylcoumarin (BFC) as substrates for CYP2B6 and CYP3A4, respectively, were performed using high-throughput kits. The inhibitory concentration pIC_{50} was 4.87 for CYP2B and 5.8 for CYP3A4, respectively.The inhibitory activity determined was analyzed as to the molecular properties of the azole compounds. A nice correlation was found with the bilinear model of log P. These results suggested that the molecular hydrophobicity of the azole compounds plays a major role in the inhibition as well as in the binding. For the binding, HOMO was significant as an additional descriptor in the correlation equations, whereas the existence of a hydroxyl group was significant for the inhibition.

Conclusion: Supplementary information

Report: CA 5.8.2/11
Rotroff D.M. et al., 2014a
Predictive endocrine testing in the 21st century using in vitro assays of estrogen receptor signaling responses
2014/1323273
Guidelines: none
GLP: no

Executive Summary: Thousands of environmental chemicals are subject to regulatory review for their potential to be endocrine disruptors (ED). In vitro high-throughput screening (HTS) assays have emerged as a potential tool for prioritizing chemicals for ED-related whole-animal tests. In this study, 1814 chemicals including pesticide active and inert ingredients, industrial chemicals, food additives, and pharmaceuticals were evaluated in a panel of 13 in vitro HTS assays. The panel of in vitro assays interrogated multiple end points related to estrogen receptor (ER) signalling, namely binding, agonist, antagonist, and cell growth responses. The results from the in vitro assays were used to create an ER Interaction Score. For the ~1,800 chemicals evaluated in this study, 82% did not display indications of interacting with the ER signalling pathway and would be low priorities for additional ER testing. If maximum sensitivity is desired, the model can be run with narrower confidence intervals around the composite curves. This would result in an increased false positive rate and a decreased false negative rate.
For this dossier the data for metconazole are relevant and thus the results are described in the following figures.




Parent.Agonist Curves
Metconazole
Generic Chemical ID: DSSTox_GSID_34497
CASRN: 125116-23-6
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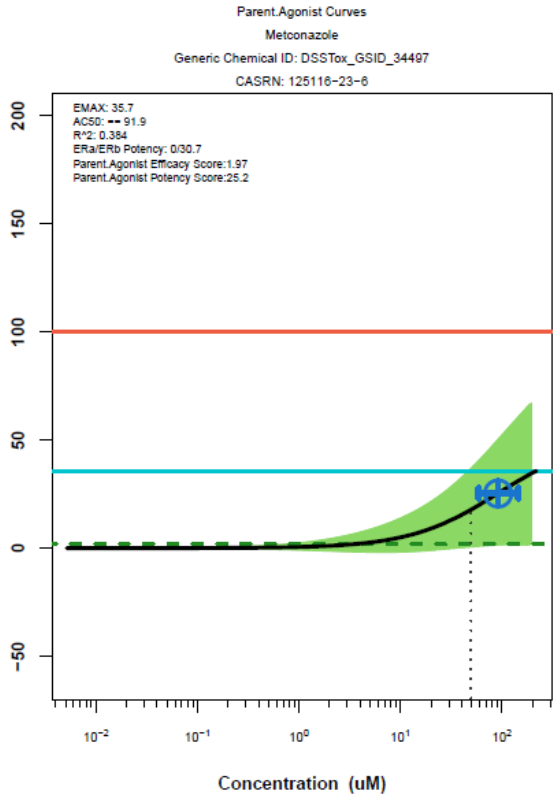
Tox21_ERa_BLA_Agonist_ratio
OT_ER_ERbERb_1440
OT_ER_ERaERb_1440
OT_ER_ERaERa_1440
ATG_ERE_CIS_perc
ATG_ERa_TRANS_perc
% E2
Concentration (uM)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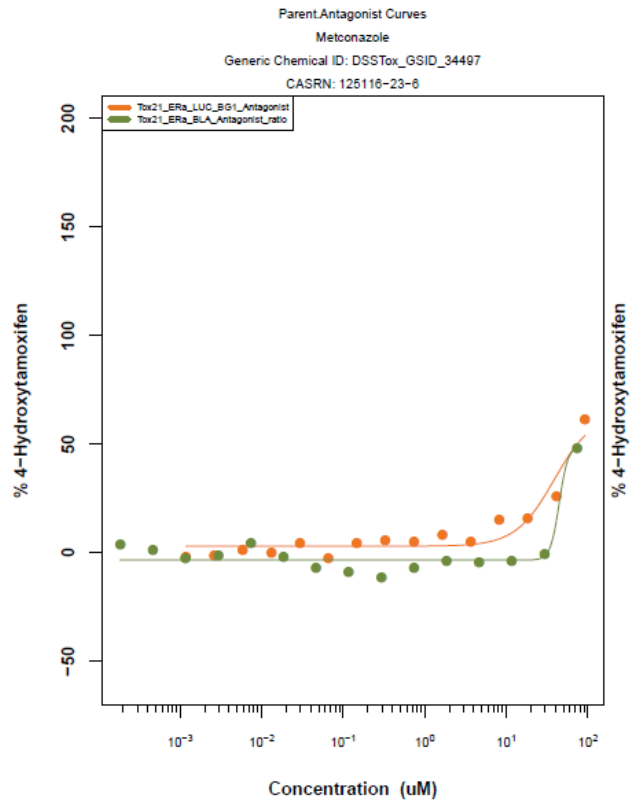
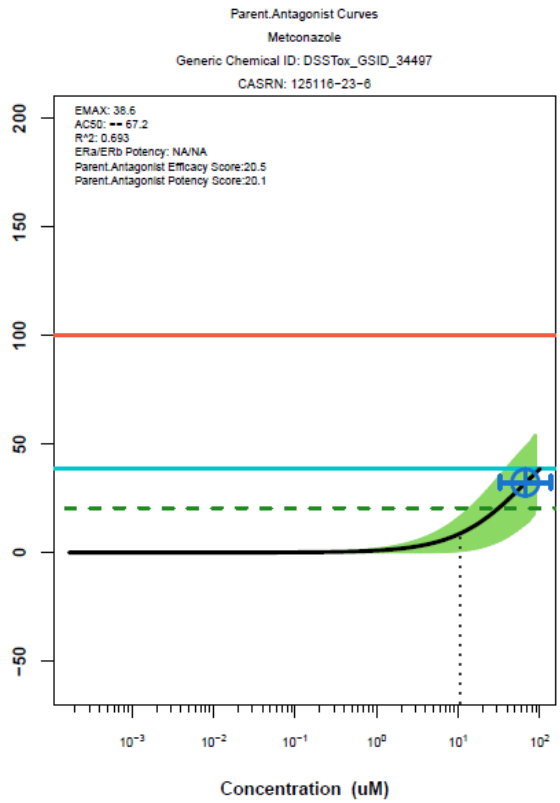
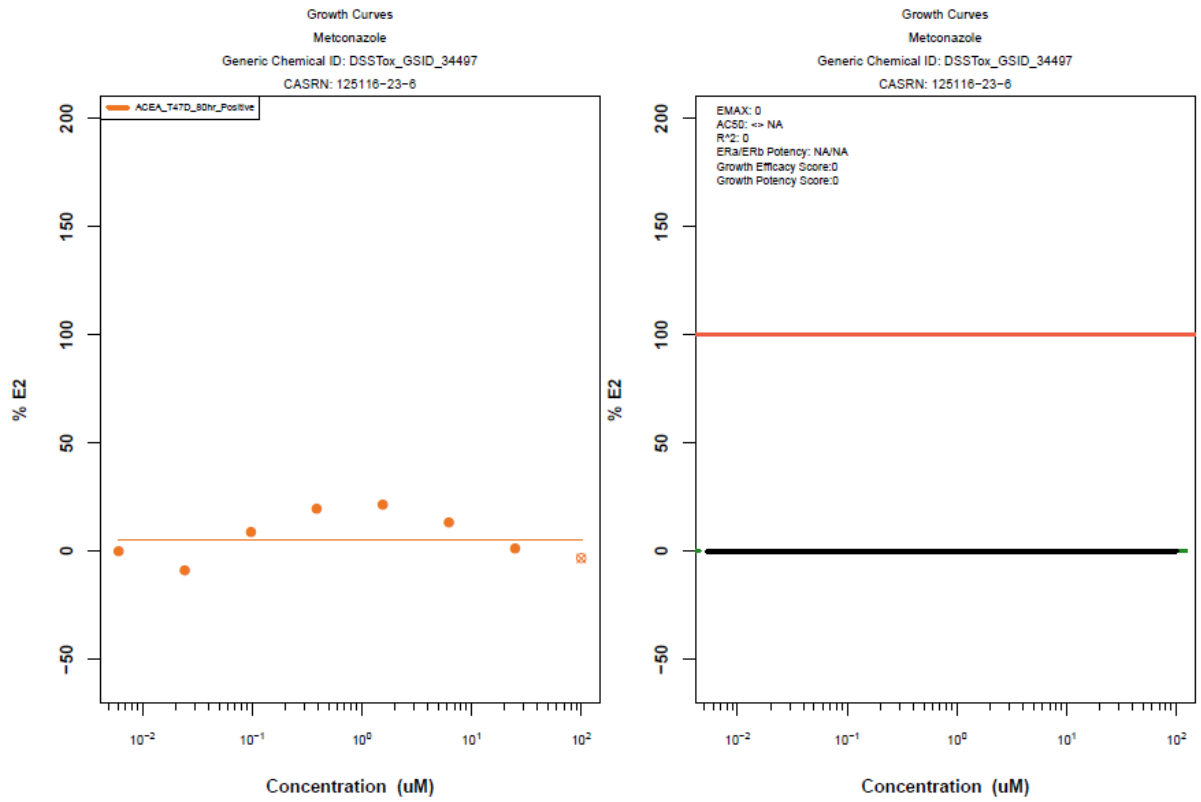
Metconazole
Generic Chemical ID: DSSTox_GSID_34497
CASRN: 125116-23-6
EMAX: 35.7
AC50: =< 91.9
R^2: 0.384
ERa/ERb Potency: 0/30.7
Parent.Agonist Efficacy Score:1.97
Parent.Agonist Potency Score:25.2
% E2
Concentration (uM)


Parent.Antagonist Curves
Metconazole
Generic Chemical ID: DSSTox_GSID_34497
CASRN: 125116-23-6
Tox21_ERa_LUC_BG1_Antagonist
Tox21_ERa_BLA_Antagonist_ratio
% 4-Hydroxytamoxifen
Concentration (uM)


Parent.Antagonist Curves
Metconazole
Generic Chemical ID: DSSTox_GSID_34497
CASRN: 125116-23-6
EMAX: 38.6
AC50: =< 67.2
R^2: 0.693
ERa/ERb Potency: NA/NA
Parent.Antagonist Efficacy Score:20.5
Parent.Antagonist Potency Score:20.1
% 4-Hydroxytamoxifen
Concentration (uM)



Conclusion of the author: An ER Interaction Score was developed by aggregating data from 13 different in vitro ER assays based on the known cellular ER signalling pathways. This model produced scores for an overall likelihood of a chemical being estrogenic, and these scores were highly correlated with in vivo data and ER reference chemical classifications, indicating that the model is capable of predicting estrogenic likelihood with a high degree of accuracy.

Conclusion of the applicant: This study analyses around 1800 substances concerning their potential to be an endocrine disruptor. Metconazole was one of them and found to be zero for the binding and growth group. In comparison the endogenous estrogen 17β-estradiol showed binding and growth scores of 83 and 63. Scores of 14, 22, and 11 were derived for the agonist group, antagonist group and the ER Interaction Score, respectively. For 17β-estradiol the scores for agonist, antagonist and ER interaction were 100, 0, and 82, respectively. Based on these screening tests Metconazole is considered to be of rather low priority for additional ER testing. This study is considered to be relevant for human risk assessment.

Classification of the study: relevant

CA 5.8.3 Endocrine disrupting properties

The most widely used definition of an endocrine disruptor is based on the WHO/IPCS (2002): 'An endocrine disrupter is an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations'. This definition is purely based on hazard identification, looking at whether the effects reported are regarded to be ED-related and supported by mechanistic information.

According to Regulation (EC) No 1107/2009, Annex II, Point 3.6.5 'an active substance shall only be approved if, (…), it is not considered to have endocrine disrupting properties that may cause adverse effect in humans.'
Nevertheless, there is no regulatory guidance available yet on how to address endocrine disruption (ED) and no final criteria are established.
Pending the adoption of the final scientific criteria for the determination of ED properties, currently the so called Interim criteria are applied. There were two Interim criteria defined within Regulation (EC) No 1107/2009, Annex II, Point 3.6.5:

1) '(…) substances that are or have to be classified, in accordance with the provisions of Regulation (EC) No 1272/2008, as carcinogenic category 2 and toxic for reproduction category 2, shall be considered to have endocrine disrupting properties.'

2) 'Substances such as those that are or have to be classified, in accordance with the provisions of Regulation (EC) No 1272/2008, as toxic for reproduction category 2 and which have toxic effects on the endocrine organs, may be considered to have such endocrine disrupting properties.'

Metconazole belongs to the triazole class of fungicide compounds acting by blockage of the sterol biosynthesis, i.e. inhibition of 14α-demethylase and aromatase, in fungal cell membranes.
Regarding CMR classification, metconazole is classified as toxic for reproduction category 2 (Repr. 2, (H361d)), (harmonized classification under Regulation (EC) No. 1272/2008 as amended by Commission Regulation (EC) No. 790/2009 (1st ATP)). Metconazole is neither classified nor classifiable for carcinogenicity (see MCA 5.5). Thus, the conditions of the Interim Criterion 1 are not met for metconazole.
Likewise, in an ED assessment published by Marx-Stoelting et al., 2014 (BASF DocID 2014/1326032) metconazole does not trigger the Interim Criterion 1.

For evaluation of the second criterion, it has to be determined whether toxic effects are observed on endocrine organs. There are no scientific criteria defined to assess adversity and what specifically is considered to be 'toxic effects on endocrine organs', which leaves space for interpretation and does not give a guidance. Additionally, no advice is given in the Interim Criteria which organs are to be regarded as endocrine. Thus, in this dossier the evaluation of effects on reproductive organs, pituitary, thyroid, and adrenals from subacute, subchronic, chronic, and reproduction studies were discussed in more detail. Marx-Stoelting et al., 2014 (BASF DocID 2014/1326032) evaluated metconazole to be of questionable outcome for the interim criterion 2 ('may-be cut off'), but unfortunately the compound specific effects and detailed criteria of this assessment are not laid down in this publication

The most prominent findings were isolated (endocrine) organ weight changes. The general observation was, that the observed organ weight changes were either not accompanied by histopathological correlates, or were observed at high dose levels only and considered to occur secondary to a stress response (as evidenced by marked systemic toxicity). Thus, they are unrelated to a specific endocrine mode of action (see also M-CA 5.3, M-CA 5.6, and M-CA 5.7), for further discussion please see below).

Furthermore, with regard to an ED risk assessment approach, the available toxicological database of metconazole was screened for possible endocrine-mediated effects, which are further discussed below. For this, mechanistic data and results of scientific literature were also considered.

Discussion of potential endocrine-related effects of metconazole

Effects on endocrine organs

A detailed evaluation on the effects of endocrine organs was made for the available subacute, subchronic, chronic studies, as well as reproduction toxicity studies (see also chapters M-CA 5.3, M-CA 5.5, M-CA 5.6, M-CA 5.7).

In rodent 28-day and 90-day studies organ weight decreases (testes, seminal vesicles, prostate, uterus, ovaries) were attributed to dose levels with marked effect of treatment on food consumption and body weight reductions (approximately by >10 - 60%), and lesions of the forestomach were observed in rats. These effects demonstrate an impaired health state in the animals, related to pain and distress. The observed organ weight reductions were either seen as isolated findings without any histopathological correlate or the respective macroscopic and microscopic effects were in line with organ size reduction, atrophy of the tissue, or anorexia of the animals. According to the guidance document for histologic evaluation of endocrine and reproductive tests in rodents (OECD Series on Testing and Assessment No. 106, 2009), such findings are considered to be an unspecific effect rather than a consequence of an endocrine mechanism. As shown for seminal vesicles and prostate, decreased body weight gains of ≥ 10% result in weight and size reduction, and a body weight gain reduction of ≥ 30% is accompanied by weight or morphology changes in testes or epididymis. Thus, the overall assessment of metconazole effects on the organs of the endocrine system at these high doses is considered to be a stress-induced secondary response to general impaired state of health as a consequence of malnutrition evident by body weight reductions and reduced food consumption and body weight reductions.

In the 2-year carcinogenicity study in Fisher 344 rats (0, 100, 300 and 1000 ppm equivalent to 4.6/5.5, 13.8/16.6, 46.5/56.2 mg/kg bw/day in males/females, respectively) a marginal increase of focal interstitial cell (Leydig cell) hyperplasia in the testis was reported at the top dose of 1000 ppm (46.5 mg/kg bw/d) at terminal sacrifice (see chapter M-CA 5.5). The spontaneous rate of testis interstitial cell tumors is quite high in the Fisher 344 rat strain used and the observed incidence of testis interstitial cell tumors was within the range of historical controls as reported by National Toxicology Program/NTP (Haseman et al., 1990; BASF DocID MK-905-011). Furthermore, no impairment of reproductive performance or sperm parameters (as measured in the 2-generation study with metconazole cis/trans) were described in the 2-generation rat studies and no gonadal tumours developed at the end of the 2-year treatment period.

An increased incidence of pituitary adenoma (pars distalis) was observed in male rats at the mid dose (300 ppm) in the 2-year carcinogenicity study. At the top-dose (1000 ppm), the incidence was similar to that of controls, and the incidence at any dose was within the incidence range which could be expected for F344-rats of this age (Haseman et al., 1998; BASF DocID 1998/1006954). Thus, the effects on the testis and pituitary in the rat cancer study are not considered to be adverse but of spontaneous origin in this strain of rats.

In the 2-generation rat studies conducted with metconazole cis increased absolute and relative ovary weights were observed at 32 mg/k g bw/d (mid dose) and 48 mg/kg bw/d (top dose) in parental animals of the F1 generation, but histopathology revealed no corroborative findings. Similarly, absolute and relative ovary weights were significantly increased in Parental and F1 females at the top dose (750 ppm corresponding to approx. 53 mg/kg bw/d), but no histopathological changes which may be related to the increased organ weight were noted in the ovary. The relative weight of seminal vesicles increased significantly in F1 males in the 750 ppm group; however, this change was considered to be incidental because histopathological alteration were neither observed in this organ nor in other male reproductive organs. In addition, no abnormalities were found in spermatogenesis.
Conversely to the isolated weight increases on ovary and seminal vesicles observed in the 2-generation studies, organ weight reductions were observed in the subacute and subchronic studies, which were secondary to excessively reduced body weights. Furthermore, in the rat carcinogenicity studies no ovarian or gonadal tumours were observed.
Significantly lower values were observed in the absolute pituitary weights of parental male animals of the F1 generation at the top dose (750 ppm group) but no significant differences were noted in the relative weights and are thus, considered to be not treatment-related.
No other changes were observed on the reproductive organs, pituitary, thyroid, and adrenals. Therefore, it was concluded that overall, no relevant adverse findings were observed on the investigated organs weights or gross pathology and histopathology in the 2-generation rat studies.

In the dog 90-day study (0, 60, 600, and 6000 ppm equivalent to 2.5/2.6, 24.4/24.3, and 225.2/206.6 mg/kg bw/day in males/females, respectively) isolated increased absolute and/or relative thyroid weights were observed in both sexes at the mid and high dose. However the organ weight changes were not significant except for the relative weight increase in females at the top dose (relative weights at 6000 ppm: +22% in males/+52% in females) without any corroborated relevant macroscopic and/or microscopic observation. In the absence of other pathological findings in this organ the increased thyroid weights are of unclear toxicological significance. Non-significantly increased testes weights at the top dose in the absence of histopathological correlates are not considered to be relevant. Effects in dogs at the top dose (6000 ppm) were seen in the presence of significantly reduced body weights (-11 to -21%) and food consumption (≥ - 10%).

In the 1-year dog study (30, 300, 1000, and 3000 ppm equivalent to 1.1/1.1, 12.0/10, 38.5/36.5, and 110/113.7 mg/kg bw/d in males/females, respectively) a minimal non-significant adjusted thyroid weight increase was noted at the top dose (+12%/+14 in males/females), but was considered to be irrelevant in the absence of dose response and other related macroscopic and histopathological observations. At this top dose in females (corresponding to 113.7 mg/kg bw/d) adjusted ovary weight was increased (+45%), but was not of statistical significance. It was noted that considerable variation in ovary weight was also observed at lower doses and control animals, probably reflecting various stages of oestrus cycle of individual animals, since some animals contained large follicles while others contained corpora lutea. A slightly increased incidence of corpora lutea was not considered to be of pathological alert. The organ weight changes (thyroid/ovary) at the top dose were the only effects on endocrine organs seen in the 1-year dog study with no histopathological correlates. It is therefore concluded that no treatment-related adverse effects on the organs of the endocrine system could be assigned in dogs after 1 year of exposure to metconazole cis/trans.

Effects on the adrenal gland

The effects on the adrenal gland are discussed separately in this paragraph, since unlike the other organ effects discussed above, the observed adrenal weight changes were accompanied by a histopathological correlate, i.e. adrenal cortex vacuolation.

Changes in the adrenal gland were observed in rat, mouse, and dog studies with metconazole (see M-CA 5.3 and M-CA 5.5). Generally these findings include relative adrenal weight increases and/or adrenal cortex vacuolation.

Regarding the rodent studies, there was adrenal cortical cell vacuolation and increased relative adrenal weights at the top dose 10000 ppm (721/784 mg/kg bw/d) in the rat 28-day study on the cis isomer. In the rat 28 day study on the cis/trans mixture there was increased vacuolation of the adrenal cortex observed at the top dose of 3000 ppm (261/287 mg/kg bw/d), but without relative organ weight changes.

In the rat 90-day study on the cis isomer, a slight reduced absolute adrenal weights were observed at the top dose (4050 ppm equivalent to 265/267 mg/kg bw/d); adjusted adrenal weight was increased in males and decreased in females at this dose. At the lower dose-levels (450ppm/29 mg/kg bw/d and 150ppm/10 mg/kg bw/d) absolute adrenal weight decrease was observed only in males without a dose-response, the magnitude was low, and no concomitant histological lesions were reported. The observed cortical vacuolation of the adrenals in the male recovery group at the top dose in the absence of similar findings in the group without recovery was unexplained. In the rat 90 day study on the cis/trans mixture cortical vacuolation of the adrenals was seen at the top dose of 3000 ppm (193/208 mg/kg bw/d) accompanied by reduced absolute and relative (only males) adrenal weights. The mouse 90-day study revealed increased relative adrenal weights only in males at the top dose (341/438 mg/kg bw/d). The observed incidences of cortical vacuolation in top dose female mice was similar to those seen in the concurrent control group females and no effect on the adrenal weight was determined.

In the two year rat carcinogenicity study the incidence of adrenal cortex vacuolation was increased in males at the mid (300 ppm, 13.8 mg/kg bw) and high dose (1000 ppm, 46.5 mg/kg bw/d) and is considered to be reflected by the increased adrenal weights in males at 1000 ppm. However, the rat chronic toxicity study which ran in parallel to the carcinogenicity study did not report adrenal changes at similar dose levels. The differences in effects between the two studies cannot be explained, group size may be an accounting factor.

The changes seen in rodents in the adrenal (on organ weights and/or cortical vacuolation) only occurred at high dose levels, which also showed impaired food consumptions and marked deficits in bodyweights. In addition, in the rat lesions of the forestomach were observed, which are consistent with irritation and inflammation and are considered to induce pain and distress. Due to this severe general toxicity findings it is considered that the primary effect of anorexia and irritation of the stomach leads via stress of these conditions to the effects observed in the adrenal. Classical stress-related changes originate from stimulation of adrenocorticoptrophic hormone (ACTH) action on the adrenal gland, resulting in increased glucocorticoid secretion from the adrenal in response to the ACTH (Harvey and Sutcliffe, 2010, DocID 2010/1233292).
If the finding of cortex vacuolation is seen in the zona fasciculate, it would represent stimulation by ACTH. Unfortunately, from the existing studies on metconazole it was not defined which zone of the adrenal cortex shows vacuolation. However, although the described effects in rodents are occasionally variable between studies they are consistent with those typically in a physiological stress response.

In the 90-day dog study a clear increase in adrenal weights and thymic involution (atrophy) were observed at the top dose level (6000 ppm, corresponding to 206.6 mg/kg bw/day) in the presence of marked reductions of food consumption and decreased body weight. Thymic involution is a well described feature of stress pathology (Harvey and Sutcliffe, 2010, DocID 2010/1233292) and it proves secretory competency of the adrenal to produce glucocorticoid (in response to stress-induced increased ACTH), which then excludes a direct adrenocortical toxicity mechanism.

Taken together, there is evidence that the toxicolocigal profile of metconazole in the adrenals is consistent with stress. This is first based on the severity of effects at the dose levels where the adrenal effects are seen, i.e. impaired food intake and body weight reductions, as well as forestomach lesions (in the rat). These effects are well known stressors. Second, the pathological findings were confined to adrenal cortical vacuolation and/or adrenal weight changes (generally increased relative adrenal weights) with no evidence of degeneration, thus demonstrating the lack of pathological alerts in the adrenal, and supporting a functional competency of the organ to produce glucocorticoids. Also, the magnitude of adrenal weight changes (in rats and dogs) was relatively small based on which a functional glucocorticoid production can be assumed. Evidence of intact glucocorticoid production of the adrenal was supported by the microscopic finding of thymic involution in the dog, which is a known marker of stress. Furthermore, the changes in the adrenals were not sufficiently adverse to affect health or survival and are therefore consistent with a physiological mechanism rather than a toxicopathological mechanism.
Although the described effects are occasionally variable between studies they are consistent with those typically seen with activation of the hypothalamo-pituitary-adrenocorticol system in the physiological stress response. In conclusion, the profile of adrenal changes does not indicate direct adrenal toxicity but the effects are considered to be secondary to a stress response.

Further findings in reproduction and developmental studies, and mechanistic data

Some effects observed in the reproduction and developmental toxicity studies performed on metconazole cis and cis/trans are potentially ED-related (see also M-CA 5.6).

Administration of metconazole cis/trans to pregnant rats in the 2-generation study led to dystocia associated with maternal deaths and extended gestation lengths only at the top dose level (750 ppm, ~53 mg/kg bw/d), resulting in decreased gestation indices for both generations in the presence of maternal toxicity (decreases in food consumption, body weight and body weight gain, as well as liver effects). Also, marginally but significantly prolonged estrous cycle (only parental females, not F1 females) were noted at this top dose. Absence of effects on the estrous cycle in the second generation and in the 2-generation study with the cis isomer raises however, doubts on the treatment-relationship of this effect.

In order to address the mechanism by which slightly prolonged duration of gestation and dystocia occurred at the high dose of cis/trans metconazole, a mechanistic 1-generation study in rats was performed investigating hormone measurements during late gestation (on gestation days 19 or 21) and liver CYP protein analysis (for details please refer to the study summary in chapter M-CA 5.8.2). In this supplemental study a significant inhibition of increases in the 17ß-estradiol/progesterone (E/P) ratio were observed on gestation days 19 and 21 only at the high dose of 750 ppm, which was also the top dose used in the 2-generation study with metconazole cis/trans. Decreases in the E/P ratio at the top dose were mainly attributed to the decreased serum 17ß-estradiol concentrations on gestation days 19 and 21, which was significantly changed only on gestation day 21. Progesterone concentrations in this 750 ppm group tended to be slightly but not statistically significantly higher than that of the control group on gestation day 21 with large standard deviations. It was suggested that corpora lutea may not have entered the stage of functional regression yet and may thus, still be active for progesterone production during late pregnancy, which was possibly in line with the finding of higher-than-the-control ratio of corpora lutea containing PCNA-positive lutein cells. However, the large variations of the measured serum progesterone levels make it difficult to draw a final conclusion. Additionally, increased hepatic CYP proteins of isozymes, which are involved in the estradiol metabolism in the liver were observed and the study author hypothesized that the inhibition of increases in the E/P ratio at this top dose may be due to an excessive acceleration of 17ß-estradiol metabolism.

The effect on difficult delivery and slight delayed onset of parturition may be associated with aromatase inhibition. Fundamental species differences exist regarding hormonal regulation during pregnancy and parturition. While in rodents and rabbits sustained corpora luteal activity is required to maintain progesterone secretion throughout gestation, in guinea pigs and in humans, the corpora lutea is only active during early pregnancy and thereafter the placenta takes over the progesterone production. Likewise, the placenta is also the primary source for estrogen production in pregnant guinea pigs and humans, while the ovaries are the sole source of estrogen in pregnant rats. (Mitchell and Taggert, 2009; BASF DocID 2009/1132342). Towards the end of the gestation, an increase in serum estradiol concentrations and a decline in progesterone is required to trigger parturition in rats (Fang et al., 1996; BASF DocID 1996/1005200). Disturbances of these serum hormone levels may be the cause for parturition difficulties in rats. The effect of slightly extended gestation length and dystocia can therefore be considered to be rat-specific without relevance to humans.

Based on the significantly decreased levels of estradiol during late pregnancy in rats, which are indicative of an aromatase inhibiting compound, the mode of action of metconazole as an aromatase inhibitor was further elucidated in an aromatase assay using recombinant CYP19 enzyme investigating the activity of rat versus human enzyme (study summary see M-CA 5.8.2). Metconazole cis/trans (IC50 (human): 7.21 x 10-7M, IC50 (rat): 1.57 x 10-7 M) inhibited both rat and human aromatase activities, but the aromatase inhibition was considerably more pronounced for the rat enzyme than for the human enzyme with a highly significant difference of 4.6-fold between the enzymes from the two species. The results suggest that metconazole is more potent towards rat aromatase than towards human aromatase. The cis isomer resulted in a similar response as for the cis/trans mixture, whereas the trans isomer showed a weaker aromatase inhibiting response. In comparison with rat and human IC50 values of letrozole, a known triazole compound designed to inhibit aromatase in humans, metconazole TGAI (cis/trans) is considerably less potent (103-/799- times) than letrozole on rat and human enzyme, respectively. This potency distinction seems to be also reflected in the severity and magnitude of the typical azole-related effects in vivo. Furthermore, with regard to binding capacities to the estrogen receptor (ER), metconazole did not display indications to interact with the ER signalling pathway and would be of low priority for additional ER testing based on data from published literature (see summary in chapter M-CA 5.8.2).

Fetotoxicity was evidenced by post-implantation losses (early and late resorption) in rats and was accompanied by reduced litter size, reduced fetal/litter weights, and increased placental weights (where measured) at maternotoxic dose levels. These effects also occurred in the developmental toxicity studies in rats and to a lesser extent in rabbits and may also be associated with aromatase inhibition. However, reproductive performance of the rats, mating and fertility indices were not affected by treatment. Furthermore, spermatogenesis in parental male rats was not affected and the females that delivered without difficulties weaned pups successfully. No relevant treatment-related abnormalities on the reproductive organs, pituitary, and adrenals were noted in the generational studies. It has also to be noted that sexual maturation (investigated by preputial separation and vaginal opening in the latest 2-gen study conducted in 2002) was comparable for all dose groups.

In conclusion, the above discussed effects of metconazole in pregnant rats, which only occur in the presence of considerable maternotoxic effects (primarily evidenced by impaired food consumption, body weight and body weight gain) at the respective high/top doses, may be associated with aromatase inhibition, a mechanism which appears to be less relevant in humans than in rats.

Final conclusion on ED assessment

Based on the harmonised classification of metconazole and the fact that no adverse effects on endocrine organs were identified, metconazole does not meet the currently applied Interim Criteria.

Taking into consideration all LOAELs of the potential endocrine–related effects on metconazole as discussed above, the most sensitive LOAEL is identified at 32 mg/kg bw/d (NOAEL at 8 mg/kg bw/d) based on slightly increased gestation length and decreased post-implantation survival in the second (F2) generation of the 2-generation study with metconazole cis (see also M-CA 5.6.1; [redacted] 1992; BASF DocID MK-430-003).

This most sensitive LOAEL for potential ED effects is greater than the overall LOAEL of the metconazole toxicological database which was set at 10 mg/kg bw/d (a non-endocrine related endpoint based on effects in the rabbit teratology studies, please refer to chapter M-CA 5.6) and based on which the reference values are derived from. In addition, it has to be noted that for all potential ED-related endpoints NOAELs have been identified. The effects discussed above from the reproduction and developmental toxicity studies imply a possible mode of action via aromatase inhibition in rat, a mechanism which is considered to be less relevant in humans. This is based on results of an aromatase assay, showing an IC50 for metconazole at considerably higher concentrations in human vs rat. Furthermore, the effects on slightly increased gestation lengths and dystocia are considered to be rat-specific and not relevant for humans. Overall, the effect levels at which these potentially endocrine-mediated effects occur are not the lead effects and an ED risk assessment can be applied.

CA 5.9 Medical Data

A search in the databases listed below - restricted to “pps=human” and “ct d human” - has been performed on August 28th, 2015 via DIMDI-host for the following terms:

- **Metconazole**
- **CAS** 125116-23-6

ME66	MEDLINE	NLM
ME0A	MEDLINE Alert	NLM
EM74	EMBASE	2005 Elsevier B.V.
EA08	EMBASE Alert	2005 Elsevier B.V.
CL63	CancerLit	NCI
CCTR93	Cochrane Library - Central	Cochrane

2. Crosscheck via ChemIDplus (http://chem2.sis.nlm.nih.gov/chemidplus/chemidlite.jsp)

3. Crosscheck via PubMed (http://www.ncbi.nlm.nih.gov/sites/entrez)

4. GUA-internal literature database “FAUST”

5. Regarding the databases HSDB (NLM) and GESTIS (BGIA)

6. Register of the internal medical ward

CA 5.9.1 Medical surveillance on manufacturing plant personnel and monitoring studies

All persons handling crop protection products are surveyed by regular medical examinations. There are no specific parameters available for effect monitoring of Metconazole. Thus, the medical monitoring programme is designed as a general health check-up, with special interest in the primary target organs presumed to be relevant by analogy from animal experiments.
The surveillance program includes a general physical examination including neurological status, red and white blood cell counts, liver enzymes. Adverse health effects suspected to be related to Metconazole exposure have not been observed.

CA 5.9.2 Data collected on humans

Two cases of eye irritation have been registered in the BASF-internal clinical incident log in employees accidently exposed to Metconazole in combination with other products. It is not clear whether Metconazole was the cause for these irritations.

CA 5.9.3 Direct observations

Some cases of slight irritation of the eyes have been reported to BASF in persons exposed to Metconazole in combination with other products. These reports could not be verified, and it is not clear whether Metconazole was the cause for these irritations.

CA 5.9.4 Epidemiological studies

Neither data on exposure of the general public nor epidemiologic studies are available for BASF SE, nor is BASF SE aware on any epidemiologic studies performed by third parties.
No epidemiological studies with Metconazole have been conducted.

No monitoring of the general population nor epidemiological studies are available for Metconazole. As such, no observations regarding health effects after exposure of the general public are known to us.

CA 5.9.5 Diagnosis of poisoning (determination of active substance, metabolites), specific signs of poisoning, clinical tests

Analytical methods in biological matrices are not established. Clinical tests are not known. No specific symptoms of poisoning are seen.

CA 5.9.6 Proposed treatment: first aid measures, antidotes, medical treatment

See safety data sheet / precautions; symptomatic and supportive treatment, no specific antidote known

CA 5.9.7 Expected effects of poisoning

The expected effects were derived for acute and subacute studies in animals.

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Metconazole

Document M-CA, Section 6

RESIDUES IN OR ON TREATED PRODUCTS, FOOD AND FEED AND PLANT METABOLISM

Compiled by:

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Version history[1]

Date	Data points containing amendments or additions and brief description	Document identifier and version number
27/Jan/2016	Additional study, summary CA 6.3.2 (BASF DocID 2006/1046074)	Document MCA Section 6 Version 2 (BASF DocID 2016/1030844

[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 How to revise an Assessment Report

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CA 6 RESIDUES IN OR ON TREATED PRODUCTS, FOOD AND FEED AND PLANT METABOLISM

CA 6.1 Storage stability of residues

The stability of residues of metconazole in plant and animal matrices was reviewed during the previous active substance approval process and no further data was requested at that time.

Plant

The following information is copied from the EFSA Conclusion (EFSA Scientific Report (2006) 64, 1-71, Conclusion on the peer review of metconazole):

Stability of residues (Annex IIA, point 6 introduction, Annex IIIA, point 8 introduction)

Residues of metconazole expressed as cis- and trans-isomers in cereal green plant, straw and grain, rape seed and rape oil, carrots and lettuce can be considered as stable under frozen storage conditions for a period of 12 months.

EFSA Reasoned Opinion on MRLs (European Food Safety Authority; Review of the existing maximum residue levels (MRLs) for metconazole according to Article 12 of Regulation (EC) No 396/2005. EFSA Journal 2011;9(10):2422. [47 pp.] doi:10.2903/j.efsa.2011.2422. Available online: www.efsa.europa.eu/efsajournal) referred to the EFSA Conclusion: "In the framework of the peer review, storage stability of metconazole was demonstrated for a period of twelve months at -18°C in commodities with high water (carrots, lettuce and cereals green plant) and high fat (rape seed) content as well as dry commodities (cereals grain)".

The tested matrices belong to the current categories high water (lettuce, cereal forage), oil (oilseed rape), and starch (carrot, cereal grain) content defined in the OECD Guideline 506.

An additional storage stability study on plant matrices was conducted to cover a period of up to 26 months. The results for metconazole isomers and its metabolites are summarized below. The results obtained in this storage stability study demonstrate that metconazole (*cis* and *trans* isomers) and its metabolites M555F021, M555F011 and M555F030 are stable in plant matrices with high water (radish tops), high oil (soya bean seed) and high starch content (wheat grain, radish/sugar beet roots) when stored frozen for up to 26 months. The study is summarized below.

Report:	CA 6.1/1 Gooding R., Saha M., 2008a Freezer storage stability of BAS 555 F (Metconazole) and its metabolites in plant samples 2008/7019330
Guidelines:	EPA 860.1380, EEC 7032/V/95 rev. 5
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: Metconazole

Lot/Batch #: AC8879-136A, BAS 555 F (Cis-Isomer): 99.3%; AC9339-122A, BAS 555 F (Trans-Isomer): 99.1%; AS 2110a, M21: 98.3%; AS 2106a, M11: 98.5%; AS 2111a, M30: 98.4%; AC10194-134, Triazole: 99%; 01893-196, Triazolyl acetic acid: 98.9%; 0541531, Triazolyl alanine: 98.8%

Purity: 99.3% (*cis*-metconazole); 99.1% (*trans*-metconazole); 98.3 (M21); 98.5% (M11); 98.4% (M30)

CAS#: 115850-27-6 (*cis*-metconazole); 115850-28-7 (*trans*-metconazole)

Spiking levels: 0.1 mg/kg

2. Test Commodity:

Crop: Wheat (grain, straw, hay), soya bean (seed), radish or sugar beet (tops and roots)

Sample size: 5 g (stored sample)

B. STUDY DESIGN

1. Test procedure

Plant matrices (wheat grain, straw and hay, soya bean seed, radish tops and radish or sugar beet roots) were fortified with metconazole and its metabolites M11 (M555F011), M21 (M555F021 and M30 (M555F030) at a concentration level of 0.1 mg/kg (10 x LOQ). The spiked samples were stored ≤-5°C in the dark and analyzed after about 0, 1, 3, 6, 12 and 26 months.

2. Description of analytical procedures

The residues of metconazole isomers and its metabolites were quantitated using BASF method No D0508. Plant material was homogenized and extracted with a mixture of methanol and water. An aliquot of the extract was diluted with methanol/water and filtered before HPLC-MS/MS analysis. The limit of quantitation of the method was 0.01 mg/kg.
Freshly fortified specimens were analyzed concurrently with specimens dosed and stored frozen. Acceptable procedural recovery data were obtained except for cis-metconazole and M11 in soya bean seed at 26 months (68% and 69%, respectively).

II. RESULTS AND DISCUSSION

The table below shows a summary of the stability data. The results are expressed as average percentage of the nominal fortification. In order to account possible variation over the time investigated, the mean corrected recovery results are given in addition in parentheses. The analytical results used for the stability calculation were corrected for recoveries.

After a storage time of 26 months the mean recovery results of the nominal in all stored samples were above 70% for *cis*- and *trans*-metconazole as well as metabolites M11 and M21 with few exceptions. *Cis*-metconazole recoveries in radish roots were at 68% after 26 months, which is only slightly below the required 70%; correction for procedural recovery gives 78%. Likewise, M11 recoveries in wheat straw and soya bean seed were at 68% and 67%, respectively, after 26 months, which is only slightly below the required 70%; correction for procedural recovery gives 95% and 97%, respectively.

Recoveries of M30 were below 70% (55-68%) in almost all samples stored for 26 months. The respective procedural recoveries were also low, ranging from 71% to 81%. Thus, the low recoveries are not likely to be caused by storage; correction for procedural recovery gives 77-85%.

The stored samples of M21 showed enhanced recoveries for all matrix types at the 12 month time intervals which declined to about 88-123% after 26 months. The reason is unclear, but could be due to some unidentified procedural errors during the analysis. However, these results have no impact in the stability of the M21 in all matrix types tested as shown by the stability data after 26 months which concluded the fact that M21 is stable for all matrix types tested.

In conclusion, storage stability could be proven for metconazole and its metabolites M11, M21 and M30 for a period of up to 26 months.

Table 6.1-1: Storage stability of metconazole and metabolites in plant matrices (mean result corrected for procedural is given in parentheses)

***Cis*-Metconazole - Mean recovery (%)**												
Storage time (months)	**Wheat grain**		**Wheat hay**		**Wheat straw**		**Soya bean seed**		**Radish roots**		**Radish tops**	
	A: in stored samples, % of nominal						B: procedural, in freshly spiked sample[3]					
	A[1)]	B[2)]	**A[1)]**	B[2)]	**A[1)]**	B[2)]	**A[1)]**	B[2)]	**A[1)]**	B[2)]	**A[1)]**	B[2)]
0	**n r.**	n r.	**95[3] (103)**	93	**n.r.**	n r.	**98[3] (93)**	106	**89[3] (89)**	91	**n.r.**	n r.
1	**n r.**	n r.	**87[3] (93)**		**n.r.**	n r.	**N/A**	N/A	**91[3] (100)**		**n.r.**	n r.
3	**n r.**	n r.	**78[3] (91)**	86	**n.r.**	n r.	**86[3] (82)**	106	**81[3] (105)**	77	**n.r.**	n r.
6	**n r.**	n r.	**89[3] (95)**	94	**n.r.**	n r.	**83[3] (85)**	98	**82[3] (83)**	99	**n.r.**	n r.
12	**n r.**	n r.	**78[3] (90)**	87	**n.r.**	n r.	**86[3] (98)**	88	**92[3] (104)**	88	**n.r.**	n r.
26	**N/A**	N/A	**71[3] (94)**	76	**N/A**	N/A	**76[3] (111)**	68	**68[3] (78)**	87	**N/A**	N/A

Table 6.1-1: Storage stability of metconazole and metabolites in plant matrices (mean result corrected for procedural is given in parentheses)

***Trans*-Metconazole - Mean recovery (%)**												
Storage time (months)	**Wheat grain**		**Wheat hay**		**Wheat straw**		**Soya bean seed**		**Radish roots**		**Radish tops**	
	A: in stored samples, % of nominal						B: procedural, in freshly spiked sample[3]					
	A[1)]	B[2)]	**A[1)]**	B[2)]	**A[1)]**	B[2)]	**A[1)]**	B[2)]	**A[1)]**	B[2)]	**A[1)]**	B[2)]
0	**n r.**	n r.	**87[3] (92)**	94	**n.r.**	n r.	**103[3] (98)**	105	**90[3] (103)**	87	**n.r.**	n r.
1	**n r.**	n r.	**85[3] (90)**		**n.r.**	n r.	**N/A**	N/A	**96[3] (111)**		**n.r.**	n r.
3	**n r.**	n r.	**89[3] (107)**	83	**n.r.**	n r.	**85[3] (81)**	105	**99[3] (117)**	84	**n.r.**	n r.
6	**n r.**	n r.	**95[3] (95)**	100	**n.r.**	n r.	**91[3] (91)**	100	**94[3] (98)**	96	**n.r.**	n r.
12	**n r.**	n r.	**89[3] (110)**	81	**n.r.**	n r.	**95[3] (110)**	86	**106[3] (128)**	83	**n.r.**	n r.
26	**N/A**	N/A	**78[3] (94)**	83	**N/A**	N/A	**83[3] (119)**	70	**75[3] (83)**	91	**N/A**	N/A
M11 - Mean recovery (%)												
Storage time (months)	**Wheat grain**		**Wheat hay**		**Wheat straw**		**Soya bean seed**		**Sugar beet roots**		**Radish tops**	
	A: in stored samples, % of nominal						B: procedural, in freshly spiked sample[3]					
	A[1)]	B[2)]	**A[1)]**	B[2)]	**A[1)]**	B[2)]	**A[1)]**	B[2)]	**A[1)]**	B[2)]	**A[1)]**	B[2)]
0	**91[3] (97)**	93	**102[3] (107)**	96	**96[3] (98)**	98	**101[3] (101)**	100	**95[3] (103)**	92	**92[3] (92)**	100
1	**96[6] (103)**		**86[3] (90)**		**85[3] (87)**		**N/A**	N/A	**91[3] (100)**		**89[3] (89)**	
3	**96[3] (93)**	104	**96[3] (105)**	91	**108[3] (105)**	102	**83[3] (83)**	100	**106[3] (109)**	98	**96[3] (87)**	111
6	**113[3] (86)**	131	**103[3] (108)**	95	**106[3] (114)**	94	**108[3] (118)**	92	**133[3] (110)**	121	**100[3] (111)**	90
12	**103[3] (106)**	97	**96[3] (115)**	83	**93[3] (106)**	88	**90[3] (106)**	85	**89[3] (97)**	92	**86[3] (96)**	90
26	**82[3] (88)**	94	**78[3] (95)**	82	**68[4] (95)[3]**	72[4]	**67[3] (97)**	69	**110[3] (92)**	120	**90[3] (105)**	85
M21 - Mean recovery (%)												
Storage time (months)	**Wheat grain**		**Wheat hay**		**Wheat straw**		**Soya bean seed**		**Sugar beet roots**		**Radish tops**	
	A: in stored samples, % of nominal						B: procedural, in freshly spiked sample[3]					
	A[1)]	B[2)]	**A[1)]**	B[2)]	**A[1)]**	B[2)]	**A[1)]**	B[2)]	**A[1)]**	B[2)]	**A[1)]**	B[2)]
0	**96[3] (105)**	92	**88[3] (99)**	90	**96[3] (107)**	89	**111[3] (106)**	105	**82[3] (93)**	89	**90[3] (106)**	85
1	**96[3] (104)**		**86[3] (96)**		**87[3] (98)**		**N/A**	N/A	**90[3] (101)**		**94[3] (110)**	
3	**104[3] (113)**	92	**101[3] (125)**	81	**114[3] (116)**	98	**104[3] (99)**	105	**103[3] (95)**	109	**98[3] (97)**	102
6	**108[3] (96)**	112	**98[3] (107)**	92	**100[2] (96)**	103	**109[3] (111)**	98	**109[3] (97)**	112	**91[3] (88)**	104
12	**180[3] (176)**	103	**157[3] (182)**	87	**157[3] (158)**	100	**167[3] (175)**	95	**166[3] (169)**	98	**148[3] (148)**	100
26	**111[3] (122)**	91	**98[3] (129)**	76	**96[4] (129)[3]**	76[4]	**88[3] (124)**	71	**108[4] (90)[3]**	102[4]	**123[3] (122)**	101

Table 6.1-1: Storage stability of metconazole and metabolites in plant matrices (mean result corrected for procedural is given in parentheses)

M30 - Mean recovery (%)												
Storage time (months)	**Wheat grain**		**Wheat hay**		**Wheat straw**		**Soya bean seed**		**Sugar beet roots**		**Radish tops**	
	A: in stored samples, % of nominal						B: procedural, in freshly spiked sample[3]					
	A[1)]	B[2)]	**A[1)]**	B[2)]	**A[1)]**	B[2)]	**A[1)]**	B[2)]	**A[1)]**	B[2)]	**A[1)]**	B[2)]
0	**97[3] (96)**	101	**92[3] (98)**	94	**94[3] (95)**	99	**101[3] (91)**	110	**91[3] (99)**	92	**91[3] (92)**	99
1	**86[3] (85)**		**89[3] (95)**		**92[3] (93)**		**N/A**	N/A	**83[3] (90)**		**91[3] (92)**	
3	**117[3] (104)**	113	**104[3] (108)**	96	**81[3] (84)**	96	**93[3] (84)**	110	**103[3] (104)**	100	**78[3] (88)**	88
6	**128[3] (111)**	115	**95[3] (99)**	97	**100[3] (108)**	93	**94[3] (89)**	105	**108[3] (94)**	115	**112[3] (114)**	98
12	**114[3] (123)**	92	**87[3] (95)**	91	**91[3] (101)**	91	**88[3] (106)**	83	**93[3] (107)**	87	**111[3] (132)**	84
26	**70[3] (78)**	89	**61[3] (86)**	71	**55[3] (77)**	72	**61[3] (81)**	76	**68[3] (84)**	81	**63[3] (85)**	74

1 A = mean % recovery in stored samples
2 B = mean % procedural recovery for freshly spiked samples
3 Average of two analyses
4 Average of four analyses
() Mean corrected recovery results are given in parentheses
N/A Not applicable

III. CONCLUSION

Recoveries (corrected for procedural recoveries) of cis and trans metconazole in stored samples after 26 month frozen storage were 78% to 119%. Recoveries (corrected for procedural recoveries) of M11 in stored samples after 26 month frozen storage were 88% to 105%. Recoveries (corrected for procedural recoveries) of M21 in stored samples after 26 month frozen storage were 90% to 129%. Recoveries (corrected for procedural recoveries) of M30 in stored samples after 26 month frozen storage were 77% to 85%. The results obtained in this storage stability study demonstrate that metconazole (*cis* and *trans* isomers) and its metabolites M21, M11 and M30 are stable in plant matrices with high water (radish tops), high oil (soya bean seed) and high starch content (wheat grain, radish/sugar beet roots) when stored frozen for up to 26 months.

Animal

A separate storage stability study was not conducted for animal matrices as the storage stability was assessed within the feeding and/or metabolism study when needed.

Ruminant:
In the cow feeding study (BASF DocID 2006/1046033, summary in M-CA 6.4.2), all samples were stored frozen at approximately -20°C until extraction and analysis. All treated samples of milk, skim milk, and cream were extracted for analysis within 19 days of collection, and thus storage stability data are not required for these matrices. The fat, muscle kidney and liver samples were analyzed for *cis*- and *trans*-metconazole within 50 days of sampling. The kidney and liver samples were analyzed for metabolites M1 (M555F001) and M12 (M555F012) within 181 days (6 months) of sampling.

Storage stability of *cis*- and *trans*-metconazole in muscle, fat, and liver after freezer storage for approximately 110 days was determined within the cow feeding study. Duplicate untreated control samples were fortified at 0.10 mg/kg *cis*- and *trans*-metconazole and analyzed at fortification to verify the dose and after storage in the freezer at approximately -20°C. Average recovery after 108 to 112 days storage was 77% in muscle, 79% in liver and 99.5% in fat.

Data supporting the freezer storage stability of M1 (free + conjugated) and M12 in kidney and liver are included in the goat metabolism report (BASF Reg. Doc. No. MK-440-013) evaluated during initial active substance approval. The overall final extraction was 101% of the initial extraction in both liver and kidney, and the metabolite profiles indicate that the major metconazole metabolites were unchanged for 195 days (approximately 7 months).

The cow feeding study was evaluated by ESFA; the following statement regarding stability of metconazole in animal matrices can be found in EFSA Reasoned Opinions (EFSA Journal 2011; 9(10):2422; EFSA Journal 2013;11(4):3185):

> "According to the RMS, the stability of metconazole was demonstrated in ruminant tissues (meat, liver and kidney) and fat for up to 3 months when stored deep frozen. No storage stability study was performed on milk and on poultry products. The storage conditions of samples of the livestock feeding study were not reported. In order to ensure acceptability of the livestock feeding study and to exclude decline of residues during the storage of the samples, information on the storage conditions for liver samples in particular is desirable."

The storage conditions for all samples are described in the cow feeding study report under Sample Handling on page 15 as follows:

> "Composite milk samples were frozen prior to overnight shipping (via Federal Express) to the Valent Technical Center. Tissue samples were also frozen prior to shipment (overnight via Federal Express) to the Valent Technical Center. Samples received at the lab were stored frozen at approximately -20°C until extraction and analysis."

Poultry:
In the hen feeding study (BASF DocID 2008/8000061, see summary in M-CA 6.4.1), all samples were stored frozen at approximately -10°C until extraction and analysis. Egg and tissue samples were extracted for *cis*- and *trans*-metconazole analysis within 26 days of collection, and thus storage stability data are not required for metconazole in these matrices. Tissue samples were extracted for M1 (M555F001) analysis within 246 days (approximately 8 months) of collection, and the liver samples were extracted for M12 (M555F012) analysis within 127 days (approximately 4 months) of collection.

Data supporting the freezer storage stability of the major metconazole metabolites (i.e. M1 and M12) in liver, muscle, fat and egg whites are included in the hen metabolism report (BASF DocID 2006/1046073, summary in M-CA 6.2.2). The overall final extraction was between 96.2 and 98.4% of the initial extraction for all of the matrices, and the metabolite profiles indicate that the major metconazole metabolites were unchanged for approximately 300 days (approximately 10 months).

The storage conditions for all samples are described in the hen feeding study report under Sample Handling on page 16 as follows:

> "Egg samples were frozen prior to overnight shipping (via Federal Express) to Morse Laboratories (in Sacramento, CA). Tissue samples were also frozen prior to shipment (overnight via Federal Express) to the Morse Laboratories. Samples received at the lab were stored frozen at approximately -10°C until extraction and analysis."

Triazole Derivative Metabolites (TDMs)

The following paragraphs are an exact copy of the relevant dossier section submitted to UK CRD. It summarizes the results of in total 10 studies which were performed by the TDMG member companies.

The stability of residues of 1,2,4-triazole , triazole alanine, triazole acetic acid and triazole lactic acid was investigated in a range of frozen crop and animal commodities.

1,2,4-triazole was demonstrated to be stable for the full duration of studies (12 - 54 months) in crops representative of the high starch, high oil and high water crop groups according to the OECD guideline 506. There were, however, some commodities in which 1,2,4-triazole was not stable for the full duration of the study; these were wheat grain from one study (12 months), wheat straw (40 months), turnip root (40 months), radish root (12 months), soybean seed (12 months) and tomato fruit (40 months). For a few other matrices the data were inconclusive since a quick degradation occurred at the very beginning of the study while thereafter 1,2,4-triazole seemed to remain fairly stable.

Triazole alanine was demonstrated to be stable for the full duration of the studies (12 - 54 months) in most crops representative of the high starch, high oil, high water and high protein crop groups according to the OECD guideline 506. Inconclusive results were obtained for oilseed rape seed and oilseed rape oil. Triazole alanine was also demonstrated to be stable in milk and eggs for the full duration of the studies (12 months).

Triazole acetic acid was demonstrated to be stable in most crop and animal commodities tested for the full duration of the studies (12 - 54 months). The crop commodities are representative of the high starch, high oil, high water and high protein crop groups according to the OECD guideline 506 and the animal commodities were milk and eggs. There were, however, some commodities in which triazole acetic acid was not stable for the full duration of the study; these were wheat straw (40 months), wheat bran (40 months) and radish tops (12 months). In one study the results were inconclusive for wheat grain but two other studies demonstrate that triazole acetic acid is stable in wheat grain for at least 25-26 months upon freezer storage

Triazole lactic acid was demonstrated to be stable in all crop commodities tested for at least 12 months. The crop commodities are representative of the high starch, high oil, high acid, high water and high protein crop groups according to the OECD guideline 506.

A summary of the stability results for all analytes and matrices is presented in the table below.

Table 6.1-2: Stability of Triazole Metabolites in Crop and Animal Commodities Following Freezer Storage

Commodities[1]	Crop	Commodity	Nominal period of stability demonstrated (months)			
			1,2,4-Triazole	Triazole alanine	Triazole acetic acid	Triazole lactic acid
Crops – high starch	Wheat	Grain	≥ 54 -- -- 12 --	≥ 54 -- ≥ 15 ≥ 26 --	NC[3] ≥ 25 -- ≥ 26 --	-- -- -- -- ≥ 12
		Straw[2]	40 -- --	≥ 54 -- ≥ 15	40 ≥ 25 --	-- -- --
		Flour	≥ 54 ≥ 12	≥ 54 ≥ 12	≥ 54 ≥ 12	-- --
		Bran[2]	NC[3]	≥ 54	40	--
	Barley	Grain	-- --	≥ 36 --	-- ≥ 36	-- --
		Straw[2]	-- --	≥ 36 --	-- ≥ 36	-- --
	Turnip	Root	40	≥ 54	≥ 54	--
	Sugar beet	Root	-- --	-- ≥ 15	≥ 25 --	-- --
	Radish	Root	12	≥ 26	≥ 26	--
Crops – high oil	Oilseed rape	Seed	NC[3] -- -- --	NC[3] ≥ 15 -- --	≥ 54 -- ≥ 24 --	-- -- -- ≥ 12
		Oil	≥ 54	NC[3]	≥ 54	--
		Meal[2]	≥ 54	≥ 54	≥ 54	--
	Soybean	Seed	12	≥ 26	≥ 26	--
	Peanut	Butter	≥ 12	≥ 12	≥ 12	--
Crops – high acid	Orange	Fruit	--	--	--	≥ 12
Crops – high water	Wheat	Forage	NC[3]	≥ 54	≥ 54	--
	Mustard	Leaves	NC[3]	≥ 54	≥ 54	--
	Tomato	Fruit	40	≥ 54	≥ 54	--
		Paste	≥ 54	≥ 54	≥ 54	--
	Apple	Fruit	≥ 12	≥ 12	≥ 12	--
	Cabbage	Head	-- --	-- ≥ 15	≥ 24 --	-- --
	Radish	Tops	≥ 26	≥ 26	12	--
	Lettuce	Lettuce	--	--	--	≥ 12
Crops – high protein	Pea	Dry Seed	-- --	-- ≥ 15	≥ 25 --	-- --
	Navy Bean	Dry Bean	--	--	--	≥ 12
Animal	na	Milk	≥ 12 ≥ 18	≥ 12 --	≥ 12 --	-- --
	na	Liver	≥ 12	--	--	--
	na	Muscle	≥ 12	--	--	--
	na	Fat	≥ 12	--	--	--
	na	Eggs	≥ 12	≥ 12	≥ 12	--

[1] Crop commodities according to the categories described in OECD guideline 506.
[2] Commodities not included in the crop categories described in OECD guideline 506.
[3] Not conclusive: for these compounds / commodities the study suggested a noticeable degradation of residues upon storage but it is unclear whether this is attributable to the study design or demotes a real stability issue.

As the storage stability study for triazole lactic acid was submitted to CRD in mid of June 2015 as a preliminary report, the final report is submitted in this dossier.

Report: CA 6.1/2
Perez R., 2015b
Freezer storage stability of Triazolyl Lactic acid in plant samples
2015/7005764
Guidelines: OECD 506 (Oct. 2007), EPA 860.1380
GLP: yes
(certified by United States Environmental Protection Agency)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: Triazolyl lactic acid (TLA)
Description: Not available
Lot/Batch #: L70-135
Purity: 97.7-98.8%
CAS#: Not available
Spiking levels: 0.1 mg/kg

2. Test Commodity:
Crop: Wheat grain, navy bean, orange, canola seed, lettuce
Type: Cereals; legume vegetables; citrus fruit; oilseeds; leafy vegetables
Variety: Not given
Botanical name: *Triticum aestivum; Phaseolus vulgaris; Citrus sinensis; Brassica napus L.; Lactuca sativa*
Crop parts(s) or processed commodity: Wheat grain, navy bean, orange, canola seed, lettuce
Sample size: 5 g

B. STUDY DESIGN

1. Test procedure

The stability of triazolyl lactic acid (TLA) in various plant matrices (wheat grain, navy bean, orange, canola seed, and lettuce) was investigated under the usual storage conditions (-25°C to -18°C) for fortified stored samples for up to 48 months. The plant matrices used were untreated control samples obtained from various field study sites.

Wheat grain, navy bean, orange, canola seed, and lettuce samples were spiked with TLA at a concentration of 0.1 mg/kg and analyzed in duplicate after storage intervals of 0, 1, 3, 6, 12, 18, 24, 30, 36, 42 and 48 months.

For frozen storage in wheat grain, navy bean, orange, canola seed and lettuce, TLA was dosed as follows: Exactly 0.1 mL of methanolic fortification solution containing the analyte TLA at a concentration of 5.0 µg/mL was dosed to pre-weighed 5 g plant specimens. Immediately after dosing, the specimens were stored frozen. The fortification standards were assayed to verify their concentration before use.

Additionally, three unfortified control samples were introduced into the analysis set, one was used as a control and two were used as procedural recovery samples, for each storage interval and matrix. Procedural recovery samples were fortified at 0.1 mg/kg on the day of extraction

2. Description of analytical procedures

Residue analysis was conducted following procedures of BASF Method No D0905. A plant sample of 5 g was extracted by homogenization with acetonitrile/water (70:30 v/v, 60 mL) using a Polytron homogenizer. An aliquot of the extract was diluted with water and evaporated to about 1.4 mL under nitrogen at 40°C to remove acetonitrile. The resulting aqueous phase was brought to volume of 5 mL with 0.1% formic acid in water. The extract was then filtered through a syringe filter into an autosampler vial for LC-MS/MS determination.

The limit of quantitation (LOQ) of the analytical method for TLA was 0.01 mg/kg in all matrix types.

II. RESULTS AND DISCUSSION

The stability results are expressed as average percentage of the nominal fortification. In order to account for possible variations over the time investigated, the mean procedural recovery results are given in addition. Stored recoveries demonstrate TLA was quantitatively recovered within acceptable limits from all plant matrices stored under freezer conditions over a period of 48 months. No significant differences were seen when comparing freshly-fortified samples to recovery samples stored for 48 months.

Table 6.1-3 shows a summary of the stability data.

Table 6.1-3: Storage stability of TLA in wheat grain, navy bean, orange, canola seed and lettuce

Mean recovery (%)										
Day	**A: mean in stored samples, % of nominal**					B: mean in procedural, freshly spiked samples				
	A	B	**A**	B	**A**	B	**A**	B	**A**	B
	Wheat grain		**Navy bean**		**Orange**		**Canola seed**		**Lettuce**	
0	**91**	93	**102**	91	**90**	115	**106**	99	**101**	111
47	**80**	93	**100**	91	**108**	115	**126**	99	**116**	111
97	**110**	93	**114**	91	**120**	115	**131**	117	**117**	111
181	**98**	87	**108**	87	**109**	109	**125**	108	**116**	104
362	**103**	113	**111**	110	**120**	108	**120**	117	**112**	110
548	**118**	107	**113**	109	**119**	111	**118**	99	**118**	104
736	**90**	82	**94**	96	**107**	109	**115**	91	**96**	88
915	**95**	90	**119**	102	**110**	104	**99**	104	**116**	95
1097	**81**	82	**84**	97	**114**	114	**97**	90	**108**	98
1310	**81**	83	**108**	114	**94**	102	**98**	108	**98**	98
1461	**86**	89	**97**	93	**107**	97	**98**	108	**102**	91

III. CONCLUSION

The results obtained in the storage stability study indicate that under freezer storage conditions, residues of TLA were stable in all matrices through the 48 month storage interval.

Stability of residues in sample extracts

Data on the storage stability of *cis*- and *trans*-metconazole in working solutions was evaluated within the Draft Assessment Report (DAR) prepared under Directive 91/414/EEC (Belgium, 2004) and was considered as suitable. Additional data on storage stability was generated and is reported within the summaries of the new analytical methods in M-CA 4.1.2 and 4.2.

In summary, from the data available it can be concluded that metconazole is stable in plant and animal sample extracts or working solutions when stored refrigerated during residue analysis.

CA 6.2 Metabolism, distribution and expression of residues

Plant

Metabolism of metconazole was investigated in the framework of the peer review of Directive 91/414/EEC for foliar application on pules and oilseed (oilseed rape) and on cereals (winter wheat) using ^{14}C-cyclopentyl, ^{14}C-chlorophenyl and ^{14}C-triazolyl metconazole (EFSA Conclusion 2006). After Annex I inclusion, an additional metabolism study on banana (fruits and fruiting vegetable group) was submitted and evaluated in the framework of a routine MRL application (EFSA Reasoned Opinion 2010). Since the banana metabolism study is not considered peer reviewed, it is submitted with this document.

The following statement was copied from the EFSA Reasoned Opinion 2011 (Article 12 review):

"In wheat grain at harvest, the TRR ranged between 0.074 mg eq/kg (study with 14C-cyclopentyl metconazole) and 0.66 mg eq/kg (study with 14C-triazolyl metconazole). The TRR in wheat straw accounted for approx. 6 mg eq/kg. In the triazole study, the main components of the TRR in wheat grain were triazole alanine (33%, 0.22 mg/kg) and triazole acetic acid (9% TRR, 0.06 mg/kg) with parent metconazole being below 2% of the TRR (<0.1 mg/kg). In wheat straw the major component of the TRR in both the cyclopentyl and triazole studies was parent metconazole accounting for 32% and 25.3% of the TRR, respectively. In wheat straw from the cyclopentyl study, stereoisomeric monohydroxy metabolites were identified, but they accounted for less than 10% of the TRR.

In rapeseed at harvest, the TRR accounted for 19.62 mg eq/kg in pods and 2.39 mg eq/kg in seeds. The characterisation of the TRR indicated that in pods the parent metconazole accounts for 16.4%, glucose conjugate of monohydroxylated metconazole for 36.5% and monohyhydroxylated metconazole for 28.2%. In seeds, parent metconazole accounted for 24% of the TRR, triazole alanine for 40.2% of the TRR and monohydroxylated metconazole for 12% of the TRR.

In bananas at harvest at the PHI of 2 hours (0.1 day) after last application (56 days after the first one), the TRR ranged from 0.93 to 1.37 mg eq/kg in the whole fruit, from 1.62 to 2.54 mg eq/kg in the banana peel and from 0.61 to 0.78 mg eq/kg in the pulp of banana. The main residue in all banana fractions was parent metconazole, accounting for more than 85% of the TRR in the triazole study. Small amounts of monohydroxylated metconazole were identified in amounts not exceeding 2.5% TRR. Triazole alanine accounted for a maximum of 3.5% TRR (0.021 mg/kg) in banana pulp. Also in a study with chlorophenyl labeled metconazole, the parent metconazole was the major component of the TRR accounting for more than 86% in all fractions of banana.

Based on the above studies EFSA concludes that the metabolism in three crop groups investigated proceeds in a similar pathway. The primary metabolic pathway of metconazole proceeds by oxidative hydroxylation of the benzylic methylene group, the methyl side chain on the cyclopentyl ring, and potentially the cyclopentyl ring to produce monohydroxylated metabolites of metconazole which are further conjugated through glycosidation. The presence of triazole alanine and triazole acetic acid suggest that the methylene group between the triazole ring and the cyclopentyl ring is also susceptible to oxidative hydroxylation. Triazole alanine, derived from 1,2,4-triazole and the o-acetyl-serine by cysteine synthases, was very well translocated and mainly detected in the non-vegetative parts of plants where it was stored. Even though studies indicate that the main residues in cereal grain and rape seeds are triazole alanine and to a lower extent also triazole acetic acid, the peer review initially considered them as of no toxicological relevance and concluded not to include these compounds in the residue definition for risk assessment."

The following endpoints are based on the EFSA Reasoned Opinion (2011).

Metabolism in plants (Annex IIA, point 6.1 and 6.7, Annex IIIA, point 8.1 and 8.6)

Plant groups covered	Wheat (C), oilseed rape (P/O), banana (fruit)
Rotational crops	Wheat (C), lettuce (L), radish (R/T) Metabolic pathway of metconazole in succeeding crops is similar to that in the target crops
Plant residue definition for monitoring	Metconazole (sum of *cis* and *trans* isomers), all crop categories
Plant residue definition for risk assessment	Metconazole (sum of *cis* and *trans* isomers), all crop categories
Conversion factor (monitoring to risk assessment)	None

Some additional detail related to metabolites found in the metabolism studies is provided here since the level of metabolites is to be considered in discussions of the toxicological relevance of metabolites. In wheat straw from the cyclopentyl study, stereoisomeric monohydroxy metabolites were identified; M11 (M555F011) accounted for 9.8% TRR (0.058 mg/kg) and M21 (M555F021) accounted for 9.7% TRR (0.057 mg/kg). Two other metabolites were identified in straw at lower levels, M1 (M555F001) coeluting with other unidentified components, at 4.6% TRR (0.27 mg/kg) and M30 (M555F030) at 2.4% TRR (0.14 mg/kg).

Livestock

The nature of metconazole residues in commodities of animal origin was investigated in the framework of Directive 91/414/EEC. Reported metabolism studies include two studies in lactating goats and two studies in laying hens using ^{14}C-cyclopentyl metconazole.

The following statement was copied from the EFSA Conclusion 2006:

Livestock metabolism was studied in dairy goats and laying hens by orally dosing the animals with ^{14}C-metconazole. Administered metconazole was rapidly metabolised and excreted. Hence radioactivity was mainly found in goat urine (33-44% of total dose) and faeces (28-46%), and in hen excreta (92%), respectively. Excretion in milk and eggs was comparatively minor and TRR in milk and eggs plateaued within 4 and 8 days of dosing, respectively. It is noted that with regard to its log Pow metconazole is characterised as fat soluble (log Pow 3.85). However, TRR were roughly one order of magnitude higher in the excretory organs liver and kidney of both species than in other animal tissue, indicating that there might be no accumulation of residues in adipose tissue.
Chromatographic analysis of goat matrices show that metconazole made up the majority of the residue in liver, fat and muscle tissues, while it was hardly detected in kidney and urine samples (<2% TRR). Therein, monohydroxy metabolites M1 (up to ca. 26% TRR) and M31 (up to ca. 35% TRR)) and the carboxylic acid metabolite M12 (ca. 20% TRR) were the major constituents. Results from the goat studies suggest that metconazole is rapidly and completely metabolised through oxidative processes, forming monohydroxylated compounds, which are further oxidized to carboxylic acid metabolites and/or conjugated subsequently. All metabolites identified in goat tissues were also found in the rat metabolism.

As the metabolic pathway of metconazole residues in laying hens was not established during the previous active substance approval, an additional metabolism study on laying hens is summarized in this document.

CA 6.2.1 Metabolism, distribution and expression of residues in plants

Report: CA 6.2.1/1
Kao L.M., 1998a
CL 900768 (Metconazole): Metabolism of CL 900768 in banana under greenhouse conditions
MK-640-008
Guidelines: EPA 40 CFR 158.240, EPA 860.1300, EEC 91/414 Annex II (Part A Section 6.1)
GLP: yes
(certified by United States Environmental Protection Agency)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description: [Triazole-3,5-^{14}C]-CL900768 (metconazole)
[Triazole-3,5-^{13}C]-CL900768 (metconazole)
[*p*-Chlorophenyl-U-^{14}C]-CL900768 (metconazole)
[1-Methylene-^{13}C]-CL900768 (metconazole)
Unlabeled metconazole
Note: the *cis/trans* isomer ratio was about 80/20

Lot/Batch #: Isotopic mixture (^{14}C, ^{13}C, ^{12}C, triazole label): MRN-8D-17
Isotopic mixture (^{14}C, ^{13}C, ^{12}C, chlorophenyl label): MRN-8D-17A
Unlabeled: AC 9339-114

Purity: Radiochemical purity: 97.26% of mixture (triazole label)
95.37% of mixture (chlorophenyl label)
Specific activity of mixture: 9.47 μCi/mg (triazole label)
10.9 μCi/mg (chlorophenyl label)

CAS#: 125116-23-6

Stability of test compound: The test item was stable over the test period.

2. Test Commodity:

Crop: Banana
Type: Fruit
Variety: Dwarf Cavendish
Botanical name: *Musa* L.
Crop parts(s) or processed commodity: Peel, pulp and whole fruit
Sample size: Not relevant

3. Soil: A sandy loam was used. The soil physicochemical properties are described below (see Table 6.2.1-1).

Table 6.2.1-1: Soil physicochemical properties

Soil series	Soil type	pH	OM %	Sand %	Silt %	Clay %	Moisture at 1/3 bar %	CEC cmol/kg
NA*	Sandy loam**	5.7	1.7	70**	19**	11**	16.2	15.2

* NA not applied ** USDA scheme

B. STUDY DESIGN

The metabolism study was conducted with [^{14}C]-metconazole (triazole label and chlorophenyl label) during 1997. The field phase of the study was performed at Plant Science Inc., Watsonville, California.

1. Test procedure

The greenhouse-grown banana plants were treated with 5 foliar applications beginning at the flowering stage and every two weeks after the initial application at a rate of 143 g a.s./ha (triazole moiety labeling) or 139 g a.s./ha (chlorophenyl moiety labeling) for a total application of about 700 g a.s./ha. A control plot was also established. The fruit samples were harvested 56 days after the first application (56 DAT1), corresponding to about 2 hours after the last application (0 DAT5). Approximately 1/3 of the harvested fruit samples from each plot was collected as the whole fruit sample; the remaining 2/3 of the fruit was separated into peel and pulp samples.

2. Description of analytical procedures

Radioanalysis: Total radioactive residue (TRR) in each fruit sample was determined by radiocombustion analysis followed by liquid scintillation counting (LSC) and by direct LSC of the extracts of the fruit samples.
Extraction: An aliquot of each homogenized frozen banana fruit sample from the treatment plots were initially extracted with 6 volumes of methanol for three times, each followed by centrifugation. The residual radioactive residue (RRR) was further extracted with 2% HCl in methanol at room temperature overnight followed by centrifugation. The methanol extracts were radioassayed individually, and were combined and concentrated for HPLC analyses. The remaining RRR was combusted to $^{14}CO_2$ for determination of the radioactivity.

3. Identification of metabolites

The [^{14}C]-metconazole derived residues in the whole fruit extracts were isolated for metabolite characterization and identification. The [^{14}C]-metconazole derived residues in the composited methanol extracts of whole fruit were isolated using HPLC. The isolated metabolites were analyzed by mass spectrometry or HPLC.

II. RESULTS AND DISCUSSION

A. TOTAL RADIOACTIVE RESIDUES (TRRs)

The TRR in control samples were below the validated detection limit of 0.005-0.007 mg/kg.

Plant: At harvest (56 DAT1 or about 2 hours after the 5th application), the total amount of radioactive residues ranged from 0.93 to 1.37 mg/kg for the whole fruit, from 1.62 to 2.54 mg/kg for the banana peel and from 0.61 to 0.78 mg/kg for the banana pulp.

Soil: The TRR in the 0-8, 8-15, and 15-30 cm (pot bottom) soil of the [triazole-3,5-^{14}C]-metconazole treated plots was 0.103, 0.011 and <0.005 mg/kg, respectively. The TRR in the 0-8, 8-15, and 15-30 cm soil of the [chlorophenyl-U-^{14}C]-metconazole treated plots was 0.762, 0.058 and 0.023 mg/kg, respectively.

Table 6.2.1-2: Total radioactive residues (TRR) of [^{14}C]-metconazole in pulp, peel and whole banana fruit and soil following 5 foliar applications

TRRs in treated banana and soil				
Matrix	**DAFT/ Soil depth [cm]**		**TRR [mg/kg]**	
			(Triazole-3,5-^{14}C)-metconazole (5 x 143 g a.s./ha)	**(*p*-Chlorophenyl-U-^{14}C)-metconazole (5 x 139 g a.s./ha)**
Pulp	56		0.61	0.78
Peel	56		1.62	2.54
Whole fruit	56		1.37	0.93
Soil	0 DAFT	0-8	<0.005	<0.004
		8-15	<0.005	<0.004
		15-30	<0.005	<0.004
	56 DAFT	0-8	0.103	0.762
		8-15	0.011	0.058
		15-30	<0.005	0.023

DAFT = Days after first treatment, corresponds to 2 h after the last treatment

B. EXTRACTION, CHARACTERISATION AND IDENTIFICATION OF RESIDUES

1. Extraction of residues in banana

The banana fruit samples were initially extracted with methanol. The extractability of the [^{14}C]-metconazole derived radioactivity using organic solvents (methanol) ranged from 86 to 93% of TRR. Further extraction of the residual radioactive residues (RRR) with 2% HC1 in methanol resulted in extracting additional 3-12% of the total radioactivity for all samples. The total extractability for all the samples ranged from 96 to 98% of TRR.

Table 6.2.1-3: Extractability of the total radioactivity of [^{14}C]-metconazole in pulp, peel and whole banana fruit following 5 foliar applications

Matrix	DAFT	TRR calc. *	Distribution of radioactive residues				ERR		RRR	
			Methanol		2% HCL in methanol					
		[mg/kg]	[mg/kg]	[%TRR]	[mg/kg]	[%TRR]	[mg/kg]	[%TRR]	[mg/kg]	[%TRR]
[Triazole-3,5-^{14}C]-metconazole: 5 x 143 g a.s./ha										
Pulp	56	0.61	0.54	89.16	0.06	9.04	0.599	98.20	0.01	1.80
Peel	56	1.62	1.52	93.77	0.05	2.86	1.565	96.63	0.05	3.37
Whole fruit	56	1.37	1.27	93.02	0.05	3.35	1.320	96.37	0.05	3.63
[*p*-Chlorophenyl-U-^{14}C]-metconazole: 5 x 139 g a.s./ha										
Pulp	56	0.78	0.67	86.22	0.09	12.11	0.767	98.33	0.01	1.67
Peel	56	2.54	2.37	93.27	0.09	3.36	2.454	96.66	0.09	3.38
Whole fruit	56	0.93	0.85	91.90	0.04	4.74	0.899	96.64	0.03	3.36

DAFT = Days after first treatment, corresponds to 2 h after the last treatment

* TRR was calculated as the sum of ERR + RRR

2. Identification and characterization of extractable residues in banana

A total of 13 [^{14}C]-metconazole derived residue components (BT-1 to BT-13 and BC-1 to BC-13) were separated by HPLC from each treatment. The identities of the main residue components were determined by HPLC retention time coincidence with known reference compounds, and where possible by mass spectrometric analysis of the isolate. The predominant residue in banana fruit was the unchanged metconazole (CL 900768) comprising up to 89% TRR (0.7 mg/kg) in pulp, up to 87% TRR (2.2 mg/kg) in peel, and up to 87% TRR (1.2 mg/kg) in whole banana fruit. In addition to the parent compound, many other minor residue components were detected, including monohydroxylated metconazole metabolites and a glucose conjugate of the monohydroxylated metconazole. Two of the monohydroxylated metabolites were identified as M1 (CL 359451) and M11 (CL 382390). Triazolyl alanine accounted for <0.02 mg/kg (<3.5% of TRR) in the pulp and was nearly undetectable in peel (<0.01 mg/kg). Other unidentified metabolites were each at <0.01-0.03 mg/kg. Approximately 96-98% TRR was identified or characterized.

Table 6.2.1-4: Residues of [triazole-3,5-^{14}C]-metconazole in pulp, peel and whole banana fruit following 5 foliar applications at a rate of 143 g a.s./ha

Test substance	[Triazole-3,5-^{14}C]-metconazole					
Sample	Pulp		Peel		Whole fruit	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
BT-12/BT-13[1] Metconazole (BAS 555F, CL 900768)	0.522	85.63	1.403	86.6	1.188	86.69
BT-1[3] (triazolyl alanine; unknown polar)	0.021	3.51	0.010	0.64	0.017	1.21
BT-2 (unknown[4])	<0.007	0.27	<0.007	0.00	<0.005	0.08
BT-3 (unknown[4])	<0.007	0.00	0.016	0.99	0.009	0.67
BT-4 (glucose conjugate of monohydroxylated metconazole	<0.007	0.00	0.012	0.74	0.006	0.47
BT-5 (unknown[4])	<0.007	0.09	0.010	0.59	<0.005	0.33
BT-6 (unknown[4])	<0.007	0.62	0.016	1.01	0.012	0.86
BT-7 (unknown[4])	<0.007	0.00	<0.007	0.13	<0.005	0.00
BT-8 (monohydroxylated metconazole) M1 (M555F001, CL 359451)	0.014	2.34	0.014	0.87	0.017	1.24
BT-9 (unknown[4])	<0.007	0.20	<0.007	0.17	<0.005	0.00
BT-10 (monohydroxylated metconazole)	<0.007	0.95	0.013	0.82	0.013	0.93
BT-11 (monohydroxylated metconazole) M11 (M555F011, CL 382390)	0.010	1.70	0.023	1.40	0.017	1.27
Others[2]	0.018	2.90	0.043	2.66	0.036	2.61
Total identified and/or characterized from ERR[5]	0.599	98.20	1.565	96.63	1.320	96.37
Unextractable (RRR)[6]	0.01	1.80	0.05	3.37	0.05	3.63
Grand total	0.61	100	1.62	100	1.37	100

1 BT-13 represents a tail end region of the BT-12. Mass spectrometry has proved that it is indeed one part of the BT-12.
2 Consists of multiple unresolved peak regions.
3 BT-1 consists of 2 components: triazolyl alanine & extremely polar unknown.
4 BT-2, BT-3, BT-5, BT-6, BT-7 and BT-9 are unknown metabolites; it is likely that these unknown metabolites are glucose conjugates of monohydroxylated metconazole and their corresponding monohydroxylated metconazole.
5 ERR = extractable radioactive residue
6 RRR = residual radioactive residue

Table 6.2.1-5: Residues of [*p*-chlorophenyl-U-^{14}C]-metconazole in pulp, peel and whole banana fruit following 5 foliar applications at a rate of 139 g a.s./ha

Test substance	[*p*-Chlorophenyl-U-^{14}C]-metconazole					
Sample	Pulp		Peel		Whole fruit	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
BC-12/BC-13[1] Metconazole (BAS 555 F, CL900768)	0.695	89.13	2.203	86.72	0.800	86.11
BC-1 (unknown polar[3])	<0.005	0.07	<0.005	0.13	<0.005	0.13
BC-2 (unknown[3])	<0.005	0.00	0.007	0.29	<0.005	0.28
BC-3 (unknown[3])	<0.005	0.00	0.032	1.28	0.012	1.25
BC-4 (unknown[3])	<0.005	0.00	0.016	0.63	<0.005	0.45
BC-5 (unknown[3])	<0.005	0.00	0.028	1.12	0.011	1.19
BC-6 (unknown[3])	<0.005	0.43	0.033	1.31	0.014	1.47
BC-7 (unknown[3])	<0.005	0.00	0.010	0.38	<0.005	0.08
BC-8 (monohydroxylated metconazole) M1 (M555F001, CL 359451)	0.014	1.74	0.022	0.87	0.009	0.96
BC-9 (unknown[3])	<0.005	0.13	0.010	0.41	<0.005	0.53
BC-10 (monohydroxylated metconazole)	0.014	1.81	0.035	0.45	0.012	1.30
BC-11 (monohydroxylated metconazole) M11 (M555F011, CL 382390)	0.016	2.05	0.040	1.56	0.016	1.72
Others[2]	0.023	2.98	0.015	1.50	0.011	1.15
Total identified and/or characterized from ERR[4]	0.767	98.33	2.454	96.66	0.899	96.64
Unextractable (RRR)[5]	0.01	1.67	0.09	3.38	0.03	3.36
Grand total	0.78	100	2.54	100.0	0.93	100

1 BC-13 represents a tail end region of the BC-12. Mass spectrometry has proved that it is indeed one part of the BC-12.
2 Consist of multiple unresolved peak regions.
3 BC-1, BC-2, BC-3, BC-4, BC-5, BC-6, BC-7 and BC-9 are unknown metabolites; it is likely that these unknown metabolites are glucose conjugates of monohydroxylated CL900768 and their corresponding monohydroxylated metconazole.
4 ERR = extractable radioactive residue
5 RRR = residual radioactive residue

3. Proposed metabolic pathway

On the basis of the identified residue components, it can be concluded that the principle route of metabolism for metconazole in banana is through oxidative hydroxylation of the methylene groups and of the methyl groups on the cyclopentyl ring, and possibly of the cyclopentyl ring itself. The detection of triazolyl alanine suggests that the methylene group between the triazole and cyclopentyl rings is also susceptible to oxidative hydroxylation. It appeared that the monohydroxylated metabolite was readily conjugated with glucose to form a glucoside as the terminal residue in the banana fruit. The proposed metabolic pathway is shown in Figure 6.2.1-1.

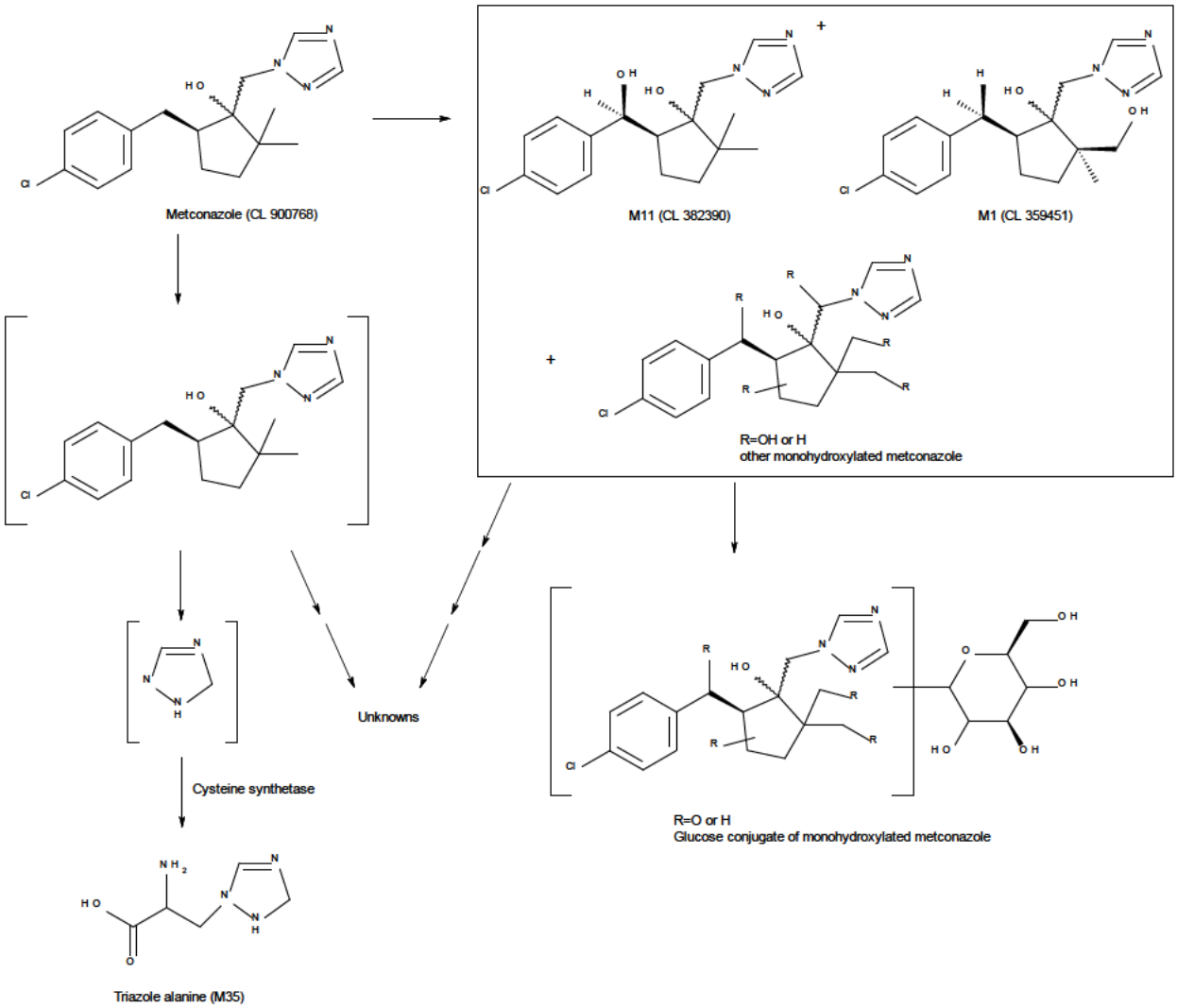
4. Storage stability

The fruit samples were analyzed after 3 to 72 days in storage at a temperature range of 0°C to -40°C. The metabolite profile of the [^{14}C]-metconazole derived residues were analyzed by HPLC 66-77 days after storage. Reanalysis of the same extract stored in a refrigerator for 171 ([chlorophenyl-U-^{14}C]-metconazole) or 196 days ([triazole-3,5-^{14}C]-metconazole) showed that the HPLC profile was nearly unchanged. The results indicate that the [^{14}C]-metconazole derived residues in banana were unchanged under the storage conditions. These intervals cover the period of storage of extracts prior to analysis.

III. CONCLUSION

Total radioactive residues in banana were found ranging from 0.93 to 1.37 mg/kg for whole fruit, from 1.62 to 2.54 mg/kg for banana peel and from 0.61 to 0.78 mg/kg for banana pulp after five foliar applications of [^{14}C]-metconazole. The [triazole-3,5^{14}C]- and [*p*-chlorophenyl-U-^{14}C]-metconazole was applied at an average treatment rate of 139-143 g a.s./ha/application at the flowering stage.
Unchanged metconazole is the predominant residue in banana fruit. Other residue components in banana were comprised of monohydroxylated metconazole which included M11 (CL 382390) and M1 (CL 359451), the glucose conjugate of monohydroxylated metconazole and triazolyl alanine.
On the basis of the identified residue components, it can be concluded that the principle route of metabolism for metconazole in banana is through oxidative hydroxylation of the methylene groups and of the methyl groups on the cyclopentyl ring, and possibly of the cyclopentyl ring itself. The detection of triazolyl alanine suggests that the methylene group between the triazole and cyclopentyl rings is also susceptible to oxidative hydroxylation. The metabolism and metabolic pathway of metconazole in banana is very similar to that found in oilseed rape, wheat and the rat. The metabolite M11 (CL 382390) was not identified in the rat metabolism study; however, it was shown to be non-toxic in an acute oral study in rats. Triazolyl alanine was also shown to be non-toxic. Thus, the unchanged metconazole is considered to be the only significant residue found in banana.

Figure 6.2.1-1: Proposed metabolic pathway of metconazole in banana



CA 6.2.2 Poultry

The nature of metconazole residues in poultry was investigated in the framework of Directive 91/414/EEC. Reported metabolism studies include two studies in laying hens using ^{14}C-cyclopentyl metconazole. Further details are presented in chapter M-CA 6.2 and in the table below.

Table 6.2.2-1: Summary of peer reviewed metabolism studies in laying hens

Group	Species	Label position	No of animals	Application details		Sample details	
				Rate (mg/kg bw/d)	Duration	Commodity	Time
Laying poultry	Hens	^{14}C-cyclopentyl, *cis*-isomer of metconazole	Five groups of 3 hens	1.5 mg/bird/day (10 mg/kg feed)	28 days	Eggs	Daily
						Excreta	Daily
						Tissues	After sacrifice (6, 18, 48, 96 and 144 h after last dose)
		^{14}C-cyclopentyl *cis*-isomer of metconazole			14 days	Eggs	Daily
						Excreta	Daily
						Tissues	After sacrifice (6 h after last dose)

As the metabolic pathway of metconazole residues in laying hens was not established during the previous active substance approval, an additional metabolism study on laying hens is summarized below.

Report: CA 6.2.2/1
[redacted] 2006a
Metconazole (KNF-S-474m): Metabolism by laying hens
2006/1046073
Guidelines: EPA 860.1300
GLP: yes
(certified by Japan Ministry of Agriculture, Forestry and Fisheries, Japan)

Executive Summary

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

Description:	Metconazole (BAS 555 F)
Lot/Batch #:	CP-2397 (cyclopentyl-^{14}C) CFQ12045 (triazole-3/5-^{14}C) AS2122a (unlabeled)
Purity:	99.9% (radiochemical), 5.71 MBq/mg (cyclopentyl-1-^{14}C); mixture of ^{14}C/^{12}C: 99.9%, 2.84 MBq/mg 99.9% (radiochemical), 6.44 MBq/mg (triazole-3/5-^{14}C); mixture of ^{14}C/^{12}C: 99.8%, 2.84 MBq/mg 98.7% (unlabeled)
CAS#:	125116-23-6
Development code:	KNF-S-474
Stability of test compound:	The test item was stable for the test period

2. Test Animals

Species:	Hen
Variety:	White Leghorn
Gender:	Female
Age:	Approximately 24 weeks
Weight at dosing:	1.427-1.799 kg (Day 1)
Number of animals:	13 (5 per label and 3 control)
Acclimation period:	14 days
Diet:	Poultry diet, offered as needed
Water:	Fresh potable water, *ad libitum*
Housing:	Individual cages
Environmental conditions -	
Temperature:	18-20°C
Humidity:	41-62%
Photoperiod:	14 h light / 10 h dark

B. STUDY DESIGN AND METHODS

1. Dosing regime

Oral:	**Amount of dose:**	10 mg/kg feed/day (nominal)
	Food consumption:	76-108 g/animal/day (treatment period)
	Vehicle:	Gelatin capsule by oral gavage
	Timing:	Twice daily
	Duration:	4.5 days

2. Sample collection

Egg collection:	Twice daily
Excreta collection:	Daily
Interval from last dose to sacrifice:	4.5 h
Tissues harvested & analyzed:	Eggs, liver, breast / thigh muscle, skin, abdominal fat, blood, excreta

3. Test system

The metabolism and distribution of metconazole was investigated in five laying hens per label following a repeated oral administration of triazole-^{14}C- or cyclopentyl-^{14}C-metconazole at a dose level of 10 mg/kg feed for 4.5 consecutive days. The test item was prepared in gelatin capsules and administered orally by gavage. The mean daily dose administered was 14.0 (cyclopentyl label) and 12.6 mg/kg food consumed (triazole label). Details of the study outline are summarized in Table 6.2.1-2.

Table 6.2.2-2: Dosing of laying hens with ^{14}C-metconazole

Animal	Treatment days	Nominal daily dose	Actual daily dose (mean)	
		mg/kg feed intake	mg/kg feed intake (treatment phase)	mg/kg bw[1]
Cyclopentyl label				
165	4.5	10	14.8	0.67
161	4.5	10	14.9	0.76
174	4.5	10	14.3	0.73
173	4.5	10	15.7	0.74
177	4.5	10	11.3	0.74
Average	4.5	10	14.0	0.73
Triazole label				
170	4.5	10	13.2	0.81
171	4.5	10	13.2	0.82
178	4.5	10	14.6	0.69
166	4.5	10	11.2	0.82
175	4.5	10	11.5	0.73
Average	4.5	10	12.6	0.75

1 Based on mean body weight on study days 1 and 5 as well as on total test item in capsule (2 x 0.6 mg)

4. Sampling and Storage

Eggs produced by each group of hens were collected for analysis twice daily (8-12 hours apart) between dosing Days 1 and 4. On dosing Day 5, the eggs were collected in the morning before termination. The afternoon eggs were collected, weighed and refrigerated overnight then combined with the weighed eggs of the following morning to generate one 24-hour (daily) sample. Eggs from each 24-hour sample from each group were separated into yolks and whites, which were weighed separately and mixed thoroughly. The egg samples were stored in a freezer until shipment.
Collections of excreta began on study Day 1 and continued until the scheduled termination of the treated hens. Excreta collected in 24-hour periods from each group of treated hens were weighed. Excreta was collected and weighed from the control group, but was not retained for analysis. Following termination, all excreta samples were separately homogenized and stored frozen until shipment.
The hens were terminated approximately 4.5 hours following the final dose. Following necropsy, samples of blood, abdominal fat, skin with fat, breast muscle, thigh muscle and liver (entire), were collected for analysis. Blood samples were collected in 10 mL sterile blood collection tubes containing sodium heparin and stored in a refrigerator. The tissues collected from each group of hens were weighed, homogenized and stored in a freezer until shipment.
The test and reference substance solutions, dosing capsules, and all animal samples were stored in freezers set at a nominal temperature of -20°C.
The processing (homogenization with dry ice) of the tissue samples was done within 2 days of termination. The combustion analysis of the hen samples started within 2 weeks, and the extraction of the tissue samples was started approximately a month after termination.

5. Description of analytical methods

For the determination of the measured TRR, tissue samples were combusted by means of a sample oxidizer before LSC measurement. To quantify ^{14}C-radioactivity in solutions, samples were mixed with a suitable scintillation cocktail before LSC analysis.
Thin layer chromatography was performed on silica gel plates. Following development and evaporation of the solvent, plates were observed under UV light to visualize the non-radiolabeled reference standard spots against the fluorescent background of the plate. The radioactive spots on TLC plates were revealed by Phosphor-imaging.
HPLC was used to determine radiochemical purity of the ^{14}C test materials, to analyze the test samples, to identify metconazole and its metabolites based on cochromatography with and retention time correspondence to standards, to quantify the percentage distribution of radioactivity between different peaks, and to separate and isolate individual radioactive peaks. LC-MS and LC-MS^n analyses of the isolated metabolites and metconazole standards were performed on a LC-MS connected to a HPLC system fitted with a UV detector (230 nm).
^1H NMR spectroscopy of the isolated metabolites and selected reference standards was performed. In addition to 1D ^1H NMR experiments, 2D COSY and NOESY experiments were carried out on the isolated metabolite samples.

Samples of tissues and eggs which contained significant radioactive residues based on TRR analysis were extracted with acetonitrile and water to determine the distribution of soluble and insoluble residues and to identify and characterize the radioactive metabolite components. The residual radioactive residue (RRR) was quantified by combustion analysis.

A number of metabolites present in the unhydrolyzed and hydrolyzed excreta extracts were isolated using a series of chromatographic steps, and were identified by combinations of ^{1}H NMR spectroscopy, mass spectrometry, and co-chromatography with standards. The metabolites in the tissue and egg samples were identified by chromatographic correlation with these identified excreta metabolites.

II. RESULTS AND DISCUSSION

A. TOTAL RADIOACTIVE RESIDUES (TRRs)

Based on total radioactive residue (TRR) concentrations, the administered radioactivity was rapidly eliminated from the treated hens via excreta. TRR level in excreta was high from treatment Day 1 and varied from 10.7 to 19.4 mg/kg during the treatment period (Table 6.2.2-3). The low level of TRR in blood samples (0.08 mg/kg in the cyclopentyl label and 0.18 mg/kg in the triazole label) was indicative of low absorption and transport of the test substance within the treated animals. TRR levels in the muscle and fat tissues (0.03-0.09 mg/kg in the cyclopentyl label and 0.13-0.14 mg/kg in the triazole label) were either lower or equal to the TRR concentration in whole blood suggesting that metconazole residue would not accumulate in these tissues. The residue levels in egg white reached a plateau in 3 days in the cyclopentyl label (0.049 mg/kg) and in 4 days in the triazole label (0.173 mg/kg). The maximum residue in egg yolk was 0.096 mg/kg in the cyclopentyl label and 0.163 mg/kg in the triazole label on Day 5. These results indicated that the transfer of radioactive residues from oral administration of [^{14}C]-metconazole into egg was low. The TRR levels in treated liver from both labels were in the range of 0.75-0.90 mg/kg.

Table 6.2.2-3: Total radioactive residues in edible matrices after dosing of laying hens with [cyclopentyl-^{14}C]- or [triazole-^{14}C]-metconazole

Matrix	TRR [mg/kg]			
	Measured	Calculated	Measured	Calculated
	Cyclopentyl label		Triazole label	
Breast muscle	0.030	0.031	0.138	0.145
Thigh muscle	0.045	0.049	0.140	0.152
Liver	0.745	0.790	0.901	0.972
Skin	0.070	0.075	0.127	0.137
Abdominal fat	0.093	0.091	0.131	0.141
Egg white day -1	<0.001	0.047	<0.001	0.186
Egg white day 1	<0.001		0.017	
Egg white day 2	0.037		0.088	
Egg white day 3	0.049		0.139	
Egg white day 4	0.041		0.173	
Egg white day 5	0.046		0.174	
Egg yolk day -1	<0.001	0.088	<0.001	0.156
Egg yolk day 1	<0.001		0.005	
Egg yolk day 2	0.011		0.036	
Egg yolk day 3	0.032		0.092	
Egg yolk day 4	0.066		0.126	
Egg yolk day 5	0.096		0.163	
Blood plasma	0.078	n r.	0.183	n r.
Blood cells	0.08	n r.	0.184	n r.
Whole blood	0.080	n r.	0.184	n r.
Excreta day 1	10.748	15.007	13.849	16.005
Excreta day 2	11.325		16.888	
Excreta day 3	12.610		17.501	
Excreta day 4	14.655		19.445	
Excreta day 5	14.685		14.463	

TRR Total radioactive residue
Calculated Sum of ERR + RRR
n.r. Not reported

B. EXTRACTION OF RESIDUES

Based on the TRR analysis, liver, breast muscle, thigh muscle, fat, skin, egg white and egg yolk samples from both labels were extracted and the extract analyzed for the identity and distribution of metabolites. Almost the entire radioactive residue in tissue and egg samples (86-99%) was extractable with acetonitrile (with or without hexane) and water (see Table 6.2.2-4).

Table 6.2.2-4: Extractability of edible matrices with solvents after dosing of laying hens with [cyclopentyl-^{14}C]- or [triazole^{14}C]-metconazole

Matrix	TRR (calculated) mg/kg	Acetonitrile/ hexane extract		Aqueous extract		Solvent extract (ERR)		RRR	
		mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
Cyclopentyl label									
Breast muscle	0.031	0.028	89.4	0.001	4.0	0.029	93.4	0.002	6.6
Thigh muscle	0.049	0.042	87.2	0.003	5.9	0.045	93.2	0.003	6.8
Liver	0.790	0.641	81.1	0.081	10.3	0.722	91.4	0.068	8.6
Skin	0.075	0.066	87.5	0.005	6.8	0.071	94.3	0.004	5.7
Fat	0.091	0.086	94.5	0.004	3.9	0.089	98.4	0.001	1.6
Egg white	0.047	0.045	95.8	0.001	2.2	0.046	98.0	0.001	2.0
Egg yolk	0.088	0.070	78.8	0.006	7.1	0.076	85.9	0.012	14.1
Excreta	15.007	9.413	62.7	5.277	35.2	14.690	97.9	0.317	2.1
Triazole label									
Breast muscle	0.145	0.128	87.8	0.013	8.6	0.140	96.4	0.005	3.6
Thigh muscle	0.152	0.138	90.5	0.010	6.6	0.148	97.1	0.004	2.9
Liver	0.972	0.798	82.1	0.098	10.1	0.895	92.1	0.077	7.9
Skin	0.137	0.124	90.9	0.007	5.1	0.131	96.0	0.005	4.0
Fat	0.141	0.126	89.3	0.012	8.7	0.138	98.0	0.003	2.0
Egg white	0.186	0.182	97.8	0.003	1.6	0.185	99.4	0.001	0.6
Egg yolk	0.156	0.138	88.7	0.007	4.2	0.145	92.9	0.011	7.1
Excreta	16.005	9.884	61.8	5.750	35.9	15.634	97.7	0.371	2.3

TRR: Total radioactive residue (sum of ERR + RRR)
ERR Extractable radioactive residue
RRR: Residual radioactive residue after solvent extraction

C. IDENTIFICATION AND CHARACTERIZATION OF RESIDUES

A summary of all identified metabolites and their distribution in egg, muscle, fat, skin and liver is given in Table 6.2.2-5 and Table 6.2.2-6.

The parent compound was a minor residue in liver, muscles and egg samples. The total residues of parent were 1.7-4.3% (0.017-0.034 mg/kg) in liver, 0.8-9.8% (0.001-0.005 mg/kg) in breast and thigh muscle, and 2.5-11.4% (0.003-0.010 mg/kg) in the egg white and egg yolk samples from both labels. In fat and skin tissues, parent compound was a major residue and accounted for 19.7-36.7% of TRR (0.02-0.05 mg/kg).

Most of the radioactive residues in hen liver were conjugated metabolites, which released their metconazole moieties after acid hydrolysis. One of the conjugates in liver, which was present as a major metabolite in excreta, was the sulfate ester of 3-hydroxy metconazole, M-32. Other sulfate esters of mono- and di-hydroxy metconazole derivatives (MHM and DHM) were present in liver samples. The metabolites M-1, M-12, M-31 and M-32, with traces of other isomers of M-12 (CM-1 to 3), were also present in liver samples. The extracts of the triazole label liver contained 1,2,4-triazole as the single major metabolite accounting for 27.3% of TRR (0.265 mg/kg).
The only significant residue in breast and thigh muscle was 1,2,4-triazole from the triazole label samples. This metabolite accounted for 77.2% of TRR (0.112 mg/kg) in the breast muscle and 75.2% of TRR (0.114 mg/kg) in the thigh muscle. None of the other metabolites, including the conjugates, individually exceeded 0.008 mg/kg of residue.
The concentration of 1,2,4-triazole was lower in fat and skin tissues compared to other hen tissues. This metabolite accounted for 13.3% of TRR (0.019 mg/kg) in the abdominal fat and 46.7% of TRR (0.064 mg/kg) in the skin. M-1 and M-31 together made up the other major residue in the fat and skin tissues. These metabolites accounted for 24.1-27.7% (0.018-0.025 mg/kg) of the total residue in the cyclopentyl label and 10.6-13.4% (0.015-0.019 mg/kg) of the total residue in the triazole label fat and skin tissues. None of the other metabolites, including the conjugates, individually exceeded 0.01 mg/kg of the residue in both labels.
The major triazole labeled residue, 1,2,4-triazole, accounted for 64.8% of TRR (0.120 mg/kg) in the egg white, 42.9% of TRR (0.067 mg/kg) in the egg yolk and 58.1% of TRR (0.104 mg/kg) in whole egg. M-1 and M-31 together accounted for 16.5% (0.010 mg/kg) of total residue in the cyclopentyl label and 8.2% (0.014 mg/kg) of total residue in the triazole label eggs. None of the other metabolites, including the conjugates, individually exceeded 0.01 mg/kg of residue in both labels.

Table 6.2.2-5: Summary of identified and characterized residues in edible matrices of laying hens after dosing with [cyclopentyl-^{14}C]-metconazole

Components	Liver (pre-hydrolysis)		Liver (post-hydrolysis)		Breast muscle		Thigh muscle		Abdominal fat		Skin with fat		Egg white[1]		Egg yolk[1]		Whole egg[1]	
	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]
Cis-metconazole	0.031	3.9	-	-	0.001	1.8	0.002	3.7	0.017	19.0	0.011	14.4	0.001	2.1	0.004	4.4	0.002	2.8
Trans-metconazole	0.003	0.4	-	-	0.001	3.6	0.003	6.0	0.016	17.7	0.010	13.4	0.002	5.2	0.006	7.1	0.004	5.8
Total metconazole	0.034	4.3	0.033	4.1	0.002	5.4	0.005	9.8	0.033	36.7	0.021	27.8	0.003	7.3	0.010	11.4	0.006	8.6
M-1	0.100	12.7	0.128	16.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
M-1 (+ M-31)	-	-	-	-	0.006	19.9	0.008	15.7	0.025	27.7	0.018	24.1	0.007	15.7	0.016	18.2	0.010	16.5
M-12 (+ CM-2/3)	0.033	4.1	0.091	11.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-
M-31	0.057	7.2	0.064	8.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
M-32 (+ CM-1)	0.047	5.9	0.032	4.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
M-32 sulfate	0.031	3.9	-	-	0.003	10.3	0.004	7.9	0.001	1.6	0.003	3.7	0.008	16.7	0.007	7.9	0.008	13.9
CM-2	-	-	-	-	0.003	8.5	0.005	10.2	0.009	9.6	0.007	9.6	0.008	17.1	0.006	6.6	0.007	13.7
DHM sulfate	0.015	1.9	-	-	0.003	9.9	0.008	16.8	-	-	0.002	2.3	-	-	0.003	2.8	0.001	0.9
MHM sulfate	0.012	1.5	-	-	0.006	18.0	0.006	12.9	0.004	4.5	0.005	6.0	0.008	16.3	0.012	13.1	0.009	15.3
MHM-4	-	-	-	-	0.002	7.7	0.003	6.1	0.003	3.5	0.004	5.3	-	-	-	-	-	-
MHM (1-2 isomers)	-	-	-	-	-	-	-	-	-	-	-	-	0.003	7.1	0.007	8.4	0.005	7.5
DCM	-	-	0.073	9.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CHM (1-4 isomers)	0.007	0.9	0.044	5.6	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DHM (2 isomers) (may contain a trace of OCM)	-	-	0.017	2.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
MHM (4-6 isomers)	0.043	5.5	0.104	13.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Total identified	**0.378**	**47.9**	**0.585**	**74.1**	**0.025**	**79.6**	**0.039**	**79.5**	**0.076**	**83.7**	**0.059**	**78.8**	**0.038**	**80.1**	**0.060**	**68.4**	**0.045**	**76.3**
Unidentified conjugates	0.303	38.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hexane-soluble unidentified	0.002	0.2	-	-	-	-	-	-	0.008	9.1	0.002	2.5	-	-	0.001	0.6	0.0002	0.2
Unidentified	0.039	5.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 6.2.2-5: Summary of identified and characterized residues in edible matrices of laying hens after dosing with [cyclopentyl-^{14}C]-metconazole

Components	Liver (pre-hydrolysis)		Liver (post-hydrolysis)		Breast muscle		Thigh muscle		Abdominal fat		Skin with fat		Egg white[1]		Egg yolk[1]		Whole egg[1]	
	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]
precipitate from - aqueous extract																		
Other unidentified components (>10)	-	-	0.137	17.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Acetonitrile-soluble unidentified	-	-	-	-	0.003	9.8	0.004	7.8	0.002	1.7	0.005	6.2	0.007	15.8	0.009	9.7	0.008	13.8
Water-soluble unidentified	-	-	-	-	0.001	4.0	0.003	5.9	0.004	3.9	0.005	6.8	0.001	2.2	0.006	7.1	0.003	3.8
EtOAC-soluble hydrolysate	-	-	0.021	2.7	-	-	-	-	-	-	-	-	-	-	0.003	3.7	-	-
Water-soluble hydrolysate	-	-	0.031	3.9	-	-	-	-	-	-	-	-	-	-	0.005	5.8	-	-
Total characterized	**0.344**	**43.5**	**0.188**	**23.9**	**0.004**	**13.8**	**0.007**	**13.7**	**0.014**	**14.7**	**0.012**	**15.5**	**0.008**	**17.9**	**0.024**	**26.9**	**0.010**	**17.8**
Total identified and/or characterized	**0.722**	**91.4**	**0.774**	**98.0**	**0.029**	**93.4**	**0.045**	**93.2**	**0.089**	**98.4**	**0.071**	**94.3**	**0.046**	**98.0**	**0.084**	**95.3**	**0.055**	**94.1**
Final residue (RRR)	0.068	8.6	0.016	2.0	0.002	6.6	0.003	6.8	0.001	1.6	0.004	5.7	0.001	2.0	0.004	4.7	0.005	5.9
Grand total	**0.79**	**100**	**0.79**	**100**	**0.031**	**100**	**0.049**	**100**	**0.091**	**100**	**0.075**	**100**	**0.047**	**100**	**0.088**	**100**	**0.060**	**100**

TRR Total radioactive residue (sum of ERR + RRR)
CHM Metconazole carboxylic acid
DCM Metconazole dicarboxylic acid
DHM Di-hydroxy metconazole
MHM Mono-hydroxy metconazole
1 Analysis was done with Day 4 and Day 5 samples combined
- Not applicable or not detected

Table 6.2.2-6: Summary of identified and characterized residues in edible matrices of laying hens after dosing with [triazole-^{14}C]-metconazole

Components	Liver (post-hydrolysis)		Breast muscle		Thigh muscle		Abdominal fat		Skin with fat		Egg white[1]		Egg yolk[1]		Whole egg[1]	
	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]
Cis-metconazole	-	-	-	-	0.003	1.7	0.025	18.0	0.014	10.2	0.001	0.8	0.003	2.0	0.002	1.2
Trans-metconazole	-	-	0.001	0.8	0.002	1.5	0.024	17.3	0.013	9.5	0.003	1.7	0.007	4.6	0.004	2.6
Total metconazole	0.017	1.7	0.001	0.8	0.005	3.3	0.050	35.3	0.027	19.7	0.005	2.5	0.010	6.6	0.006	3.7
1,2,4-triazole	0.265	27.3	0.112	77.2	0.114	75.2	0.019	13.3	0.064	46.7	0.120	64.8	0.067	42.9	0.104	58.1
M-1	0.094	9.7	-	-	-	-	-	-	-	-	-	-	-	-	-	-
M-1 (+ M-31)	-	-	0.006	3.8	0.006	4.0	0.019	13.4	0.015	10.6	0.011	6.2	0.020	12.8	0.014	8.2
M-12 (+ CM-2/3)	0.081	8.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-
M-31	0.049	5.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
M-32 (+ CM-1)	0.039	4.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
M-32 sulfate	-	-	0.003	2.1	0.006	3.8	0.014	9.7	0.004	2.8	0.007	3.9	0.005	3.1	0.007	3.7
CM-2	-	-	0.002	1.6	0.003	1.9	0.007	5.0	0.004	2.6	0.009	4.8	0.004	2.8	0.008	4.2
DHM sulfate	-	-	0.003	1.9	0.002	1.3	0.006	4.0	-	-	-	-	0.002	1.3	0.001	0.4
MHM sulfate	-	-	0.005	3.7	0.004	2.4	0.009	6.4	0.003	2.5	0.010	5.3	0.013	8.3	0.011	6.3
MHM-4	-	-	0.002	1.4	0.002	1.5	0.004	2.6	0.003	1.9	0.005	2.5	-	-	-	-
MHM (1-2 isomers)	-	-	-	-	-	-	-	-	-	-	-	-	0.007	4.5	0.005	3.1
DCM	0.070	7.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CHM (1-4 isomers)	0.048	4.9	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DHM (2 isomers) (may contain a trace of OCM)	0.018	1.9	-	-	-	-	-	-	-	-	-	-	-	-	-	-
MHM (4-6 isomers)	0.094	9.7	-	-	-	-	-	-	-	-	-	-	-	-	-	-
OCM	0.005	0.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Total identified	**0.781**	**80.3**	**0.134**	**92.5**	**0.142**	**93.3**	**0.126**	**89.7**	**0.119**	**86.8**	**0.167**	**90.0**	**0.128**	**82.3**	**0.155**	**87.7**
Hexane-soluble unidentified	-	-	-	-	-	-	0.011	7.8	0.002	1.3	-	-	0.000	0.2	0.0001	0.1
Other unidentified components (>10)	0.115	11.8	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 6.2.2-6: Summary of identified and characterized residues in edible matrices of laying hens after dosing with [triazole-^{14}C]-metconazole

Components	Liver (post-hydrolysis)		Breast muscle		Thigh muscle		Abdominal fat		Skin with fat		Egg white[1]		Egg yolk[1]		Whole egg[1]	
	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]
Acetonitrile-soluble unidentified	-	-	0.002	1.6	0.003	1.7	0.001	0.5	0.004	2.8	0.014	7.8	0.010	6.2	0.013	7.3
Water-soluble unidentified	-	-	0.003	2.4	0.003	2.1	-	-	0.007	5.1	0.003	1.6	0.007	4.2	0.004	2.4
EtOAC-soluble hydrolysate	0.025	2.6	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Water-soluble hydrolysate	0.036	3.7	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Total characterized	**0.176**	**18.1**	**0.006**	**3.9**	**0.006**	**3.8**	**0.012**	**8.4**	**0.013**	**9.2**	**0.018**	**9.4**	**0.017**	**10.6**	**0.017**	**9.7**
Total identified and/or characterized	**0.957**	**98.4**	**0.140**	**96.4**	**0.148**	**97.1**	**0.138**	**98.0**	**0.131**	**96.0**	**0.185**	**99.4**	**0.145**	**92.9**	**0.173**	**97.4**
Final residue (RRR)	0.015	1.6	0.005	3.6	0.004	2.9	0.003	2.0	0.005	4.0	0.001	0.6	0.011	7.1	0.004	2.6
Grand total	**0.972**	**100**	**0.145**	**100**	**0.152**	**100**	**0.141**	**100**	**0.137**	**100**	**0.186**	**100**	**0.156**	**100**	**0.177**	**100**

TRR Total radioactive residue (sum of ERR + RRR)
1 Analysis was done with Day 4 and Day 5 samples combined
- Not applicable or not detected

1. Metabolic pathway

The proposed metabolic pathway of metconazole in hens is shown in Figure 6.2.2-1. Metconazole was extensively metabolized in hen by several major pathways, which involved (i) oxidation of the methyl group at position 2 in the cyclopentyl ring forming either the carboxylic acid derivative, M-12, and its isomers (M-13, CM-1 to 3), or the hydroxymethyl derivative, M-1, and its isomers (one or more of MHM-1 to 6); (ii) oxidation of the methylene group at position 3 of the cyclopentyl ring, forming M-31 and M-32 and their isomers (one or more of MHM-1 to 6); (iii) oxidation of M-1, M-12, M-31, M-32 and their isomers to dihydroxy metconazole (DHM), monohydroxy metconazole carboxylic acid (CHM) and metconazole dicarboxylic acid (DCM); (iv) sulfate ester formation of the hydroxylated metconazole derivatives; and (v) C-N bond cleavage to release 1,2,4-triazole from metconazole and likely from its metabolites. The metabolites were more water-soluble than the parent resulting in their rapid elimination from the hens via excreta.

2. Enantiomer ratio

The test materials used in this study contained a nominal *cis*:*trans* ratio of 85:15 (83-85 : 15-17 based on radiochemical analysis). The *trans* and the *cis* isomers of metconazole were present in approximately equal ratios in fat although the test substance administered to the hens contained a much higher ratio of the *cis* isomer (83-85%) compared to that of the *trans* isomer (15-17%). The results indicated that the *cis* isomer was preferentially metabolized in hen.

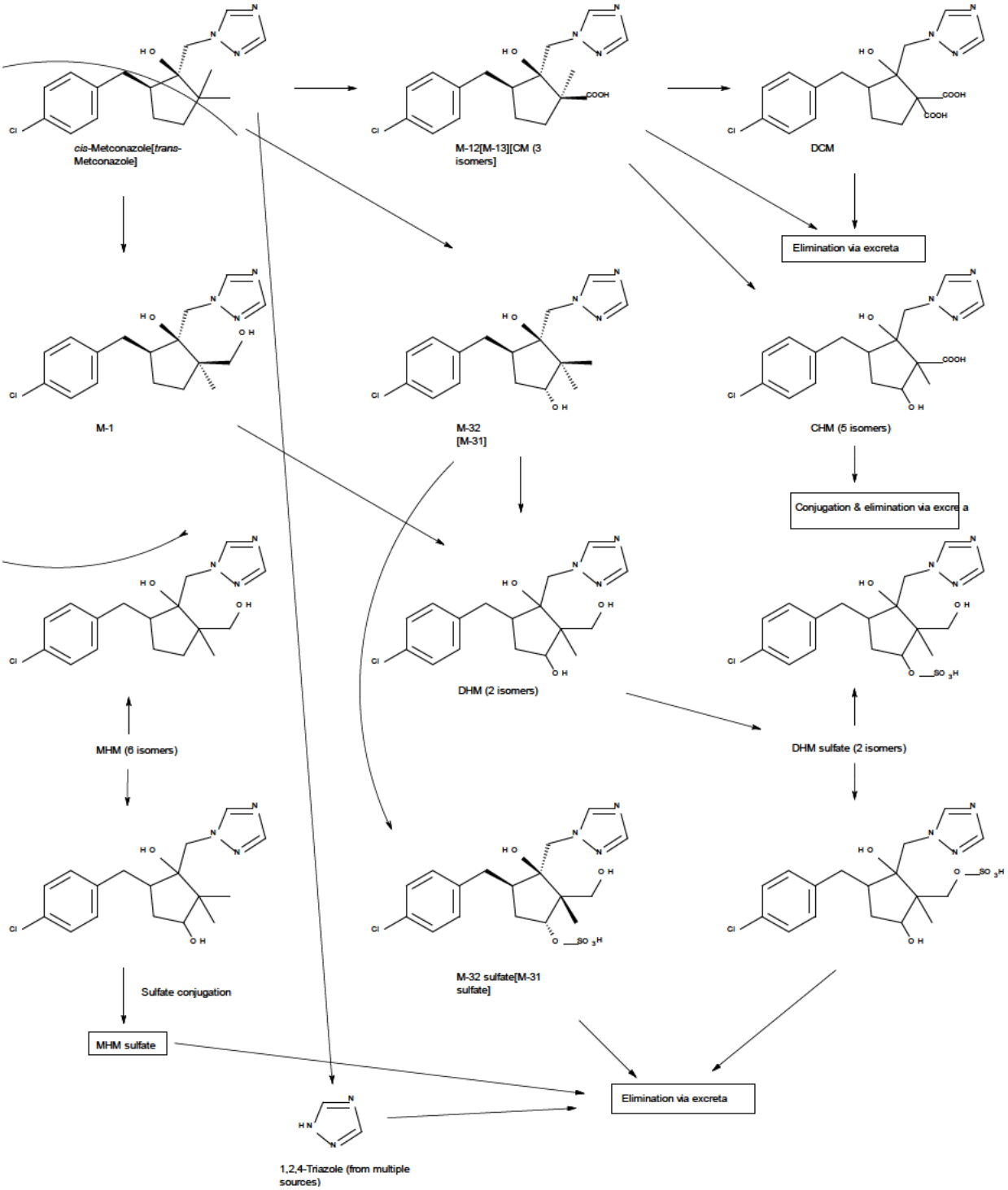
3. Storage stability

To determine longer-term storage stability, representative tissue samples were re-extracted and analyzed at the end of the analytical phase of the study after the samples were stored for about 10 months in the freezer. The metconazole residues in hen tissue and egg samples were stable in frozen storage during the 10-month course of the study.

III. CONCLUSION

Combustion analysis showed a large amount of the administered radiolabeled test substance was eliminated from the hens via excreta. The total radioactive residues of [^{14}C]-metconazole from both labels were low in egg white and yolk (<0.001-0.17 mg/kg), breast and thigh muscle (0.03-0.14 mg/kg), and fat and skin tissues (0.07-0.13 mg/kg). The TRR levels in treated liver were in the range of 0.75-0.90 mg/kg. The liver, breast muscle, thigh muscle, abdominal fat, skin, egg white and egg yolk from both labels were extracted and analyzed for the identity and distribution of metabolites.
The parent compound, metconazole, was a minor residue in liver, muscles and egg samples. The total residues of parent were 1.7-4.3% (0.017-0.034 mg/kg) in liver, 0.8-9.8% (0.001-0.005 mg/kg) in breast and thigh muscle, and 2.5-11.4% (0.003-0.010 mg/kg) in the egg white and yolk samples from both labels. Fat and skin contained 19.7-36.7% of TRR (0.021-0.050 mg/kg) as the parent residue. 1,2,4-triazole was a major residue in the triazole label samples and accounted for 13.3-77.2% of TRR (0.02-0.27 mg/kg). Hen samples contained M-1, M-12, M-31, M-32 and a number of minor hydroxylated and carboxylated metabolites, which formed sulfate ester conjugates before being eliminated from the hens via excreta. The metconazole residues in liver and kidney samples were stable in frozen storage during the 10-month course of the study.

Figure 6.2.2-1: Proposed metabolic pathway of BAS 555 F in laying hen



CA 6.2.3 Lactating ruminants

The nature of metconazole residues in commodities of animal origin was investigated in the framework of Directive 91/414/EEC. Reported metabolism studies include two studies in lactating goats using ^{14}C-cyclopentyl metconazole. Further details are presented in chapter M-CA 6.2 above and in the table below.

Table 6.2.3-1: Summary of peer reviewed metabolism studies in lactating goats

Group	Species	Label position	No of animals	Application details		Sample details	
				Rate (mg/kg bw/d)	Duration	Commodity	Time
Lactating ruminants	Goat	^{14}C-cyclopentyl *cis:trans* metconazole, *80:20*	2	Nominal 10 mg/kg feed Actual: 13.7 mg/kg (0.477 mg/kg bw/d) 25 mg/kg (0.479 mg/kg bw/d)	3 days (goat 1) 4 days (goat 2)	Milk	Twice daily (morning and afternoon)
						Urine and feces	Daily
						Tissues	After sacrifice (18 h after last administration)
		^{14}C-cyclopentyl *cis*-isomer of metconazole	1	Nominal 10 mg/kg feed Actual: 10.6 mg/kg (0.64 mg/kg bw/d)	4 days	Milk	Twice daily (morning and afternoon)
						Urine and feces	Daily
						Tissues	After sacrifice (16 h after last administration)

In the Conclusion 2006, EFSA noted that with regard to its log P_{ow}, metconazole is characterized as fat soluble (log P_{ow} 3.85 at 20°C, pH 7.2-8; effect of pH was not investigated since there is no dissociation in water in the environmentally relevant pH-range). However, TRR were roughly one order of magnitude higher in the excretory organs liver and kidney of lactating goats and laying hens than in other animal tissue, indicating that there might be no accumulation of residues in adipose tissue.

Due to the request of the RMS Belgium to submit all available livestock studies, a further goat metabolism study, performed with ^{14}C-triazole metconazole, is presented below.

Report:	CA 6.2.3/1 [redacted] 2006 a Metconazole (KNF-S-474m): Metabolism by lactation goats 2006/1046074
Guidelines:	EPA 860.1300
GLP:	yes (certified by United States Environmental Protection Agency)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test material

Description:		^{14}C-metconazole (triazole-^{14}C-label; cis:trans; 87:13) ^{12}C-metconazole (unlabeled; cis:trans; 85:15)
Lot/batch #:		CFQ12045 (triazole-^{14}C-label) AS2122a (unlabeled)
Purity:	Radiochemical purity:	99.9% (triazole-^{14}C-label)
	Chemical purity:	98.7% (unlabeled)
	Specific activity:	triazole-^{14}C-label 2.07 GBq/mmol (before isotopic dilution) 0.921 GBq/mmol (after isotopic dilution)
CAS#:		125116-23-6
Development code:		Not reported
Stability of test compound:		The test item was stable for the test period

2. Test animals

Species:	Goat
Variety:	Crossbred
Gender:	Female
Age:	2-4 years
Weight at dosing:	44 kg
Number of animals:	1 (+ 1 control)
Acclimation period:	14 days
Diet:	Goat Pellet 16% (Smith Feed Service/Vita Plus-Loyal, 213 E. Mill Street, Loyal, WI 54446) + alfalfa hay cubes (Manzanola Feeds, Manzanola, CO 81058) via permanent bins + fresh feed (grain and hay cubes) was offered twice daily.
Water:	potable water, *ad libitum*
Housing:	Metabolism cage

Environmental conditions	
Temperature:	13-28°C
Humidity:	67-86%
Photoperiod:	14 h light/10 h dark

B. STUDY DESIGN AND METHODS

1. Dosing regime

Oral:
Amount of dose: 10 mg/kg feed/day (nominal), 10.36 mg/kg feed /day (mean actual: 0.23 mg/kg bw per day)
Food consumption: 2.0 kg feed/animal/day
Vehicle: Gelatin capsule administered by using a balling gun
Timing: Twice daily
Duration: 4 consecutive days

2. Sample collection

Milk collection: Twice daily prior to treatment
Urine and feces collection: Daily prior to treatment
Interval from last dose to sacrifice: 17 h
Tissues harvested & analyzed: Blood, urine, feces, milk, bile, liver, kidney, muscle, fat and cage wash

3. Test system

The metabolism and distribution of metconazole was investigated in one lactating goat following repeated oral administration of triazole-^{14}C-metconazole (BAS 555 F) at an actual dose level of 10.36 mg/kg feed per day for four consecutive days. The test item was prepared in gelatin capsules and administered orally by a balling gun. Details of the study outline are summarized in Table 6.2.3-2.

Table 6.2.3-2: Dosing of lactating goat with ^{14}C-metconazole

Animal	Treatment days	Nominal daily dose		Actual daily dose		Sacrifice time after last dose (hours)
		mg/kg feed intake	mg/kg bw [1]	mg/kg feed intake	mg/kg bw [1]	
1	4	10 (2x5)	0.22 (2x0.11)	10.36 (2x5.18)	0.23 (2x0.115)	17

1 Body weight mean calculated based on weight on 1 day before dosing and day 4 of dosing = 45 kg

4. Sampling and storage

Blood was collected at termination. Urine and feces samples were collected for the 24 h period prior to first dose and for each 24 h period until sacrifice. The goat was milked twice daily and immediately prior to sacrifice. At approximately 17 h post final dose, the goat was sacrificed and edible tissues (liver, kidney, muscle and fat), bile, blood and the GI tract were removed post mortem. All samples were homogenized and stored at ca. –20°C.

5. Description of analytical methods

The radioactivity of homogenized extracts of the liquid samples (milk, bile, urine, blood) was determined by LSC (liquid scintillation counting) analysis. Solid debris (feces, tissues) were analyzed for radioactivity content by combustion analysis followed by LSC.
Homogenized liver and kidney samples containing significant amounts of radioactivity were extracted three times with acetonitrile and once with water (subsequently rinsed with ethanol).
Further investigation of non-solvent extractable residues (RRR) was not conducted.
Samples were analyzed using three HPLC methods; one for purity check of the test substance, one for routine analysis and fractionation and one for the analysis of polar extract fractions. A mix of the reference standards was prepared in order to identify radiolabeled components based on co-chromatography by HPLC and TLC using authentic reference items. Additionally, LC-MS and NMR (1D and 2D) analyses were performed for metabolite identification and peak assignment.

II. RESULTS AND DISCUSSION

A. TOTAL RADIOACTIVE RESIDUES (TRRs)

The total radioactive residues (TRR) in all investigated matrices are summarized in Table 6.2.3-3. Rapid excretion was observed. Until sacrifice, the radioactive residues excreted via urine and feces amounted to approximately 20.6-28.3% and 22.6-50.8% of the actual administered daily dose, respectively. Approximately 41.1% of the actual administered daily dose were recovered in bile and <0.1% in whole blood. Radioactivity associated with edible portions (milk and tissues) accounted for <0.1% of the administered daily dose, except for liver and kidney (2.2% and 1.1% of the dose, respectively). Radioactive residues in milk were low and ranged from 0.003 mg/kg to 0.005 mg/kg. The radioactive residues in muscle and fat were low and accounted for 0.004 mg/kg and 0.003 mg/kg, respectively. The residues in the liver were 0.223 mg/kg and in kidney 0.110 mg/kg.

Table 6.2.3-3: Total radioactive residues measured in matrices and excreta after dosing of lactating goat with ^{14}C-metconazole

Day	Milk		Urine		Feces		Urine + Feces	
	mg/kg	% dose	mg/kg	% dose	mg/kg	% dose	mg/kg	% dose
-1	<0.001	-	<0.001	-	<0.001	-	-	-
1	0.003	<0.1	2.132	20.6	2.345	22.6	4.477	43.2
2	0.004	<0.1	2.933	28.3	5.261	50.8	8.194	79.1
3	0.005	<0.1	2.594	25.0	5.208	50.3	7.802	75.3
4	0.005	<0.1	2.730	26.4	5.025	48.5	7.755	74.9

Day	Whole blood (plasma+cells)		Bile		Muscle		Fat		Liver		Kidney		Cage rinse	
	mg/kg	% dose	mg/kg	% dose	mg/kg	% dose	mg/kg	% dose	mg/kg	% dose	mg/kg	% dose	mg/kg	% dose
4	0.016 (0.023+0.006)	<0.1	4.260	41.1	0.004	<0.1	0.003	<0.1	0.223	2.2	0.110	1.1	0.131	1.3

B. EXTRACTION OF RESIDUES

The extractability of the edible tissues was high (≥93.6% TRR; see Table 6.2.3-4). The residues after solvent extraction of liver and kidney were low, accounting for 6.4% TRR and 3.7% TRR, respectively, and were therefore not further investigated.

Table 6.2.3-4: Residues after ^{14}C-metconazole treatment in goat matrices extracted with acetonitrile and water

Matrix	TRR measured		ERR [1]		RRR [2]		TRR calculated [3]
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	%
Liver	0.223	100.0	0.226	93.6	0.015	6.4	108.0
Kidney	0.110	100.0	0.112	96.3	0.004	3.7	106.1

1 Extractable radioactive residue
2 Radioactive residues after solvent extraction (post-extraction solid)
3 Sum of ERR and RRR

C. IDENTIFICATION AND CHARACTERIZATION OF RESIDUES

Summaries of identified and characterized residues are shown in Table 6.2.3-5 and Table 6.2.3-6. Identification was accomplished by HPLC or TLC co-chromatography with reference standards, LC-MS and NMR analyses. Peak assignment was conducted by comparison of the retention time, HPLC or TLC co-chromatography.

For quantification of labeled components, the concentrated liver extract was analyzed by HPLC (see Table 6.2.3-5). The parent compound, metconazole, was the major residue in liver, where its cis-isomer accounted for 22.1% TRR and its trans-isomer for 15.5% TRR. The second most abundant metabolite was identified as glucuronic acid cis-conjugate of M-31 (M555F031; 13.5% TRR). Further, identified metabolites M-12 (M555F012), M-1 (M555F001), M-31 (M555F031, cis- and trans-isomer, respectively) and M-32 (M555F032, cis-isomer) and glucuronic acid conjugates (M-1 glucuronide, M-32 glucuronide and trans-isomer of M-31 glucuronide) accounted for ≤6.0% TRR. A number of minor metabolites (20), most of which were polar and chromatographically similar to the identified glucuronic acid conjugates, were present in liver extracts. These unidentified compounds were most likely the structural isomers of M-1, M-31 and other mono-hydroxylated analogues of metconazole. None of these metabolites individually exceeded 10% of TRR or 0.01 mg/kg in liver.

For quantification of labeled components, the concentrated kidney extract was analyzed by HPLC (see Table 6.2.3-6). Main metabolites in goat kidney were M-12 (M555F012) and glucuronic acid conjugates of M-1 and M-31 (cis-isomer) accounting for 20.6% TRR, 11.7% TRR and 24.2% TRR, respectively. Further, identified metabolites M-1 (M555F001) and glucuronic acid conjugated M-32 accounted for ≤5.7% TRR. Free M-31 (M555F031) and M-32 (M555F032) as well as M-31 glucuronide (M555F031 conjugated with glucuronic acid, trans-isomer) were not detected in goat kidney. In addition, only trace amounts of cis- and trans-metconazole were present accounting for 1% TRR, respectively.
A number of minor metabolites (14), most of which were polar and chromatographically similar to the glucuronic acid conjugates of M-1, M-31 and M-32, were present in kidney extracts. These unidentified compounds were most likely the structural isomers of M-1, M-31 and other mono-hydroxylated analogues of metconazole. None of these metabolites individually exceeded 10% of TRR or 0.01 mg/kg in liver.

Radiolabeled metconazole residue in milk reached its highest level (0.005 mg/kg) on day 3 and stayed at a similar level on day 4. Although the residue level was low, the milk sample obtained on day 4 was analyzed in order to characterize the nature of the residue. About 95.5% TRR (0.004 mg/kg) remained dissolved in the aqueous acetonitrile extract, when milk was extracted with acetonitrile. The extract was partitioned against ethyl acetate and only about 29% TRR were transferred into the organic phase, while over 66% TRR remained in the aqueous fraction. Chromatographic analysis of the milk residue was difficult. When the pH of this turbid solution was adjusted to 4.0, a large mass of the colloidal proteins, together with the major part of the radioactivity, precipitated out of the solution. The clear supernatant obtained after centrifugation contained only 22% TRR. The polar radioactive residues seem to be directly associated with proteins and other natural components of milk.

Table 6.2.3-5: Summary of identified and characterized residues in liver of lactating goat treated with ^{14}C-metconazole

Components	Liver	
	[mg/kg]	[% TRR]
Metconazole (BAS 555 F)	0.091	37.6
Cis-metconazole	0.053	22.1
Trans-metconazole	0.037	15.5
M-12 (M555F012)	0.004	1.7
M-1 (M555F001) / M-31 (M555F031, cis)	0.003	1.3
M-32 (M555F032, cis)	0.002	0.8
M-31 (M555F031, trans)	0.015	6.0
M-1 glucuronide (M555F001 conjugated with glucuronic acid)	0.011	4.4
M-31 glucuronide (M555F031 conjugated with glucuronic acid)	0.033	13.5
M-32 glucuronide (M555F032 conjugated with glucuronic acid)	0.006	2.3
M-31 glucuronide (M555F031 conjugated with glucuronic acid, trans)	0.006	2.6
Total identified in extractable radioactivity (ERR)	**0.169**	**70.2**
Total characterized in extractable radioactivity (ERR) [1]	**0.056**	**23.4**
Sum identified/characterized (from ERR)	**0.226**	**93.6**
Final residue	**0.015**	**6.4**
Grand total	**0.241**	**100.0**

1 Each component/peak accounted for 0.001-0.010 mg/kg

Table 6.2.3-6: Summary of identified and characterized residues in kidney of lactating goat treated with ^{14}C-metconazole

Components	Kidney	
	[mg/kg]	[% TRR]
Metconazole (BAS 555 F)	0.003	2.3
Cis-metconazole	0.001	1.2
Trans-metconazole	0.001	1.1
M-12 (M555F012)	0.024	20.6
M-1 (M555F001)	0.001	0.9
M-1 glucuronide (M555F001 conjugated with glucuronic acid)	0.014	11.7
M-31 glucuronide (M555F031 conjugated with glucuronic acid)	0.028	24.2
M-32 glucuronide (M555F032 conjugated with glucuronic acid)	0.007	5.7
Total identified in extractable radioactivity (ERR)	**0.076**	**65.4**
Total characterized in extractable radioactivity (ERR) [1]	**0.036**	**31.0**
Sum identified/characterized (from ERR)	**0.112**	**96.3**
Final residue	**0.004**	**3.7**
Grand total	**0.117**	**100.0**

1 Each component/peak accounted for 0.001-0.010 mg/kg

2. Storage stability

Initial analyses of the tissue and excreta extracts were carried out within 7 months of sacrifice and storage at -20°C. Liver and kidney samples were re-extracted and profiled by HPLC after storage for at least 7 months after initial analysis. The profiles were comparable showing stability over the course of the study in both tissues and extracts.

3. Metabolic pathway

The proposed metabolic pathway of metconazole (BAS 555 F) in the lactating goat is provided in Figure 6.2.3-1. The unchanged parent was extensively metabolized in lactating goat.

One of the major pathways of metconazole metabolism in goat was the oxidation of one of its methyl groups at position 2 in the cyclopentyl ring. A carboxyl derivative, M-12, and a hydroxymethyl derivative, M-1, were produced and M-12 being mainly present in kidney and excreted via urine. Metabolite M-1 is rapidly conjugated with glucuronic acid through its hydroxymethyl group at position 2 and eliminated via urine. The other major metabolic pathway of metconazole involved hydroxylation of the methylene group at position 3 of the cyclopentyl ring, followed by conjugation of glucuronic acid via the 3-hydroxyl group. The predominant hydroxylation product, M-31 rapidly formed the 3-O-glucuronic acid conjugate. The glucuronide conjugate was then eliminated from the animal body via urine. A parallel, but minor, metabolic pathway in goat involved hydroxylation of the methylene group at position 3 of the cyclopentyl ring of trans-metconazole. The product, M-31 (trans-isomer), produced the 3-O-glucuronic acid conjugate, which was then also eliminated via urine. Another minor metabolite, glucuronic acid conjugate of M-32, was produced in goat liver and kidney and was eliminated via urine.

Through hydroxylation and subsequent glucuronidation, the animal body apparently fashioned a mechanism to convert metconazole into highly water-soluble compounds for facile elimination via urine.

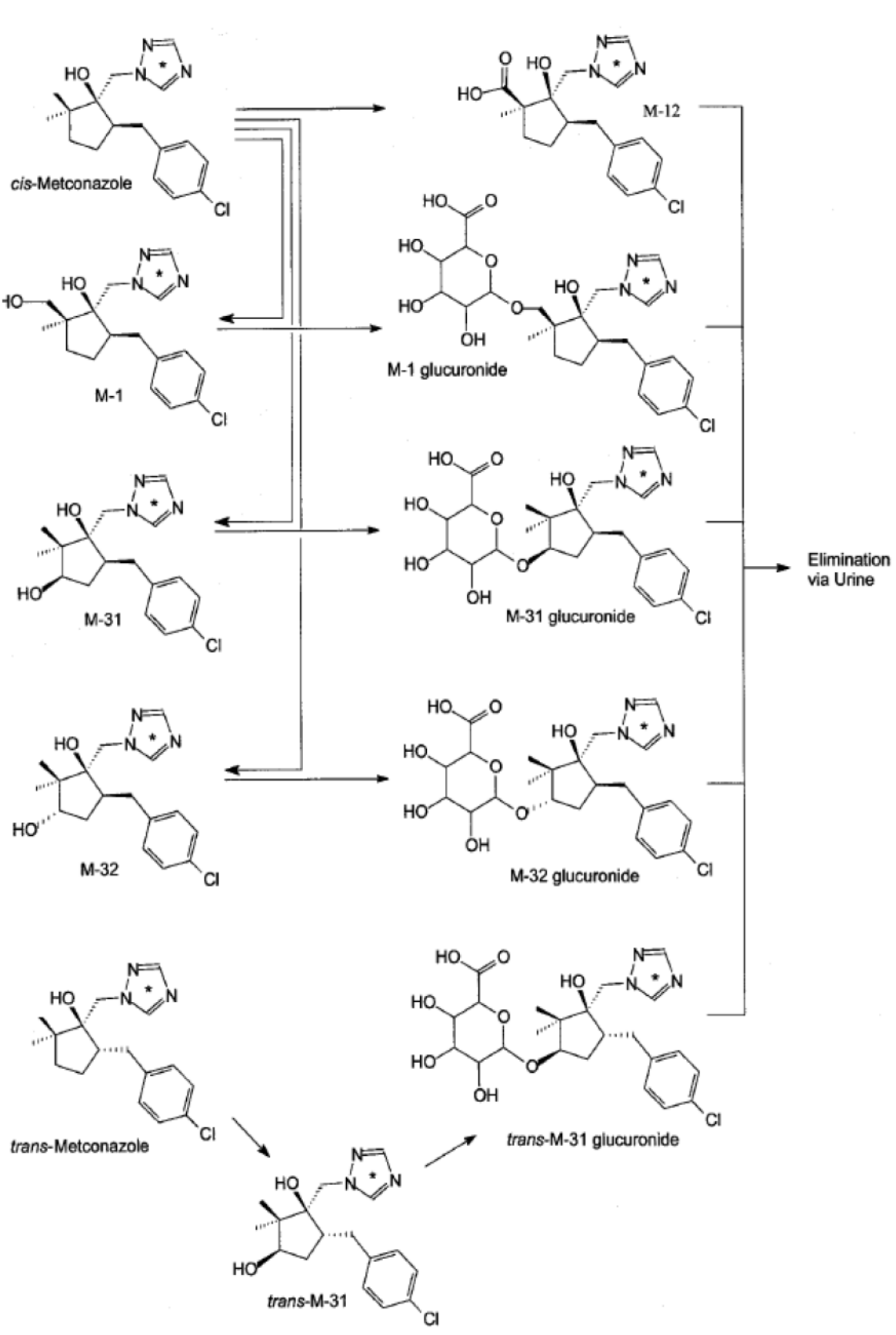
4. Stereoisomer analysis

The isomer ratio of metconazole was investigated in the treatment solution and in extracts of liver and kidney. The average cis-trans ratio of metconazole was 83:17 in the treatment solution. The cis-trans ratio of metconazole was approximately in the same ratio referring the absolute amount in liver. This outcome indicates a preference for biotransformation of the cis-isomer.

III. CONCLUSION

The TRR results showed that most of the radiolabeled residues of administered test substance was eliminated from the treated goat via feces and urine. The TRR of metconazole was low in milk, reached its highest level (0.005 ppm) on day 3 and stayed at this level until sacrifice. The major components of the milk residues were polar materials, which remained associated with proteins in aqueous solution and precipitated out together with proteins, when the solution was concentrated or the pH of the solution was lowered. The TRRs were significantly low in muscle and fat (≤0.004 ppm) and were not analyzed further. The TRR levels in treated kidney and liver were in the range of 0.11-0.22 mg/kg. These samples were extracted and analyzed for the identity and distribution of metabolites. The parent compound, metconazole, was the major residue in liver. Its cis-isomer accounted for 22.1% TRR, while the trans-isomer accounted for about 15.5% TRR. Liver also contained glucuronic acid conjugate of M-31 as a major component (13.5% TRR). A large number of minor metabolites, including M-12, M-1 and its glucuronide, M-31, M-32 and its glucuronide and trans-M-31 and its glucuronide, were also present in liver. None of these minor metabolites individually exceeded 6% TRR. The parent compound, metconazole, was present in kidney only in trace amounts (about 1% TRR of each isomer). The major metabolites in kidney were M-12 (20.6% TRR) and glucuronic acid conjugates of M-31 (24.2% TRR) and M-1 (11.7% TRR). Kidney also contained a number of minor metabolites, none of which individually exceeded 9% TRR.

Figure 6.2.3-1: Proposed biotransformation pathway for [^{14}C]-metconazole in the lactating goat



CA 6.2.4 Pigs

A pig metabolism study was not performed since similar metabolic patterns of metconazole in rats and in ruminants were observed.

CA 6.2.5 Fish

A fish metabolism study was not performed. According to the Commission Regulations (EU) No 283/2013 (active substances) and 284/2013 (plant protection products) as of 1 March 2013, metabolism studies on fish and fish feeding studies might be required in future (latest by 31 Dec 2015), if residues occur in crops that are intended as feed items for fish. A fish feeding study may be required where residues at levels above 0.01 mg/kg may be reasonably expected in edible tissues, based on the findings of the fish metabolism study and the estimated maximum residues which might occur in fish feed. Particular attention should be laid on lipophilic substances with an intrinsic tendency for accumulation. Feeding studies shall not be required where intake is below 0.004 mg/kg bw/day, except in cases where the residue, that is to say the active substance, its metabolites or breakdown products, as defined in the residue definition for risk assessment, tends to accumulate. As metconazole is used in several crops being fed to fish, a fish feed burden calculation was performed. Calculation of the feed burden (M-CA 6.4) indicated intake below the trigger for a feeding study as defined in the guidance.

With regard to its log P_{ow} (3.85) metconazole is characterized as fat soluble. However the following was noted in the EFSA Conclusion (EFSA 2006):

> "With regard to its log P_{ow} (3.85) metconazole is characterized as fat soluble. However, TRR were roughly one order of magnitude higher in the excretory organs liver and kidney of goat and hen than in other animal tissue, indicating that there might be no accumulation of residues in adipose tissue."

In two bioconcentration studies evaluated in context of the Annex I inclusion process (M-CA 8.2.2.3), the bioconcentration factor was found to be low, 114-119 based on total radioactive residues and 51-80 based on parent residues. Metconazole had a clearance time of < day.

Thus, in the absence of a need for a fish feeding study, a metabolism study is not justified.

CA 6.3 Magnitude of residues trials in plants

The dossier is intended to support the renewal of approval of metconazole (BAS 555 F). Within this dossier chapter, residue data are only provided for the representative uses of cereals and oilseed rape. The solo EC formulation BAS 555 01 F has been selected as the representative formulation. BASF proposes to change the representative formulation from BAS 555 00 F submitted in Annex I to BAS 555 01 F. The formulations have only minor differences in the antifoaming agent and solvent as discussed in Document J of the formulated product dossier, so the change should have no impact on residue levels in treated crops. The amount of applied active ingredient is the same. Within this chapter and MCA 6.7, BASF presents data which demonstrates that the level of residues for cereals and oilseed rape using the new representative formulation are covered by the current approved MRLs.

Discussion of change in formulation description from SL to EC

The representative formulation for residue studies submitted within Annex I was generally described as a "soluble concentrate (SL)". The formulation description was changed to a emulsifiable concentrate (EC) with the following explanation provided in a statement (Reg. Doc. No 2009/1072242) as discussed in M-CP 1.5.

The main part of the statement on the classification change of the formulation type of CARAMBA 60® and CARAMBA 90® (Metconazole) is quoted in this chapter for ease of reference.

> "Due to the intrinsic physico-chemical properties of Metconazole the classification of the formulation type of CARAMBA 60® and CARAMBA 90® as SL (Soluble Concentrate) is not appropriate. In fact these products are best described as emulsifiable concentrates (EC).
>
> BASF intention is to chance the formulation description and misleading trade names in all countries where the classification is relevant and where it distributes and sells CARAMBA® formulations.
> This change will be integrated during the course of national Annex III re-submissions in the European Region (mid 2009).
>
> There has been no change to the composition of CARAMBA 60® and CARAMBA 90® formulations. Therefore, the physical-chemical properties of these two formulations will remain unchanged as will the toxicological and ecotoxicological endpoints and consequently the (eco-) toxicological classification. All existing studies performed with CARAMBA 60® and CARAMBA 90® will remain valid. The properties and benefits for the customers remain exactly the same."

In this dossier section the relevant data for cereals and oilseed rape which were evaluated in the DAR (Belgium, 2004) and by EFSA (EFSA Conclusion 2006) are very briefly summarized. In addition, residue studies with BAS 555 00 F and other formulations are submitted for wheat, barley and oilseed rape to demonstrate that metconazole has been tested in a large number of field trials over many seasons. The data is submitted to provide a weight of the evidence approach that no additional residue studies with BAS 555 01 F are needed to support the designation of BAS 555 01 F as the representative formulation in place of BAS 555 00 F.

General remark: Total metconazole refers to the sum of *cis*- and *trans*-metconazole. Possible slight differences in results are due to rounding.

Triazole Derivative Metabolites

Residues for the triazole derivative metabolites (TDMs) were quantitated and reported for wheat CA 6.3.1/4, barley CA 6.3.2/6 and oilseed rape CA 6.3.3/1.

CA 6.3.1 Wheat, triticale and rye

Table 6.3.1-1: Summary of the critical GAP for the proposed use of BAS 555 01 F (90 g/L EC) in wheat, rye and triticale in the EU

Crop	Outdoor/ Protected	Growth stage (BBCH)	Maximum number of applications	Minimum application interval (days)	Maximum		Minimum PHI (days)
					Rate (kg a.s./ha)	Water (L/ha)	
Cereals (wheat, rye triticale)	Outdoor	30-69	2	14	0.090	110-400	PHI is covered by the time remaining between application and harvest or by PHI of 35 days

Table 6.3.1-2: Summary of the cGAP used for trials evaluated in the DAR for use in wheat, rye and triticale with BAS 555 00 F (60 g/L EC)

Crop	Outdoor/ Protected	Growth stage (BBCH)	Maximum number of applications	Minimum application interval (days)	Maximum		Minimum PHI (days)
					Rate (kg a.s./ha)	Water (L/ha)	
Cereals (wheat, rye triticale)	Outdoor	29-69	2	14	0.090	200-400	PHI of 35 days

Table 6.3.1-3: Number of residue trials conducted with BAS 555 00 F (60 g/L EC) according to the cGAP per geographical region and vegetation period and evaluated in the DAR for use in wheat, rye and triticale

Crop	Vegetation period	Number of trials					Reference
		EU North	Country	EU South	Country	Total	
Wheat	1995	2	DE			2	MK -730-033 MK -730-035
	1996	4	DE, UK			4	MK -730-041 MK -730-036 MK -730-037 MK -730-039
	1998	2	DE			2	MK -730-045
	2000			4	FR	4	MK -730-049
	2001	2	UK	4	FR	6	MK -730-050
Rye	1995	1	DE			1	MK -730-027
Triticale	1996	1	DE			1	MK -730-030
Total number of trials per region		**10**		**8**	**Total number of trials**	**18**	

Residue trials in wheat, rye and triticale evaluated in the DAR and by EFSA

Residue trials on wheat, rye and triticale (see Table 6.3.1-3) were performed for the previous active substance approval procedure and were evaluated in the DAR (Belgium, 2004) and by EFSA (EFSA Conclusion 2006). All the submitted studies were carried out under GLP conditions. A total number of 16 supervised residue trials treated with BAS 555 00 F (EC formulation) were considered for wheat covering Northern and Southern Europe. One trial each was conducted with BAS 555 00 F in rye and triticale in Northern Europe. The trials were performed according to the cGAP with two applications at a nominal rate of 90 g/ha as defined in Table 6.3.1-2. For most of the available trials, samples of whole plants were taken at different growth stages up to harvest and for all studies grain and straw were sampled at maturity, as defined by BBCH growth stage 89, targeting a PHI of 35 days. Samples were analyzed for metconazole (sum of *cis*- and *trans*-) using Method FAMS 050-01 with a method LOQ of 0.01 mg/kg for each isomer.

For the submitted and peer evaluated trials in wheat, the application parameters and results as reported in the EFSA Conclusion 2006 are shown below.

Winter wheat
North (Actual application rate: 85-90 g a.s./ha, 2 applications – BBCH: 32-39; 65-79, PHI: 35 days)
Metconazole (sum of isomers)
- Grain: <0.002, <0.01 (4x), <0.02 (2x), 0.04 mg/kg
- Straw: 0.25, 0.44, 0.53, 0.57, 0.64, 0.75, 0.76, 0.87 mg/kg

South (Actual application rate: 82-95 g a.s./ha, 2 applications – BBCH: 33-39; 65-77, PHI: 35 days:
- Grain: <0.02 (6x), 0.03, 0.05 mg/kg
- Straw: 0.16, 0.23 (3x), 0.27, 0.30 (2x), 0.57 mg/kg

Winter rye
North (trial performed in accordance with the critical GAP)
- Grain: <0.01 mg/kg
- Straw: 0.37 mg/kg

South: no residue trials performed

Triticale
North (trial performed in accordance with the critical GAP)
- Grain: <0.01 mg/kg
- Straw: 0.16 mg/kg

South: no residue trials performed

The following study was already evaluated in the Annex I process. However, two trials performed with the formulated product BAS 555 01 F were not included in the dossier since BAS 555 00 F was the representative formulation in the Annex I approval process. The results from trials performed at two sites in southern France site (Sarians and Expas) which included side by side trials with BAS 555 00 F and BAS 555 01 F are reported in this dossier to provide a comparison with side by side trials of residue levels from treatment at the cGAP with BAS 555 01 F and with BAS 555 00 F.

Within the already evaluated study (Young H.E., MK-730-049, 2002), two supervised residue trials were performed in Southern France according to the cGAP with one plot receiving two applications of BAS 555 00 F (60 g a.s./L EC) and one plot receiving two applications of BAS 555 01 F (90 g. a.s./L EC) at a nominal rate of 90 g/ha with an application interval of 21 days as defined in Table 6.3.1-2. The two trials with the formulated product BAS 555 01 F were not included in the dossier since BAS 555 00F was the representative formulation in the Annex I approval process. The actual application rates were 85-92 g a.s./ha, with the first application at BBCH: 33-36 and the second at 64-77. Samples of whole plants were taken at different growth stages up to harvest and grain and straw were sampled at maturity targeting a PHI of 35 days and also at 42-45 days after treatment. Samples were analyzed for parent compound using Method FAMS 050-01. The results of the analyses are summarized below.

Table 6.3.1-4: Comparison of residue levels in wheat treated at the cGAP with BAS 555 00 F or BAS 555 01 F in side-by side plots

	Wheat	PHI	BAS 555 00 F	BAS 555 01 F
Sarrians FR F-84260 00-731-639	grain	35	<0.02	<0.02
	grain	42	<0.02	<0.02
	straw	35	0.16	0.29
	straw	42	0.39	0.37
Espas FR F-32370 00-731-283	grain	34	0.03	0.03
	grain	45	<0.02	<0.02
	straw	34	0.30	0.22
	straw	45	0.38	0.29

Conclusion: Two side by side trials were performed in wheat with BAS 555 00 F and BAS 555 01 F in 2000 in south of France. After treatment at the cGAP residues in the grain were the same

Residue studies in wheat not evaluated as part of the Annex 1 approval process

Residue studies performed in wheat according to the cGAP with BAS 555 00 F and other BAS 555 F-containing formulations such as BAS 627 00 F and BAS 627 02 F (each a mixture of metconazole and epoxiconazole) are submitted in this dossier to demonstrate that metconazole has been tested in a large number of field trials over many seasons. The data is submitted to provide a weight of the evidence approach that a change in the formulation has little impact on the residue levels and to support the BASF proposal that no additional residue studies with BAS 555 01 F are needed to support the designation of BAS 555 01 F as the representative formulation in place of BAS 555 00 F. For the studies performed with mixtures, the residue results for the mixing partner are not included in this dossier.

Table 6.3.1-5: Summary of the cGAP used for trials (not EU-wide peer evaluated) for use in wheat, rye and triticale with BAS 555 00 F, BAS 627 00 F or BAS 627 02 F

Crop	Outdoor/ Protected	Growth stage (BBCH)	Maximum number of applications	Minimum application interval (days)	Maximum		Minimum PHI (days)
					Rate (kg a.s./ha)	Water (L/ha)	
Cereals (wheat, rye triticale)	Outdoor	First 32-39 Second application 64-79	2	21	0.090	200-400	PHI is covered by the time remaining between application and harvest or by PHI of 35 days

Report:	CA 6.3.1/1 Erdmann H.-P., 2007a Study on the residue behaviour of Metconazole and Epoxiconazole in wheat and triticale after application of BAS 627 00 F, BAS 480 31 F and BAS 555 00 F under field condition in France and Germany, 2007 2007/1050102
Guidelines:	EEC 1607/VI/97 rev. 2 10.06.1999, EEC 91/414 Annex II (Part A Section 6), EEC 91/414 Annex III (Part A Section 8), EEC 7029/VI/95 rev. 5, EEC 7525/VI/95 rev. 7
GLP:	yes (certified by Land Brandenburg Ministerium fuer Laendliche Entwicklung, Umwelt und Verbraucherschutz, Potsdam, Germany)

In this study, also epoxiconazole residues were determined, following application of mix formulation BAS 627 00 F and solo formulation BAS 480 31 F. These results are not summarized below.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	BAS 627 00 F (EC): epoxiconazole: 37.5 g/L nominal; metconazole: 27.5 g/L nominal BAS 555 00 F (EC): metconazole: 60 g/L nominal
Lot/Batch #:	BAS 627 00 F (EC): 1568 BAS 555 00 F (EC): 1089, 1072
Purity:	
CAS#:	BAS 555 F (metconazole): 125116-23-6
Development code:	
Spiking levels:	*Cis*-metconazole: 0.005-0.5 mg/kg *Trans*-metconazole: 0.005-0.5 mg/kg

2. Test Commodity:

Crop:	Wheat; triticale
Type:	Cereals
Variety:	Tommi, Brilliant, Kalango, Andalou, Caphorn; Talentro
Botanical name:	*Triticum aestivum; Triticale*
Crop parts(s) or processed commodity:	Whole plant without root, ears, rest of plant without root, grain, straw
Sample size:	Straw: 0.5 kg / grain: 1.0 kg

B. STUDY DESIGN

1. Test procedure

During the 2007 growing season, six field trials were conducted in different representative wheat and triticale growing areas in Germany and France (North and South) to determine the magnitude of the residues in or on raw agricultural commodities (RAC).
The fungicidal combination product BAS 627 00 F, containing epoxiconazole and metconazole, was compared to the solo formulations of both active substances, BAS 480 31 F (epoxiconazole) and BAS 555 00 F (metconazole). Only the residue data for metconazole are reported in this document.
In all cases, the test item was foliar applied two times in a spray volume of 200 L/ha. BAS 627 00 F was applied in plot 2 at a target rate corresponding to 0.0825 kg/ha metconazole. BAS 555 00 F was applied in plot 4 at a target rate of 2 x 0.090 kg/ha of metconazole.
The application timing was varied in the trials in order to cover pre-harvest intervals (PHI) defined in specific days before harvest or defined by the growth stage of the last application. In four trials, each formulation was applied 56 and 35 days before harvest resulting in a 21 day interval between treatments and a 35 day PHI. In two trials, the treatment was based on the growth stage with the first treatment targeted for 21 days before growth stage BBCH 69 and the second treatment at growth stage BBCH 69.
Wheat whole plant without root specimens were collected directly after the last application. Both ears and rest of plant, or grain and straw were taken depending on the crop maturity at about 35, 42 and 49 days after the last application. In those cases when growth stage BBCH 89 was not reached after 49 days, grain and straw were taken later as soon as growth stage BBCH 89 was reached.
All specimens were frozen within 24 hours of being taken and remained frozen (≤-18°C) until analysis including transportation (except slight increases of temperature at loading time for transportation, but material was always frozen). The maximum storage interval from harvest until extraction for analysis of metconazole was approximately 124 days for grain and straw samples and 175 days for whole plant samples.

Table 6.3.1-6: Target application rates and timings for wheat

Year	No of trials	No of appl.	F P or G	Test item	Active substance	Appl. rate (kg a.s./ha)	Water volume (L/ha)	Target date/ Timing[1]
2007	6	2	F	BAS 627 00 F (EC)	BAS 480 F	0.1125	200	1st appl.: 56±1 DBH 2nd appl.: 35±1 DBH or 1st appl.: 21 days before 2nd appl. 2nd appl.: 69 BBCH
					BAS 555 F	0.0825		
		2	F	BAS 480 31 F (SC)	BAS 480 F	0.125	200	
		2	F	BAS 555 00 F (EC)	BAS 555 F	0.090	200	

1 DBH = days before harvest

2. Description of analytical procedures

Samples were analyzed for metconazole residues using BASF method No 550/0 (L0019/01). The homogenized specimens were extracted using a mixture of methanol, water and hydrochloric acid. An aliquot of the extract is centrifuged and partitioned with dichloromethane, and the analytes in the final extract were determined by LC-MS/MS. The limit of quantitation in plant material was 0.005 mg/kg for each *cis*- and *trans*-metconazole (0.01 mg/kg for the sum of both isomers).

Table 6.3.1-7: Summary of procedural recovery data for metconazole

Matrix	Fortification level (mg/kg)	Summary recoveries					
		n	Mean (%)	RSD (%)	n	Mean (%)	RSD (%)
BASF method No L0019/01; LOQ = 0.005 mg/kg		***cis*-Metconazole**			***trans*-Metconazole**		
Whole plant without roots,	0.005 - 10	6	92.2	6.8	6	92.4	10.7
Ears	0.005 - 10	10	88.7	14.4	10	91.5	13.1
Rest of plant without roots	0.005 - 10	9	81.9	11.3	9	85.1	8.7
Grain	0.005 - 1.0	8	94.9	5.5	8	95.6	7.5
Straw	0.005 - 10	14	95.1	9.6	14	102.8	12.3

II. RESULTS AND DISCUSSION

The residue ranges of metconazole in/on wheat treated either with formulation BAS 627 00 F or BAS 555 00 F are shown in Table 6.3.1-8. Details are presented in Table 6.3.1-9 to Table 6.3.1-12.

Comparison of the residue levels in the whole plant immediately after treatment, indicate comparable residues with 1.37-2.02 mg/kg after treatment with BAS 627 00 F (plot 2) and 1.33-1.85 mg/kg after treatment with BAS 555 00 F (plot 4). The residue levels in the RAC were also comparable at harvest. For a pre-harvest interval of 34-36 days or 41-43 days, the levels in grain were <0.01-0.01 mg/kg after treatment with BAS 627 00 F and <0.01 mg/kg after treatment with BAS 555 00 F. For a pre-harvest interval of 49 days or 54-65 days the levels in grain were <0.01 mg/kg after treatment with either BAS 627 00 F or BAS 555 00 F.

For some European regions especially in the North, the interval between the last application and harvest is defined in the GAP according to the growth stage of application. The last application was done at growth stage 69 for both sites in the North of France. For these sites residues in grain were <0.01-0.02 mg/kg after treatment with BAS 627 00 F.

For a pre-harvest interval of 34-36 days, the levels in straw were 0.36-1.13 mg/kg after treatment with BAS 627 00 F and 0.26-0.88 mg/kg after treatment with BAS 555 00 F. For a pre-harvest interval of 41-43 days, the levels in straw were 0.29-1.72 mg/kg after treatment with BAS 627 00 F and 0.34-1.29 mg/kg after treatment with BAS 555 00 F. For a pre-harvest interval of 49 days the levels in straw were 0.25-2.83 mg/kg after treatment with BAS 627 00 F or 0.33-1.18 mg/kg after treatment with BAS 555 00 F. For a pre-harvest interval of 54-65 days the levels in straw were 0.20-0.49 mg/kg after treatment with BAS 627 00 F and 0.12-0.56 mg/kg after treatment with BAS 555 00 F.

In one trial in the South of France (L070186) samples of grain and straw at 49 DALA were taken after the commercial harvest date for the region of June 29. The residues in these samples were not considered relevant in summarizing the data.

No residues of metconazole at or above the limit of quantitation (0.01 mg/kg) were found in any of the analyzed untreated specimens.

Table 6.3.1-8: Summary of residues in wheat treated with BAS 627 00 F or BAS 555 00 F

Crop	Year	DALA[1]	Growth stage[2] (BBCH)	Metconazole residues (sum of *cis*- and *trans*-isomer; mg/kg)		
				Matrix	BAS 627 00 F	BAS 555 00 F
Wheat/ triticale	2007	0	69-85	Whole plant without roots	1.37-2.02	1.33-1.85
		35-36	85	Ears	0.10-0.16	0.09-0.12
		41-42	87	Ears	0.09-0.12	0.05-0.10
		48	87-89	Ears	0.09-0.10	0.08-0.09
		35-36	85	Rest of plant without roots	0.26	0.18-0.33
		41-42	87	Rest of plant without roots	0.17	0.13-0.25
		48	87-89	Rest of plant without roots	0.12-0.36	0.11-0.20
		34-36	87-89	Grain	<0.01-0.01	<0.01-0.01
		41-43	87-89	Grain	<0.01-0.01	<0.01
		49	89	Grain	<0.01	<0.01-0.01
		54-65	89	Grain	<0.01	<0.01
		34-36	87-89	Straw	0.36-1.13	0.26-0.88
		41-43	87-89	Straw	0.29-1.72	0.34-1.29
		49	89	Straw	0.25-2.83	0.33-1.18
		54-65	89	Straw	0.20-0.49	0.12-0.56

1 Days after last application
2 At sampling

III. CONCLUSION

The data is submitted to provide a weight of the evidence approach to support the BASF proposal that no additional residue studies in wheat with BAS 555 01 F are needed to support the designation of BAS 555 01 F as the representative formulation in place of BAS 555 00 F. Five trials were performed in wheat and one in triticale in 2007, two in Germany, one in north France, two in south France and one in triticale (north of France). After application of BAS 627 00 F or BAS 555 00 F, metconazole residues in wheat or triticale grain samples at all harvests were <0.01-0.01 mg/kg. Residues of metconazole in wheat straw measured after treatment with BAS 627 00 F or BAS 555 00 F were respectively 0.36-1.13 or 0.26-0.88 mg/kg at 35 DALA, 0.29-1.72 or 0.34-1.29 mg/kg at 42 DALA, 0.25-2.83 or 0.33-1.18 mg/kg at 49 DALA and 0.20-0.49 or 0.12-0.56 mg/kg at 54-65 DALA. The residues after treatment with the mixture formulation BAS 627 00 F are comparable to the residue levels measured after treatment with the solo formulation BAS 555 00 F.
The residue levels in grain are consistent with the levels in grain evaluated in the peer review and listed in the EFSA Conclusion (2006) as follows:
North <0.01-0.04 mg/kg; South <0.02-0.05 mg/kg

Table 6.3.1-9: Residues of metconazole in wheat after two applications of BAS 627 00 F in Northern Europe

Trial details		Crop	Country	Formulation, Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1)]	Residues found (mg/kg)	
							Matrix	Metconazole (sum of *cis*- and *trans*-isomer)
Study code:	255298	Wheat	Germany	BAS 627 00 F	73	0	Whole plant without root	1.37
Doc ID:	2007/1050102			2 x 0.0825		35	Ears	0.01
Trial No:	L070184					35	Rest of plant	1.31
GLP:	Yes					42	Grain	<u>0.01</u>
Year:	2007					42	Straw	1.72
						49	Grain	<0.01
						49	Straw	*2.83*
Study code:	255298	Wheat	Germany	BAS 627 00 F	69	0	Whole plant without root	1.68
Doc ID:	2007/1050102			2 x 0.0825		36	Grain	<0.01
Trial No:	L070185					36	Straw	*1.15*
GLP:	Yes					41	Grain	<0.01
Year:	2007					41	Straw	0.75
						49	Grain	<0.01
						49	Straw	1.15
Study code:	255298	Triticale	France	BAS 627 00 F	69	0	Whole plant without root	2.02
Doc ID:	2007/1050102			2 x 0.0825		36	Ears	0.16
Trial No:	L070188					36	Rest of plant	0.26
GLP:	Yes					41	Ears	0.12
Year:	2007					41	Rest of plant	0.17
						48	Ears	0.09
						48	Rest of plant	0.36
						54	Grain	<0.01
						54	Straw	*0.20*
Study code:	255298	Wheat	France	BAS 627 00 F	69	0	Whole plant without root	1.49
Doc ID:	2007/1050102			2 x 0.0825		35	Ears	0.10
Trial No:	L070189					35	Rest of plant	0.26
GLP:	Yes					42	Ears	0.09
Year:	2007					42	Rest of plant	0.17
						48	Ears	0.10
						48	Rest of plant	0.12
						65	Grain	<0.01
						65	Straw	0.49

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
<u>Underlined values</u> were used for MRL derivation
Values in *italic* were used for HR and STMR determination (needed for feed burden calculation)

Table 6.3.1-10: Residues of metconazole in wheat after two applications of BAS 627 00 F in Southern Europe

Trial details		Crop	Country	Formulation, Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1)]	Residues found (mg/kg)	
							Matrix	Metconazole (sum of *cis*- and *trans*-isomer)
Study code:	255298	Wheat	France	BAS 627 00 F	85	0	Whole plant without root	1.73
Doc ID:	2007/1050102			2 x 0.0825		34	Grain	<0.01
Trial No:	L070186					34	Straw	*0.36*
GLP:	Yes					42	Grain	<0.01
Year:	2007					42	Straw	0.29
						49	Grain	<0.01
						49	Straw	0.25
Study code:	255298	Wheat	France	BAS 627 00 F	69	0	Whole plant without root	1.59
Doc ID:	2007/1050102			2 x 0.0825		35	Grain	0.01
Trial No:	L070187					35	Straw	*0.55*
GLP:	Yes					43	Grain	0.01
Year:	2007					43	Straw	0.52
						49	Grain	<0.01
						49	Straw	0.34

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
Values in *italic* were used for HR and STMR determination (needed for feed burden calculation)

Table 6.3.1-11: Residues of metconazole in wheat after two applications of BAS 555 00 F in Northern Europe

Trial details		Crop	Country	Formulation, Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1)]	Residues found (mg/kg)	
							Matrix	Metconazole (sum of *cis*- and *trans*-isomer)
Study code:	255298	Wheat	Germany	BAS 555 00 F	73	0	Whole plant without root	1.33
Doc ID:	2007/1050102			2 x 0.090		35	Ears	<0.01
Trial No:	L070184					35	Rest of plant	0.88
GLP:	Yes					42	Grain	<0.01
Year:	2007					42	Straw	1.29
						49	Grain	<0.01
						49	Straw	1.18
Study code:	255298	Wheat	Germany	BAS 555 00 F	69	0	Whole plant without root	1.85
Doc ID:	2007/1050102			2 x 0.090		36	Grain	<u><0.01</u>
Trial No:	L070185					36	Straw	0.62
GLP:	Yes					41	Grain	<0.01
Year:	2007					41	Straw	0.97
						49	Grain	<0.01
						49	Straw	0.72
Study code:	255298	Triticale	France	BAS 555 00 F	69	0	Whole plant without root	1.38
Doc ID:	2007/1050102			2 x 0.090		36	Ears	0.12
Trial No:	L070188					36	Rest of plant	0.33
GLP:	Yes					41	Ears	0.05
Year:	2007					41	Rest of plant	0.25
						48	Ears	0.08
						48	Rest of plant	0.20
						54	Grain	<u><0.01</u>
						54	Straw	0.12
Study code:	255298	Wheat	France	BAS 555 00 F	69	0	Whole plant without root	1.69
Doc ID:	2007/1050102			2 x 0.090		35	Ears	0.09
Trial No:	L070189					35	Rest of plant	0.18
GLP:	Yes					42	Ears	0.10
Year:	2007					42	Rest of plant	0.13
						48	Ears	0.09
						48	Rest of plant	0.11
						65	Grain	<u><0.01</u>
						65	Straw	*0.56*

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
<u>Underlined values</u> were used for MRL derivation
Values in *italic* were used for HR and STMR determination (needed for feed burden calculation)

Table 6.3.1-12: Residues of metconazole in wheat after two applications of BAS 555 00 F in Southern Europe

Trial details		Crop	Country	Formulation, Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1)]	Residues found (mg/kg)	
							Matrix	Metconazole (sum of *cis*- and *trans*-isomer)
Study code:	255298	Wheat	France	BAS 555 00 F	85	0	Whole plant without root	1.60
Doc ID:	2007/1050102			2 x 0.090		34	Grain	<0.01
Trial No:	L070186					34	Straw	0.26
GLP:	Yes					42	Grain	<u><0.01</u>
Year:	2007					42	Straw	0.34
						49	Grain	<0.01
						49	Straw	0.33
Study code:	255298	Wheat	France	BAS 555 00 F	69	0	Whole plant without root	1.39
Doc ID:	2007/1050102			2 x 0.090		35	Grain	<u>0.01</u>
Trial No:	L070187					35	Straw	0.34
GLP:	Yes					43	Grain	<0.01
Year:	2007					43	Straw	0.46
						49	Grain	0.01
						49	Straw	0.37

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
<u>Underlined values</u> were used for MRL derivation

Report:	CA 6.3.1/2 Schroth E.,Martin T., 2010a Residue behavior of BAS 480 F and BAS 555 F in wheat after the application of BAS 480 31 F, BAS 555 00 F and BAS 627 00 F under field conditions in France (North), Germany, Denmark, United Kingdom, France (South), Greece, Italy, Spain, 2009 2010/1075867
Guidelines:	EEC 91/414 Annex II (Part A Section 6), EEC 91/414 Annex III (Part A Section 8), EEC 1607/VI/97 rev. 2 10.06.1999, EEC 7029/VI/95 rev. 5, EEC 7525/VI/95 rev. 7
GLP:	yes (certified by ENAC, Entidad Nacional de Acreditación, Madrid Spain)

In this study, also epoxiconazole residues were determined, following application of mix formulation BAS 627 00 F and solo formulation BAS 480 31 F. These results are not summarized below.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	BAS 627 00 F (EC): epoxiconazole: 37.5 g/L nominal; metconazole: 27.5 g/L nominal BAS 555 00 F (EC): metconazole: 60 g/L nominal
Lot/Batch #:	BAS 627 00 F (EC): 1568 BAS 555 00 F (EC): 1093
Purity:	
CAS#:	BAS 555 F (metconazole): 125116-23-6
Development code:	
Spiking levels:	Metconazole: 0.01 – 50 mg/kg

2. Test Commodity:

Crop:	Wheat
Type:	Cereals
Variety:	Hattrick, Consort, Frument, Maxwell, Bologna, Panifor, Califa Sur, Rosario, Orvantis, Premio
Botanical name:	*Triticum aestivum*
Crop parts(s) or processed commodity:	Whole plant w/o roots, ears, rest of plant w/o roots, grain, straw
Sample size:	0.5 – 1.0 kg

B. STUDY DESIGN

1. Test procedure

During the 2009 growing season, 10 trials in wheat were conducted in different representative growing areas in Denmark, France, Germany, Greece, Italy, Spain and United Kingdom to determine the residue level of epoxiconazole and metconazole in or on wheat raw agricultural commodities (RAC).

The fungicidal combination product BAS 627 00 F, containing epoxiconazole and metconazole, was compared to the solo formulations of both active substances, BAS 480 31 F (epoxiconazole) and BAS 555 00 F (metconazole). Only the residue data for metconazole are reported in this document.

In all cases, the test item was foliar applied two times in a spray volume of 200 L/ha. BAS 627 00 F was applied in plot 2 at a target rate corresponding to 0.0825 kg/ha metconazole. BAS 555 00 F was applied in plot 4 at a target rate of 0.090 kg/ha of metconazole.

The application timing was varied in the trials in order to cover preharvest intervals (PHI) defined in specific days before harvest or defined by the growth stage of the last application. In seven trials (trial numbers L090073, L090074, L090075, L090076, L090077, L090078 and L090079), the applications were made at crop stage BBCH 39-49 and BBCH 69, and in three trials (trial numbers L090080, L090081 and L090082) the applications were made 56(±1) and 35(±1) days before harvest.

Wheat "whole plant without roots" specimens were collected at the day of the last application and specimens of wheat grain and straw, or ear and rest of plant in case of immaturity, were taken about 28, 35 and 42 days thereafter (in case of immaturity an additional sampling of grain and straw was done at growth stage BBCH 89). Samples were generally stored frozen at or below -18°C from harvest until analysis for a maximum of 375 days.

Table 6.3.1-13: Target application rates and timings for wheat

Year	No of trials	No of appl.	F P or G	Test item	Active substance	Appl. rate (kg a.s./ha)	Water volume (L/ha)	Target date/ Timing[1]
2009	10	2	F	BAS 627 00 F (EC)	BAS 480 F	0.1125	200	1st appl.: 65±1 DBH 2nd appl.: 35±1 DBH or 1st appl.: 39-49 BBCH 2nd appl.: 69 BBCH
					BAS 555 F	0.0825		
		2	F	BAS 480 31 F (SC)	BAS 480 F	0.125	200	
		2	F	BAS 555 00 F (EC)	BAS 555 F	0.060	200	

1 DBH = days before harvest

2. Description of analytical procedures

Samples were analyzed for metconazole residues using BASF method No 535/1 (L0076/01). The homogenized specimens were extracted using a mixture of methanol, water and hydrochloric acid. An aliquot of the extract was centrifuged and partitioned at alkaline conditions against cyclohexane, and the analytes in the final extract were determined by LC-MS/MS. The limit of quantitation in plant material was 0.01 mg/kg.

The method was validated concurrently with field sample analyses and the results are given in Table 6.3.1-14 below. In all control samples analyzed (except in the isolated cases noted) no residues of the analyte at or above the limit of quantitation were found which proves that no interferences of the specimen material with the analytical procedure occurred. Control samples were fortified with metconazole analyzed simultaneously with the treated samples in order to determine the efficiency of the method. Fortification results were corrected for apparent residues in the control samples.

Table 6.3.1-14: Summary of procedural recovery data for metconazole

Matrix	Fortification level (mg/kg)	Summary recoveries					
		n	Mean (%)	RSD (%)	n	Mean (%)	RSD (%)
BASF method No L0076/01; LOQ = 0.01 mg/kg		**Metconazole (m/z 320 → 70)**			**Metconazole (m/z 320 → 125)**		
Whole plant without roots	0.005 - 10	12	92	12	12	96	9
Ears	0.005 - 10	6	96	4	6	103	5
Grain	0.005 - 1.0	8	94	7	8	96	8
Straw	0.005 - 10	9	96	9	9	99	6

II. RESULTS AND DISCUSSION

The residue ranges of metconazole in/on wheat treated either with formulation BAS 627 00 F or BAS 555 00 F are shown in Table 6.3.1-15. Details are presented in Table 6.3.1-16 to Table 6.3.1-19.

Comparison of the residue levels in the whole plant immediately after treatment, indicate comparable residues of metconazole at 1.3-4.5 mg/kg after treatment with BAS 627 00 F (plot 2) and 1.8-4.9 mg/kg after treatment with BAS 555 00 F (plot 4).

The residue levels were also comparable at harvest. Residues of metconazole found in grain were <0.01-0.025 mg/kg (plot 2) and <0.01-0.016 mg/kg (plot 4) at 34-35 DALA. Residues decreased to levels of <0.01-0.018 mg/kg (plot 2) <0.01-0.013 mg/kg (plot 4) at 41-50 DALA.

The residues in straw are dependent on the ripening stage / water content of the matrix, and at 34-35 DALA metconazole residues were 0.71-6.6 mg/kg (plot 2) and 0.35-3.5 mg/kg (plot 4). At longer post-treatment intervals, residues in straw were 1.0-8.4 mg/kg (plot 2) and 0.44-4.4 mg/kg (plot 4) at 41-43 DALA, and were 1.4 mg/kg (plot 2) and 1.4 mg/kg (plot 4) in the one sample harvested at 50 DALA.

No residues ≥0.010 mg/kg were present in any of the analyzed untreated samples, with the following exceptions: Whole plant, ears and straw samples originating from one trial in France (L090081) seemed to be contaminated with metconazole (range, 0.06-0.33 mg/kg).

Table 6.3.1-15: Summary of residues in wheat treated with BAS 627 00 F or BAS 555 00 F

Crop	Year	DALA[1]	Growth stage[2] (BBCH)	Metconazole residues (sum of *cis*- and *trans*-isomer; mg/kg)		
				Matrix	Plot 2 (BAS 627 00 F)	Plot 4 (BAS 555 00 F)
Wheat	2009	0	69-71	Whole plant without roots	1.3-4.5	1.8-4.9
		27-29	75-87	Ears	0.12-1.8	0.11-0.85
		34-35	83-87	Ears	0.23-0.74	0.16-0.52
		42	87	Ears	0.45	0.37
		27-29	75-87	Rest of plant	0.33-4.0	0.41-3.1
		34-35	83-87	Rest of plant	0.29-4.2	0.56-2.6
		42	87	Rest of plant	0.93	0.73
		28-29	83-89	Grain	<0.01-0.047	<0.01-0.023
		34-35	87-89	Grain	<0.01-0.025	<0.01-0.016
		41-43	89	Grain	<0.01-0.018	<0.01-0.013
		50	89	Grain	<0.01	<0.01
		28-29	83-89	Straw	0.44-1.6	0.26-1.3
		34-35	87-89	Straw	0.71-6.6	0.35-3.5
		41-43	89	Straw	1.0-8.4	0.44-4.4
		50	89	Straw	1.4	1.4

1 Days after last application
2 At sampling

III. CONCLUSION

The data is submitted to provide a weight of the evidence approach to support the BASF proposal that no additional residue studies in wheat with BAS 555 01 F are needed to support the designation of BAS 555 01 F as the representative formulation in place of BAS 555 00 F. Ten trials were performed in wheat in 2009, six in north of Europe and four in south of Europe. After application of BAS 627 00 F, metconazole residues in wheat grain samples at all harvests at or after the PHI were <0.01-0.03 mg/kg. After application of BAS 555 00 F, metconazole residues in wheat grain samples at all harvests at or after the PHI were <0.01-0.02 mg/kg. Residues of metconazole in wheat straw measured after treatment with BAS 627 00 F or BAS 555 00 F were, respectively, 0.71-6.6 or 0.35-3.5 mg/kg at 35 DALA, 1.0-8.4 or 0.44-4.4 mg/kg at 42 DALA and 1.4 or 1.4 mg/kg at 50 DALA. The residues after treatment with the mixture formulation BAS 627 00 F are comparable to the residue levels measured after treatment with the solo formulation BAS 555 00 F.
The residue levels in grain are consistent with the levels in grain evaluated in the peer review and listed in the EFSA Conclusion (2006) as follows:
North <0.01-0.04 mg/kg; South <0.02-0.05 mg/kg

Table 6.3.1-16: Residues of metconazole in wheat after two applications of BAS 627 00 F in Northern Europe

Trial details		Crop	Country	Formulation, Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1)]	Residues found (mg/kg)	
							Matrix	Metconazole (sum of *cis*- and *trans*-isomer)
Study code:	358573	Wheat	Germany	BAS 627 00 F	69	0	Whole plant without root	2.3
Doc ID:	2010/1075867			2 x 0.0825		27	Ears	0.12
Trial No:	L090073					27	Rest of plant	1.3
GLP:	Yes					35	Grain	0.025
Year:	2009					35	Straw	*2.7*
						43	Grain	<0.01
						43	Straw	1.2
Study code:	358573	Wheat	United Kingdom	BAS 627 00 F	69	0	Whole plant without root	1.3
Doc ID:	2010/1075867			2 x 0.0825		28	Grain	0.047
Trial No:	L090074					28	Straw	1.6
GLP:	Yes					35	Grain	0.01
Year:	2009					35	Straw	*1.4*
						41	Grain	<0.01
						41	Straw	1.3
Study code:	358573	Wheat	Denmark	BAS 627 00 F	69	0	Whole plant without root	1.9
Doc ID:	2010/1075867			2 x 0.0825		27	Ears	0.29
Trial No:	L090075					27	Rest of plant	1.6
GLP:	Yes					35	Grain	0.011
Year:	2009					35	Straw	2
						41	Grain	<0.01
						41	Straw	*2.2*
Study code:	358573	Wheat	France	BAS 627 00 F	69	0	Whole plant without root	2
Doc ID:	2010/1075867			2 x 0.0825		29	Ears	0.31
Trial No:	L090076					29	Rest of plant	0.33
GLP:	Yes					35	Ears	0.23
Year:	2009					35	Rest of plant	0.29
						42	Grain	0.012
						42	Straw	*2.1*
Study code:	358573	Wheat	Germany	BAS 627 00 F	71	0	Whole plant without root	1.8
Doc ID:	2010/1075867			2 x 0.0825		29	Grain	<0.01
Trial No:	L090080					29	Straw	0.44
GLP:	Yes					34	Grain	0.011
Year:	2009					34	Straw	0.71
						42	Grain	0.012
						42	Straw	*1*
Study code:	358573	Wheat	France	BAS 627 00 F	71	0	Whole plant without root	2.6
Doc ID:	2010/1075867			2 x 0.0825		28	Ears	0.19
Trial No:	L090081					28	Rest of plant	2
GLP:	Yes					35	Grain	0.023
Year:	2009					35	Straw	2.3
						42	Grain	0.018
						42	Straw	2.5

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
Underlined values were used for MRL derivation
Values in *italic* were used for HR and STMR determination (needed for feed burden calculation)

Table 6.3.1-17: Residues of metconazole in wheat after two applications of BAS 627 00 F in Southern Europe

Trial details		Crop	Country	Formulation, Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1)]	Residues found (mg/kg)	
							Matrix	Metconazole (sum of *cis*- and *trans*-isomer)
Study code:	358573	Wheat	Italy	BAS 627 00 F	69	0	Whole plant without root	1.9
Doc ID:	2010/1075867			2 x 0.0825		28	Ears	0.19
Trial No:	L090077					28	Rest of plant	1.2
GLP:	Yes					35	Ears	0.23
Year:	2009					35	Rest of plant	1.2
						42	Grain	<0.01
						42	Straw	1.2
Study code:	358573	Wheat	Greece	BAS 627 00 F	69	0	Whole plant without root	1.7
Doc ID:	2010/1075867			2 x 0.0825		29	Ears	0.23
Trial No:	L090078					29	Rest of plant	0.98
GLP:	Yes					35	Ears	0.32
Year:	2009					35	Rest of plant	0.89
						42	Ears	0.45
						42	Rest of plant	0.93
						50	Grain	<0.01
						50	Straw	1.4
Study code:	358573	Wheat	Spain	BAS 627 00 F	69	0	Whole plant without root	4.5
Doc ID:	2010/1075867			2 x 0.0825		28	Ears	1.8
Trial No:	L090079					28	Rest of plant	4
GLP:	Yes					35	Grain	<0.01
Year:	2009					35	Straw	6.6
						41	Grain	<0.01
						41	Straw	*8.4*
Study code:	358573	Wheat	France	BAS 627 00 F	71	0	Whole plant without root	2.8
Doc ID:	2010/1075867			2 x 0.0825		28	Ears	0.68
Trial No:	L090082					28	Rest of plant	2.5
GLP:	Yes					34	Ears	0.74
Year:	2009					34	Rest of plant	4.2
						41	Grain	<0.01
						41	Straw	*3*

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
Values in *italic* were used for HR and STMR determination (needed for feed burden calculation)

Table 6.3.1-18: Residues of metconazole in wheat after two applications of BAS 555 00 F in Northern Europe

Trial details		Crop	Country	Formulation, Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1)]	Residues found (mg/kg)	
							Matrix	Metconazole (sum of *cis*- and *trans*-isomer)
Study code:	358573	Wheat	Germany	BAS 555 00 F	69	0	Whole plant without root	2.9
Doc ID:	2010/1075867			2 x 0.090		27	Ears	0.11
Trial No:	L090073					27	Rest of plant	0.9
GLP:	Yes					35	Grain	0.016
Year:	2009					35	Straw	1.4
						43	Grain	<0.01
						43	Straw	1.1
Study code:	358573	Wheat	United Kingdom	BAS 555 00 F	69	0	Whole plant without root	1.8
Doc ID:	2010/1075867			2 x 0.090		28	Grain	0.023
Trial No:	L090074					28	Straw	1.3
GLP:	Yes					35	Grain	<0.01
Year:	2009					35	Straw	0.86
						41	Grain	<0.01
						41	Straw	0.84
Study code:	358573	Wheat	Denmark	BAS 555 00 F	69	0	Whole plant without root	2.2
Doc ID:	2010/1075867			2 x 0.090		27	Ears	0.34
Trial No:	L090075					27	Rest of plant	1.4
GLP:	Yes					35	Grain	<0.01
Year:	2009					35	Straw	2.8
						41	Grain	<0.01
						41	Straw	2.4
Study code:	358573	Wheat	France	BAS 555 00 F	69	0	Whole plant without root	3.3
Doc ID:	2010/1075867			2 x 0.090		29	Ears	0.44
Trial No:	L090076					29	Rest of plant	0.41
GLP:	Yes					35	Ears	0.52
Year:	2009					35	Rest of plant	0.56
						42	Grain	<0.01
						42	Straw	1.4
Study code:	358573	Wheat	Germany	BAS 555 00 F	71	0	Whole plant without root	1.9
Doc ID:	2010/1075867			2 x 0.090		29	Grain	<0.01
Trial No:	L090080					29	Straw	0.26
GLP:	Yes					34	Grain	<0.01
Year:	2009					34	Straw	0.35
						42	Grain	<0.01
						42	Straw	0.44
Study code:	358573	Wheat	France	BAS 555 00 F	71	0	Whole plant without root	2.7
Doc ID:	2010/1075867			2 x 0.090		28	Ears	0.85
Trial No:	L090081					28	Rest of plant	1.1
GLP:	Yes					35	Grain	0.013
Year:	2009					35	Straw	*3.5*
						42	Grain	0.013
						42	Straw	2

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
Values in *italic* were used for HR and STMR determination (needed for feed burden calculation)

Table 6.3.1-19: Residues of metconazole in wheat after two applications of BAS 555 00 F in Southern Europe

Trial details		Crop	Country	Formulation, Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1)]	Residues found (mg/kg)	
							Matrix	Metconazole (sum of *cis*- and *trans*-isomer)
Study code:	358573	Wheat	Italy	BAS 555 00 F	69	0	Whole plant without root	2.4
Doc ID:	2010/1075867			2 x 0.090		28	Ears	0.14
Trial No:	L090077					28	Rest of plant	1.1
GLP:	Yes					35	Ears	0.16
Year:	2009					35	Rest of plant	1.2
						42	Grain	<u><0.01</u>
						42	Straw	*1.2*
Study code:	358573	Wheat	Greece	BAS 555 00 F	69	0	Whole plant without root	2
Doc ID:	2010/1075867			2 x 0.090		29	Ears	0.2
Trial No:	L090078					29	Rest of plant	0.61
GLP:	Yes					35	Ears	0.22
Year:	2009					35	Rest of plant	0.58
						42	Ears	0.37
						42	Rest of plant	0.73
						50	Grain	<u><0.01</u>
						50	Straw	*1.4*
Study code:	358573	Wheat	Spain	BAS 555 00 F	69	0	Whole plant without root	4.9
Doc ID:	2010/1075867			2 x 0.090		28	Ears	0.76
Trial No:	L090079					28	Rest of plant	3.1
GLP:	Yes					35	Grain	<u><0.01</u>
Year:	2009					35	Straw	3.5
						41	Grain	<0.01
						41	Straw	4.4
Study code:	358573	Wheat	France	BAS 555 00 F	71	0	Whole plant without root	2.8
Doc ID:	2010/1075867			2 x 0.090		28	Ears	0.51
Trial No:	L090082					28	Rest of plant	1.8
GLP:	Yes					34	Ears	0.43
Year:	2009					34	Rest of plant	2.6
						41	Grain	<u><0.01</u>
						41	Straw	2.1

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
<u>Underlined values</u> were used for MRL derivation
Values in *italic* were used for HR and STMR determination (needed for feed burden calculation)

Report:	CA 6.3.1/3 Schroth E.,Martin T., 2010b Study on the residue behavior of Epoxiconazole (BAS 480 F) and Metconazole (BAS 555 F) in wheat after the application of either BAS 627 00 F or BAS 627 02 F under field conditions in Germany, United Kingdom, France (South) and Spain, 2010 2010/1144333
Guidelines:	EEC 91/414 Annex II (Part A Section 6), EEC 91/414 Annex III (Part A Section 8), EEC 1607/VI/97 rev. 2 10.06.1999, EEC 7029/VI/95 rev. 5, EEC 7525/VI/95 rev. 7
GLP:	yes (certified by ENAC, Entidad Nacional de Acreditación, Madrid Spain)

In this study, also epoxiconazole residues were determined, following application of mix formulations BAS 627 00 F and BAS 627 02 F. These results are not summarized below.

I. MATERIAL AND METHODS

A. MATERIALS

Test Material:	
Description:	BAS 627 00 F (EC)
Lot/Batch #:	1615, BAS 627 02 F, metconazole: 27.5 g/L nominal; epoxiconazole: 37.5 g/L nominal
Purity:	
CAS#:	BAS 555 F: 125116-23-6 (metconazole)
Development code:	
Spiking levels:	0.005-5.0 mg/kg (metconazole)
Test Commodity:	
Crop:	Wheat
Type:	Cereals
Variety:	Winnetou, Hereward, Apache, Prospero
Botanical name:	*Triticum aestivum*
Crop parts(s) or processed commodity:	Whole plant w/o root, ears, rest plant w/o root, grain, straw
Sample size:	Min. 0.5-1.0 kg

B. STUDY DESIGN

1. Test procedure

During the 2010 growing season, 4 field trials were conducted in wheat in different representative growing areas in Germany, United Kingdom, France (South) and Spain in order to determine the magnitude of the residues of metconazole and epoxiconazole in or on raw agricultural commodities (RAC) and other parts. The formulation BAS 627 00 F (EC) applied on plot 3 at 82.5 g a.s./ha of metconazole and 112.5 g a.s./ha of epoxiconazole, water volume 200 L/ha with an application rate of 3.0 L product/ha, was compared to the formulation BAS 627 02 F (EC), applied on plot 2. Plot 1 remained untreated. In both cases, two foliar applications were done with the first application at growth stage BBCH 49 and the second application at BBCH 69 with an interval between the applications of 11 to 23 days.
Specimens of whole plant without roots were collected at the day of the last application and specimens of grain and straw (or ear and rest of plant in case of immaturity) were taken about 35, 42 and 49 days thereafter. In case of immaturity an additional sampling of grain and straw was done at growth stage BBCH 89.

Table 6.3.1-20: Target application rates and timings for wheat

Year	No of trials	No of appl.	F P or G	Test item	Active substance	Appl. rate (kg a.s./ha)	Water volume (L/ha)	Target date/ Timing
2010	4	2	F	BAS 627 00 F (EC) or BAS 627 02 F (EC)	Metconazole	0.0825	200	1st appl.: 49 BBCH 2nd appl.: 69 BBCH
					Epoxiconazole	0.1125		

2. Description of analytical procedures

All treated specimens were analyzed for metconazole (detected separately as *cis* and *trans* isomers) using BASF method No L0019/01 (550/0) with a limit of quantitation (LOQ) of 0.005 mg/kg for each isomer (or 0.01 mg/kg for the sum of *cis* and *trans* isomer).

Principle of BASF method No 550/0 (L0019/01)

BAS 555 F is extracted using a mixture of methanol, water and hydrochloric acid. An aliquot of the extract is centrifuged and partitioned against dichloromethane. The final determination of BAS 555 F is performed by HPLC-MS/MS. Limit of quantitation (LOQ): 0.01 mg/kg (sum of *cis* and *trans* isomer, which are determined separately, each at with a LOQ of 0.005 mg/kg).

Table 6.3.1-21: Summary of recoveries of metconazole (BAS 555 F) in wheat

Matrix	Fortification level (mg/kg)	Summary recoveries			
		n	Mean (%)	SD (+/-)	RSD (%)
Method No L0019/01 (550/0)		**Metconazole (*cis*/*trans*)**			
Whole/rest plants*	0.005-10	3/3	100/96.1	6.8/7.8	6.7/8.1
Ears	0.005-0.5	3/3	90.6/88.8	14/10	16/12
Grain	0.005-0.5	6/6	98.1/96.6	9.8/12	9.9/13
Straw	0.005-10	6/6	100/94.0	6.5/6.1	6.5/6.5

* Without roots

II. RESULTS AND DISCUSSION

For metconazole, residues in whole plants without roots were 2.4-4.5 mg/kg and 2.4-5.1 mg/kg directly after the last application.
In wheat grain at the PHI of 35 days and later, metconazole residues were between LOQ and 0.016 mg/kg for BAS 627 00 F and between LOQ and 0.014 mg/kg for BAS 627 02 F. Residues in straw were found up to 5.1 mg/kg for BAS 627 00 F and up to 7.3 mg/kg for BAS 627 02 F.

The summarized results are given in Table 6.3.1-22. The detailed results are given in Table 6.3.1-23 and Table 6.3.1-24.

Table 6.3.1-22: Summary of metconazole residues in wheat after application of BAS 627 00 F and BAS 627 02 F

Crop	Year	Application	DALA[1]	BBCH[2]	Metconazole (sum of *cis* and *trans*) found (mg/kg)		
					Matrix	BAS 627 00 F	BAS 627 02 F
Wheat	2010	BAS 627 00 F (EC) or BAS 627 02 F (EC)	0	69	Whole plant*	2.4-4.5	2.4 - 5.1
			35	83-85	Ears	0.31	0.47
			35	83-85	Rest of plant*	0.64	0.67
			34-35	87	Grain	<0.010-0.011	<0.010 - 0.011
			41-42	87-89	Grain	<0.010-0.010	< 0.010 - 0.010
			48-49	89	Grain	<0.010-0.016	< 0.010 - 0.014
			34-35	87	Straw	1.7-4.5	1.8 - 6.4
			41-42	87-89	Straw	0.98-5.1	0.62 - 5.4
			48-49	89	Straw	1.0-4.0	1.0 - 7.3

1 Days after last application
2 BBCH stage at respective sampling
* Without root

III. CONCLUSION

The data is submitted to provide a weight of the evidence approach to support the BASF proposal that no additional residue studies in wheat with BAS 555 01 F are needed to support the designation of BAS 555 01 F as the representative formulation in place of BAS 555 00 F. Four trials were performed in wheat in 2010 with BAS 627 00 F and BAS 627 02 F, two in north of Europe and two in south of Europe. After application of BAS 627 00 F or BAS 627 02 F , metconazole residues in wheat grain samples at all harvests at or after the PHI were <0.01-0.02 mg/kg. Residues of metconazole in wheat straw measured after treatment with BAS 627 00 F or BAS 627 02 F were, respectively, 1.7-4.5 or 1.8-6.4 mg/kg at 35 DALA, 0.98-5.1 or 0.62-5.4 mg/kg at 42 DALA and 1.0-4.0 or 1.0-7.3 mg/kg at 49 DALA. The residues after treatment with the mixture formulation BAS 627 02 F are comparable to the residue levels measured after treatment with the formulation BAS 627 00 F.
The residue levels in grain are consistent with the levels in grain evaluated in the peer review and listed in the EFSA Conclusion (2006) as follows:
North <0.01-0.04 mg/kg; South <0.02-0.05 mg/kg

Table 6.3.1-23: Residues of metconazole (BAS 555 F) in wheat after two applications of BAS 627 00 F or BAS 627 02 in Northern Europe

Study details		Crop	Country Trial No	Formulation Application rate (kg a.s./ha)	GS[1] BBCH	DALA[2]	Metconazole found (mg/kg)		
							Matrix	BAS 62700F	BAS 62702F
Study code:	339326	Wheat	Germany L100105	BAS 627 00 F 2 x 0.0825	69	0	Whole plant[3]	2.4	2.4
Doc ID:	2010/1144333					34	Grain	0.011	<u>0.011</u>
Author:	Schroth E.					34	Straw	1.7	*1.8*
GLP:	yes					42	Grain	0.010	< 0.010
Year:	2010					42	Straw	1.7	0.90
						49	Grain	0.010	0.010
						49	Straw	1.0	1.1
Study code:	339326	Wheat	United Kingdom L100106	BAS 627 00 F 2 x 0.0825	69	0	Whole plant[3]	2.9	2.9
Doc ID:	2010/1144333					35	Grain	<0.010	< 0.010
Author:	Schroth E.					35	Straw	4.5	6.4
GLP:	yes					41	Grain	<0.010	0.010
Year:	2010					41	Straw	5.1	5.4
						49	Grain	0.016	<u>0.014</u>
						49	Straw	4.0	*7.3*

1 Growth stage at last application
2 Days after last application
3 Without root
<u>Underlined values</u> were used for MRL derivation
Values in *italic* were used for HR and STMR determination (needed for feed burden calculation)

Table 6.3.1-24: Residues of metconazole (BAS 555 F) in wheat after two applications of BAS 627 00 F and BAS 627 02 F in Southern Europe

Study details		Crop	Country Trial No	Formulation Application rate (kg a.s./ha)	GS[1] BBCH	DALA[2]	Metconazole found (mg/kg)		
							Matrix	BAS 62700F	BAS 62702F
Study code:	339326	Wheat	France L100107	BAS 627 00 F 2 x 0.0825	69	0	Whole plant[3]	2.5	2.8
Doc ID:	2010/1144333					35	Ears	0.31	0.47
Author:	Schroth E.					35	Rest plant[3)]	0.64	0.67
GLP:	yes					42	Grain	0.010	< 0.010
Year:	2010					42	Straw	0.98	0.62
						49	Grain	0.010	<u>0.012</u>
						49	Straw	*1.4*	1.0
Study code:	339326	Wheat	Spain L100108	BAS 627 00 F 2 x 0.0825	69	0	Whole plant[3]	4.5	5.1
Doc ID:	2010/1144333					35	Grain	<0.010	<u>0.010</u>
Author:	Schroth E.					35	Straw	2.9	3.8
GLP:	yes					42	Grain	<0.010	< 0.010
Year:	2010					42	Straw	2.6	*4.0*
						48	Grain	<0.010	< 0.010
						48	Straw	2.2	3.0

1 Growth stage at last application
2 Days after last application
3 Without root
<u>Underlined values</u> were used for MRL derivation
Values in *italic* were used for HR and STMR determination (needed for feed burden calculation)

Report:	CA 6.3.1/4 Tandy R., 2012b Study on the residue behaviour of Pyraclostrobin (BAS 500 F) and Metconazole (BAS 555 F) in winter wheat after treatment with either BAS 556 03 F, BAS 500 06 F or BAS 555 00 F in Northern andSouthern Europe during 2011 2012/1194991
Guidelines:	EEC 91/414 (1607/VI/97), SANCO/3029/99 rev. 4 (11 July 2000), EEC 91/414 Annex II (Part A Section 6), EEC 91/414 Annex III (Part A Section 8), EEC 1607/VI/97 rev. 2 10.06.1999
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Report:	CA 6.3.1/5 Tandy R., 2014a Amendment No. 1: Study on the residue behaviour of Pyraclostrobin (BAS 500 F) and Metconazole (BAS 555 F) in winter wheat after treatment with either BAS 556 03 F, BAS 500 06 F or BAS 555 00 F in Northern and Southern Europe during 2011 2014/1090810
Guidelines:	EEC 91/414 (1607/VI/97), SANCO/3029/99 rev. 4 (11 July 2000), EEC 91/414 Annex II (Part A Section 6), EEC 91/414 Annex III (Part A Section 8), EEC 1607/VI/97 rev. 2 10.06.1999
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

This study contains also residue data on pyraclostrobin which are not reported in this document.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description: BAS 556 03 F (EC): pyraclostrobin: 130 g/L nominal; metconazole: 80 g/L nominal
BAS 500 06 F (EC): pyraclostrobin: 200 g/L nominal
BAS 555 00 F (EC): metconazole: 60 g/L nominal

Lot/Batch #: BAS 556 03 F (EC): 380009
BAS 500 06 F (EC): 0003223026
BAS 555 00 F (EC): 0003255328

Purity:

CAS#: BAS 500 F (pyraclostrobin): 175013-18-0
BAS 555 F (metconazole): 125116-23-6

Development code:

Spiking levels: Pyraclostrobin: 0.01-20 mg/kg
Cis-metconazole: 0.005-10 mg/kg
Trans-metconazole: 0.005-10 mg/kg

2. Test Commodity:

Crop: Wheat

Type: Cereals

Variety: Julius, Oakley, PR22 R58, Bologna

Botanical name: *Triticum aestivum*

Crop parts(s) or processed commodity: Whole plant w/o root, ears, rest of plant w/o root, grain, straw

Sample size: Whole plant w/o root, straw: ≥0.5 kg / ears, rest of plant w/o root, grain: ≥1.0 kg

B. STUDY DESIGN

1. Test procedure

During the 2011 growing season, 4 trials in wheat were conducted in different representative growing areas in Germany, United Kingdom, France and Italy to determine the residue level of pyraclostrobin and metconazole, including the triazole derivative metabolites, in or on wheat raw agricultural commodities (RAC).
The fungicidal combination product BAS 556 03 F, containing pyraclostrobin and metconazole, was compared to the solo formulations of both active substances, BAS 500 06 F (pyraclostrobin) and BAS 555 00 F (metconazole). Only the residue data for metconazole are reported in this document.
In all cases, the test item was foliar applied two times in a spray volume of 200 L/ha. BAS 556 03 F was applied in plot 2 at a target rate corresponding to 0.088 kg/ha metconazole. BAS 555 00 F was applied in plot 4 at a target rate of 0.090 kg/ha of metconazole.

Whole plant without root specimens were collected after the last application at growth stage 69 BBCH. At 27-29 days after the last application (DALA), 34-36 DALA and 39-43 DALA, either ears and rest of plant without roots or grain or straw specimens were taken depending on the maturity. At trials S11-00711-01 and 02 (L110188 and L110189) where BBCH 89 was not reached at 42±1 DALA, grain and straw specimens were taken 57 DALA when maturity was reached.
All specimens were frozen within 24 hours of being taken and remained frozen (≤-18°C) until analysis including transportation. The maximum storage interval from harvest until extraction for analysis of metconazole residues was 343 days.

Table 6.3.1-25: Target application rates and timings for wheat

Year	No of trials	No of appl.	F P or G	Test item	Active substance	Appl. rate (kg a.s./ha)	Water volume (L/ha)	Target date/ Timing[1]
2007	6	2	F	BAS 556 03 F (EC)	BAS 500 F	0.143	200	1st appl.: 49 BBCH 2nd appl.: 69 BBCH
					BAS 555 F	0.088		
		2	F	BAS 500 06 F (EC)	BAS 500 F	0.250	200	
		2	F	BAS 555 00 F (EC)	BAS 555 F	0.090	200	

2. Description of analytical procedures

Samples were analyzed for metconazole residues using BASF method No 535/1 (L0076/01). The homogenized specimens were extracted using a mixture of methanol, water and hydrochloric acid. An aliquot of the extract was centrifuged and partitioned at alkaline conditions against cyclohexane, and the analytes in the final extract were determined by LC-MS/MS. The limit of quantitation in plant material was 0.005 mg/kg for each cis- and trans-metconazole (0.01 mg/kg for the sum of both isomers).

Table 6.3.1-26: Summary of procedural recovery data for metconazole

Matrix	Fortification level (mg/kg)	Summary recoveries					
		n	Mean (%)	SD (%)	N	Mean (%)	SD (%)
BASF method No L0076/01; LOQ = 0.005 mg/kg		**cis-Metconazole**			**trans-Metconazole**		
Whole plant, rest plant without roots	0.005-10	8	82	8	8	78	9
Ears	0.005-1.0	8	85	2	8	84	2
Grain	0.005-0.50	8	87	4	8	83	3
Straw	0.005-4.0	8	82	6	8	80	5

Triazole derivative metabolites

Bayer Crop Science Method 01062/M003 was adapted to determine the triazole derivative metabolites (TDM) T, TA, TAA and TLA. Validation of the method in a range of matrices is reported (MCA 4.1.2). The analytes were extracted from the specimen matrix with methanol/water (4/1, v/v). An aliquot was filtered, concentrated and cleaned-up by dispersive C18-SPE prior to analysis by LC-MS/MS using internal standardization based on stable isotope standards. The limit of quantitation (LOQ) is 0.01 mg/kg for each analyte.

Table 6.3.1-27: Summary of recoveries of triazole derivative metabolites in wheat

Matrix	Fortification level (mg/kg)	Summary recoveries[1]					
		n	Mean (%)	RSD (%)	n	Mean (%)	RSD (%)
Bayer Crop Science Method 01062/M003		**T**[2]			**TA**		
Whole plant, rest plant without roots	0.01-1.0	8	77	9	8	106	8
Ears	0.01-1.0	8	90	10	8	100	16
Grain	0.01-1.0	8	88	20	8	92	14
Straw	0.01-1.0	8	92	11	8	84	14
Bayer Crop Science Method 01062/M003		**TAA**			**TLA**		
Whole plant, rest plant without roots	0.01-1.0	7	83	22	8	97	17
Ears	0.01-1.0	8	88	12	8	85	8
Grain	0.01-1.0	7	78	12	8	86	15
Straw	0.01-1.0	8	94	12	8	91	10

1 T=1,2,4-triazole, TA=triazolylalanine, TAA=triazole acetic acid, TLA=triazole lactic acid

II. RESULTS AND DISCUSSION

The residue ranges of metconazole in/on wheat treated either with formulation BAS 556 03 F or BAS 555 00 F are shown in Table 6.3.1-28. Details are presented in Table 6.3.1-31 to Table 6.3.1-34.
Comparison of the residue levels in the whole plant immediately after treatment, indicate comparable residues of metconazole at 1.1-2.9 mg/kg after treatment with BAS 556 03 F (plot 2) and 1.3-3.4 mg/kg after treatment with BAS 555 00 F (plot 4).
The residue levels were also comparable at harvest. Residues of metconazole found in grain were <0.01-0.011 mg/kg (plot 2) and <0.01 mg/kg (plot 4) at 36 DALA.
Metconazole residues in straw reached a maximum of 2.9 mg/kg at growth stage 89 BBCH (42 DALA). No residues of metconazole at or above the limit of quantitation (0.01 mg/kg) were found in any of the analyzed untreated specimens.

Table 6.3.1-28: Summary of residues in wheat treated with BAS 556 03 F or BAS 555 00 F

Crop	Year	DALA[1]	Growth stage[2] (BBCH)	Metconazole residues (sum of cis- and trans-isomer; mg/kg)		
				Matrix	BAS 556 03 F	BAS 555 00 F
Wheat	2011	0	69	Whole plant without roots	1.1-2.9	1.3-3.4
		27-29	77-87	Ears	0.10-0.18	0.12-0.28
		34-36	82-87	Ears	0.06-0.24	0.25-0.34
		43	85	Ears	0.17	0.27
		27-29	77-87	Rest of plant without roots	0.53-3.2	0.69-3.6
		34-36	82-87	Rest of plant without roots	0.67-1.8	0.79-2.6
		43	85	Rest of plant without roots	0.56	0.15-0.72
		36	83	Grain	0.011	<0.01
		39-42	85-89	Grain	<0.01	<0.01
		57	89	Grain	<0.01	<0.01
		36	83	Straw	<0.01	0.88
		39-42	85-89	Straw	0.81-1.2	1.1-2.9
		57	89	Straw	0.63-0.71	0.57-0.84

1 Days after last application
2 At sampling

Triazole derivative metabolites

The maximum storage interval from harvest until extraction for analysis of metconazole including the TDM residues was 343 days. Freezer storage stability is established for TDMs through a minimum interval of 12 months (MCA 6.1).

T residues were below the LOQ of 0.01 mg/kg in wheat matrices. TA residues ranged between <0.01 mg/kg and 0.38 mg/kg. TAA residues were 0.012-0.20 mg/kg. Residues of TLA were below the LOQ in ears and grain. In all other samples, residues ranged from 0.019 to 0.17 mg/kg.

Table 6.3.1-29: Summary of residues of triazole derivative metabolites in wheat treated with BAS 556 03 F or BAS 555 00 F

Crop	Year	Application	GS[1]	DALA[2]	Matrix	Residues[3] (mg/kg)			
						T	TA	TAA	TLA
Wheat (EU North)	2011	BAS 556 03 F (EC)	69	0	Whole plant	<0.01	0.051-0.076	0.058-0.12	0.090-0.12
			79-81	29	Ears	<0.01	0.24-0.25	0.11-0.16	<0.01
			82	36	Ears	<0.01	0.27	0.12	<0.01
			85	43	Ears	<0.01	0.28	0.12	<0.01
			79-81	29	Rest of plant	<0.01	<0.01-0.010	0.020-0.094	0.12-0.15
			82	36	Rest of plant	<0.01	<0.01	0.030	0.11
			85	43	Rest of plant	<0.01	0.011	0.040	0.082
			83-85	36	Grain	<0.01	0.35	0.20	<0.01
			85-87	42	Grain	<0.01	0.31	0.18	<0.01
			89	57	Grain	<0.01	0.27-0.36	0.12-0.19	<0.01
			83-85	36	Straw	<0.01	0.013	0.12	0.12
			85-87	42	Straw	<0.01	<0.01	0.19	0.15
			89	57	Straw	<0.01	<0.01-0.024	0.057-0.11	0.037-0.042
		BAS 555 00 F (EC)	69	0	Whole plant	<0.01	0.050-0.077	0.040-0.088	0.067-0.11
			79-81	29	Ears	<0.01	0.19-0.21	0.073-0.13	<0.01
			82	36	Ears	<0.01	0.17	0.088	<0.01
			85	43	Ears	<0.01	0.17	0.065	<0.01
			79-81	29	Rest of plant	<0.01	<0.01-0.01	0.015-0.084	0.078-0.13
			82	36	Rest of plant	<0.01	<0.01	0.028	0.065
			85	43	Rest of plant	<0.01	<0.01	0.031	0.056
			83-85	36	Grain	<0.01	0.25	0.13	<0.01
			85-87	42	Grain	<0.01	0.38	0.16	<0.01
			89	57	Grain	<0.01	0.23-0.30	0.075-0.16	<0.01
			83-85	36	Straw	<0.01	0.013	0.10	0.11
			85-87	42	Straw	<0.01	0.017	0.17	0.17
			89	57	Straw	<0.01	0.011-0.012	0.039-0.094	0.021-0.031

Table 6.3.1-29: Summary of residues of triazole derivative metabolites in wheat treated with BAS 556 03 F or BAS 555 00 F

Crop	Year	Application	GS[1]	DALA[2]	Matrix	Residues[3] (mg/kg)			
						T	TA	TAA	TLA
Wheat (EU South)	2011	BAS 556 03 F (EC)	69	0	whole plant	<0.01	0.014-0.10	0.014-0.059	0.022-0.071
			77-87	27-28	ears	<0.01	0.054-0.095	0.036-0.042	<0.01
			85-87	34-35	ears	<0.01	0.044-0.18	0.029-0.10	<0.01
			77-87	27-28	rest of plant	<0.01	<0.01	0.018-0.027	0.047-0.10
			85-87	34-35	rest of plant	<0.01	<0.01-0.015	0.012-0.026	0.019-0.078
			89	39-42	grain	<0.01	0.092-0.23	0.039-0.099	<0.01
			89	39-42	straw	<0.01	<0.01-0.020	0.025-0.11	0.019-0.021
		BAS 555 00 F (EC)	69	0	whole plant	<0.01	0.014-0.027	0.014-0.027	0.024-0.027
			77-87	27-28	ears	<0.01	0.066-0.099	0.037-0.038	<0.01
			85-87	34-35	ears	<0.01	0.054-0.075	0.032-0.048	<0.01
			77-87	27-28	rest of plant	<0.01	<0.01	0.016-0.018	0.057-0.066
			85-87	34-35	rest of plant	<0.01	<0.01-0.010	0.012-0.023	0.021-0.064
			89	39-42	grain	<0.01	0.079-0.14	0.034-0.056	<0.01
			89	39-42	straw	<0.01	<0.01	0.031-0.038	0.022-0.082

1 Growth stage at sampling (BBCH)
2 Days after last application
3 T=1,2,4-triazole, TA=triazolylalanine, TAA=triazole acetic acid, TLA=triazole lactic acid

Apparent residues of T were <LOQ (<0.01 mg/kg) in/on all the untreated control samples analysed. Even in the absence of maintenance treatments with triazole-containing plant protection products, many residue trials showed measurable residues of TDMs in the control samples. These residues are attributed to the use of triazole-containing plant products on the test plots during previous seasons. It is assumed that besides plant protection products other sources (such as fertilizers) potentially contribute to the presence of 1,2,4-triazole residues in agricultural soils. In the absence of maintenance treatments with triazole-containing plant protection products, the residues of triazole derivative metabolites (TDMs) in control samples reflect the residues that result from the use of triazole-containing plant protection products during previous growing seasons as well as the residues from other sources than plant protection products. Therefore, the levels of TDM residues measured in the treated samples are considered to properly reflect the residues that result from the test item and from the use of triazole-containing plant protection products during previous growing seasons. Apparent residues of TA, TAA and TLA were <LOQ (<0.01 mg/kg) in the untreated control samples with the exceptions shown in Table 6.3.2-32. Residues of TA, TAA and TLA were similar in treated and untreated samples indicating that treatment with BAS 556 03 F or BAS 555 00 F had little impact on the residue level of these metabolites.

Table 6.3.1-30: Summary of TDM residues in untreated wheat control samples

Crop	Field Report	Application	Residues Found[1] (mg/kg)				
			Matrix	T	TA	TAA	TLA
Wheat	L110188	Metconazole	plant	<0.01	0.051	0.049	0.084
			ears	<0.01	0.16	0.090	<0.01
			rest	<0.01	<0.01	0.024	0.087
			ears	<0.01	0.16	0.088	<0.01
			rest	<0.01	<0.01	0.038	0.067
			ears	<0.01	0.16	0.097	<0.01
			rest	<0.01	<0.01	0.041	0.053
			grain	<0.01	0.28	0.12	<0.01
			straw	<0.01	<0.01	0.059	0.035
	L110189		plant	<0.01	0.090	0.14	0.16
			ears	<0.01	0.19	0.16	<0.01
			rest	<0.01	0.041	0.15	0.13
			grain	<0.01	0.28	0.23	<0.01
			straw	<0.01	<0.01	0.17	0.11
			grain	<0.01	0.35	0.26	<0.01
			straw	<0.01	0.015	0.28	0.14
			grain	<0.01	0.31	0.29	<0.01
			straw	<0.01	0.014	0.10	0.023
	L110190		ears	<0.01	0.021	0.021	<0.01
			rest	<0.01	<0.01	0.010	0.030
			ears	<0.01	0.020	0.021	<0.01
			rest	<0.01	<0.01	<0.01	0.012
			grain	<0.01	0.035	0.021	<0.01
			straw	<0.01	<0.01	0.020	0.010
	L110191		plant	<0.01	0.031	0.021	<0.01
			ears	<0.01	0.059	0.022	<0.01
			rest	<0.01	<0.01	0.014	0.070
			ears	<0.01	0.067	0.044	<0.01
			rest	<0.01	<0.01	0.018	0.035
			grain	<0.01	0.068	0.024	<0.01
			straw	<0.01	<0.01	0.031	0.045

1 T = 1,2,4 triazole
TA = triazole alanine
TAA = triazole acetic acid
TLA = triazole lactic acid

III. CONCLUSION

After application of BAS 556 03 F, metconazole residues in wheat grain samples at intended harvest were <0.01-0.01 mg/kg. Residues of metconazole in wheat grain measured after treatment with the solo formulation BAS 555 00 F were comparable to the residue levels measured after treatment with the formulated product BAS 556 03 F.
Residues of 1,2,4-triazole (T) were below the LOQ of 0.01 mg/kg in wheat matrices. TA residues ranged between <0.01 mg/kg and 0.38 mg/kg. TAA residues were 0.012-0.20 mg/kg. Residues of TLA were below the LOQ in ears and grain. In all other samples, residues ranged from 0.019 to 0.17 mg/kg. Residues of TA, TAA and TLA were similar in treated and untreated samples indicating that treatment with BAS 556 03 F or BAS 555 00 F had little impact on the residue level of these metabolites.

Table 6.3.1-31: Residues of metconazole in wheat after two applications of BAS 556 03 F in Northern Europe

Trial details		Crop	Country	Formulation, Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1)]	Residues found (mg/kg)	
							Matrix	Metconazole (sum of cis- and trans-isomer)
Study code:	S11-00711	Wheat	Germany	BAS 556 03 F	69	0	Whole plant without root	2.3
Doc ID:	2012/1194991			2 x 0.088		29	Ears	0.12
Trial No:	L110188					29	Rest of plant without root	0.53
GLP:	Yes					36	Ears	0.16
Year:	2011					36	Rest of plant without root	0.67
						43	Ears	0.17
						43	Rest of plant without root	0.56
						57	Grain	<0.01
						57	Straw	0.71
Study code:	S11-00711	Wheat	United	BAS 556 03 F	69	0	Whole plant without root	1.1
Doc ID:	2012/1194991		Kingdom	2 x 0.088		29	Ears	0.14
Trial No:	L110189					29	Rest of plant without root	0.54
GLP:	Yes					36	Grain	<u>0.011</u>
Year:	2011					36	Straw	<0.01
						42	Grain	<0.01
						42	Straw	*1.2*
						57	Grain	<0.01
						57	Straw	0.63

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
<u>Underlined values</u> were used for MRL derivation
Values in *italic* were used for HR and STMR determination (needed for feed burden calculation)

Table 6.3.1-32: Residues of metconazole in wheat after two applications of BAS 556 03 F in Southern Europe

Trial details		Crop	Country	Formulation, Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1)]	Residues found (mg/kg)	
							Matrix	Metconazole (sum of cis- and trans-isomer)
Study code:	S11-00711	Wheat	France	BAS 556 03 F	69	0	Whole plant without root	2.2
Doc ID:	2012/1194991			2 x 0.088		28	Ears	0.18
Trial No:	L110190					28	Rest of plant without root	3.2
GLP:	Yes					35	Ears	0.24
Year:	2011					35	Rest of plant without root	1.8
						42	Grain	<0.01
						42	Straw	0.91
Study code:	S11-00711	Wheat	Italy	BAS 556 03 F	69	0	Whole plant without root	2.9
Doc ID:	2012/1194991			2 x 0.088		27	Ears	0.10
Trial No:	L110191					27	Rest of plant without root	1.0
GLP:	Yes					34	Ears	0.06
Year:	2011					34	Rest of plant without root	1.0
						39	Grain	<0.01
						39	Straw	0.81

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application

Table 6.3.1-33: Residues of metconazole in wheat after two applications of BAS 555 00 F in Northern Europe

Trial details		Crop	Country	Formulation, Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1)]	Residues found (mg/kg)	
							Matrix	Metconazole (sum of cis- and trans-isomer)
Study code:	S11-00711	Wheat	Germany	BAS 555 00 F	69	0	Whole plant without root	2.8
Doc ID:	2012/1194991			2 x 0.090		29	Ears	0.23
Trial No:	L110188					29	Rest of plant without root	0.85
GLP:	Yes					36	Ears	0.27
Year:	2011					36	Rest of plant without root	0.79
						43	Ears	0.27
						43	Rest of plant	0.72
						57	Grain	<u><0.01</u>
						57	Straw	*0.84*
Study code:	S11-00711	Wheat	United Kingdom	BAS 555 00 F	69	0	Whole plant without root	1.3
Doc ID:	2012/1194991			2 x 0.090		29	Ears	0.12
Trial No:	L110189					29	Rest of plant without root	0.69
GLP:	Yes					36	Grain	<0.01
Year:	2011					36	Straw	0.88
						42	Grain	<0.01
						42	Straw	1.1
						57	Grain	<0.01
						57	Straw	0.57

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
<u>Underlined values</u> were used for MRL derivation
Values in *italic* were used for HR and STMR determination (needed for feed burden calculation)

Table 6.3.1-34: Residues of metconazole in wheat after two applications of BAS 555 00 F in Southern Europe

Trial details		Crop	Country	Formulation, Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1)]	Residues found (mg/kg)	
							Matrix	Metconazole (sum of cis- and trans-isomer)
Study code:	S11-00711	Wheat	France	BAS 555 00 F	69	0	Whole plant without root	2.4
Doc ID:	2012/1194991			2 x 0.090		28	Ears	0.28
Trial No:	L110190					28	Rest of plant without root	3.6
GLP:	Yes					35	Ears	0.25
Year:	2011					35	Rest of plant without root	2.6
						42	Grain	<u><0.01</u>
						42	Straw	*2.9*
Study code:	S11-00711	Wheat	Italy	BAS 555 00 F	69	0	Whole plant without root	3.4
Doc ID:	2012/1194991			2 x 0.090		27	Ears	0.17
Trial No:	L110191					27	Rest of plant without root	1.8
GLP:	Yes					34	Ears	0.34
Year:	2011					34	Rest of plant without root	1.7
						39	Grain	<u><0.01</u>
						39	Straw	*2.56*

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
<u>Underlined values</u> were used for MRL derivation
Values in *italic* were used for HR and STMR determination (needed for feed burden calculation)

Table 6.3.1-35: Residues of TDMs in wheat after two applications of BAS 556 03 F in Northern Europe

Trial details		Crop	Country	Formulation, Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1)]	Residues found[3] (mg/kg)				
							Matrix	T	TA	TAA	TLA
Study code:	S11-00711	Wheat	Germany	BAS 556 03 F	69	0	Whole plant[4]	<0.01	0.051	0.058	0.090
Doc ID:	2012/1194991			2 x 0.088		29	Ears	<0.01	0.25	0.11	<0.01
Trial No:	L110188					29	Rest of plant[4]	<0.01	<0.01	0.020	0.12
GLP:	Yes					36	Ears	<0.01	0.27	0.12	<0.01
Year:	2011					36	Rest of plant[4]	<0.01	<0.01	0.030	0.11
						43	Ears	<0.01	0.28	0.12	<0.01
						43	Rest of plant[4]	<0.01	0.011	0.040	0.082
						57	Grain	<0.01	0.36	0.12	<0.01
						57	Straw	<0.01	0.024	0.057	0.037
Study code:	S11-00711	Wheat	United Kingdom	BAS 556 03 F	69	0	Whole plant[4]	<0.01	0.076	0.12	0.12
Doc ID:	2012/1194991			2 x 0.088		29	Ears	<0.01	0.24	0.16	<0.01
Trial No:	L110189					29	Rest of plant[4]	<0.01	0.010	0.094	0.15
GLP:	Yes					36	Grain	<0.01	0.35	0.20	<0.01
Year:	2011					36	Straw	<0.01	0.013	0.12	0.12
						42	Grain	<0.01	0.31	0.18	<0.01
						42	Straw	<0.01	<0.01	0.19	0.15
						57	Grain	<0.01	0.27	0.19	<0.01
						57	Straw	<0.01	<0.01	0.11	0.042

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
3 T=1,2,4-triazole, TA=triazolylalanine, TAA=triazole acetic acid, TLA=triazole lactic acid
4 Without root

Table 6.3.1-36: Residues of TDMs in wheat after two applications of BAS 556 03 F in Southern Europe

Trial details		Crop	Country	Formulation, Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1)]	Residues found[3] (mg/kg)				
							Matrix	T	TA	TAA	TLA
Study code:	S11-00711	Wheat	France	BAS 556 03 F	69	0	Whole plant[4]	<0.01	0.014	0.014	0.022
Doc ID:	2012/1194991			2 x 0.088		28	Ears	<0.01	0.054	0.036	<0.01
Trial No:	L110190					28	Rest of plant[4]	<0.01	<0.01	0.018	0.047
GLP:	Yes					35	Ears	<0.01	0.044	0.029	<0.01
Year:	2011					35	Rest of plant[4]	<0.01	<0.01	0.012	0.019
						42	Grain	<0.01	0.092	0.039	<0.01
						42	Straw	<0.01	<0.01	0.025	0.019
Study code:	S11-00711	Wheat	Italy	BAS 556 03 F	69	0	Whole plant[4]	<0.01	0.10	0.059	0.071
Doc ID:	2012/1194991			2 x 0.088		27	Ears	<0.01	0.095	0.042	<0.01
Trial No:	L110191					27	Rest of plant[4]	<0.01	<0.01	0.027	0.10
GLP:	Yes					34	Ears	<0.01	0.18	0.10	<0.01
Year:	2011					34	Rest of plant[4]	<0.01	0.015	0.026	0.078
						39	Grain	<0.01	0.23	0.099	<0.01
						39	Straw	<0.01	0.020	0.11	0.021

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
3 T=1,2,4-triazole, TA=triazolylalanine, TAA=triazole acetic acid, TLA=triazole lactic acid
4 Without root

Table 6.3.1-37: Residues of TDMs in wheat after two applications of BAS 555 00 F in Northern Europe

Trial details		Crop	Country	Formulation, Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1)]	Residues found[3] (mg/kg)				
							Matrix	T	TA	TAA	TLA
Study code:	S11-00711	Wheat	Germany	BAS 555 00 F	69	0	Whole plant[4]	<0.01	0.050	0.040	0.067
Doc ID:	2012/1194991			2 x 0.090		29	Ears	<0.01	0.19	0.073	<0.01
Trial No:	L110188					29	Rest of plant[4]	<0.01	<0.01	0.015	0.078
GLP:	Yes					36	Ears	<0.01	0.17	0.088	<0.01
Year:	2011					36	Rest of plant[4]	<0.01	<0.01	0.028	0.065
						43	Ears	<0.01	0.17	0.065	<0.01
						43	Rest of plant[4]	<0.01	<0.01	0.031	0.056
						57	Grain	<0.01	0.23	0.075	<0.01
						57	Straw	<0.01	0.011	0.039	0.021
Study code:	S11-00711	Wheat	United Kingdom	BAS 555 00 F	69	0	Whole plant[4]	<0.01	0.077	0.088	0.11
Doc ID:	2012/1194991			2 x 0.090		29	Ears	<0.01	0.21	0.13	<0.01
Trial No:	L110189					29	Rest of plant[4]	<0.01	0.01	0.084	0.13
GLP:	Yes					36	Grain	<0.01	0.25	0.13	<0.01
Year:	2011					36	Straw	<0.01	0.013	0.10	0.11
						42	Grain	<0.01	0.38	0.16	<0.01
						42	Straw	<0.01	0.017	0.17	0.17
						57	Grain	<0.01	0.30	0.16	<0.01
						57	Straw	<0.01	0.012	0.094	0.031

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
3 T=1,2,4-triazole, TA=triazolylalanine, TAA=triazole acetic acid, TLA=triazole lactic acid
4 Without root

Table 6.3.1-38: Residues of TDMs in wheat after two applications of BAS 555 00 F in Southern Europe

Trial details		Crop	Country	Formulation, Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1)]	Residues found[3] (mg/kg)				
							Matrix	T	TA	TAA	TLA
Study code:	S11-00711	Wheat	France	BAS 555 00 F	69	0	Whole plant[4]	<0.01	0.014	0.014	0.024
Doc ID:	2012/1194991			2 x 0.090		28	Ears	<0.01	0.066	0.038	<0.01
Trial No:	L110190					28	Rest of plant[4]	<0.01	<0.01	0.016	0.057
GLP:	Yes					35	Ears	<0.01	0.054	0.032	<0.01
Year:	2011					35	Rest of plant[4]	<0.01	<0.01	0.012	0.021
						42	Grain	<0.01	0.079	0.034	<0.01
						42	Straw	<0.01	<0.01	0.031	0.022
Study code:	S11-00711	Wheat	Italy	BAS 555 00 F	69	0	Whole plant[4]	<0.01	0.027	0.027	0.027
Doc ID:	2012/1194991			2 x 0.090		27	Ears	<0.01	0.099	0.037	<0.01
Trial No:	L110191					27	Rest of plant[4]	<0.01	<0.01	0.018	0.066
GLP:	Yes					34	Ears	<0.01	0.075	0.048	<0.01
Year:	2011					34	Rest of plant[4]	<0.01	0.010	0.023	0.064
						39	Grain	<0.01	0.14	0.056	<0.01
						39	Straw	<0.01	<0.01	0.038	0.082

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
3 T=1,2,4-triazole, TA=triazolylalanine, TAA=triazole acetic acid, TLA=triazole lactic acid
4 Without root

CA 6.3.2 Barley

Table 6.3.2-1: Summary of the critical GAP for the proposed use of BAS 555 01 F in barley in the EU

Crop	Outdoor/ Protected	Growth stage (BBCH)	Maximum number of applications	Minimum application interval (days)	Maximum		Minimum PHI (days)
					Rate (kg a.s./ha)	Water (L/ha)	
Cereals (barley)	Outdoor	30-69	2	14	0.090	110-400	PHI is covered by the time remaining between application and harvest or by PHI of 35 days

Table 6.3.2-2: Summary of the cGAP used for trials evaluated in the DAR for use in barley with BAS 555 00 F

Crop	Outdoor/ Protected	Growth stage (BBCH)	Maximum number of applications	Minimum application interval (days)	Maximum		Minimum PHI (days)
					Rate (kg a.s./ha)	Water (L/ha)	
Cereals (barley)	Outdoor	29-69	2	14	0.090	150-400	PHI of 35 days

Table 6.3.2-3: Number of residue trials conducted with BAS 555 00 F according to the cGAP per geographical region and vegetation period and evaluated in the DAR for use in barley (4 trials in Addendum of August 2004)

Crop	Vegetation period	Number of trials					Reference
		EU North	Country	EU South	Country	Total	
Barley	1994	1	DE			1	MK -730-025
	1995	1	DE			1	MK -730-026
	1996	12	DE, UK			12	MK -730-028 MK -730-029 MK -730-031 MK -730-032 MK -730-033 MK -730-034 MK -730-036 MK -730-038 MK -730-040 MK -730-043
	1997	2	UK			2	MK -730-042
	2004			4	FR	4	2004/1000755
Total number of trials per region		**16**		**4**	**Total number of trials**	**20**	

Residue trials on barley (see Table 6.3.2-3) performed with BAS 555 00 F were submitted for the previous active substance approval procedure and were evaluated in the DAR (Belgium, 2004) and by EFSA (EFSA Conclusion 2006). All the submitted studies were carried out under GLP conditions. A total number of 16 supervised residue trials in Northern Europe and four trials for Southern Europe were considered for winter and spring barley. The trials were performed according to the cGAP with two applications at a nominal rate of 90 g/ha with an application interval of 21 days as defined in Table 6.3.2-2. Samples of whole green plants at day 0 as well as samples of ears, plants with ears removed were sampled up to normal harvest time and straw and grain were sampled at the targeted PHI of 35 days as well as later PHIs. All samples were analyzed for metconazole using Method FAMS 050-01 with a method LOQ of 0.01 mg/kg for each isomer.

The requirement for additional trials in barley in Southern Europe was noted in the EFSA Conclusion as cited from the Summary.

> "For barley uses in Southern Europe additional trial data need to be submitted to confirm the compliance with currently proposed MRL based on Northern European trials."

For the submitted and peer evaluated trials in barley, the application parameters and results as reported in the EFSA Conclusion 2006 are shown below (residues are rearranged here in increasing concentration).

Winter/spring barley (possible extrapolation to oat (for Northern Europe only)

North (89-96 g a.s./ha, 2 applications BBCH 33; 71-75, PHI: 35-37 days)

- Grain: <0.01 (4x), 0.01 (3x), 0.02, 0.03 (5x), 0.05 (2x), 0.09 mg/kg
- Straw: 0.03, 0.13, 0.15, 0.18, 0.22, 0.23, 0.32, 0.37, 0.73, 0.74, 0.82, 0.99, 1.06, 1.33, 1.37 1.61 mg/kg

South (target dose of 90 g a.s./ha, 2 applications (BBCH 51-83), PHI: 35 days)
-Grain: 0.03 0.03 0.04, 0.05 mg/kg
-Straw 1.3, 1.4, 1.5, 2.6 mg/kg

Residue studies in barley not peer evaluated as part of the Annex 1 approval process

The four additional trials in barley matching the critical GAP to complete the requirement for Southern Europe were performed in 2007 and are summarized below (see CA 6.3.2/1). The trials were performed with BAS 555 01 F (90 g a.s./L EC).

Additional residue studies performed in barley according to the cGAP (6.3.2/3 to 6/3.2/5) with BAS 555 00 F and other BAS 555 F-containing formulations such as BAS 627 00 F and BAS 627 02 F (each a mixture of metconazole and epoxiconazole) are submitted in this dossier to demonstrate that metconazole has been tested in a large number of field trials over many seasons. The data is submitted to provide a weight of the evidence approach to support the BASF proposal that no additional residue studies with BAS 555 01 F are needed to support the designation of BAS 555 01 F as the representative formulation in place of BAS 555 00 F. For the studies performed with mixtures, the residue results for the mixing partner are not included in this dossier.

The data generated in these studies, in addition to the studies evaluated in the Annex I process, were submitted and evaluated by CRD in accordance with Regulation (EC) No 396/2005 to raise the MRL in barley grain. EFSA concluded the data was sufficient to derive a MRL (EFSA Reasoned Opinion 2013), and the modified MRL for barley grain, extrapolated to oat grain, of 0.4 mg/kg was approved by the European Commission June 2014 (published July 10, 2014).

Report:	CA 6.3.2/1 Schroth E.,Martin T., 2008a Study on the residue behavior of Metconazole on barley after the application of BAS 555 01 F under field conditions in Italy and Spain, 2007 2008/1009268
Guidelines:	EEC 91/414 Annex II (Part A Section 6), EEC 91/414 Annex III (Part A Section 8), EEC 1607/VI/97 rev. 2 10.06.1999, EEC 7029/VI/95 rev. 5, EEC 7525/VI/95 rev. 7
GLP:	yes (certified by ENAC, Entidad Nacional de Acreditación, Madrid, Spain)

Report:	CA 6.3.2/2 Schroth E., 2009a Amendment No. 1: Study on the residue behavior of Metconazole on barley after the application of BAS 555 01 F under field conditions in Italy and Spain, 2007 2009/1102126
Guidelines:	EEC 91/414 Annex II (Part A Section 6), EEC 91/414 Annex III (Part A Section 8), EEC 1607/VI/97 rev. 2 10.06.1999, EEC 7029/VI/95 rev. 5, EEC 7525/VI/95 rev. 7
GLP:	yes (certified by ENAC, Entidad Nacional de Acreditación, Madrid, Spain)

I. MATERIAL AND METHODS

A. MATERIALS

Test Material:

Description:	BAS 555 01 F (SL)
Lot/Batch #:	200001, metconazole: 90 g/L nominal
Purity:	Not relevant
CAS#:	Metconazole-125116-23-6
Development code:	
Spiking levels:	Metconazole-0.005-1.0 mg/kg

Test Commodity:

Crop:	Barley
Type:	Cereals
Variety:	Belen, Cecilia, Aliseo, Trasimeno
Botanical name:	*Hordeum vulgare*
Crop parts(s) or processed Commodity:	Grain, straw, whole plant without root, ears, rest of plant without root
Sample size:	Grain 1 kg, straw, 0.5 kg

B. STUDY DESIGN

1. Test procedure

During the 2007 growing season, four field trials were conducted in different representative barley growing areas in Italy and Spain to determine the magnitude of the residues in or on raw agricultural commodities (RAC).
The fungicidal product BAS 555 01 F (90 g/L of metconazole, SL) was applied two times at 0.090 kg a.s./ha in a spray volume of 150 L/ha, resulting in a maximum seasonal target rate of 0.18 kg/ha.
The first application was done about 21 days before growth stage 69 BBCH, the second treatment was targeted at growth stage 69.
Barley whole plant without root specimens were collected directly after the last application. Either ears and rest of plant, or grain and straw were taken depending on the crop maturity at 35, 42 and 49 days after the last application.
All specimens were frozen within 24 hours after being taken and remained frozen (≤-18°C) until analysis including transportation.

Table 6.3.2-4: Target application rates and timings for barley

Year	No of trials	No of Appl.	F P or G	Test item	Active substance	Appl. rate (kg a.s./ha)	Water volume (L/ha)	Target date/ Timing[1]
2007	4	2	F	BAS 555 01 F (SL)	BAS 555 F	0.090	150	2nd appl.: 69 BBCH or 35 day PHI/ 21 day interval

1 EU-wide the cGAP PHI is 35days if the GAP defines a PHI in days after last treatment. For the northern regions such as UK and IE, the PHI is defined by the latest application, which can occur through growth stage BBCH 69.

2. Description of analytical procedures

Samples were analyzed for *cis*- and *trans*-metconazole with BASF method No 550/0 (L0019/01) which allows the determination of each isomer with a limit of quantitation of 0.005 mg/kg. The results of the recovery experiments ranged from 79% to 96% with an average of 88% for the *trans*-isomer (n=13, S.D.=5.3), and ranged from 83% to 97% with an average of 89% for the *cis*-isomer (n=13, S.D.=4.6) at fortification levels between 0.005 and 1.0 mg/kg. The coefficient of variation was below 5.9% and 5.1%. The results for each matrix are shown below.

Table 6.3.2-5: Summary of recoveries of metconazole (BAS 555 F) in barley

Matrix	Fortification level (mg/kg)	Summary of recoveries							
		n	Mean (%)	SD (+/-)	RSD (%)	n	Mean (%)	SD (+/-)	RSD (%)
Method No 550/0		***trans*-metconazole**				***cis*-metconazole**			
Ears and grain	0.005	2	91.7	3.0	3.3	2	92.6	2.9	3.1
	Higher levels	4	85.5	2.2	2.6	4	85.0	2.0	2.3
Plant and straw	0.005	3	87.7	8.4	9.6	3	94.1	2.7	2.9
	Higher levels	4	89.8	5.9	6.6	4	86.7	3.0	3.4
total		13	88.3	5.3	6.0	13	88.8	4.6	5.1

II. RESULTS AND DISCUSSION

Directly after the last application, the sum of *cis*- and *trans*-metconazole ranged between 0.882 and 2.069 mg/kg in barley whole plant without root.

In **barley grain,** the levels of total metconazole were 0.019-0.053 mg/kg after 35-36 days, 0.011-0.040 mg/kg after 42 days and 0.014-0.045 mg/kg after 48-50 days.

In **barley straw,** the residues 0.047-0.184 mg/kg after 35-36 days, 0.082-0.389 mg/kg after 42 days and 0.063-0.343 mg/kg after 48-50 days.

Table 6.3.2-6: Summary of residues in barley from trials according to critical GAP after application of BAS 555 01 F

Sampling No.	Portion analyzed	n	DALA [1]	Growth stage	Range of metconazole residues (sum of *trans* and *cis*) (mg/kg)
1	whole plant 2)	4	0	69 - 71	0.882 - 2.069
2	ears rest of plant 2)	2	35 - 36	87	0.036 - 0.039 0.194 - 0.308
	grain straw	2		83 - 87	0.019 - 0.053 0.047 - 0.184
3	grain straw	4	42	87 - 89	0.011 - 0.040 0.082 - 0.389
4	grain straw	4	48 - 50	89 - 92	0.014 - 0.045 0.063 - 0.343

1 Days after last application
2 Without root

III. CONCLUSION

Four trials were performed according to the critical GAP in barley in 2007 with BAS 555 01 F in south of Europe. Metconazole residues in barley grain samples at the targeted PHI of 35 days were 0.02-0.05 mg/kg and decreased at later sampling intervals. Residues of metconazole in barley straw were 0.05-0.18 mg/kg at 35 DALA, 0.08-0.39 mg/kg at 42 DALA and 0.06-0.34 mg/kg at 49 DALA.
The residue levels in barley grain are consistent with the levels in grain and straw after treatment with BAS 555 00 F evaluated in the peer review and listed in the EFSA Conclusion (2006) as follows:

North grain	<0.01-0.09 mg/kg;	straw 0.03-1.6 mg/kg
South	0.03-0.05 mg/kg	straw 1.3-2.6 mg/kg

Table 6.3.2-7: Residues of metconazole (BAS 555 F) in barley after two applications of BAS 555 01 F in Southern Europe

Study details		Crop	Country Trial No	Formulation Application rate (kg a.s./ha)	GS[1] BBCH	DALA[2]	Residues found (mg/kg)	
							Matrix	BAS 555 F
Study code:	272866	Barley	Spain L070190	BAS 555 01 F 2 x 0.090	69	0	Whole plant[3]	2.07
Doc ID:	2008/1009268					36	Ears	0.04
Author:	Schroth E.					36	Rest of plant[3]	0.31
GLP:	yes					42	Grain	<u>0.02</u>
Year:	2007					42	Straw	*0.39*
						49	Grain	0.02
						49	Straw	0.33
Study code:	272866	Barley	Spain L070191	BAS 555 01 F 2 x 0.090	69	0	Whole plant[3]	1.97
Doc ID:	2008/1009268					36	Ears	0.04
Author:	Schroth E.					36	Rest of plant[3]	0.19
GLP:	yes					42	Grain	0.01
Year:	2007					42	Straw	0.25
						49	Grain	<u>0.01</u>
						49	Straw	*0.34*
Study code:	272866	Barley	Italy L070192	BAS 555 01 F 2 x 0.090	69	0	Whole plant[3]	0.88
Doc ID:	2008/1009268					36	Grain	<u>0.05</u>
Author:	Schroth E.					36	Straw	*0.18*
GLP:	yes					42	Grain	0.04
Year:	2007					42	Straw	0.15
						49	Grain	0.05
						49	Straw	0.13
Study code:	272866	Barley	Italy L070193	BAS 555 01 F 2 x 0.090	69	0	Whole plant[3]	1.72
Doc ID:	2008/1009268					36	Grain	0.02
Author:	Schroth E.					36	Straw	0.05
GLP:	yes					42	Grain	<u>0.02</u>
Year:	2007					42	Straw	*0.08*
						49	Grain	0.02
						49	Straw	0.06

1 Growth stage at last application
2 Days after last application
3 Without root
<u>Underlined values</u> were used for MRL derivation
Values in *italic* were used for HR and STMR determination (needed for feed burden calculation)

Report:	CA 6.3.2/3 Erdmann H.-P., 2007b Study on the residue behaviour of Metconazole and Epoxiconazole in barley after application of BAS 627 00 F, BAS 480 31 F and BAS 555 00 F under field condition in France and Germany, 2007 2007/1050101
Guidelines:	EEC 91/414 Annex II (Part A Section 6), EEC 91/414 Annex III (Part A Section 8), EEC 1607/VI/97 rev. 2 10.06.1999, EEC 7029/VI/95 rev. 5, EEC 7525/VI/95 rev. 7
GLP:	yes (certified by Land Brandenburg Ministerium fuer Laendliche Entwicklung, Umwelt und Verbraucherschutz, Potsdam, Germany)

This study contains also residue data on epoxiconazole which are not reported in this document.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	BAS 627 00 F (EC): epoxiconazole: 37.5 g/L nominal; metconazole: 27.5 g/L nominal BAS 480 31 F (SC): epoxiconazole 125 g/L nominal BAS 555 00 F (EC): metconazole: 60 g/L nominal
Lot/Batch #:	BAS 627 00 F (EC): 1568 BAS 480 31 F (SC): 7324 BAS 555 00 F (EC): 1089 and 1072
Purity:	
CAS#:	BAS 480 F (epoxiconazole): 133855-98-8 BAS 555 F (metconazole): 125116-23-6
Development code:	
Spiking levels:	Epoxiconazole: 0.01 – 10 mg/kg *Cis*-metconazole: 0.005 – 5.0 mg/kg *Trans*-metconazole: 0.005 – 5.0 mg/kg

2. Test Commodity:

Crop:	Barley
Type:	Cereals
Variety:	Braemar, Campanile, Platine, Nikel, Regalia
Botanical name:	*Hordeum vulgare*
Crop parts(s) or processed commodity:	Grain, straw, whole plant without root, ears, rest of plant without root
Sample size:	Straw 0.5 kg / grain 1.0 kg

B. STUDY DESIGN

1. Test procedure

During the 2007 growing season, six field trials were conducted in different representative barley growing areas in Germany and France to determine the magnitude of the residues in or on raw agricultural commodities (RAC).
The fungicidal combination product BAS 627 00 F, containing epoxiconazole and metconazole, was compared to the solo formulations of both active substances, BAS 480 31 F (epoxiconazole) and BAS 555 00 F (metconazole). Only the residue data for metconazole are reported in this document.
In all cases, the test item was foliar applied two times in a spray volume of 200 L/ha. BAS 627 00 F was applied in plot 2 at a target rate of 0.0825 kg/ha metconazole. BAS 555 00 F was applied in plot 4 at a target rate of 0.090 kg/ha of metconazole. The actual application rates were within 10% of the target except for trial L070183 (France in which the first application was made at growth stage 33 and the interval between applications was 18 rather than 21±1 days).
The application timing was varied in the trials in order to cover pre-harvest intervals (PHI) defined in specific days before harvest or defined by the growth stage of the last application. In four trials, each formulation was applied 56 and 35 days before harvest resulting in a 21 day interval between treatments and a 35 day PHI. In two trials, the treatment was based on the growth stage with the first treatment targeted for 21 days before growth stage 69 BBCH and the second treatment at growth stage 69.
Barley whole plant without root specimens were collected directly after the last application. Either ears and rest of plant, or grain and straw were taken depending on the crop maturity at 35, 42 and 49 days after the last application. In those cases when growth stage BBCH 89 was not reached after 49 days, grain and straw were taken later as soon as growth stage BBCH 89 was reached. In some cases additional samples of grain and straw were taken after growth stage BBCH 89 was reached and after the commercial harvest date for that region.
All specimens were frozen within 24 hours after being taken and remained frozen (≤-18°C) until analysis including transportation. The maximum storage interval from harvest until extraction for analysis of metconazole was approximately 139 days for grain and straw samples and 207 days for whole plant samples.

Table 6.3.2-8: Target application rates and timings for barley

Year	No of trials	No of appl.	F P or G	Test item	Active substance	Appl. rate (kg a.s./ha)	Water volume (L/ha)	Target date/ Timing
2007	6	2	F	BAS 627 00 F (EC)	BAS 555 F	0.0825	200	2nd appl.: 69 BBCH or 35 day PHI/ 21 day interval
					BAS 480 F	0.1125		
		2	F	BAS 480 31 F (SC)	BAS 480 F	0.125	200	2nd appl.: 69 BBCH or 35 day PHI/ 21 day interval
		2	F	BAS 555 00 F (SL)	BAS 555 F	0.090	200	2nd appl.: 69 BBCH or 35 day PHI/ 21 day interval

2. Description of analytical procedures

Samples were analyzed for metconazole residues using BASF method No 550/0 (L0019/01). The homogenized specimens were extracted using a mixture of methanol, water and hydrochloric acid. An aliquot of the extract is centrifuged and partitioned with dichloromethane, and the analytes in the final extract were determined by LC-MS/MS. The limit of quantitation in plant material was 0.005 mg/kg for each *cis*- and *trans*-metconazole (0.01 mg/kg for the sum of both isomers).

Table 6.3.2-9: Summary of procedural recovery data for metconazole

Matrix	Fortification level (mg/kg)	Summary recoveries					
		n	Mean (%)	RSD (%)	n	Mean (%)	RSD (%)
BASF method No L0019/01; LOQ = 0.005 mg/kg		***cis*-Metconazole**			***trans*-Metconazole**		
Whole plant w/o root	0.005-10	6	96	9.6	6	93	11.6
Ears	0.005-10	3	88	16	3	85	9.3
Rest of plant	0.005-10	3	86	6.8	3	90	6.9
Grain	0.005-1.0	4	79	9.7	4	84	5.2
Straw	0.005-10	5	94	7.7	5	97	5.4

II. RESULTS AND DISCUSSION

The residue ranges of metconazole in/on barley treated either with formulation BAS 627 00 F or BAS 555 00 F are shown in Table 6.3.2-10. Details are presented in Table 6.3.2-11 to Table 6.3.2-14.

Comparison of the residue levels in the whole plant immediately after treatment indicate comparable residues with 1.21-2.73 mg/kg after treatment with BAS 627 00 F (plot 2) and 1.47-2.22 mg/kg after treatment with BAS 555 00 (plot 4). The residue levels in the RAC were also comparable at harvest. For a pre-harvest interval of 34-36 days the levels in barley grain were 0.01-0.19 mg/kg after treatment with BAS 627 00 F and 0.01-0.16 mg/kg after treatment with BAS 555 00 F. For a pre-harvest interval of 41-43 days the levels in barley grain were 0.01-0.15 mg/kg after treatment with BAS 627 00 F and 0.01-0.14 mg/kg after treatment with BAS 555 00 F.

Residues measured at one trial site (L070181) were significantly higher than at the other five trial sites. In the two trials in the south of France, samples of grain and straw taken at 49 DALA were taken after the crops had reached maturity (growth stage BBCH 89) and after the commercial harvest date for the region.

The residues in straw are dependent on the ripening stage / water content of the matrix. For a pre-harvest interval of 34-36 days, the levels in barley straw were 0.09-0.79 mg/kg after treatment with BAS 627 00 F and 0.07-0.57 mg/kg after treatment with BAS 555 00 F. For a pre-harvest interval of 41-43 days, the levels in straw were 0.05-1.01 mg/kg after treatment with BAS 627 00 F and 0.08-1.04 mg/kg after treatment with BAS 555 00 F.

For some regions including the United Kingdom and Ireland, the interval between the last application and harvest is defined in the GAP according to the growth stage of last application, in this case in growth stages BBCH 25-69. Trials for plot 2 with BAS 627 00 F had the last application at growth stage BBCH 61-69 for one site in Germany and both sites in the North of France. For these sites residues in grain at growth stage 89 were 0.01-0.02 mg/kg after treatment with either BAS 627 00 F or BAS 555 00 F.

No residues of metconazole at or above the limit of quantitation (0.01 mg/kg) were found in any of the analyzed untreated specimens.

Table 6.3.2-10: Summary of residues in barley treated with BAS 627 00 F or BAS 555 00 F

Crop	Year	DALA[1]	Growth stage[2] (BBCH)	Metconazole residues (sum of *cis*- and *trans*-isomer; mg/kg)		
				Matrix	Plot 2 (BAS 627 00 F)	Plot 4 (BAS 555 00 F)
Barley	2007	0	61-83	Whole plant without roots	1.21-2.73	1.47-2.22
		35	83-85	Ears	0.02-0.03	0.02-0.03
		42	87	Ears	0.02	0.03
		35	83-85	Rest of plant	0.16-0.24	0.20-0.32
		42	87	Rest of plant	0.34	0.33
		34-36	89	Grain	0.01-0.19	0.01-0.16
		41-43	85-89	Grain	0.01-0.15	0.01-0.14
		48-50	89	Grain	0.01-0.16	0.01-0.13
		34-36	89	Straw	0.09-0.79	0.07-0.57
		41-43	85-89	Straw	0.05-1.01	0.08-1.04
		48-50	89	Straw	0.07-0.65	0.04-1.08

1 Days after last application
2 At sampling

III. CONCLUSION

Six trials (4 in northern Europe and 2 in southern Europe) were performed in 2007 in barley in side by side trials with BAS 627 00 F and with BAS 555 00F according to the critical GAP. After treatment with BAS 627 00 F or BAS 555 00 F, metconazole residues in barley grain samples at the targeted PHI of 35 days were 0.01-0.19 or 0.01-0.16 mg/kg, respectively, and decreased at later sampling intervals. Residues of metconazole in barley straw were 0.09-0.79 or 0.07-0.57mg/kg at 35 DALA, respectively, 0.05-1.0 or 0.08-1.0 mg/kg at 42 DALA, respectively, and 0.06-0.65 or 0.04-1.1mg/kg at 49 DALA, respectively. Residues of metconazole in barley grain and straw measured after treatment with the formulated product BAS 627 00 F were comparable to the residue levels after treatment with the solo formulation BAS 555 00 F.
The residue levels in grain are slightly higher than the levels in barley grain evaluated in the peer review and listed in the EFSA Conclusion (2006) as follows:
North <0.01-0.09 mg/kg; South 0.03-0.05 mg/kg
The study was submitted and evaluated by CRD with other studies in accordance with Regulation (EC) No 396/2005 to raise the MRL in barley grain.

Table 6.3.2-11: Residues of metconazole in barley after two applications of BAS 627 00 F in Northern Europe

Trial details		Crop	Country	Formulation, Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1)]	Residues found (mg/kg)	
							Matrix	Metconazole (sum of *cis*- and *trans*-isomer)
Study code:	255301	Barley	Germany	BAS 627 00 F	61	0	Whole plant without root	1.71
Doc ID:	2007/1050101			2 x 0.0825		35	Ears	0.02
Trial No:	L070178					35	Rest of plant	0.24
GLP:	Yes					42	Grain	0.01
Year:	2007					42	Straw	*1.01*
						48	Grain	0.01
						48	Straw	0.50
Study code:	255301	Barley	Germany	BAS 627 00 F	77-83	0	Whole plant without root	1.21
Doc ID:	2007/1050101			2 x 0.0825		34	Grain	0.05
Trial No:	L070179					34	Straw	*0.09*
GLP:	Yes					41	Grain	0.04
Year:	2007					41	Straw	0.07
						48	Grain	0.04
						48	Straw	0.08
Study code:	255301	Barley	France	BAS 627 00 F	69	0	Whole plant without root	1.37
Doc ID:	2007/1050101			2 x 0.0825		34	Grain	0.01
Trial No:	L070182					34	Straw	*0.33*
GLP:	Yes					41	Grain	0.01
Year:	2007					41	Straw	0.21
						50	Grain	0.01
						50	Straw	0.07
Study code:	255301	Barley	France	BAS 627 00 F	69	0	Whole plant without root	1.85
Doc ID:	2007/1050101			2 x 0.0825		35	Ears	0.03
Trial No:	L070183					35	Rest of plant	0.16
GLP:	Yes					42	Ears	0.02
Year:	2007					42	Rest of plant	0.34
						50	Grain	0.02
						50	Straw	*0.27*

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
Values in *italic* were used for HR and STMR determination (needed for feed burden calculation)

Table 6.3.2-12: Residues of metconazole in barley after two applications of BAS 627 00 F in Southern Europe

Trial details		Crop	Country	Formulation, Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1)]	Residues found (mg/kg)	
							Matrix	Metconazole (sum of *cis*- and *trans*-isomer)
Study code:	255301	Barley	France	BAS 627 00 F	83	0	Whole plant without root	1.65
Doc ID:	2007/1050101			2 x 0.0825		35	Grain	0.02
Trial No:	L070180					35	Straw	0.14
GLP:	Yes					42	Grain	0.03
Year:	2007					42	Straw	0.05
						49	Grain	0.02
						49	Straw	0.09
Study code:	255301	Barley	France	BAS 627 00 F	83	0	Whole plant without root	2.73
Doc ID:	2007/1050101			2 x 0.0825		36	Grain	<u>0.19</u>
Trial No:	L070181					36	Straw	0.79
GLP:	Yes					43	Grain	0.15
Year:	2007					43	Straw	0.80
						49	Grain	0.16
						49	Straw	0.65

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
<u>Underlined values</u> were used for MRL derivation

Table 6.3.2-13: Residues of metconazole in barley after two applications of BAS 555 00 F in Northern Europe

Trial details		Crop	Country	Formulation, Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1)]	Residues found (mg/kg)	
							Matrix	Metconazole (sum of *cis*- and *trans*-isomer)
Study code:	255301	Barley	Germany	BAS 555 00 F	61	0	Whole plant without root	1.98
Doc ID:	2007/1050101			2 x 0.090		35	Ears	0.03
Trial No:	L070178					35	Rest of plant	0.20
GLP:	Yes					42	Grain	<u>0.01</u>
Year:	2007					42	Straw	0.63
						48	Grain	0.01
						48	Straw	0.45
Study code:	255301	Barley	Germany	BAS 555 00 F	77-83	0	Whole plant without root	1.47
Doc ID:	2007/1050101			2 x 0.090		34	Grain	0.05
Trial No:	L070179					34	Straw	0.07
GLP:	Yes					41	Grain	<u>0.06</u>
Year:	2007					41	Straw	0.08
						48	Grain	0.05
						48	Straw	0.07
Study code:	255301	Barley	France	BAS 555 00 F	69	0	Whole plant without root	1.63
Doc ID:	2007/1050101			2 x 0.090		34	Grain	<u>0.01</u>
Trial No:	L070182					34	Straw	0.15
GLP:	Yes					41	Grain	0.01
Year:	2007					41	Straw	0.09
						50	Grain	0.01
						50	Straw	0.04
Study code:	255301	Barley	France	BAS 555 00 F	69	0	Whole plant without root	1.99
Doc ID:	2007/1050101			2 x 0.090		35	Ears	0.02
Trial No:	L070183					35	Rest of plant	0.32
GLP:	Yes					42	Ears	0.03
Year:	2007					42	Rest of plant	0.33
						50	Grain	<u>0.02</u>
						50	Straw	0.20

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
<u>Underlined values</u> were used for MRL derivation

Table 6.3.2-14: Residues of metconazole in barley after two applications of BAS 555 00 F in Southern Europe

Trial details		Crop	Country	Formulation, Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1)]	Residues found (mg/kg)	
							Matrix	Metconazole (sum of *cis*- and *trans*-isomer)
Study code:	255301	Barley	France	BAS 555 00 F	83	0	Whole plant without root	2.22
Doc ID:	2007/1050101			2 x 0.090		35	Grain	<u>0.03</u>
Trial No:	L070180					35	Straw	*0.29*
GLP:	Yes					42	Grain	0.02
Year:	2007					42	Straw	0.13
						49	Grain	0.02
						49	Straw	0.28
Study code:	255301	Barley	France	BAS 555 00 F	83	0	Whole plant without root	2.11
Doc ID:	2007/1050101			2 x 0.090		36	Grain	0.16
Trial No:	L070181					36	Straw	0.57
GLP:	Yes					43	Grain	0.14
Year:	2007					43	Straw	1.04
						49	Grain	0.13
						49	Straw	*1.08*

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
<u>Underlined values</u> were used for MRL derivation
Values in *italic* were used for HR and STMR determination (needed for feed burden calculation)

Report:	CA 6.3.2/4 Schroth E.,Martin T., 2010c Residue behavior of BAS 480 F and BAS 555 F in barley after the application of BAS 480 31 F, BAS 555 00 F and BAS 627 00 F under field conditions in France (North, South), Germany, Denmark, Netherlands United Kingdom Greece Italy Spain 2009 2010/1110643
Guidelines:	EEC 91/414 Annex II (Part A Section 6), EEC 91/414 Annex III (Part A Section 8), EEC 1607/VI/97 rev. 2 10.06.1999, EEC 7029/VI/95 rev. 5, EEC 7525/VI/95 rev. 7
GLP:	yes (certified by ENAC, Entidad Nacional de Acreditación, Madrid Spain)

This study contains also residue data on epoxiconazole which are not reported in this document.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	BAS 627 00 F (EC): epoxiconazole: 37.5 g/L nominal; metconazole: 27.5 g/L nominal BAS 555 00 F (EC): metconazole: 60 g/L nominal
Lot/Batch #:	BAS 627 00 F (EC): 1568 BAS 555 00 F (EC): 1093
Purity:	
CAS#:	BAS 480 F (epoxiconazole): 133855-98-8 BAS 555 F (metconazole): 125116-23-6
Development code:	
Spiking levels:	Metconazole: 0.01-50 mg/kg

2. Test Commodity:

Crop:	Barley
Type:	Cereals
Variety:	Campanele, Sequel, Suzuka, Quench, Moutso, Otis, Cecilia, Prestige, Alinghi, Franzi
Botanical name:	*Hordeum vulgare*
Crop parts(s) or processed commodity:	Whole plant w/o roots, ears, rest of plant w/o roots, grain, straw
Sample size:	0.5 – 1.0 kg

B. STUDY DESIGN

1. Test procedure

During the 2009 growing season, 10 trials in barley were conducted in different representative growing areas in Denmark, France (North and South), Germany, Greece, Italy, the Netherlands, Spain and United Kingdom to determine the residue level of epoxiconazole and metconazole in or on barley raw agricultural commodities (RAC).

The fungicidal combination product BAS 627 00 F, containing epoxiconazole and metconazole, was compared to the solo formulations of both active substances, BAS 480 31 F (epoxiconazole) and BAS 555 00 F (metconazole). Only the residue data for metconazole are reported in this document.

In all cases, the test item was foliar applied two times in a spray volume of 200 L/ha. BAS 627 00 F was applied in plot 2 at a target rate of 0.0825 kg/ha metconazole. BAS 555 00 F was applied in plot 4 at a target rate of 0.090 kg/ha of metconazole.

The application timing was varied in the trials in order to cover pre-harvest intervals (PHI) defined in specific days before harvest or defined by the growth stage of the last application. In seven trials (trial numbers L090063, L090064, L090065, L090066, L090067, L090068 and L090069), the applications were made at crop stage BBCH 39-49 and BBCH 69. In three trials (trial numbers L090070, L090071 and L090072) the applications were made 56(±1) and 35(±1) days before harvest.

Barley "whole plant without roots" specimens were collected at the day of the last application and specimens of barley grain and straw, or ear and rest of plant in case of immaturity, were taken about 28, 35 and 42 days thereafter (in case of immaturity an additional sampling of grain and straw was done at growth stage BBCH 89). Generally the specimens were stored frozen at or below -18°C for a maximum of 410 days.

Table 6.3.2-15: Target application rates and timings for barley

Year	No of trials	No of appl.	F P or G	Test item	Active substance	Appl. rate (kg a.s./ha)	Water volume (L/ha)	Target date/ Timing[1]
2009	10	2	F	BAS 627 00 F (EC)	BAS 555 F	0.085	200	1st appl.: 39-49 BBCH 2nd appl.: 69 BBCH/ 21 day interval or 1st appl.: 56±1 DBH 2nd appl.: 35±1 DBH*
					BAS 480 F	0.1125		
		2	F	BAS 480 31 F (SC)	BAS 480 F	0.125	200	
		2	F	BAS 555 00 F (SL)	BAS 555 F	0.090	200	

1 DBH = days before harvest

2. Description of analytical procedures

Samples were analyzed for metconazole residues using BASF method No 535/1 (L0076/01). The homogenized specimens were extracted using a mixture of methanol, water and hydrochloric acid. An aliquot of the extract was centrifuged and partitioned at alkaline conditions against cyclohexane, and the analytes in the final extract were determined by LC-MS/MS. The limit of quantitation in plant material was 0.005 mg/kg for each *cis*- and *trans*-metconazole (0.01 mg/kg for the sum of both isomers).

Table 6.3.2-16: Summary of procedural recovery data for metconazole

Matrix	Fortification level (mg/kg)	Summary recoveries					
		n	Mean (%)	RSD (%)	n	Mean (%)	RSD (%)
BASF method No L0019/01; LOQ = 0.01 mg/kg		**Metconazole (m/z 320 → 70)**			**Metconazole (m/z 320 → 125)**		
Whole plant without roots	0.01 - 50	9	96	7	6	96	8
Ears	0.01 - 50	6	98	9	3	100	6
Grain	0.01 - 50	7	95	6	4	92	10
Straw	0.01 - 50	8	99	10	5	98	10

II. RESULTS AND DISCUSSION

The residue ranges of metconazole in/on barley treated either with formulation BAS 627 00 F or BAS 555 00 F are shown in Table 6.3.2-17. Details are presented in Table 6.3.2-18 to Table 6.3.2-21. Side-by-side bridging trials covering both North and South EU verify that treatment with the mixture product, BAS 627 00 F results in residue levels of metconazole comparable to those from treatment with the solo product BAS 555 00 F.

In whole plant specimens collected directly after the last application residues of metconazole were 1.4-3.6 mg/kg (plot 2) and 1.1-3.3 mg/kg (plot 4).

Residues of metconazole found in grain were 0.017-0.087 mg/kg (plot 2) and 0.014-0.079 mg/kg (plot 4) at 34-36 DALA, 0.012-0.20 mg/kg (plot 2) and <0.01-0.077 mg/kg (plot 4) at 41-43 DALA. At the longest post-treatment intervals, 48-56 DALA, residues in grain were <0.01-0.16 mg/kg (plot 2) and 0.011-0.075 mg/kg (plot 4). After treatment with BAS 627 00 F residues in barley at maturity (growth stage BBCH 89) were <0.01–0.16 mg/kg for North EU and 0.02-0.07 mg/kg for South EU. After treatment with BAS 555 00 F residues in barley at maturity (growth stage 89) were <0.01-0.07 mg/kg for north EU and 0.01-0.04 mg/kg for south EU.

Residues of metconazole found in straw were 0.28-2.5 mg/kg (plot 2) and 0.20-2.5 mg/kg (plot 4) at 34-36 DALA, 0.22-2.2 mg/kg (plot 2) and 0.15-2.3 mg/kg (plot 4) at 41-43 DALA. At the longest post-treatment intervals, 48-56 DALA, residues in straw were 0.16-1.4 mg/kg (plot 2) and 0.12-0.60 mg/kg (plot 4).

Table 6.3.2-17: Summary of residues in barley treated with BAS 627 00 F or BAS 555 00 F

Crop	Year	DALA[1]	Growth stage[2] (BBCH)	Metconazole residues (sum of *cis*- and *trans*-isomer; mg/kg)		
				Matrix	BAS 627 00 F	BAS 555 00 F
Barley	2009	0	69-77	Whole plant without roots	1.4-3.6	1.1-3.3
		28-29	77-89	Ears	0.19-0.55	0.085-0.43
		36	77/83	Ears	0.17	0.065
		41	85/87	Ears	0.23	0.075
		28-29	77-89	Rest of plant	0.64-2.9	0.19-3.5
		36	77/83	Rest of plant	0.69	0.14
		41	85/87	Rest of plant	0.96	0.23
		27-29	85-89	Grain	0.023-0.091	0.019-0.12
		34-36	87-89	Grain	0.017-0.087	0.014-0.079
		41-43	83-92	Grain	0.012-0.2	<0.01-0.077
		48-56	89	Grain	<0.01-0.16	0.011-0.075
		27-29	85-89	Straw	0.24-0.59	0.18-0.95
		34-36	87-89	Straw	0.28-2.5	0.2-2.5
		41-43	83-92	Straw	0.22-2.2	0.15-2.3
		48-56	89	Straw	0.16-1.4	0.12-0.6

1 Days after last application
2 At sampling

III. CONCLUSION

Ten trials (6 in northern Europe and 4 in southern Europe) were performed in 2009 in barley in side by side trials with BAS 627 00 F and with BAS 555 00F according to the critical GAP. After treatment with BAS 627 00 F or BAS 555 00 F, metconazole residues in barley grain samples at the targeted PHI of 35 days were 0.02-0.09 or 0.01-0.08 mg/kg, respectively, and were 0.01-0.2 or <0.01-0.8 at 42 DALA and <0.01-0.16 or 0.01-0.08 at 48-56 DALA, respectively Residues of metconazole in barley straw were 0.28-2.5 or 0.2-2.5mg/kg at 35 DALA, respectively, 0.22-2.2 or 0.15-2.3 mg/kg at 42 DALA, respectively, and 0.16-1.4 or 0.12-0.6 mg/kg at 48-56 DALA, respectively. Residues of metconazole in barley grain and straw measured after treatment with the formulated product BAS 627 00 F were comparable to the residue levels after treatment with the solo formulation BAS 555 00 F.
The residue levels in grain are slightly higher than the levels in barley grain evaluated in the peer review and listed in the EFSA Conclusion (2006) as follows:
North <0.01-0.09 mg/kg; South 0.03-0.05 mg/kg
The study was submitted and evaluated by CRD with other studies in accordance with Regulation (EC) No 396/2005 to raise the MRL in barley grain.

Table 6.3.2-18: Residues of metconazole in barley after two applications of BAS 627 00 F in Northern Europe

Trial details		Crop	Country	Formulation, Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1)]	Residues found (mg/kg)	
							Matrix	Metconazole (sum of *cis*- and *trans*-isomer)
Study code:	366605	Barley	Germany	BAS 627 00 F	69	0	Whole plant without root	3.6
Doc ID:	2010/1110643			2 x 0.0825		43	Grain	<u>0.20</u>
Trial No:	L090063					43	Straw	*1.6*
GLP:	Yes					49	Grain	0.11
Year:	2009					49	Straw	1.1
						56	Grain	0.16
						56	Straw	1.4
Study code:	366605	Barley	The Nether-lands	BAS 627 00 F	69	0	Whole plant without root	2.2
Doc ID:	2010/1110643			2 x 0.0825		42	Grain	0.012
Trial No:	L090064					42	Straw	0.35
GLP:	Yes					48	Grain	0.013
Year:	2009					48	Straw	0.41
						56	Grain	<0.01
						56	Straw	0.16
Study code:	366605	Barley	United Kingdom	BAS 627 00 F	69	0	Whole plant without root	1.8
Doc ID:	2010/1110643			2 x 0.0825		29	Grain	0.023
Trial No:	L090065					29	Straw	0.25
GLP:	Yes					34	Grain	<u>0.017</u>
Year:	2009					34	Straw	*0.28*
						41	Grain	0.015
						41	Straw	0.22
Study code:	366605	Barley	Denmark	BAS 627 00 F	69	0	Whole plant without root	2.4
Doc ID:	2010/1110643			2 x 0.0825		28	Ears	0.55
Trial No:	L090066					28	Rest of plant	1.2
GLP:	Yes					34	Grain	<u>0.058</u>
Year:	2009					34	Straw	*1.3*
						41	Grain	0.056
						41	Straw	1.2
Study code:	366605	Barley	France	BAS 627 00 F	71	0	Whole plant without root	1.4
Doc ID:	2010/1110643			2 x 0.0825		27	Grain	<u>0.065</u>
Trial No:	L090070					27	Straw	0.24
GLP:	Yes							
Year:	2009							
Study code:	366605	Barley	Germany	BAS 627 00 F	77	0	Whole plant without root	2.3
Doc ID:	2010/1110643			2 x 0.0825		28	Grain	0.091
Trial No:	L090071					28	Straw	0.59
GLP:	Yes					36	Grain	<u>0.087</u>
Year:	2009					36	Straw	0.34
						42	Grain	0.064
						42	Straw	0.26

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
<u>Underlined values</u> were used for MRL derivation
Values in *italic* were used for HR and STMR determination (needed for feed burden calculation)

Table 6.3.2-19: Residues of metconazole in barley after two applications of BAS 627 00 F in Southern Europe

Trial details		Crop	Country	Formulation, Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1)]	Residues found (mg/kg)	
							Matrix	Metconazole (sum of *cis*- and *trans*-isomer)
Study code:	366605	Barley	Greece	BAS 627 00 F	69	0	Whole plant without root	2.4
Doc ID:	2010/1110643			2 x 0.0825		29	Ears	0.19
Trial No:	L090067					29	Rest of plant	0.64
GLP:	Yes					36	Ears	0.17
Year:	2009					36	Rest of plant	0.69
						41	Ears	0.23
						41	Rest of plant	0.96
						50	Grain	<u>0.02</u>
						50	Straw	*0.76*
Study code:	366605	Barley	Italy	BAS 627 00 F	69	0	Whole plant without root	2.1
Doc ID:	2010/1110643			2 x 0.0825		28	Ears	0.22
Trial No:	L090068					28	Rest of plant	2.1
GLP:	Yes					35	Grain	0.04
Year:	2009					35	Straw	*2.5*
						42	Grain	0.025
						42	Straw	1.4
Study code:	366605	Barley	Spain	BAS 627 00 F	69	0	Whole plant without root	2.9
Doc ID:	2010/1110643			2 x 0.0825		29	Ears	0.23
Trial No:	L090069					29	Rest of plant	2.9
GLP:	Yes					36	Grain	0.02
Year:	2009					36	Straw	1.9
						42	Grain	0.025
						42	Straw	2.2
Study code:	366605	Barley	France	BAS 627 00 F	71	0	Whole plant without root	1.8
Doc ID:	2010/1110643			2 x 0.0825		28	Ears	0.4
Trial No:	L090072					28	Rest of plant	2.3
GLP:	Yes					36	Grain	<u>0.067</u>
Year:	2009					36	Straw	1.2
						43	Grain	0.06
						43	Straw	0.97

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
<u>Underlined values</u> were used for MRL derivation
Values in *italic* were used for HR and STMR determination (needed for feed burden calculation)

Table 6.3.2-20: Residues of metconazole in barley after two applications of BAS 555 00 F in Northern Europe

Trial details		Crop	Country	Formulation, Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1)]	Residues found (mg/kg)	
							Matrix	Metconazole (sum of *cis*- and *trans*-isomer)
Study code:	366605	Barley	Germany	BAS 555 00 F	69	0	Whole plant without root	2.9
Doc ID:	2010/1110643			2 x 0.090		43	Grain	0.077
Trial No:	L090063					43	Straw	0.56
GLP:	Yes					49	Grain	0.075
Year:	2009					49	Straw	0.43
						56	Grain	0.056
						56	Straw	0.6
Study code:	366605	Barley	The Nether-lands	BAS 555 00 F	69	0	Whole plant without root	2.3
Doc ID:	2010/1110643			2 x 0.090		42	Grain	<u>0.021</u>
Trial No:	L090064					42	Straw	*0.65*
GLP:	Yes					48	Grain	0.017
Year:	2009					48	Straw	0.59
						56	Grain	0.013
						56	Straw	0.25
Study code:	366605	Barley	United Kingdom	BAS 555 00 F	69	0	Whole plant without root	2.0
Doc ID:	2010/1110643			2 x 0.090		29	Grain	0.019
Trial No:	L090065					29	Straw	0.18
GLP:	Yes					34	Grain	0.014
Year:	2009					34	Straw	0.20
						41	Grain	<0.01
						41	Straw	0.15
Study code:	366605	Barley	Denmark	BAS 555 00 F	69	0	Whole plant without root	2.1
Doc ID:	2010/1110643			2 x 0.090		28	Ears	0.24
Trial No:	L090066					28	Rest of plant	0.86
GLP:	Yes					34	Grain	0.031
Year:	2009					34	Straw	0.76
						41	Grain	0.032
						41	Straw	0.74
Study code:	366605	Barley	France	BAS 555 00 F	71	0	Whole plant without root	1.1
Doc ID:	2010/1110643			2 x 0.090		27	Grain	0.055
Trial No:	L090070					27	Straw	*0.45*
GLP:	Yes							
Year:	2009							
Study code:	366605	Barley	Germany	BAS 555 00 F	77	0	Whole plant without root	3.3
Doc ID:	2010/1110643			2 x 0.090		28	Grain	0.12
Trial No:	L090071					28	Straw	0.95
GLP:	Yes					36	Grain	0.079
Year:	2009					36	Straw	*0.59*
						42	Grain	0.074
						42	Straw	0.55

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
<u>Underlined values</u> were used for MRL derivation
Values in *italic* were used for HR and STMR determination (needed for feed burden calculation)

Table 6.3.2-21: Residues of metconazole in barley after two applications of BAS 555 00 F in Southern Europe

Trial details		Crop	Country	Formulation, Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1)]	Residues found (mg/kg)	
							Matrix	Metconazole (sum of *cis*- and *trans*-isomer)
Study code:	366605	Barley	Greece	BAS 555 00 F	69	0	Whole plant without root	2.7
Doc ID:	2010/1110643			2 x 0.090		29	Ears	0.085
Trial No:	L090067					29	Rest of plant	0.19
GLP:	Yes					36	Ears	0.065
Year:	2009					36	Rest of plant	0.14
						41	Ears	0.075
						41	Rest of plant	0.23
						50	Grain	0.011
						50	Straw	0.12
Study code:	366605	Barley	Italy	BAS 555 00 F	69	0	Whole plant without root	2.3
Doc ID:	2010/1110643			2 x 0.090		28	Ears	0.29
Trial No:	L090068					28	Rest of plant	2.6
GLP:	Yes					35	Grain	<u>0.041</u>
Year:	2009					35	Straw	1.2
						42	Grain	0.024
						42	Straw	1.2
Study code:	366605	Barley	Spain	BAS 555 00 F	69	0	Whole plant without root	2.6
Doc ID:	2010/1110643			2 x 0.090		29	Ears	0.3
Trial No:	L090069					29	Rest of plant	3.5
GLP:	Yes					36	Grain	<u>0.037</u>
Year:	2009					36	Straw	*2.5*
						42	Grain	0.033
						42	Straw	2.3
Study code:	366605	Barley	France	BAS 555 00 F	71	0	Whole plant without root	2.6
Doc ID:	2010/1110643			2 x 0.090		28	Ears	0.43
Trial No:	L090072					28	Rest of plant	1.7
GLP:	Yes					36	Grain	0.035
Year:	2009					36	Straw	*2.0*
						43	Grain	0.027
						43	Straw	1.6

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
<u>Underlined values</u> were used for MRL derivation
Values in *italic* were used for HR and STMR determination (needed for feed burden calculation)

Report:	CA 6.3.2/5 Schroth E.,Martin T., 2010d Study on the residue behavior of Epoxiconazole (BAS 480 F) and Metconazole (BAS 555 F) in barley after the application of either BAS 627 00 F or BAS 627 02 F under field conditions in Germany, United Kingdom, France (South) and Spain, 2010 2010/1144334
Guidelines:	EEC 91/414 Annex II (Part A Section 6), EEC 91/414 Annex III (Part A Section 8), EEC 1607/VI/97 rev. 2 10.06.1999, EEC 7029/VI/95 rev. 5, EEC 7525/VI/95 rev. 7
GLP:	yes (certified by ENAC, Entidad Nacional de Acreditación, Madrid, Spain)

This study contains also residue data on epoxiconazole which are not reported in this document.

I. MATERIAL AND METHODS

A. MATERIALS

Test Material:	
Description:	1) BAS 627 00 F (EC) 2) BAS 627 02 F (EC)
Lot/Batch #:	1) 1615, BAS 627 00 F, metconazole: 27.5 g/L nominal; epoxiconazole: 37.5 g/L nominal 2) 241238, BAS 627 02 F, metconazole: 41.25 g/L nominal; epoxiconazole: 56.25 g/L nominal
CAS#:	BAS 555 F: 125116-23-6 (metconazole)
Spiking levels:	0.005-5.0 mg/kg
Test Commodity:	
Crop:	Barley
Type:	Cereals
Variety:	Braemar, Suzaka, Diadéme, Henley
Botanical name:	*Hordeum vulgare*
Crop parts(s) or processed commodity:	Whole plant w/o root, ears, rest plant w/o root, grain, straw
Sample size:	Min. 0.5-1.0 kg

B. STUDY DESIGN

1. Test procedure

During the 2010 growing season, 4 field trials were conducted in barley in different representative growing areas in Germany, United Kingdom, France (South) and Spain in order to determine the magnitude of the residues of metconazole and epoxiconazole in or on raw agricultural commodities (RAC) and other parts.
The formulation BAS 627 02 F (56.25 g/L of epoxiconazole, 41.25 g/L of metconazole, EC) was compared to BAS 627 00 F (27.5 g/L of epoxiconazole, 37.5 g/l of metconazole, EC). Both products were applied at 82.5 g a.s./ha of metconazole and 112.5 g a.s./ha of epoxiconazole, water volume 200 L/ha with a application rate of 3.0 L product/ha. Plot 1 remained untreated. Two foliar applications were done with the first application at growth stage BBCH 49 and the second application at BBCH 69 with an interval between the applications of 14 to 22 days.
Specimens of whole plant without roots were collected at the day of the last application and specimens of grain and straw (or ear and rest of plant in case of immaturity) were taken about 28, 35 and 42 days thereafter. In case of immaturity an additional sampling of grain and straw was done at growth stage BBCH 89.

Table 6.3.2-22: Target application rates and timings for barley

Year	No of trials	No of appl.	F P or G	Test item	Active substance	Appl. rate (kg a.s./ha)	Water volume (L/ha)	Target date/ Timing
2010	4	2	F	BAS 627 00 F (EC) or BAS 627 02 F (EC)	metconazole	0.0825	200	1st appl.: 49 BBCH 2nd appl.: 69 BBCH
					epoxiconazole	0.1125		

2. Description of analytical procedures

All treated specimens were analyzed for metconazole (detected separately as *cis* and *trans* isomers) using BASF method No L0019/01 (550/0) with a limit of quantitation (LOQ) of 0.005 mg/kg for each isomer (or 0.01 mg/kg for the sum of *cis* and *trans* isomer).

Principle of BASF method No 550/0 (L0019/01)

BAS 555 F is extracted using a mixture of methanol, water and hydrochloric acid. An aliquot of the extract is centrifuged and partitioned against dichloromethane. The final determination of BAS 555 F is performed by HPLC-MS/MS. Limit of quantitation (LOQ): 0.01 mg/kg (sum of *cis* and *trans* isomer, which are determined separately, each at with a LOQ of 0.005 mg/kg).

Table 6.3.2-23: Summary of recoveries of metconazole (BAS 555 F) in barley

Matrix	Fortification level (mg/kg)	Summary Recoveries			
		n	Mean (%)	SD (+/-)	RSD (%)
Method No L0019/01 (550/0) or L0076/01 (535/1)		**Metconazole (*cis*/*trans*)**			
Whole/rest plants*	0.005-5.0	4/4	90.5/84.2	6.0/7.5	6.6/8.9
Ears	0.005-0.5	3/3	96.7/93.9	6.7/6.1	6.9/6.5
Grain	0.005-0.5	8/8	97.0/95.6	11/10	11/11
Straw	0.005-5.0	6/6	81.1/88.2	7.3/5.5	9.0/6.2

* Without roots

II. RESULTS AND DISCUSSION

The summarized results are given in Table 6.3.2-24. The detailed results are given in Table 6.3.2-25 and Table 6.3.2-26.

Table 6.3.2-24: Summary of metconazole residues in barley after application of BAS 627 00 F and BAS 627 02 F

Crop	Year	Application	DALA[1]	BBCH[2]	Metconazole (sum of *cis* and *trans*) found (mg/kg)		
					Matrix	BAS 627 00 F	BAS 627 02 F
Barley	2010	BAS 627 00 F (EC) or BAS 627 02 F (EC)	0	69	Whole plant*	1.4-4.1	1.5-4.2
			28	79-83	Ears	0.075	0.068
			28	79-83	Rest of plant*	0.23	0.33
			27-28	85-87	Grain	0.023-0.13	0.016-0.075
			34-35	87-89	Grain	0.023-0.26	0.019-0.18
			41-42	89	Grain	0.020-0.19	0.021-0.10
			27-28	85-87	Straw	1.2-2.8	1.7-2.5
			34-35	87-89	Straw	0.38-5.6	0.58-6.7
			41-42	89	Straw	0.20-4.1	0.41-4.5

1 Days after last application
2 BBCH stage at respective sampling
* Without root

III. CONCLUSION

The data is submitted to provide a weight of the evidence approach to support the BASF proposal that no additional residue studies in barley with BAS 555 01 F are needed to support the designation of BAS 555 01 F as the representative formulation in place of BAS 555 00 F.Four trials (2 in northern Europe and 2 in southern Europe) were performed in 2010 in barley in side by side trials with BAS 627 00 F and with BAS 627 02 F according to the critical GAP. After treatment with BAS 627 00 F or BAS 627 02 F, metconazole residues in barley grain samples at the targeted PHI of 35 days were 0.02-0.26 or 0.02-0.18 mg/kg, respectively, and were 0.02-0.19 or 0.02-0.10 at 42 DALA, respectively Residues of metconazole in barley straw were 0.38-5.6 or 0.58-6.7 mg/kg at 35 DALA, respectively, and 0.20-4.1 or 0.41-4.5 mg/kg at 42 DALA, respectively. The residue levels of metconazole obtained in barley after two applications of BAS 627 02 F are comparable to those obtained with BAS 627 00 F.
The residue levels in grain are slightly higher than the levels in barley grain evaluated in the peer review and listed in the EFSA Conclusion (2006) as follows:
North <0.01-0.09 mg/kg; South 0.03-0.05 mg/kg
The study was submitted and evaluated by CRD with other studies in accordance with Regulation (EC) No 396/2005 to raise the MRL in barley grain.

Table 6.3.2-25: Residues of metconazole (BAS 555 F) in barley after two applications of BAS 627 00 F or BAS 627 02 in Northern Europe

Study details		Crop	Country Trial No	Formulation Application rate (kg a.s./ha)	GS[1] BBCH	DALA[2]	Metconazole found (mg/kg)		
							Matrix	BAS 62700F	BAS 62702F
Study code:	339327	Barley	Germany L100101	BAS 627 00 F 2 x 0.0825	69	0	Whole plant[3]	2.4	3.3
Doc ID:	2010/1144334					28	Grain	0.023	0.016
Author:	Schroth E.					28	Straw	1.9	2.5
GLP:	yes					34	Grain	<u>0.023</u>	0.019
Year:	2010					34	Straw	1.8	2.3
						42	Grain	0.020	0.021
						42	Straw	2.2	*3.1*
Study code:	339327	Barley	United Kingdom L100102	BAS 627 00 F 2 x 0.0825	69	0	Whole plant[3]	1.9	2.3
Doc ID:	2010/1144334					27	Grain	0.073	0.058
Author:	Schroth E.					27	Straw	1.2	1.7
GLP:	yes					35	Grain	0.044	0.050
Year:	2010					35	Straw	0.86	1.2
						41	Grain	0.087	<u>0.056</u>
						41	Straw	1.6	*2.5*

1 Growth stage at last application
2 Days after last application
3 Without root
Residues <u>underlined</u> were the highest residue which fulfilled the GAP and were within the commercial harvest date

Table 6.3.2-26: Residues of metconazole (BAS 555 F) in barley after two applications of BAS 627 00 F or BAS 627 02 in Southern Europe

Study details		Crop	Country Trial No	Formulation Application rate (kg a.s./ha)	GS[1] BBCH	DALA[2]	Metconazole found (mg/kg)		
							Matrix	BAS 62700F	BAS 62702F
Study code:	339327	Barley	France L100103	BAS 627 00 F 2 x 0.0825	69	0	Whole plant[3]	1.4	1.5
Doc ID:	2010/1144334					28	Ears	0.075	0.068
Author:	Schroth E.					28	Rest plant[3)]	0.23	0.33
GLP:	yes					35	Grain	0.039	0.033
Year:	2010					35	Straw	0.38	*0.58*
						42	Grain	<u>0.036</u>	0.035
						42	Straw	0.20	0.41
Study code:	339327	Barley	Spain L100104	BAS 627 00 F 2 x 0.0825	69	0	Whole plant[3]	4.1	4.2
Doc ID:	2010/1144334					27	Grain	0.13	0.075
Author:	Schroth E.					27	Straw	2.8	2.0
GLP:	yes					34	Grain	0.26	0.18
Year:	2010					34	Straw	5.6	*6.7*
						41	Grain	<u>0.19</u>	0.10
						41	Straw	4.1	4.5

1 Growth stage at last application
2 Days after last application
3 Without root
Residues <u>underlined</u> were the highest residue which fulfilled the GAP and were within the commercial harvest date

Report:	CA 6.3.2/6 Tandy R., 2012c Study on the residue behaviour of Pyraclostrobin (BAS 500 F) and Metconazole (BAS 555 F) in winter barley after treatment with either BAS 556 03 F, BAS 500 06 F or BAS 555 00 F in Northern and Southern Europe during 2011 2012/1194990
Guidelines:	EEC 91/414 (1607/VI/97), SANCO/3029/99 rev. 4 (11 July 2000), EEC 91/414 Annex II (Part A Section 6), EEC 91/414 Annex III (Part A Section 8), EEC 1607/VI/97 rev. 2 10.06.1999
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

This study contains also residue data on pyraclostrobin which are not reported in this document.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	BAS 556 03 F (EC): metconazole: 80 g/L nominal; pyraclostrobin: 130 g/L nominal BAS 555 00 F (EC): metconazole 60 g/L nominal BAS 500 06 F (EC): pyraclostrobin: 200 g/L nominal
Lot/Batch #:	BAS 556 03 F (EC): 380009 BAS 555 00 F (EC): 0003255328 BAS 500 06 F (EC): 0003223026
Purity:	
CAS#:	BAS 555 F (metconazole): 125116-23-6 BAS 500 F (pyraclostrobin): 175013-18-0
Development code:	
Spiking levels:	Cis-metconazole: 0.005-12.5 mg/kg Trans-metconazole: 0.005-12.5 mg/kg Pyraclostrobin: 0.01-10 mg/kg

2. Test Commodity:

Crop:	Barley
Type:	Cereals
Variety:	Souleyka, Cavia, Azurel, Amorosa
Botanical name:	*Hordeum vulgare*
Crop parts(s) or processed commodity:	Whole plant without root, ears, rest of plants without roots, grain, straw
Sample size:	Whole plant, straw: ≥ 0.5 kg / ears, rest of plant, grain: ≥ 1.0 kg

B. STUDY DESIGN

1. Test procedure

During the growing season of 2011, four field trials with barley were conducted in Germany, United Kingdom, Southern France and Italy to determine the magnitude of the residues of BAS 555 F (metconazole) BAS 500 F (pyraclostrobin) after treatment with BAS 556 03 F, BAS 500 06 F and BAS 555 00 F. Only the residue data for metconazole are reported in this document.

Plot 2 was treated twice, each time with 1.1 L/ha of BAS 556 03 F, corresponding to 0.088 kg a.s./ha of metconazole. Plot 4 was treated twice, each time with 1.5 L/ha of BAS 555 00 F, corresponding to 0.09 kg/ha of metconazole. The applications took place at BBCH 49 and BBCH 69 with an application rate of 200 L/ha of spray.

Whole plant without root specimens were collected directly after the last application. At 28-29 days after the last application (DALA), 34-36 DALA and 41-42 DALA either, ears and rest of plant without roots, or grain and straw specimens were sampled depending on the maturity. At trial L110193 where maturity was not reached at 42 DALA, grain and straw specimens were collected 54 DALA when BBCH 89 was reached.

All specimens were frozen within 24 hours of being taken and remained frozen (≤-18°C) until analysis including transportation. The maximum storage interval from harvest until extraction for analysis of metconazole residues was 343 days.

Table 6.3.2-27: Target application rates and timings for barley

Year	No of trials	No of appl.	F P or G	Test item	Active substance	Appl. rate (kg a.s./ha)	Water volume (L/ha)	Target date/ Timing
2011	4	2	F	BAS 556 03 F (EC)	BAS 555 F	0.088	200	1st appl.: 49 BBCH 2nd appl.: 69 BBCH
					BAS 500 F	0.143		
	4	2	F	BAS 500 06 F (EC)	BAS 500 F	0.250	200	1st appl.: 49 BBCH 2nd appl.: 69 BBCH
	4	2	F	BAS 555 00 F (EC)	BAS 555 F	0.090	200	1st appl.: 49 BBCH 2nd appl.: 69 BBCH

2. Description of analytical procedures

Residues of metconazole (BAS 555 F) were determined based on BASF method No L0076/01 (535/1). The analytes were extracted with a mixture of methanol, water and hydrochloric acid. An aliquot of the extract was centrifuged and partitioned at alkaline conditions against cyclohexane for determination by LC-MS/MS. The limit of quantitation in plant material was 0.005 mg/kg for each cis- and trans-metconazole (0.01 mg/kg for the sum of both isomers).

Table 6.3.2-28: Summary of procedural recovery data for metconazole

Matrix	Fortification level (mg/kg)	Summary recoveries					
		n	Mean (%)	RSD (%)	n	Mean (%)	RSD (%)
BASF method No L0076/01; LOQ = 0.005 mg/kg		**cis-Metconazole**			**trans-Metconazole**		
Whole plant, rest of plant without roots	0.005, 12.5	8	81	3	8	80	2
Ears	0.005, 0.5	8	82	6	8	77	5
Grain	0.005, 0.5	8	71	6	8	67	5
Straw	0.005, 0.5, 5.0	9	84	2	9	83	3

Triazole derivative metabolites

Bayer Crop Science Method 01062/M003 was adapted to determine the triazole derivative metabolites (TDM) T, TA, TAA and TLA. Validation of the method in a range of matrices is reported (MCA 4.1.2). The analytes were extracted from the specimen matrix with methanol/water (4/1, v/v). An aliquot was filtered, concentrated and cleaned-up by dispersive C18-SPE prior to analysis by LC-MS/MS using internal standardization based on stable isotope standards. The limit of quantitation (LOQ) is 0.01 mg/kg for each analyte.

Table 6.3.2-29: Summary of recoveries of triazole derivative metabolites in barley

Matrix	Fortification level (mg/kg)	Summary recoveries[1]					
		n	Mean (%)	RSD (%)	n	Mean (%)	RSD (%)
Bayer Crop Science Method 01062/M003		**T**[2]			**TA**		
Whole plant, rest plant without roots	0.01-1.0	8	84	13	8	104	6
Ears	0.01-1.0	8	92	10	8	84	12
Grain	0.01-1.0	8	97	11	8	94	10
Straw	0.01-1.0	8	92	11	8	95	8
Bayer Crop Science Method 01062/M003		**TAA**			**TLA**		
Whole plant, rest plant without roots	0.01-1.0	8	72	6	8	85	6
Ears	0.01-1.0	8	87	9	8	80	6
Grain	0.01-1.0	8	92	11	8	82	9
Straw	0.01-1.0	8	85	8	8	85	5

1 T=1,2,4-triazole, TA=triazolylalanine, TAA=triazole acetic acid, TLA=triazole lactic acid

II. RESULTS AND DISCUSSION

The residue ranges of metconazole in/on barley treated either with formulation BAS 556 03 F or BAS 555 00 F are shown in Table 6.3.2-30. Details are presented in Table 6.3.2-33 to Table 6.3.2-36.
After application of BAS 556 03 F, metconazole residues in whole plant specimens at 0 DALA ranged between 1.3-3.4 mg/kg. At 28-29 DALA residues in ear specimens ranged between 0.021-0.087 mg/kg, while residues in rest of plant specimens ranged between 0.12-0.90 mg/kg. At BBCH 87-89, residues in grain ranged between 0.010-0.017 mg/kg, while residues in straw ranged between 0.12-1.1 mg/kg.
After application of BAS 555 00 F, metconazole residues in whole plant specimens at 0 DALA ranged between 1.5-3.5 mg/kg. At 28-29 DALA residues in ear specimens ranged between 0.029-0.060 mg/kg, while residues in rest of plant specimens ranged between 0.20-0.90 mg/kg. At BBCH 87-89, residues in grain ranged between <0.01-0.016 mg/kg, while residues in straw ranged between 0.16-1.1 mg/kg.

Bridging trials demonstrated that metconazole residues in barley samples treated with BAS 555 00 F were in the same range compared to application of BAS 556 03 F. No residues of metconazole were found in the untreated control samples above the LOQ.

Table 6.3.2-30: Summary of residues in barley treated with BAS 556 03 F or BAS 555 00 F

Crop	Year	DALA[1]	Growth stage[2] (BBCH)	Metconazole residues (sum of cis- and trans-isomer; mg/kg)		
				Matrix	BAS 556 03 F	BAS 555 00 F
Barley	2011	0	69-73	Whole plant without roots	1.3-3.4	1.5-3.5
		28-29	77-85	Ears	0.021-0.087	0.029-0.060
		34-36	81-89	Ears	0.020-0.064	0.026-0.061
		28-29	77-85	Rest of plant without roots	0.12-0.90	0.20-0.90
		34-36	81-89	Rest of plant without roots	0.12-0.73	0.21-0.74
		35	87-89	Grain	0.013	0.014
		41-42	83-89	Grain	0.010-0.017	<0.01-0.014
		54	89	Grain	0.011	0.016
		35	87-89	Straw	0.65	0.39
		41-42	83-89	Straw	0.17-1.1	0.26-1.1
		54	89	Straw	0.12	0.16

1 Days after last application
2 At sampling

Triazole derivative metabolites

The maximum storage interval from harvest until extraction for analysis of metconazole including the TDM residues was 343 days. Freezer storage stability is established for TDMs through a minimum interval of 12 months (M-CA 6.1).

T residues were below the LOQ of 0.01 mg/kg in barley matrices. TA residues ranged between <0.01 mg/kg and 0.26 mg/kg. TAA residues were <0.01-0.055 mg/kg. Residues of TLA were below the LOQ in ears and grain. In all other samples, residues ranged from 0.012 to 0.075 mg/kg.

Even in the absence of maintenance treatments with triazole-containing plant protection products, many residue trials showed measurable residues of TDMs in the control samples. These residues are attributed to the use of triazole-containing plant products on the test plots during previous seasons. It is assumed that besides plant protection products other sources (such as fertilizers) potentially contribute to the presence of 1,2,4-triazole residues in agricultural soils. In the absence of maintenance treatments with triazole-containing plant protection products, the residues of triazole derivative metabolites (TDMs) in control samples reflect the residues that result from the use of triazole-containing plant protection products during previous growing seasons as well as the residues from other sources than plant protection products. Therefore, the levels of TDM residues measured in the treated samples are considered to properly reflect the residues that result from the test item and from the use of triazole-containing plant protection products during previous growing seasons. Residues of TA, TAA and TLA were similar in treated and untreated samples indicating that treatment with BAS 556 03 F or BAS 555 00 F had little impact on the residue level of these metabolites.

Table 6.3.2-31: Summary of residues of triazole derivative metabolites in barley treated with BAS 556 03 F or BAS 555 00 F

Crop	Year	Application	GS[1]	DALA[2]	Matrix	Residues[3] (mg/kg)			
						T	TA	TAA	TLA
Barley (EU North)	2011	BAS 556 03 F (EC)	69-73	0	Whole plant	<0.01	0.022-0.062	0.01-0.016	0.014-0.032
			79-85	28	Ears	<0.01	0.043-0.14	0.026-0.035	<0.01
			75-85	34-36	Ears	<0.01	0.044-0.13	0.025-0.031	<0.01
			79-85	28	Rest of plant	<0.01	<0.01-0.015	<0.01-0.018	0.021-0.034
			75-85	34-36	Rest of plant	<0.01	<0.01-0.016	0.015-0.016	0.024-0.025
			83-89	41-42	Grain	<0.01	0.061-0.22	0.037-0.044	<0.01
			89	54	Grain	<0.01	0.057	0.041	<0.01
			83-89	41-42	Straw	<0.01	<0.01-0.019	0.015-0.039	0.023-0.029
			89	54	Straw	<0.01	0.018	0.021	0.016
		BAS 555 00 F (EC)	69-73	0	Whole plant	<0.01	0.022-0.083	<0.01-0.019	0.012-0.04
			79-85	28	Ears	<0.01	0.047-0.14	0.026-0.037	<0.01
			75-85	34-36	Ears	<0.01	0.044-0.18	0.027-0.031	<0.01
			79-85	28	Rest of plant	<0.01	<0.01-0.024	<0.01-0.018	0.031-0.04
			75-85	34-36	Rest of plant	<0.01	<0.01-0.034	<0.01-0.018	0.023-0.03
			83-89	41-42	Grain	<0.01	0.058-0.26	0.046-0.047	<0.01
			89	54	Grain	<0.01	0.058	0.042	<0.01
			83-89	41-42	Straw	<0.01	0.015-0.042	0.022-0.048	0.02-0.039
			89	54	Straw	<0.01	0.028	0.023	0.014
Barley (EU South)	2011	BAS 556 03 F (EC)	69-71	0	Whole plant	<0.01	<0.01-0.02	<0.01	0.014-0.021
			77-85	28-29	Ears	<0.01	0.027-0.029	0.013-0.022	<0.01
			87-89	35	Ears	<0.01	0.025-0.03	0.017-0.026	<0.01
			77-85	28-29	Rest of plant	<0.01	<0.01	<0.01	0.019-0.046
			87-89	35	Rest of plant	<0.01	<0.01	0.011-0.013	0.021-0.042
			87-89	35-42	Grain	<0.01	0.036-0.057	0.026-0.035	<0.01
			87-89	35-42	Straw	<0.01	<0.01	0.014-0.017	0.015-0.026
		BAS 555 00 F (EC)	69-71	0	Whole plant	<0.01	<0.01-0.046	<0.01-0.02	0.013-0.024
			77-85	28-29	Ears	<0.01	0.024-0.055	0.018-0.035	<0.01
			87-89	35	Ears	<0.01	0.018-0.096	0.019-0.055	<0.01

Table 6.3.2-31: Summary of residues of triazole derivative metabolites in barley treated with BAS 556 03 F or BAS 555 00 F

Crop	Year	Application	GS[1]	DALA[2]	Matrix	Residues[3] (mg/kg)			
						T	TA	TAA	TLA
			77-85	28-29	Rest of plant	<0.01	<0.01	<0.01-0.012	0.019-0.034
			87-89	35	Rest of plant	<0.01	<0.01-0.024	<0.01-0.019	0.033-0.059
			87-89	35-42	Grain	<0.01	0.03-0.12	0.023-0.055	<0.01
			87-89	35-42	Straw	<0.01	<0.01-0.011	0.013-0.041	0.015-0.075

1 Growth stage at sampling (BBCH)
2 Days after last application
3 T=1,2,4-triazole, TA=triazolylalanine, TAA=triazole acetic acid, TLA=triazole lactic acid

Apparent residues of T were <LOQ (<0.01 mg/kg) in/on all the untreated control samples analysed. Apparent residues of TA, TAA and TLA were <LOQ (<0.01 mg/kg) in the untreated control samples with the following exceptions:

Table 6.3.2-32: Summary of TDM residues in untreated barley control samples

Crop	Field Report	Application	Residues Found[1] (mg/kg)				
			Matrix	T	TA	TAA	TLA
Barley	L110192	Metconazole	plant	<0.01	0.049	0.010	0.048
			ears	<0.01	0.14	0.041	<0.01
			rest	<0.01	0.048	0.017	0.034
			ears	<0.01	0.17	0.033	<0.01
			rest	<0.01	0.041	0.015	0.032
			grain	<0.01	0.25	0.048	<0.01
			straw	<0.01	0.057	0.037	0.035
	L110193		plant	<0.01	0.023	0.011	0.015
			ears	<0.01	0.043	0.028	<0.01
			rest	<0.01	<0.01	<0.01	0.027
			ears	<0.01	0.044	0.029	<0.01
			rest	<0.01	<0.01	<0.01	0.022
			grain	<0.01	0.056	0.032	<0.01
			straw	<0.01	<0.01	0.014	0.018
			grain	<0.01	0.044	0.038	<0.01
			straw	<0.01	0.011	0.020	0.015
	L110194		plant	<0.01	<0.01	<0.01	0.011
			ears	<0.01	0.012	0.013	<0.01
			rest	<0.01	<0.01	<0.01	0.021
			ears	<0.01	0.011	0.010	<0.01
			rest	<0.01	<0.01	<0.01	0.017
			grain	<0.01	0.014	0.015	<0.01
	L110195		ears	<0.01	0.017	0.013	<0.01
			ears	<0.01	0.017	0.018	<0.01
			rest	<0.01	<0.01	<0.01	0.012
			grain	<0.01	0.21	0.15	<0.01
			straw	<0.01	<0.01	0.068	0.11

1 T = 1,2,4 triazole
TA = triazole alanine
TAA = triazole acetic acid
TLA = triazole lactic acid

III. CONCLUSION

The residues of metconazole in barley were between 1.3-3.5 mg/kg in whole plant specimens at 0 DALA. At maturity (BBCH 83-89), residues in grain ranged from <0.01-0.016 mg/kg (DALA 35-54), while residues in straw ranged from 0.16-1.1 mg/kg.
The bridging trials showed that both formulations yielded comparable residue levels.
T residues were below the LOQ of 0.01 mg/kg in barley matrices. TA residues ranged between <0.01 mg/kg and 0.26 mg/kg. TAA residues were <0.01-0.055 mg/kg. Residues of TLA were below the LOQ in ears and grain. In all other samples, residues ranged from 0.012 to 0.075 mg/kg. Residues of TA, TAA and TLA were similar in treated and untreated samples indicating that treatment with BAS 556 03 F or BAS 555 00 F had little impact on the residue level of these metabolites.

Table 6.3.2-33: Residues of metconazole in barley after two applications of BAS 556 03 F in Northern Europe

Trial details		Crop	Country	Formulation, Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1)]	Residues found (mg/kg)	
							Matrix	Metconazole (sum of cis- and trans-isomer)
Study code:	401831_1	Barley	Germany	BAS 556 03 F	69-73	0	Whole plant without root	3.4
Doc ID:	2012/1194990			2 x 0.088		28	Ears	0.087
Trial No:	L110192					28	Rest of plant without root	0.89
GLP:	Yes					36	Ears	0.064
Year:	2011					36	Rest of plant without root	0.50
						42	Grain	<u>0.017</u>
						42	Straw	1.1
Study code:	401831_1	Barley	United Kingdom	BAS 556 03 F	69	0	Whole plant without root	1.3
Doc ID:	2012/1194990			2 x 0.088		28	Ears	0.021
Trial No:	L110193					28	Rest of plant without root	0.12
GLP:	Yes					34	Ears	0.020
Year:	2011					34	Rest of plant without root	0.12
						41	Grain	0.010
						41	Straw	0.17
						54	Grain	0.011
						54	Straw	0.12

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
<u>Underlined values</u> were used for MRL derivation

Table 6.3.2-34: Residues of metconazole in barley after two applications of BAS 556 03 F in Southern Europe

Trial details		Crop	Country	Formulation, Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1)]	Residues found (mg/kg)	
							Matrix	Metconazole (sum of cis- and trans-isomer)
Study code:	401831_1	Barley	France	BAS 556 03 F	69	0	Whole plant without root	2.4
Doc ID:	2012/1194990			2 x 0.088		28	Ears	0.040
Trial No:	L110194					28	Rest of plant without root	0.63
GLP:	Yes					35	Ears	0.036
Year:	2011					35	Rest of plant without root	0.62
						42	Grain	<u>0.012</u>
						42	Straw	0.49
Study code:	401831_1	Barley	Italy	BAS 556 03 F	69-71	0	Whole plant without root	2.6
Doc ID:	2012/1194990			2 x 0.088		29	Ears	0.040
Trial No:	L110195					29	Rest of plant without root	0.90
GLP:	Yes					35	Ears	0.038
Year:	2011					35	Rest of plant without root	0.73
						35	Grain	0.013
						35	Straw	*0.65*

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
<u>Underlined values</u> were used for MRL derivation
Values in *italic* were used for HR and STMR determination (needed for feed burden calculation)

Table 6.3.2-35: Residues of metconazole in barley after two applications of BAS 555 00 F in Northern Europe

Trial details		Crop	Country	Formulation, Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1)]	Residues found (mg/kg)	
							Matrix	Metconazole (sum of cis- and trans-isomer)
Study code:	401831_1	Barley	Germany	BAS 555 00 F	69-73	0	Whole plant without root	3.5
Doc ID:	2012/1194990			2 x 0.090		28	Ears	0.060
Trial No:	L110192					28	Rest of plant without root	0.90
GLP:	Yes					36	Ears	0.047
Year:	2011					36	Rest of plant without root	0.57
						42	Grain	0.014
						42	Straw	*1.1*
Study code:	401831_1	Barley	United Kingdom	BAS 555 00 F	69	0	Whole plant without root	1.5
Doc ID:	2012/1194990			2 x 0.090		28	Ears	0.029
Trial No:	L110193					28	Rest of plant without root	0.20
GLP:	Yes					34	Ears	0.061
Year:	2011					34	Rest of plant without root	0.21
						41	Grain	0.014
						41	Straw	*0.26*
						54	Grain	<u>0.016</u>
						54	Straw	0.16

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
<u>Underlined values</u> were used for MRL derivation
Values in *italic* were used for HR and STMR determination (needed for feed burden calculation)

Table 6.3.2-36: Residues of metconazole in barley after two applications of BAS 555 00 F in Southern Europe

Trial details		Crop	Country	Formulation, Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1)]	Residues found (mg/kg)	
							Matrix	Metconazole (sum of cis- and trans-isomer)
Study code:	401831_1	Barley	France	BAS 555 00 F	69	0	Whole plant without root	1.9
Doc ID:	2012/1194990			2 x 0.090		28	Ears	0.033
Trial No:	L110194					28	Rest of plant without root	0.65
GLP:	Yes					35	Ears	0.026
Year:	2011					35	Rest of plant without root	0.74
						42	Grain	<0.01
						42	Straw	*0.71*
Study code:	401831_1	Barley	Italy	BAS 555 00 F	69-71	0	Whole plant without root	2.7
Doc ID:	2012/1194990			2 x 0.090		29	Ears	0.044
Trial No:	L110195					29	Rest of plant without root	0.35
GLP:	Yes					35	Ears	0.042
Year:	2011					35	Rest of plant without root	0.31
						35	Grain	<u>0.014</u>
						35	Straw	0.39

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
<u>Underlined values</u> were used for MRL derivation
Values in *italic* were used for HR and STMR determination (needed for feed burden calculation)

Table 6.3.2-37: Residues of TDMs in barley after two applications of BAS 556 03 F in Northern Europe

Trial details		Crop	Country	Formulation, Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1)]	Residues found[3] (mg/kg)				
							Matrix	T	TA	TAA	TLA
Study code:	401831_1	Barley	Germany	BAS 556 03 F	69-73	0	Whole plant[4]	<0.01	0.062	0.016	0.032
Doc ID:	2012/1194990			2 x 0.088		28	Ears	<0.01	0.14	0.035	<0.01
Trial No:	L110192					28	Rest of plant[4]	<0.01	0.015	0.018	0.034
GLP:	Yes					36	Ears	<0.01	0.13	0.025	<0.01
Year:	2011					36	Rest of plant[4]	<0.01	0.016	0.016	0.024
						42	Grain	<0.01	0.22	0.037	<0.01
						42	Straw	<0.01	0.019	0.039	0.029
Study code:	401831_1	Barley	United	BAS 556 03 F	69	0	Whole plant[4]	<0.01	0.022	0.01	0.014
Doc ID:	2012/1194990		Kingdom	2 x 0.088		28	Ears	<0.01	0.043	0.026	<0.01
Trial No:	L110193					28	Rest of plant[4]	<0.01	<0.01	<0.01	0.021
GLP:	Yes					34	Ears	<0.01	0.044	0.031	<0.01
Year:	2011					34	Rest of plant[4]	<0.01	<0.01	0.015	0.025
						41	Grain	<0.01	0.061	0.044	<0.01
						41	Straw	<0.01	<0.01	0.015	0.023
						54	Grain	<0.01	0.057	0.041	<0.01
						54	Straw	<0.01	0.018	0.021	0.016

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
3 T=1,2,4-triazole, TA=triazolylalanine, TAA=triazole acetic acid, TLA=triazole lactic acid
4 Without root

Table 6.3.2-38: Residues of TDMs in barley after two applications of BAS 556 03 F in Southern Europe

Trial details		Crop	Country	Formulation, Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1)]	Residues found[3] (mg/kg)				
							Matrix	T	TA	TAA	TLA
Study code:	401831_1	Barley	France	BAS 556 03 F 2 x 0.088	69	0	Whole plant[4]	<0.01	<0.01	<0.01	0.021
Doc ID:	2012/1194990					28	Ears	<0.01	0.029	0.022	<0.01
Trial No:	L110194					28	Rest of plant[4]	<0.01	<0.01	<0.01	0.046
GLP:	Yes					35	Ears	<0.01	0.025	0.026	<0.01
Year:	2011					35	Rest of plant[4]	<0.01	<0.01	0.013	0.042
						42	Grain	<0.01	0.036	0.026	<0.01
						42	Straw	<0.01	<0.01	0.014	0.015
Study code:	401831_1	Barley	Italy	BAS 556 03 F 2 x 0.088	69-71	0	Whole plant[4]	<0.01	0.02	<0.01	0.014
Doc ID:	2012/1194990					29	Ears	<0.01	0.027	0.013	<0.01
Trial No:	L110195					29	Rest of plant[4]	<0.01	<0.01	<0.01	0.019
GLP:	Yes					35	Ears	<0.01	0.03	0.017	<0.01
Year:	2011					35	Rest of plant[4]	<0.01	<0.01	0.011	0.021
						35	Grain	<0.01	0.057	0.035	<0.01
						35	Straw	<0.01	<0.01	0.017	0.026

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
3 T=1,2,4-triazole, TA=triazolylalanine, TAA=triazole acetic acid, TLA=triazole lactic acid
4 Without root

Table 6.3.2-39: Residues of TDMs in barley after two applications of BAS 555 00 F in Northern Europe

Trial details		Crop	Country	Formulation, Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1)]	Residues found[3] (mg/kg)				
							Matrix	T	TA	TAA	TLA
Study code:	401831_1	Barley	Germany	BAS 555 00 F 2 x 0.090	69-73	0	Whole plant[4]	<0.01	0.083	0.019	0.04
Doc ID:	2012/1194990					28	Ears	<0.01	0.14	0.037	<0.01
Trial No:	L110192					28	Rest of plant[4]	<0.01	0.024	0.018	0.04
GLP:	Yes					36	Ears	<0.01	0.18	0.031	<0.01
Year:	2011					36	Rest of plant[4]	<0.01	0.034	0.018	0.03
						42	Grain	<0.01	0.26	0.046	<0.01
						42	Straw	<0.01	0.042	0.048	0.039
Study code:	401831_1	Barley	United Kingdom	BAS 555 00 F 2 x 0.090	69	0	Whole plant[4]	<0.01	0.022	<0.01	0.012
Doc ID:	2012/1194990					28	Ears	<0.01	0.047	0.026	<0.01
Trial No:	L110193					28	Rest of plant[4]	<0.01	<0.01	<0.01	0.031
GLP:	Yes					34	Ears	<0.01	0.044	0.027	<0.01
Year:	2011					34	Rest of plant[4]	<0.01	<0.01	<0.01	0.023
						41	Grain	<0.01	0.058	0.047	<0.01
						41	Straw	<0.01	0.015	0.022	0.02
						54	Grain	<0.01	0.058	0.042	<0.01
						54	Straw	<0.01	0.028	0.023	0.014

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
3 T=1,2,4-triazole, TA=triazolylalanine, TAA=triazole acetic acid, TLA=triazole lactic acid
4 Without root

Table 6.3.2-40: Residues of TDMs in barley after two applications of BAS 556 03 F in Southern Europe

Trial details		Crop	Country	Formulation, Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1)]	Residues found[3] (mg/kg)				
							Matrix	T	TA	TAA	TLA
Study code:	401831_1	Barley	France	BAS 555 00 F 2 x 0.090	69	0	Whole plant[4]	<0.01	<0.01	<0.01	0.013
Doc ID:	2012/1194990					28	Ears	<0.01	0.024	0.018	<0.01
Trial No:	L110194					28	Rest of plant[4]	<0.01	<0.01	<0.01	0.034
GLP:	Yes					35	Ears	<0.01	0.018	0.019	<0.01
Year:	2011					35	Rest of plant[4]	<0.01	<0.01	<0.01	0.033
						42	Grain	<0.01	0.03	0.023	<0.01
						42	Straw	<0.01	<0.01	0.013	0.015
Study code:	401831_1	Barley	Italy	BAS 555 00 F 2 x 0.090	69-71	0	Whole plant[4]	<0.01	0.046	0.02	0.024
Doc ID:	2012/1194990					29	Ears	<0.01	0.055	0.035	<0.01
Trial No:	L110195					29	Rest of plant[4]	<0.01	<0.01	0.012	0.019
GLP:	Yes					35	Ears	<0.01	0.096	0.055	<0.01
Year:	2011					35	Rest of plant[4]	<0.01	0.024	0.019	0.059
						35	Grain	<0.01	0.12	0.055	<0.01
						35	Straw	<0.01	0.011	0.041	0.075

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
3 T=1,2,4-triazole, TA=triazolylalanine, TAA=triazole acetic acid, TLA=triazole lactic acid
4 Without root

CA 6.3.3 Oilseed rape

Table 6.3.3-1: Summary of the critical GAP for the proposed use of BAS 555 01 F (90 g/L EC) in oilseed rape in the EU

Crop	Outdoor/ Protected	Growth stage (BBCH)	Maximum number of applications	Minimum application interval (days)	Maximum		Minimum PHI (days)
					Rate (kg a.s./ha)	Water (L/ha)	
Winter oilseed rape	Outdoor	Autumn: 13-20 and spring: 21-71	1	14	0.072	110-440	56
			1				
		or spring: 21-71	2				

Table 6.3.3-2: Summary of the cGAP used for trials evaluated in the DAR for use in oilseed with BAS 555 00 F (60 g/L EC)

Crop	Outdoor/ Protected	Growth stage (BBCH)	Maximum number of applications	Minimum application interval (days)	Maximum		Minimum PHI (days)
					Rate (kg a.s./ha)	Water (L/ha)	
Oilseed rape	Outdoor	14-73	2	14	0.090	200-400	PHI of 56 days

Table 6.3.3-3: Number of residue trials conducted with BAS 555 00 F (60 g/L EC) according to the cGAP per geographical region and vegetation period and evaluated in the DAR for use in oilseed rape

Crop	Vegetation period	Number of trials					Reference
		EU North	Country	EU South	Country	Total	
Oilseed rape	1995	2	FR			2	MK -750-003
	1996	6	FR	8[1]		14	MK -750-004 MK -750-005 MK -750-006 MK -750-007
	1998[2]			2	FR	2	MK -750-009
Total number of trials per region		**8**		**10**	**Total number of trials**	**18**	

1 The results from one trial with samples taken at PHI of 39 day were not considered in the EFSA conclusion.
2 Trials were performed with BAS 555 01 F

Residue trials in oilseed rape evaluated in the DAR and by EFSA

Residue trials on oilseed rape (see Table 6.3.3-3) were performed for the previous active substance approval procedure and were evaluated in the DAR (Belgium, 2004) and by EFSA (EFSA Conclusion 2006). All the submitted studies were carried out under GLP conditions. A total number of 15 supervised residue trials (one trial results were not considered since seed were sampled at 39 days) treated with BAS 555 00 F (EC formulation) and two trials treated with BAS 555 01 F were considered for oilseed rape covering Northern and Southern Europe. The trials were performed according to the cGAP with two applications at a nominal rate of 90 g/ha and targeted PHI of 56 days as defined in Table 6.3.3-2. For oilseed rape most of the available trials, samples of whole plants were taken at different growth stages (PHIs) up to harvest and seed was sampled at maturity at a PHI of 56-70 days. Results at 0.11 mg/kg from one trial in south of France with seed sampled at 39 days after treatment were included in the EFSA Conclusion. Samples were analyzed for Method FAMS 050-01 or FAMS-050-02, each with a method LOQ of 0.01 mg for each isomer.

For the submitted and peer evaluated trials in oilseed rape, the application parameters and results as reported in the EFSA Conclusion 2006 are shown below.

Oilseed rape

North (90 g a.s./ha, 2 applications – BBCH: 63-67; 69-71 , PHI: 56-70 days)
- Seed: <0.01 (5x), 0.04, 0.06, 0.07 mg/kg

South (79-90 g a.s./ha, 2 applications – BBCH: 65; 69-75, PHI: 56-63 days)
- Seed: <0.01, 0.02 (3x), 0.03, 0.04, 0.05 (2x), 0.11 mg/kg

Residue studies in oilseed rape not evaluated as part of the Annex 1 approval process

Additional residue studies performed in oilseed rape according to the cGAP with other BAS 555 F-containing formulations such as BAS 556 03 F (a mixture of metconazole and pyraclostrobin) are submitted in this dossier to demonstrate that metconazole has been tested in a number of field trials over a number of seasons. The data is submitted to provide a weight of the evidence approach to support the BASF proposal that no additional residue studies with BAS 555 01 F are needed to support the designation of BAS 555 01 F as the representative formulation in place of BAS 555 00 F. For the studies performed with mixtures, the residue results for the mixing partner are not included in this dossier.

Report:	CA 6.3.3/1 Tandy R., 2012a Study on the residue behaviour of Pyraclostrobin (BAS 500 F) and Metconazole (BAS 555 F) in oilseed rape after treatment with BAS 556 03 F in Northern and Southern Europe during 2011 2012/1255033
Guidelines:	EEC 1607/VI/97 rev. 2 10.06.1999, SANCO/3029/99 rev. 4 (11 July 2000), EEC 91/414 Annex II (Part A Section 6), EEC 91/414 Annex III (Part A Section 8)
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	BAS 556 03 F (EC)
Lot/Batch #:	380009 (80 g/L metconazole, 130 g/L pyraclostrobin, nominal)
Purity:	Not reported
CAS#:	125116-23-6
Development code:	Not reported
Spiking levels:	0.005-12.5 mg/kg

2. Test Commodity:

Crop:	Oilseed rape
Type:	Oilseeds
Variety:	Petrol, DK Cabernet, Ovation, Cabernet, Coklicot, Nelson, Vectra, Artist
Botanical name:	*Brassica napus* subsp. *napus*
Crop parts(s) or processed commodity:	Whole plant, seed, rest of plant, pods with seeds
Sample size:	≥0.5 kg / ≥12 units

B. STUDY DESIGN

1. Test procedure

During the 2011 growing season 8 field trials in oilseed rape were conducted in different representative growing areas in Northern and Southern EU (Germany, United Kingdom, France, Denmark, Greece, Italy and Spain) to determine the residue level of metconazole in or on raw agricultural commodities (RAC). BAS 556 03 F was foliar applied twice at individual rates equivalent to 0.080 kg metconazole/ha in a spray volume of 200 L/ha. One untreated plot of each trial served as control. The applications were performed at BBCH 65 and 71-75. Samples of whole plants were taken immediately after the last application (0 DALA) and seeds and rest of plants were taken at about BBCH 89 at each site; at three sites, seeds, rest of plants and pods with seeds were collected at about 56 DALA in addition. Samples were generally stored deep-frozen at -18°C for a maximum of 392 days until analysis.

Table 6.3.3-4: Target application rates and timings for oilseed rape

Year	No of trials	No of appl.	F P or G	Test item	Active substance	Appl. rate (kg a.s./ha)	Water volume (L/ha)	Target date/ Timing
2011	8	2	F	BAS 556 03 F (EC)	BAS 555 F BAS 500 F	0.080 Not relevant	200	BBCH 65 BBCH 71

2. Description of analytical procedures

The specimens were analyzed for metconazole (*cis*- and *trans*-isomer determined separately) with BASF method No 535/1 (L0076/01) quantifying the analytes with a limit of quantitation (LOQ) of 0.005 mg/kg. The analytes were extracted with a mixture of methanol, water and hydrochloric acid. An aliquot of the extract was centrifuged and partitioned at alkaline conditions against cyclohexane. The final determination was performed using HPLC-MS/MS.

Table 6.3.3-5: Summary of procedural recovery data for metconazole (BAS 555 F)

Matrix	Fortification level (mg/kg)	Summary recoveries					
		n	Mean (%)	RSD (%)	n	Mean (%)	RSD (%)
BASF method No 535/1; LOQ = 0.005 mg/kg		***Cis*-isomer**			***Trans*-isomer**		
Whole plant and rest of plant	0.005, 12.5	10	78	5	10	75	5
Seeds and pods with seeds	0.005, 0.50	10	78	8	10	73	6

Triazole derivative metabolites

Bayer Crop Science Method 01062/M003 was adapted to determine the triazole derivative metabolites (TDM) T, TA, TAA and TLA. Validation of the method in a range of matrices is reported (MCA 4.1.2). The analytes were extracted from the specimen matrix with methanol/water (4/1, v/v). An aliquot was filtered, concentrated and cleaned-up by dispersive C18-SPE prior to analysis by LC-MS/MS using internal standardization based on stable isotope standards. The limit of quantitation (LOQ) is 0.01 mg/kg for each analyte.

Table 6.3.3-6: Summary of recoveries of triazole derivative metabolites in oilseed rape

Matrix	Fortification level (mg/kg)	Summary recoveries[1]					
		n	Mean (%)	RSD (%)	n	Mean (%)	RSD (%)
Bayer Crop Science Method 01062/M003		T[2]			TA		
Whole plant and rest of plant	0.01-1.0	10	88	5	10	102	11
Seeds and pods with seeds	0.01-1.5	16	83	7	15	97	12
Bayer Crop Science Method 01062/M003		TAA			TLA		
Whole plant and rest of plant	0.01-1.0	10	83	7	10	88	9
Seeds and pods with seeds	0.01-1.5	16	84	10	16	78	16

1 T=1,2,4-triazole, TA=triazolylalanine, TAA=triazole acetic acid, TLA=triazole lactic acid

II. RESULTS AND DISCUSSION

The residue ranges for the different trials are shown in Table 6.3.3-7, detailed residue levels are shown in Table 6.3.3-10 and Table 6.3.3-11.
Residues of metconazole (sum of both isomers) analyzed in whole plant specimens taken 0 DALA ranged between 0.56 and 2.6 mg/kg. At 55-57 DALA residues in seed specimens ranged between 0.019 and 0.072 mg/kg. At 35-57 DALA residues ranged between 0.041 and 0.56 mg/kg in rest of plant specimens and between 0.050 and 0.31 mg/kg in pods with seeds specimens. At BBCH 89, residues in seed specimens ranged between 0.014 and 0.046 mg/kg, and residues in rest of plant specimens ranged between 0.051 and 0.24 mg/kg.
No residues of metconazole at or above the limit of quantitation (0.01 mg/kg) were found in any of the analyzed untreated specimens.

Table 6.3.3-7: Summary of residues in oilseed rape treated with BAS 556 03 F

Region	Year	DALA[1]	Growth stage[2] (BBCH)	Range of residues (mg/kg)			
				Matrix	*Cis*-isomer	*Trans*-isomer	Total metconazole
Northern EU	2011	0	71	Whole plant[3]	0.78-2.1	0.15-0.41	0.93-2.6
		56	87-89	Pods with seeds	0.042-0.26	0.0078-0.046	0.050-0.31
		56-57	87-89	Seed	0.014-0.065	<0.005-0.0073	0.019-0.072
		67-69	89	Seed	0.0093-0.040	<0.005-0.0060	0.014-0.046
		56-57	87-89	Rest of plant[3]	0.056-0.15	0.012-0.028	0.068-0.18
		67-69	89	Rest of plant[3]	0.042-0.12	0.0087-0.024	0.051-0.14
Southern EU	2011	0	71-75	Whole plant[3]	0.47-1.1	0.094-0.23	0.56-1.34
		35	79-83	Pods with seeds	0.11	0.021	0.13
		41-57	89	Seed	0.010-0.05	<0.005	0.015-0.051
		35	79-83	Rest of plant[3]	0.085	0.018	0.10
		41-57	89	Rest of plant[3]	0.03-0.45	0.0067-0.095	0.04-0.56

1 Days after last application
2 At sampling
3 Without roots

Triazole derivative metabolites

The maximum storage interval from harvest until extraction for analysis of metconazole including the TDM residues was 392 days. Freezer storage stability in oil seed is established for T through an interval of 12 months, for TAA and TA through an interval of >15 month and for TLA through an interval of 48 months (MCA 6.1).

T residues were below the LOQ of 0.01 mg/kg in oilseed rape matrices. TAA residues were below the LOQ of 0.01 mg/kg in whole plants, rest of plants and seeds at BBCH 89. In seeds and pods with seeds taken at 56 DALA, TAA residues ranged from <0.01-0.010 mg/kg and 0.016-0.023 mg/kg, respectively. TA residues were above the LOQ in all samples but one. Residues of TA in those samples ranged from 0.012 mg/kg to 1.46 mg/kg. Residues of TLA were below the LOQ in whole plants and rest of plants before crop maturity. In all other samples, residues ranged from <0.01 to 0.030 mg/kg.

Even in the absence of maintenance treatments with triazole-containing plant protection products, many residue trials showed measurable residues of TDMs in the control samples. These residues are attributed to the use of triazole-containing plant products on the test plots during previous seasons. It is assumed that besides plant protection products other sources (such as fertilizers) potentially contribute to the presence of 1,2,4-triazole residues in agricultural soils. In the absence of maintenance treatments with triazole-containing plant protection products, the residues of triazole derivative metabolites (TDMs) in control samples reflect the residues that result from the use of triazole-containing plant protection products during previous growing seasons as well as the residues from other sources than plant protection products. Therefore, the levels of TDM residues measured in the treated samples are considered to properly reflect the residues that result from the test item and from the use of triazole-containing plant protection products during previous growing seasons. Residues of TA, TAA and TLA were similar in treated and untreated samples indicating that treatment with BAS 556 03 F or BAS 555 00 F had little impact on the residue level of these metabolites.

Table 6.3.3-8: Summary of residues of triazole derivative metabolites in oilseed rape treated with BAS 556 03 F

Crop	Year	Application	GS[1]	DALA[2]	Matrix	Residues[3] (mg/kg)			
						T	TA	TAA	TLA
Oilseed rape (EU North)	2011	BAS 556 03 F (EC)	71	0	Whole plant	<0.01	0.013-0.153	<0.01	<0.01
			87-89	56	Seed	<0.01	0.602-1.46	<0.01-0.010	0.018-0.030
			89	56-69	Seed	<0.01	0.106-1.24	<0.01	<0.01-0.024
			87-89	56	Pod w. Seed	<0.01	0.327-0.601	0.016-0.023	0.031
			87-89	56	Rest of plant	<0.01	0.055-0.083	<0.01	<0.01
			89	56-69	Rest of plant	<0.01	0.016-0.109	<0.01	<0.01-0.012
Oilseed rape (EU South)	2011	BAS 556 03 F (EC)	71-75	0	Whole plant	<0.01	0.012-0.061	<0.01	<0.01
			89	41-57	Seed	<0.01	0.227-0.476	<0.01	<0.01-0.016
			79-83	35	Pod w. Seed	<0.01	0.124	<0.01	<0.01
			79-83	35	Rest of plant	<0.01	0.017	<0.01	<0.01
			89	41-57	Rest of plant	<0.01	<0.01-0.031	<0.01	<0.01

1 Growth stage at sampling (BBCH)
2 Days after last application
3 T=1,2,4-triazole, TA=triazolylalanine, TAA=triazole acetic acid, TLA=triazole lactic acid

Apparent residues of T were <LOQ (<0.01 mg/kg) in/on all the untreated control samples analysed. Apparent residues of TA, TAA and TLA were <LOQ (<0.01 mg/kg) in the untreated control samples with the following exceptions:

Table 6.3.3-9: Summary of TDM residues in untreated oilseed rape control samples

Crop	Field Report	Application	Residues Found[1] (mg/kg)				
			Matrix	T	TA	TAA	TLA
Oilseed rape	L110180	Metconazole	plant	<0.01	0.230	<0.01	<0.01
			seed	<0.01	1.34	0.011	0.033
			rest	<0.01	0.174	<0.01	<0.01
			pods	<0.01	0.774	0.021	0.044
			seed	<0.01	1.08	<0.01	0.022
			rest	<0.01	0.254	0.016	<0.01
	L110181		plant	<0.01	0.255	<0.01	0.018
			seed	<0.01	1.04	0.011	0.029
			rest	<0.01	0.093	<0.01	<0.01
			pods	<0.01	0.539	0.030	0.043
			seed	<0.01	1.05	<0.01	0.028
			rest	<0.01	0.138	0.012	0.013
	L110182		plant	<0.01	0.059	<0.01	<0.01
			seed	<0.01	0.266	<0.01	0.011
			rest	<0.01	0.020	<0.01	<0.01
	L110183		plant	<0.01	0.016	<0.01	<0.01
			seed	<0.01	0.131	<0.01	<0.01
			rest	<0.01	0.023	<0.01	<0.01
	L110184		plant	<0.01	0.041	<0.01	<0.01
			seed	<0.01	0.209	<0.01	<0.01
			rest	<0.01	0.017	<0.01	<0.01
	L110185		seed	<0.01	0.034	<0.01	<0.01
	L110186		plant	<0.01	0.093	<0.01	<0.01
			rest	<0.01	0.021	<0.01	<0.01
			pods	<0.01	0.188	<0.01	<0.01
			seed	<0.01	0.920	0.012	0.028
	L110187		plant	<0.01	0.050	<0.01	0.010
			seed	<0.01	0.321	<0.01	0.011

1 T = 1,2,4 triazole
TA = triazole alanine
TAA = triazole acetic acid
TLA = triazole lactic acid

III. CONCLUSION

The data is submitted to provide a weight of the evidence approach to support the BASF proposal that no additional residue studies in oilseed rape with BAS 555 01 F are needed to support the designation of BAS 555 01 F as the representative formulation in place of BAS 555 00 F. Eight trials (4 in northern Europe and 4 in southern Europe) were performed in 2011 in oilseed rape with BAS 556 03 (mixture of metconazole and pyraclostrobin) according to the critical GAP that is consistent with the cGAP for BAS 555 01F. After treatment with BAS 556 03 F, metconazole residues in seed samples in northern EU or southern EU at the targeted PHI of 56 days were 0.02-0.07 or 0.02-0.05 mg/kg, respectively. Residues in seed samples from northern EU were 0.01- 0.05 at 67-69 DALA; seed samples from all southern trails were mature and harvested in the earlier sampling.

The residue levels in seed are consistent with the levels in seed evaluated in the peer review and listed in the EFSA Conclusion (2006) as follows:
North <0.01-0.07 mg/kg; South <0.01-0.11 mg/kg. (Note the residue at 0.11 had a 39 day PHI.)

T residues were below the LOQ of 0.01 mg/kg in oilseed rape matrices. TAA residues were below the LOQ of 0.01 mg/kg in whole plants, rest of plants and seeds at BBCH 89. In seeds and pods with seeds taken at 56 DALA, TAA residues ranged from <0.01-0.010 mg/kg and 0.016-0.023 mg/kg, respectively. TA residues were above the LOQ in all samples but one. Residues of TA in those samples ranged from 0.012 mg/kg to 1.46 mg/kg. Residues of TLA were below the LOQ in whole plants and rest of plants before crop maturity. In all other samples, residues ranged from <0.01 to 0.030 mg/kg. Residues of TA, TAA and TLA were similar in treated and untreated samples indicating that treatment with BAS 556 03 F had little impact on the residue level of these metabolites.

Table 6.3.3-10: Residues of metconazole in oilseed rape after two applications of BAS 556 03 F in Northern Europe

Trial details		Crop	Country	Formulation, Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1)]	Residues found (mg/kg)			
							Matrix	*Cis*-isomer	*Trans*-isomer	Total metconazole
Study code:	364929	Oilseed rape	Germany	BAS 556 03 F	71	0	Plant[3]	1.4	0.26	1.6
Doc ID:	2012/1255033			2 x 0.080	87-89	56	Seed	0.019	<0.005	0.024
Trial No.:	L110180				87-89	56	Rest[4]	0.056	0.012	0.068
GLP:	yes				87-89	56	Pods[5]	0.042	0.0078	0.050
Year:	2011				89	67	Seed	0.0093	<0.005	<u>0.014</u>
					89	67	Rest[4]	0.042	0.0087	0.051
Study code:	364929	Oilseed rape	United Kingdom	BAS 556 03 F	71	0	Plant[3]	2.1	0.41	2.6
Doc ID:	2012/1255033			2 x 0.080	87	56	Seed	0.014	<0.005	0.019
Trial No.:	L110181				87	56	Rest[4]	0.15	0.028	0.18
GLP:	yes				87	56	Pods[5]	0.26	0.046	0.31
Year:	2011				89	69	Seed	0.040	0.0060	<u>0.046</u>
					89	69	Rest[4]	0.12	0.024	0.15
Study code:	364929	Oilseed rape	France	BAS 556 03 F	71	0	Plant[3]	0.99	0.19	1.2
Doc ID:	2012/1255033			2 x 0.080	89	57	Seed	0.065	0.0073	<u>0.072</u>
Trial No.:	L110182				89	57	Rest[4]	0.15	0.028	0.18
GLP:	yes									
Year:	2011									
Study code:	364929	Oilseed rape	Denmark	BAS 556 03 F	71	0	Plant[3]	0.78	0.15	0.93
Doc ID:	2012/1255033			2 x 0.080	89	56	Seed	0.020	<0.005	<u>0.025</u>
Trial No.:	L110183				89	56	Rest[4]	0.12	0.022	0.15
GLP:	yes									
Year:	2011									

0 Actual application rates varied by 10% at most
1 Days after last application
2 At sampling
3 Whole plant without roots
4 Rest of plant without roots
5 With seeds
<u>Underlined values</u> were used for MRL derivation

Table 6.3.3-11: Residues of metconazole in oilseed rape after two applications of BAS 556 03 F in Southern Europe

Trial details		Crop	Country	Formulation, Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1)]	Residues found (mg/kg)			
							Matrix	*Cis*-isomer	*Trans*-isomer	Total metconazole
Study code:	364929	Oilseed rape	France	BAS 556 03 F	71	0	Plant[3]	1.1	0.23	1.34
Doc ID:	2012/1255033			2 x 0.080	89	57	Seed	0.032	<0.005	<u>0.037</u>
Trial No.:	L110184				89	57	Rest[4]	0.45	0.095	0.56
GLP:	yes									
Year:	2011									
Study code:	364929	Oilseed rape	Greece	BAS 556 03 F	71	0	Plant[3]	1.1	0.22	1.30
Doc ID:	2012/1255033			2 x 0.080	89	55	Seed	0.05	<0.005	<u>0.051</u>
Trial No.:	L110185				89	55	Rest[4]	0.03	0.0067	0.04
GLP:	yes									
Year:	2011									
Study code:	364929	Oilseed rape	Italy	BAS 556 03 F	71	0	Plant[3]	0.67	0.14	0.81
Doc ID:	2012/1255033			2 x 0.080	79-83	35	Rest[4]	0.085	0.018	0.10
Trial No.:	L110186				79-83	35	Pods[5]	0.11	0.021	0.13
GLP:	yes				89	43	Seed	0.011	<0.005	<u>0.016</u>
Year:	2011				89	43	Rest[4]	0.095	0.019	0.11
Study code:	364929	Oilseed rape	Spain	BAS 556 03 F	75	0	Plant[3]	0.47	0.094	0.56
Doc ID:	2012/1255033			2 x 0.080	89	41	Seed	0.010	<0.005	<u>0.015</u>
Trial No.:	L110187				89	41	Rest[4]	0.20	0.041	0.24
GLP:	yes									
Year:	2011									

0 Actual application rates varied by 10% at most
1 Days after last application
2 At sampling
3 Whole plant without roots
4 Rest of plant without roots
5 With seeds
<u>Underlined values</u> were used for MRL derivation

Table 6.3.3-12: Residues of TDMs in oilseed rape after two applications of BAS 556 03 F in Northern Europe

Trial details		Crop	Country	Formulation, Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1)]	Residues found[6] (mg/kg)				
							Matrix	T	TA	TAA	TLA
Study code:	364929	Oilseed rape	Germany	BAS 556 03 F 2 x 0.080	71	0	Plant[3]	<0.01	0.153	<0.01	<0.01
Doc ID:	2012/1255033				87-89	56	Seed	<0.01	1.46	0.010	0.030
Trial No.:	L110180				87-89	56	Rest[4]	<0.01	0.055	<0.01	<0.01
GLP:	yes				87-89	56	Pods[5]	<0.01	0.601	0.016	0.031
Year:	2011				89	67	Seed	<0.01	1.24	<0.01	0.024
					89	67	Rest[4]	<0.01	0.079	<0.01	<0.01
Study code:	364929	Oilseed rape	United Kingdom	BAS 556 03 F 2 x 0.080	71	0	Plant[3]	<0.01	0.093	<0.01	<0.01
Doc ID:	2012/1255033				87	56	Seed	<0.01	0.602	<0.01	0.018
Trial No.:	L110181				87	56	Rest[4]	<0.01	0.083	<0.01	<0.01
GLP:	yes				87	56	Pods[5]	<0.01	0.327	0.023	0.031
Year:	2011				89	69	Seed	<0.01	0.653	<0.01	0.017
					89	69	Rest[4]	<0.01	0.109	<0.01	0.012
Study code:	364929	Oilseed rape	France	BAS 556 03 F 2 x 0.080	71	0	Plant[3]	<0.01	0.061	<0.01	<0.01
Doc ID:	2012/1255033				89	57	Seed	<0.01	0.439	<0.01	0.012
Trial No.:	L110182				89	57	Rest[4]	<0.01	0.016	<0.01	<0.01
GLP:	yes										
Year:	2011										
Study code:	364929	Oilseed rape	Denmark	BAS 556 03 F 2 x 0.080	71	0	Plant[3]	<0.01	0.013	<0.01	<0.01
Doc ID:	2012/1255033				89	56	Seed	<0.01	0.106	<0.01	<0.01
Trial No.:	L110183				89	56	Rest[4]	<0.01	0.019	<0.01	<0.01
GLP:	yes										
Year:	2011										

0 Actual application rates varied by 10% at most
1 Days after last application
2 At sampling
3 Whole plant without roots
4 Rest of plant without roots
5 With seeds
6 T=1,2,4-triazole, TA=triazolylalanine, TAA=triazole acetic acid, TLA=triazole lactic acid

Table 6.3.3-13: Residues of TDMs in oilseed rape after two applications of BAS 556 03 F in Southern Europe

Trial details		Crop	Country	Formulation, Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1)]	Residues found[6] (mg/kg)				
							Matrix	T	TA	TAA	TLA
Study code:	364929	Oilseed rape	France	BAS 556 03 F 2 x 0.080	71	0	Plant[3]	<0.01	0.034	<0.01	<0.01
Doc ID:	2012/1255033				89	57	Seed	<0.01	0.365	<0.01	<0.01
Trial No.:	L110184				89	57	Rest[4]	<0.01	0.031	<0.01	<0.01
GLP:	yes										
Year:	2011										
Study code:	364929	Oilseed rape	Greece	BAS 556 03 F 2 x 0.080	71	0	Plant[3]	<0.01	0.012	<0.01	<0.01
Doc ID:	2012/1255033				89	55	Seed	<0.01	0.227	<0.01	<0.01
Trial No.:	L110185				89	55	Rest[4]	<0.01	<0.01	<0.01	<0.01
GLP:	yes										
Year:	2011										
Study code:	364929	Oilseed rape	Italy	BAS 556 03 F 2 x 0.080	71	0	Plant[3]	<0.01	0.044	<0.01	<0.01
Doc ID:	2012/1255033				79-83	35	Rest[4]	<0.01	0.017	<0.01	<0.01
Trial No.:	L110186				79-83	35	Pods[5]	<0.01	0.124	<0.01	<0.01
GLP:	yes				89	43	Seed	<0.01	0.350	<0.01	<0.01
Year:	2011				89	43	Rest[4]	<0.01	<0.01	<0.01	<0.01
Study code:	364929	Oilseed rape	Spain	BAS 556 03 F 2 x 0.080	75	0	Plant[3]	<0.01	0.013	<0.01	<0.01
Doc ID:	2012/1255033				89	41	Seed	<0.01	0.106	<0.01	<0.01
Trial No.:	L110187				89	41	Rest[4]	<0.01	0.019	<0.01	<0.01
GLP:	yes										
Year:	2011										

0 Actual application rates varied by 10% at most
1 Days after last application
2 At sampling
3 Whole plant without roots
4 Rest of plant without roots
5 With seeds
6 T=1,2,4-triazole, TA=triazolylalanine, TAA=triazole acetic acid, TLA=triazole lactic acid

CA 6.4 Feeding studies

Data/information on the active substance metconazole in livestock were reviewed during the Annex I inclusion procedure and were considered to be acceptable. The following endpoints were copied from the EFSA Conclusion (2006; EFSA addendum January 2006).

Residues from Livestock feeding studies (Annex IIA, point 6.4, Annex IIIA, point 8.3)

Intakes by livestock ≥0.1 mg/kg diet/day	Ruminant: Yes	Poultry: No	Pig: No
Muscle	<0.01	no data, not assessed, not required	no data, not assessed, not required
Liver	0.013-0.019		
Kidney	<0.01		
Fat	<0.01		
Milk	<0.01		
Eggs		no data, not assessed, not required	

During the review of the MRLs including import tolerances, new feed burdens were calculated to take into account the imported commodities. Accordingly, a feeding study with ruminants was required, and a feeding study in lactating cows was submitted in conjunction with the MRL dossier (2009). The feeding study was evaluated and accepted as documented in the EFSA Reasoned Opinion (2010): "Metabolism studies and a new feeding study demonstrated that no measurable metconazole residues above the LOQ are expected in swine and ruminant food commodities at the calculated maximum dietary burdens" (see M-CA 6.4.2 below). The study is submitted and summarized in this dossier since it is not considered peer-reviewed. New feed burden calculations shown in the EFSA Reasoned Opinion 2011 and EFSA Reasoned Opinion 2013 resulted in exceedance of the trigger value of 0.1 mg/kg DM for pigs based on imported sugar beet. However, due to a similar metabolism in rat and goat, the EFSA statement for ruminants is also applicable for pigs (see M-CA 6.4.3 below).

CA 6.4.1 Poultry

The evaluation of the expected dietary burden for metconazole in poultry was done using the agreed European methodology in conjunction with the request for modification of existing MRLs due to requested import tolerances in 2009, and the calculated dietary burden did not exceed the trigger value. A dietary burden calculation using the agreed European methodology was done in the evaluation of modification of the barley MRL (EFSA Reasoned opinion on the modification of the existing MRLs for metconazole in barley and oats. EFSA Journal 2013) and showed that the trigger in poultry was not exceeded. A copy of the results in shown in the table below.

Table 6.4.1-1: Results of the dietary burden calculation copied from EFSA Reasoned opinion 2013

	Maximum dietary burden (mg/kg bw per d)	**Median dietary burden** (mg/kg bw per d)	**Highest contributing commodity**(a)	**Max dietary burden** (mg/kg DM)	**Trigger exceeded** (Y/N)
Risk assessment residue definition: Metconazole					
Dairy ruminants	0.055	0.009	Barley straw	1.529	Y
Meat ruminants	0.158	0.023	Barley straw	3.670	Y
Poultry	0.005	0.004	Wheat grain	0.080	N
Pigs	0.006	0.004	Sugar beet	0.152	Y

The dietary burden was recalculated for the representative uses using the spreadsheet provided in the OECD Livestock Guidance document dated September 4, 2013. The residues in the layer hen exceed the trigger, driven by metconazole residues in wheat straw. The residues in wheat straw at the maximum allowed portion in feed (10%) comprise 0.050 mg/kg bw per day.

	Broiler	Layer	Turkey
Region	EU	EU	EU
Body weight (kg)	1.7	1.9	7
Daily intake (kg DM)	0.12	0.13	0.5
Dietary Burden (mg/kg bw)	**0.002**	**0.052**	**0.002**
Feed Burden (mg/kg DM)	**0.034**	**0.761**	**0.026**

Due to the exceedance of the trigger based on the OECD consumption specified for laying hens, a hen metabolism study which includes a metabolic pathway is submitted and summarized in Chapter M-CA 6.2 and a hen feeding study is submitted and summarized below.

Report: CA 6.4.1/1
[redacted] 2008a
Magnitude of the residues of Metconazole in chicken eggs and tissues
2008/8000061
Guidelines: EPA 860.1480
(certified by United States Environmental Protection Agency)

In this study, residues of 1,2,4-triazole were also measured but are not included here. TDMs are metabolites of several parent compounds including metconazole. Results of animal feeding studies compiled by the TDM task force using agreed upon methods should provide expected residue levels in animal matrices.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	Metconazole
Lot/Batch #:	AS 2122a
Purity:	84.8% *cis*-metconazole, 14.7% *trans*-metconazole
CAS#:	125116-23-6
Spiking levels:	0.02-0.2 mg/kg

2 Test Animals:

Species:	White Leghorn
Gender:	Female
Age:	approximately 7 months (28 weeks)
Weight at dosing:	1.3-1.7 kg (study initiation)
Number of animals:	48
Acclimation period:	14 days
Diet:	Genesis Midwest Poultry I, *ad libitum*
Water:	Potable well water, *ad libitum*
Housing:	In groups of 4 birds in 4' x 4' x 2' (1.2 m x 1.2 x 0.6 m) pens

Environmental conditions:

Temperature:	11-29°C
Humidity:	41-100%
Air change:	Not reported
Photoperiod:	≥10 hours of daylight per day according to study protocol

B. STUDY DESIGN

1. Dosing regime-

Oral: Amount of dose: Control (12 hens), 0 mg/kg, subgroups A, B and C (4 hens)
1X (12 hens), 2 mg/kg, subgroups A, B and C (4 hens)
3X (12 hens), 6 mg/kg, subgroups A, B and C (4 hens)
10X (12 hens), 20 mg/kg, subgroups A, B and C (4 hens)

Food consumption: Group feed consumption was recorded daily.
Vehicle: Gelatin capsules, administration via balling gun; control group received empty capsules
Timing: Once daily
Duration: 28 consecutive days
Observations: The appearance and behavior was observed at least once daily.

2. Sample collection

Egg collection: Twice daily; evening and morning eggs pooled per subgroup
Eggs to be analyzed taken on study days:
-1, 1, 3, 7, 10, 14, 17, 20, 24, 26 and 28;

Interval from last dose to sacrifice: <24 hours
Samples collected and analyzed: Liver (entire), muscle (composites of thigh and breast muscle in approximately equal portions) and fat (composites of abdominal and subcutaneous fat in approximately equal portions)

3. Storage of samples:

Egg and tissue samples were stored frozen at or below -10°C and extracted for analysis within 26 days for metconazole and within 246 days for metabolites.

4. Extraction and characterization-

Analytical method & type: Valent method RM-41M-2 (tissues and eggs); Valent method RM-41M-3 (metabolites M1 in tissues and M12 in liver)

Valent analytical method RM-41M-2: In principle, residues of *cis*- and *trans*-metconazole are extracted from the tissues using acetonitrile, the acetonitrile is partitioned with hexane to remove oil and fat, and the acetonitrile layer is evaporated. The residues are dissolved in hexane/ethyl acetate and partitioned with aqueous sodium chloride. The hexane/ethyl acetate layer is then partitioned with acetonitrile, the acetonitrile layer is evaporated, the residues are dissolved in methanol/water, and the residues are passed through a C18 cartridge. The eluant is evaporated, and the residues are dissolved in methanol/water and analyzed by triple-quadrupole LC-MS/MS. For both *cis*- and *trans*-metconazole in tissue, the limit of detection (LOD) is 0.01 mg/kg and the limit of quantitation (LOQ) is 0.02 mg/kg.
Valent method RM-41M-3: Residues of M1 and M12 are extracted using methanol and methanol/water. The resulting extract is evaporated to aqueous, partitioned with acetonitrile/methanol followed by hexane. The acetonitrile/methanol layer is then divided to give Fraction A for C18 cleanup and LC-MS/MS analysis for M12 and Fraction B for hydrolysis using HCl, C18 cleanup, and LC-MS/MS analysis for M1. For both M1 and M12, the limit of detection (LOD) is 0.01 mg/kg and the limit of quantitation (LOQ) is 0.02 mg/kg.

The analytical method used to quantify metconazole residues (both *cis* and *trans* isomers)was validated on control samples of eggs (albumin+yolk, no shell) prior to analysis of the egg samples generated in this study. Fortifications of untreated samples were made at 0.02 mg/kg and 0.2 mg/kg. Recoveries from fortified egg samples (n = 3 per analyte) ranged from 85 to 89%, giving average recoveries of 86% and 87% for *cis*- and *trans*-metconazole, respectively, with standard deviations of 0.8% and 1.5%, respectively.
Analytical method performance was monitored through concurrent analysis of freshly fortified control samples along with the field samples. Depending on the analysis, one procedural control sample and two procedural recovery samples per analytical set were typically analyzed. The individual recoveries and overall means are summarized below.

Table 6.4.1-2: Accuracy and precision data obtained during the study (procedural recoveries) for metconazole

Matrix	Fortification level [mg/kg]	Mean recovery *cis*-metconazole [%]	*cis*-metconazole RSD [%]	Mean recovery *trans*-metconazole [%]	*trans*-metconazole RSD [%]
Eggs	0.02-0.2	84	4.4 (n=22)	84	3.3 (n=22)
Muscle	0.02-0.2	90	N/A (n=2)	92	N/A (n=2)
Fat	0.02-0.2	84	N/A (n=2)	84	N/A (n=2)
Liver	0.02-0.2	88	N/A (n=2)	88	N/A (n=2)

RSD Relative standard deviation
N/A Not applicable

Table 6.4.1-3: Accuracy and precision data obtained during the study (procedural recoveries) for metabolites M555F001 (M1) and M555F012 (M12)

Matrix	Fortification level [mg/kg]	Mean recovery M1 [%]	M1 RSD [%]	Mean recovery M12 [%]	M12 RSD [%]
Eggs	0.02-0.2	n.a.	n.a.	n.a.	n.a.
Muscle	0.02-0.2	76	N/A (n=2)	n.a.	n.a.
Fat	0.02-0.2	82	N/A (n=2)	n.a.	n.a.
Liver	0.02-0.2	78	9.3 (n=4)	88	N/A (n=2)

RSD Relative standard deviation
N/A Not applicable
n.a. Not analyzed

II. RESULTS AND DISCUSSION

Animals were dosed once daily by administering the target amounts of both test items in gelatin capsules, which were prepared weekly. The actual daily dose was calculated based on the average group feed consumption from the previous week.
The actual dose levels achieved in the study in terms of mg/kg feed (dry matter) and mg/kg body weight/day are shown for each test item in the following table.

Table 6.4.1-4: Actual dose levels of metconazole achieved in the study

Group No	Nominal dose (mg/kg feed)	Actual concentration (mg/kg body weight)	Actual concentration (mg/kg feed)
1X	2	0.15	2.05
3X	6	0.45	6.24
10X	20	1.55	20.30

Animal health, feed intake, body weights and egg production

All birds were observed to be healthy and normal throughout the acclimation, quarantine and study periods.
Body weights were considered normal throughout the study. Feed consumption for all groups throughout the study appeared normal for laying hens. Review of feed consumption averages showed that the test groups were comparable to the control throughout the test period. Feed consumption during the study period averaged 0.11 kg/bird/day in the Control, 1X, 3X and 10X groups, respectively. Egg production appeared to be consistent throughout the study and did not appear to be affected by treatment with the test substance.

Residues in eggs

Eggs were analyzed for cis- and trans-metconazole. Residues were <limit of detection (LOD = 0.01 mg/kg) in samples from the control group. No residues were detected in eggs from the animals dosed at 1X (2 mg/kg feed) or 3X (6 mg/kg feed). Residues of *cis*-metconazole were found in eggs of the 10X (20 mg/kg feed) group from the third day of dosing, with group mean residues ranging from 0.024 to 0.052 mg/kg and the highest average subgroup mean of 0.065 mg/kg at the 10 day sampling. Average *trans*-metconazole residues in these samples were near or below the LOD (0.01 mg/kg) or below the LOQ (0.02 mg/kg). Total metconazole residues (group mean) in egg ranged from 0.034 mg/kg at dose day 3 to 0.065 mg/kg at dose day 20 decreasing to 0.030 mg/kg at the last dosing.

Table 6.4.1-5: Residues of metconazole in eggs

Days	Metconazole (mg/kg)							
	Control		1X (2 mg/kg)		3X (6 mg/kg)		10X (20 mg/kg)	
	cis	*trans*	*cis*	*trans*	*cis*	*trans*	*cis*	*trans*
-1	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
1	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01 (0.02)	<0.01
3	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.033 (0.060)	<0.01 (0.014)
7	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.035 (0.047)	<0.01 (0.011)
10	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.045 (0.065)	<0.01 (0.015)
14	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.044 (0.055)	<0.01 (0.013)
17	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.043 (0.057)	0.012 (0.013)
20	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.052 (0.062)	0.013 (0.015)
24	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.031 (0.044)	<0.01
26	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.024 (0.027)	<0.01
28	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.029 (0.035)	<0.01

<0.01 denotes LOD; LOQ is 0.02 mg/kg

Residues in tissues (muscle, liver and fat)

The tissues of muscle, liver and fat were analyzed for *cis*- and *trans*-metconazole and M555F001 (M1). The liver tissues were also analyzed for M555F012 (M12). No residues of analytes were detected in the matrices of the control animals. Residues of *cis*- or *trans*-metconazole were not detected (<LOD of 0.01 mg/kg for each analyte) in muscle, fat or liver of animals dosed at any level.
Residues of the metabolite M1 were not detected in the muscle and fat samples from the 10X group (20 mg/kg) so no further analysis was done for these metabolites in samples from the 1X (2 mg/kg feed) or 3X (6 mg/kg feed) dose levels. For liver samples from the 10X (20 mg/kg feed) group, M1 residues averaged 0.04 mg/kg and the maximum residue was 0.05 mg/kg. . Average M1 residues in liver samples from the 3X group were at the LOQ (0.02 mg/kg), and residues were below the LOD for the 1X group. For liver samples from the 10X group, M12 residues averaged <LOD (0.01 mg/kg) with a maximum residue of 0.016 mg/kg feed. No further analysis for M12 was done in the 1X or 3X dose groups.

Table 6.4.1-6: Residues of metconazole in tissues

Treatment group	Group mean (and maximum individual) residues in tissue for metconazole (mg/kg)					
	Muscle		Liver		Fat	
	cis	*trans*	*cis*	*trans*	*cis*	*trans*
Control	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
1X (2 mg/kg)	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
3X (6 mg/kg)	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
10X (20 mg/kg)	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

<0.01 denotes LOD; LOQ is 0.02 mg/kg

Table 6.4.1-7: Residues of M1, M12 and 1,2,4-triazole in tissues

Treatment group	Group mean (and maximum individual) residues in tissue for metabolites (mg/kg)					
	Muscle		Liver		Fat	
	M1	M12	M1	M12	M1	M12
Control	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
1X (2 mg/kg)	n.a.	n.a.	<0.01	n.a.	n.a.	n.a.
3X (6 mg/kg)	n.a.	n.a.	0.02 (0.03)	n.a.	n.a.	n.a.
10X (20 mg/kg)	<0.01	n.a.	0.04 (0.05)	<0.01 (0.016)	n.a.	n.a.

n.a. Not analyzed
<0.01 denotes LOD; LOQ is 0.02 mg/kg

Egg samples and tissue samples were frozen prior to shipment. Samples received at the lab were stored frozen at approximately -10° C until extraction and analysis. Egg and tissue samples were extracted for *cis*- and trans-metconazole analysis within 26 days of collection. Egg and tissue samples were extracted for triazole analysis within 135 days of collection. Tissue samples were extracted for M-1 analysis within 246 days of collection, and the liver samples were extracted for M-12 analysis within 127 days of collection. Data generated in the hen metabolism study support freezer storage stability of the major metconazole metabolites in liver, muscle, fat, and egg whites. The metabolite profiles indicate that the major metconazole metabolites were unchanged for approximately 10 months (approximately 300 days).

III. CONCLUSION

A residue transfer study with metconazole (85% *cis*-, 15% *trans*-) was conducted in laying hens. The animals were dosed each day with a residue level equivalent to 2 mg/kg, 6 mg/kg or 20 mg/kg feed (dry matter) per day for a period of 28 days. Residues of *cis*-metconazole were found in eggs of the 10X (20 mg/kg feed) group from the third day of dosing, with group mean residues ranging from 0.024 to 0.052 mg/kg and the highest average subgroup mean of 0.065 mg/kg at the 10 day sampling. Average *trans*-metconazole residues in these samples generally <LOD (0.01 mg/kg) and never exceeded LOQ (0.02 mg/kg). Total metconazole residues (group mean) in egg ranged from 0.034 mg/kg at dose day 3 to 0.065 mg/kg at dose day 20 decreasing to 0.030 mg/kg at the last dosing. No parent metconazole residues were found in tissues (muscle, liver, fat) of any dose group. Mean residues of metabolite M1 were found only in liver of animals in the 6 mg/kg and the 20 mg/kg feed dose group at 0.02 and 0.04 mg/kg, respectively, and not in muscle or fat. Metabolite M12, analyzed only in liver, was below the LOQ in liver of the highest dose group (20 mg/kg feed).

Determination of metconazole residues in matrices of animal origin – method validation

The methods for the determination of metconazole (BAS 555 F) are described in the analytical phase reports of the respective study report [see *M-CA 6.4.1, BASF DocID 2008/8000061]*.
Executive summaries on the original method validations are given below.

Executive summary - Validation of method RM-41M-2

Principle of the method

The analytical method RM-41M-2 was validated for the determination of metconazole (BAS 555 F) *cis*- and *trans*-isomers in liver. The study was performed by Valent U.S.A. Corporation, CA, USA.
Residues were extracted from a liver sample by blending three times with acetonitrile, and combining the extracts. Residues were partitioned using hexane to remove oil and fat and the acetonitrile layer is evaporated. The residues were dissolved in hexane/ethyl acetate (90:10, v/v), partitioned with aqueous sodium chloride solution and filtered through sodium sulfate. Using acetonitrile, the residues were partitioned again, evaporated, dissolved and washed in methanol/water (5:1, v/v), passed through a C18 cartridge and evaporated. Residues were dissolved in methanol/water (2:1, v/v) and analyzed by triple-quadrupole LC-MS/MS. Analysis was performed on an Agilent Zorbax SB-C8 column using a water-methanol gradient with formic acid as modifier.

Recovery findings

The mean recovery values were between 70% and 110% for *cis*- and *trans*-metconazole. The detailed results are given in the table below.

Table 6.4.1-8: Validation results of method RM-41M-2 using LC-MS/MS: metconazole (BAS 555 F) isomers in liver

Matrix	Analyte	Mass transition	Fortification level (mg/kg)	No of replicates	Mean recovery (%)	RSD (%)
Liver	*Cis*-metconazole	320→125	0.02	5	86	17
			0.1	6	80	3.7
			Overall	11	83	12
	Trans-metconazole	320→125	0.02	5	87	17
			0.1	6	81	3.6
			Overall	11	84	12

Linearity
The linearity was tested using four standards at concentrations between of 0.01 to 0.1 µg/mL. For BAS 555 F (*cis* and *trans*), linear correlations with coefficients of determination (R^2) greater than 0.99 were obtained. Calibration solutions were prepared in methanol/water.

Specificity
LC-MS/MS is a highly specific self-confirmatory technique. Due to the high selectivity and specificity of LC-MS/MS an additional confirmatory technique is not necessary.

Interference
No interferences from the reagent blank at the relevant retention times have been reported. No residues were detected in control samples.

Limit of Quantitation
The limit of quantitation (LOQ) is defined as the lowest fortification level successfully tested. During this study, a limit of quantitation of 0.02 mg/kg for BAS 555 F (each isomer, *cis* and *trans*) was obtained in liver.

Limit of Detection
For BAS 555 F (*cis* and *trans*), the limit of detection (LOD) was 0.01 mg/kg in liver.

Repeatability
The relative standard deviations (RSD, %) for all fortification levels were below 20%.

Stability of solutions
The stability of the analytes in working solutions was no subject of this study. Test and reference substance solutions were stored in a refrigerator and were refrigerated during their use in this study. Standards were generally not used for more than one week.

Conclusion

The analytical method RM-41M-2 for the analysis of metconazole *cis*- and *trans*-isomers in liver uses LC-MS/MS for final determination, with an LOQ of 0.04 mg/kg (as sum of isomers).
It could be demonstrated that the method generally fulfils the requirements with regard to repeatability, specificity, LOQ and recoveries and is therefore considered suitable for the analysis of metconazole (*cis*- and *trans*-isomer) liver.

Executive summary - Validation of method RM-41M-3

Principle of the method

The analytical method RM-41M-3 was validated for the determination of metconazole metabolites M1 (M555F001) and M12 (M555F012) in kidney. The study was performed by VALENT U.S.A. CORPORATION, CA, USA.
Residues were extracted from the tissues using methanol and methanol/water (9:1, v/v). The Extract was evaporated to obtain an aqueous residue, and residues were partitioned with acetonitrile/methanol (2:1, v/v) and hexane. The acetonitrile/methanol layer was split to Fraction A (for C18 cleanup and LC-MS/MS analysis for M12) and Fraction B (for hydrolysis, C18 cleanup, and LC-MS/MS analysis for M1).
For Fraction A (M12), an aliquot of the extract was evaporated, the residues were dissolved in methanol/water (5:1, v/v), passed through a Cl8 cartridge and evaporated. Residues were dissolved in methanol/water (2:1, v/v). Analysis was performed by LC-MS/MS.
For Fraction B (M1), an aliquot of the extract was evaporated, the residues were transferred into a screw-top test tube, capped, and the residues hydrolyzed in 3 N HCl at 85°C. The mixture was transferred through a C 18 cartridge, washed and rinsed with methanol/water (5:1, v/v). The eluate was evaporated, and residues were dissolved in methanol/water (2: 1, v/v) and analyzed by LC-MS/MS. Analysis was performed on a Phenomenex Luna C18 column using a methanol/water gradient with formic acid as modifier.

Recovery findings

The mean recovery values were between 77% and 70% for M1, 74% and 78% for M-12 at 0.02 and 0.1 mg/kg fortification and 72% and 76% overall, respectively. The detailed results are given in the table below.

Table 6.4.1-9: Validation results of method RM-41M-3 using LC-MS/MS: metconazole (BAS 555 F) metabolites M1 and M12 in kidney

Matrix	Analyte	Mass transition	Fortification level (mg/kg)	No of replicates	Mean recovery (%)	RSD (%)
Kidney	M-1	336→125	0.02	4	77	10
			0.1	6	70	2.3
			Overall	10	72	8.1
	M-12	350→125	0.02	4	74	6.0
			0.1	6	78	3.6
			Overall	10	76	5.1

Linearity
The linearity was tested using four standards at concentrations between of 0.005 to 0.1 µg/mL. For M1 and M12 linear correlations with coefficients of determination (R^2) greater than 0.99 were obtained. Calibration standards were prepared in methanol/water.

Specificity
LC-MS/MS is a highly specific self-confirmatory technique. Due to the high selectivity and specificity of LC-MS/MS an additional confirmatory technique is not necessary.

Interference
No interferences from the reagent blank at the relevant retention times have been reported. No residues were detected in control samples.

Limit of Quantitation
The limit of quantitation (LOQ) is defined as the lowest fortification level successfully tested. During this study, a limit of quantitation of 0.02 mg/kg for metconazole metabolites M-1 and M-12 was obtained in kidney.

Limit of Detection
For metconazole metabolites M1 and M12, the limit of detection (LOD) was 0.01 mg/kg in kidney.

Repeatability
The relative standard deviations (RSD, %) for all fortification levels were ≤10%.

Stability of solutions
The stability of the analytes in working solutions was no subject of this study. Test and reference substance solutions were stored in a refrigerator and were refrigerated during their use in this study. Standards were generally not used for more than one week.

Conclusion
The analytical method RM-41M-3 for the analysis of metconazole metabolites M1 and M12 in kidney uses LC-MS/MS for final determination, with an LOQ of 0.02 mg/kg per analyte. It could be demonstrated that the method generally fulfils the requirements with regard to repeatability, specificity, LOQ and recoveries and is therefore considered suitable for the analysis of metconazole metabolites M1 and M12 in kidney.

CA 6.4.2 Ruminants

The following sections were copied from the EFSA Reasoned Opinion (2011).

"Assessing the livestock metabolism studies, it is concluded that metconazole residue above the LOQ will not occur in milk, fat, muscle and kidney. Concerns were raised on the possible metconazole residue in liver which might occur at levels exceeding the LOQ for the calculated maximum dietary burden (for meat ruminants, 1.6 mg/kg DM/d) and a livestock study was required in order to derive a reliable MRL in liver."

After the peer review under Directive 91/414/EEC, a livestock feeding study was submitted by the applicant to the RMS Belgium but was not considered sufficient by the RMS due to data gaps regarding the validation of the analytical method in cream, meat, fat and kidney. Despite these deficiencies, the study demonstrates that metconazole residues in liver do not exceed the LOD of 0.01 mg/kg (for *cis*- and *trans*-metconazole) at the medium feeding level of 15 mg/kg DM which is significantly higher than the calculated maximum dietary burden (1.6 mg/kg DM)."

"It is therefore concluded that, according to the available livestock metabolism and feeding studies, residues exceeding enforcement LOQ of 0.02 mg/kg will not occur in swine and ruminant food commodities at the calculated maximum dietary burdens."

Report: CA 6.4.2/1
[redacted] 2006a
Magnitude of the residues of Metconazole in dairy cattle and meat
2006/1046033
Guidelines: EPA 860.1460
GLP: yes
(certified by United States Environmental Protection Agency)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	Metconazole
Lot/Batch #:	AS 2122a
Purity:	83.8%
CAS#:	125116-23-6
Spiking levels:	0.02-0.20 mg/kg

2. Test Animals:

Species:	Holstein
Gender:	Female
Age:	3-7 years
Weight at dosing:	470 - 720 kg (at the start of dosing phase)
Number of animals:	12
Acclimation period:	15 days
Diet:	2 x 4 kg dairy ration (Genesis Midwest Dairy Ration), 2 x 5 kg alfalfa hay cubes and 2 x 3 kg baled hay
Water:	Fresh potable well water, *ad libitum*
Housing:	Individual 4' x 7' (1.2 m x 2.1 m) concrete stalls with stanchions, located in a large dairy barn

Environmental conditions:

Temperature:	13-18°C
Humidity:	67-83%
Air change:	Natural ventilation
Photoperiod:	12 hours light and 12 hours dark

B. STUDY DESIGN

1. Dosing regime

Oral: Amount of dose: Group 0 (3 cows), control, 0 mg/kg
Group T-I (3 cows), 1X level, 5 mg/kg
Group T-II (3 cows), 3X level, 15 mg/kg
Group T-III (3 cows), 10X level, 50 mg/kg

Food consumption: Feed consumption was recorded daily.
Vehicle: Gelatin capsules, administration via balling gun; control group received empty capsules
Timing: Once daily, after morning milking
Duration: 29 consecutive days
Observations: The appearance and behavior was observed at least twice daily.

2. Sample collection

Milk collection: Twice daily; evening and morning milk pooled per cow
Milk to be analyzed taken on study days:
-1, 1, 2, 4, 7, 10, 14, 17, 21, 24 and 28;
Separation into skim milk and cream on study day 24

Interval from last dose to sacrifice: <24 hours

Samples collected and analyzed: Liver (composites of full thickness slices from each lobe), kidney (composites of representative portions of the center and both ends), muscle (composites of hind-quarter, pectoral, and abductor muscle in approximately equal portions) and fat (composites of perinephic, abdominal, and subcutaneous fat in approximately equal portions)

3. Storage of samples:

Milk and tissue samples were stored frozen at or below -10°C and analyzed within 50 days storage for metconazole and within 181 days for metabolites

4. Extraction and characterization

Analytical method & type: Valent method RM-41M-1 (milk and cream); Valent method RM-41M-2 (tissues); Valent method RM-41M-3 (metabolites M1 and M12 in kidney and liver)

Valent method RM-41M-1: In principle, residues of *cis*- and *trans*-metconazole are extracted from the milk using ethyl acetate/methanol. The extract is filtered, concentrated and partitioned with dichloromethane/aqueous sodium chloride solution. The concentrated organic phase is partitioned with acetonitrile/hexane, the organic acetonitrile is concentrated, and the residues are dissolved in toluene for quantitation by gas chromatography with a nitrogen-phosphorus detector (GC/NPD). An alternate analysis by liquid chromatography with mass selective detection with a triple-quadruple detector (LC-MS/MS) can be used for verification. For both *cis*- and *trans*-metconazole in milk, the limit of detection (LOD) is 0.01 mg/kg and the limit of quantitation (LOQ) is 0.02 mg/kg. For the analysis of cream, the LOD is 0.02 mg/kg and the LOQ is 0.04 mg/kg.

Valent analytical method RM-41M-2: In principle, residues are extracted from the tissues using acetonitrile; the acetonitrile is partitioned with hexane to remove oil and fat, and the acetonitrile layer is evaporated. The residues are dissolved in hexane/ethyl acetate and partitioned with aqueous sodium chloride. The hexane/ethyl acetate layer is then partitioned with acetonitrile, the acetonitrile layer is evaporated, the residues are dissolved in methanol/water, and the residues are passed through a C18 cartridge. The eluant is evaporated, and the residues are dissolved in methanol/water with quantitation using LC-MS/MS. For both *cis*- and *trans*-metconazole in tissue, the limit of detection (LOD) is 0.01 mg/kg and the limit of quantitation (LOQ) is 0.02 mg/kg.

Valent method RM-41M-3: Residues are extracted using methanol and methanol/water. The resulting extract is evaporated to aqueous, partitioned with acetonitrile/methanol followed by hexane. The acetonitrile/methanol layer is then divided to give Fraction A for C18 cleanup and LC-MS/MS analysis for M12 and Fraction B for hydrolysis using HCl, C18 cleanup, and LC-MS/MS analysis for M1. For both M1 and M12 in kidney and liver, the limit of detection (LOD) is 0.01 mg/kg and the limit of quantitation (LOQ) is 0.02 mg/kg.
Fortifications of untreated whole and skim milk samples were made at 0.02 and 0.10 mg/kg, and fortifications of untreated cream samples were made at 0.04 and 0.20 mg/kg. Recoveries from fortified whole milk samples ranged from 91 to 104%, giving average recoveries of 97% and 95% for *cis*- and *trans*-metconazole, respectively. Recoveries for *cis*- and *trans*-metconazole from fortified cream samples ranged from 92 to 105% and from fortified skim milk samples ranged from 93 to 96%.
Concurrent recoveries obtained from control samples spiked with *cis*- and *trans*-metconazole, M1 and M12 were analyzed to demonstrate the validity of the method. They are shown in the tables below. Recoveries of M1 and M12 for liver were <70% but were considered acceptable due to the consistency as shown by the correlation coefficient.

Table 6.4.2-1: Accuracy and precision data obtained during the study (procedural recoveries)

Matrix	Fortification level [mg/kg]	Mean recovery *cis*-metconazole [%]	*cis*-metconazole RSD [%]	Mean recovery *trans*-metconazole [%]	*trans*-metconazole RSD [%]
Milk	0.02/0.10	96.5	4.9 (n=10)	95.2	4.2 (n=10)
Skim milk	0.02/0.10	92.7	N/A (n=2)	95.1	N/A (n=2)
Cream	0.04/0.20	94.3	N/A (n=2)	102	N/A (n=2)
Muscle	0.02/0.10	77.6	N/A (n=2)	76.8	N/A (n=2)
Fat	0.02/0.10	82.1	N/A (n=2)	83.4	N/A (n=2)
Kidney	0.02/0.10	78.0	N/A (n=2)	80.2	N/A (n=2)
Liver	0.02/0.10	74.9	6.8 (n=4)	76.0	5.2 (n=4)

RSD Relative standard deviation
N/A Not applicable

Table 6.4.2-2: Accuracy and precision data for metabolites obtained during the study (procedural recoveries)

Matrix	Fortification level [mg/kg]	Mean recovery M1 [%]	M1 RSD [%]	Mean recovery M12 [%]	M12 RSD [%]
Kidney	0.02/0.10	74	5.0 (n=4)	70	4.0 (n=4)
Liver	0.02/0.10	60	3.1 (n=4)	67	N/A (n=2)

RSD Relative standard deviation
N/A Not applicable

II. RESULTS AND DISCUSSION

Animals were dosed once daily by administering the target amounts of both test items in gelatin capsules, which were prepared weekly. The amount of metconazole weighed into each capsule for a specific cow was dependent on the highest feed consumption for that cow during the first 13 days of the initial quarantine and acclimation period. The calculated dose was based on dry weight of the feed consumed. The moisture content of the feed was determined prior to study start and used throughout the study to calculate the feed intake on a dry matter basis.
The actual dose levels achieved in the study in terms of mg/kg feed (dry matter) and mg/kg body weight/day are shown for each test item in the following table.

Table 6.4.2-3: Actual dose levels of metconazole achieved in the study

Animal ID	Nominal dose (mg/kg feed)	Actual concentration (mg/kg body weight)	Actual concentration (mg/kg feed)
Mean group T-I	5	0.20	4.73
Mean group T-II	15	0.57	14.58
Mean group T-III	50	1.66	45.65

Animal health, feed intake, body weights and milk production
Most animals were observed to be healthy and normal throughout the study. Due to an accident on the site, one cow in the T-I treatment group (treated at 5 mg/kg) was severely injured and was euthanized on Day 11. Any observed health conditions were unrelated to the test chemical.
Body weights were considered normal throughout the study. Feed consumption for all groups throughout the study appeared normal for dairy cows. Feed consumption on a dry weight basis during quarantine and the 29-day dosing period averaged 19.2, 20.3, 20.6 and 20.3 kg per animal in the control, 1X, 3X, and l0X groups, respectively. Milk production appeared to be consistent throughout the study and did not appear to be affected by treatment with the test substance.

Residues in milk, skim milk and cream

No residues were detected in samples from the control group. No residues were detected in the animals in Group T-III (10x dose), so analyses of the milk, skim milk and cream from the animals in the lower dose groups were not needed.

Table 6.4.2-4: Residues of metconazole in milk, skim milk, and cream

Study day	Group mean residues for metconazole (mg/kg)							
	Group 0 (Control)		Group T-I 1X (5 mg/kg)		Group T-II 3X (15 mg/kg)		Group T-III 10X (50 mg/kg)	
	cis	*trans*	*cis*	*trans*	*cis*	*trans*	*cis*	*trans*
-1	<0.01	<0.01	n.a.	n.a.	n.a.	n.a.	<0.01	<0.01
1	<0.01	<0.01	n.a.	n.a.	n.a.	n.a.	<0.01	<0.01
2	<0.01	<0.01	n.a.	n.a.	n.a.	n.a.	<0.01	<0.01
4	<0.01	<0.01	n.a.	n.a.	n.a.	n.a.	<0.01	<0.01
7	<0.01	<0.01	n.a.	n.a.	n.a.	n.a.	<0.01	<0.01
10	<0.01	<0.01	n.a.	n.a.	n.a.	n.a.	<0.01	<0.01
14	<0.01	<0.01	n.a.	n.a.	n.a.	n.a.	<0.01	<0.01
17	<0.01	<0.01	n.a.	n.a.	n.a.	n.a.	<0.01	<0.01
21	<0.01	<0.01	n.a.	n.a.	n.a.	n.a.	<0.01	<0.01
24	<0.01	<0.01	n.a.	n.a.	n.a.	n.a.	<0.01	<0.01
28	<0.01	<0.01	n.a.	n.a.	n.a.	n.a.	<0.01	<0.01
Skim milk (Day 24)	<0.01	<0.01	n.a.	n.a.	n.a.	n.a.	<0.01	<0.01
Cream (Day 24)	<0.02	<0.02	n.a.	n.a.	n.a.	n.a.	<0.02	<0.02

n.a. Not analyzed
<0.01 denotes LOD (<0.02 mg/kg in case of cream); LOQ is 0.02 mg/kg (0.04 mg/kg in case of cream)

Residues in tissues (muscle, liver, kidney and fat)

No residues were detected in the matrices of the control animals. Residues of *cis*- or *trans*-metconazole were not detected (<LOD of 0.01 mg/kg for each analyte) in muscle, fat or kidney for animals in Group T-III, the 10x feeding level. Therefore, analysis was not performed for these analytes in animals at the lower feeding levels. Residues of *cis*- or *trans*-metconazole were not detected (<LOD of 0.01 mg/kg for each analyte) in liver for animals in Group T-II so analysis was not performed at the lower feeding levels. Residues of *cis*-metconazole were observed between 0.01 and 0.02 mg/kg in the liver from the Group T-III cows.

Residues of the metabolite M1 were detected at the LOD of 0.01 mg/kg in liver and kidney of one of three animals in Group T-II. No residues of M1 were detected in the liver or kidney of the animals at the 1x feeding level. In Group T-III, mean M1 residues in liver and kidney were 0.02 mg/kg, respectively.

Residues of the metabolite M12 were detected in the kidney of animals at Group T-II. No residues of M12 were detected in the kidney of the animals at the 1x feeding level. No residues of the metabolite M12 were detected in the liver of animals at Group T-II. In Group T-III, mean M12 residues in liver and kidney were 0.01 and 0.04 mg/kg, respectively.

Table 6.4.2-5: Residues of metconazole in tissues

Treatment group	Group mean (and maximum individual) residues in tissue for metconazole (mg/kg)							
	Muscle		Liver		Kidney		Fat	
	cis	***trans***	***cis***	***trans***	***cis***	***trans***	***cis***	***trans***
0 (control)	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
T-I (1X, 5 mg/kg)	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
T-II (3X, 15 mg/kg)	n.a.	n.a.	<0.01	<0.01	n.a.	n.a.	n.a.	n.a.
T-III (10X, 50 mg/kg)	<0.01	<0.01	0.01 (0.02)	<0.01	<0.01	<0.01	<0.01	<0.01

n.a. Not analyzed
<0.01 denotes LOD; LOQ is 0.02 mg/kg

Table 6.4.2-6: Residues of M1 and M12 in tissues

Treatment group	Group mean (and maximum individual) residues in tissue for metabolites (mg/kg)							
	Muscle		Liver		Kidney		Fat	
	M1	**M12**	**M1**	**M12**	**M1**	**M12**	**M1**	**M12**
0 (control)	n.a.	n.a.	<0.01	<0.01	<0.01	<0.01	n.a.	n.a.
T-I (1X, 5 mg/kg)	n.a.	n.a.	<0.01	n.a.	<0.01	<0.01	n.a.	n.a.
T-II (3X, 15 mg/kg)	n.a.	n.a.	<0.01 (0.01)	<0.01	<0.01 (0.01)	0.01 (0.02)	n.a.	n.a.
T-III (10X, 50 mg/kg)	n.a.	n.a.	0.02 (0.05)	0.01 (0.01)	0.02 (0.03)	0.04 (0.06)	n.a.	n.a.

n.a. Not analyzed
<0.01 denotes LOD; LOQ is 0.02 mg/kg

III. CONCLUSION

A residue transfer study with metconazole (85% *cis*-, 15% *trans*-metconazole) was conducted in lactating cows. The animals were dosed each day with a residue level the equivalent to 5 mg/kg, 15 mg/kg or 50 mg/kg feed (dry matter) per day for a period of 28 days. In milk and skim milk parent metconazole residues were <0.01 mg/kg for each isomer and <0.02 mg/kg for each isomer in cream. In the low dose group (5 mg/kg feed) and in the middle dose group (15 mg/kg feed), no residues could be detected (<0.01 mg/kg) in tissues, muscle, fat, liver or kidney. In the highest dose group (50 mg/kg feed), residues of *cis*-metconazole were a maximum of 0.02 mg/kg and mean of 0.01 mg/kg and residues of trans-metconazole were <0.01 mg/kg in liver; parent metconazole residues were <0.01 mg/kg (LOD) in all other tissues. In liver, residues of M1 and M12 were 0.02 mg/kg and 0.01 mg/kg at 10X (group mean), respectively, and <0.01 mg/kg (group mean) for each in the 3X group. In kidney, residues of M1 and M12 were 0.02 and 0.04 mg/kg at 10 X (group mean), respectively, and <0.01 and 0.01 mg/kg in the 3X group (group mean), respectively.

Determination of residues of animal origin of metconazole – method validation

The methods for the determination of metconazole (BAS 555 F) are described in the analytical phase reports of the respective study report [see *CA 6.4.2, BASF DocID 2006/1046033*].
Executive summaries on the original method validations are given below.

Executive summary - Validation of method RM-41M-1

Principle of the method

The analytical method RM-41M-1 was validated for the determination of metconazole (BAS 555 F) *cis*- and *trans*-isomers in milk. The study was performed by VALENT U.S.A. CORPORATION, CA, USA.
Residues were extracted from a milk sample using ethyl acetate/methanol mixture (2:1, v/v), filtered, rotary evaporated to an aqueous oily residue and partitioned with methylene chloride/aqueous sodium chloride solution. The organic phase, containing the residues, was evaporated, residues were cleaned up by acetonitrile-hexane partition and acetonitrile was removed by rotary evaporation. Residues were dissolved in toluene for analysis by gas chromatography with a nitrogen-phosphorus detector (GC-NPD) for the determination of metconazole (*cis* and *trans* isomer). Analysis was performed on an Agilent HP-5 column with helium as carrier gas.

Recovery findings

The mean recovery values were between 70% and 110% for both *cis*- and *trans*-metconazole. Details are presented in the table below.

Table 6.4.2-7: Validation results of method RM-41M-1 using GC-NPD: metconazole (BAS 555 F) isomers in milk

Matrix	Analyte	Fortification level (mg/kg)	No of replicates	Mean recovery (%)	RSD (%)
Milk	*Cis*-metconazole	0.02	3	93	2.3
		0.1	6	88	2.1
		Overall	9	89	3.3
	Trans-metconazole	0.02	3	95	1.9
		0.1	6	90	2.4
		Overall	9	92	3.7

Table 6.4.2-8: Validation results of method RM-41M-1 using LC-MS/MS: metconazole (BAS 555 F) isomers in milk

Matrix	Analyte	Mass transition	Fortification level (mg/kg)	No of replicates	Mean recovery (%)	RSD (%)
Milk	*Cis*-metconazole	320→125	0.02	3	85	3.7
	Trans-metconazole	320→125	0.02	3	84	1.3

Linearity
The linearity was tested using four standards at concentrations between of 0.1 to 2.0 µg/mL for GC and between 0.005 and 0.100 µg/mL for HPLC analysis. For BAS 555 F (*cis* and *trans*), linear correlations with coefficients of determination (R^2) greater than 0.99 were obtained. Calibration standards were prepared in acetone for GC and in methanol/water for HPLC analysis.

Specificity
LC-MS/MS was used as confirmatory technique in the positive ionization mode on an Agilent Zorbax SB-C18 column. A water-methanol gradient was used with formic acid as modifier.

Interference
No interferences from the reagent blank at the relevant retention times have been reported. No residues were detected in control samples.

Limit of Quantitation
The limit of quantitation (LOQ) is defined as the lowest fortification level successfully tested. During this study, a limit of quantitation of 0.02 mg/kg for BAS 555 F (each isomer, *cis* and *trans*) was obtained in milk.

Limit of Detection
For BAS 555 F (*cis* and *trans*), the limit of detection (LOD) was 0.01 mg/kg in milk.

Repeatability
The relative standard deviations (RSD, %) for all fortification levels were within the respective repeatability criteria (<10%).

Stability of solutions
The stability of the analytes in working solutions was no subject of this study. Test and reference substance solutions were stored in a refrigerator and were refrigerated during their use in this study.

Conclusion
The analytical method RM-41M-1 for the analysis of metconazole *cis*- and *trans*-isomers in milk uses GC-NPD for final determination, with an LOQ of 0.04 mg/kg (as sum of isomers). It could be demonstrated that the method generally fulfils the requirements with regard to repeatability, specificity, LOQ and recoveries and is therefore considered suitable for the analysis of metconazole (*cis*- and *trans*-isomer) in milk.

Executive summary - Validation of method RM-41M-2
Already described in chapter M-CA 6.4.1

Executive summary - Validation of method RM-41M-3
Already described in chapter M-CA 6.4.1

CA 6.4.3 Pigs

Feeding studies in pigs are not required. For the active substance metconazole, rat and goat metabolism studies revealed comparable metabolic pathways. The following wording was copied from the EFSA Reasoned Opinion (2011).

"Metabolism in lactating ruminants was sufficiently investigated and findings can be extrapolated to pigs as well. The relevant residue definition for both enforcement and risk assessment in pigs and ruminants was therefore defined as metconazole. Available studies also demonstrated that residues of metconazole are not expected in significant amounts and MRLs in pigs and ruminants can be therefore set at the LOQ."

CA 6.4.4 Fish

According to the Commission Regulations (EU) No 283/2013 (active substances) and 284/2013 (plant protection products) as of 1 March 2013, metabolism studies on fish and fish feeding studies might be required in future (latest by 31 Dec 2015), if residues occur in crops that are intended as feed items for fish. A fish feeding study may be required where residues at levels above 0.01 mg/kg may be reasonably expected in edible tissues, based on the findings of the fish metabolism study and the estimated maximum residues which might occur in fish feed. Particular attention should be laid on lipophilic substances with an intrinsic tendency for accumulation. Feeding studies shall not be required where intake is below 0.004 mg/kg bw/day, except in cases where the residue, that is to say the active substance, its metabolites or breakdown products, as defined in the residue definition for risk assessment, tends to accumulate.

The following was noted in the EFSA Conclusion (EFSA Scientific Report (2006) 64, 1-71, Conclusion on the peer review of metconazole).

"With regard to its log P_{ow} (3.85) metconazole is characterized as fat soluble. However, TRR were roughly one order of magnitude higher in the excretory organs liver and kidney of goat and hen than in other animal tissue, indicating that there might be no accumulation of residues in adipose tissue "

It should also be noted that fish bioconcentration studies show very short clearance times of <1 day as noted in the list of endpoints (EFSA Scientific Report (2006) 64, 1-71, Conclusion on the peer review of metconazole Appendix 1 – list of endpoints).

As metconazole is used in several crops being fed to fish, a fish feed burden calculation was performed

Report: CA 6.4.4/1
Panek M., Kampke-Thiel K., 2015a
Metconazole: Evaluation of the expected dietary burden in fish
2014/7000204
Guidelines: None
GLP: no

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: Metconazole

B. STUDY DESIGN AND METHODS

The exposure of fish to metconazole residues was calculated using the fish dietary burden calculation Version 1.0.4, developed by Fraunhofer (Klein, J., Schlechtriem, C., 2014, DietaryBurdenCalculator Version 1.0.4 of February 06, 2015, Fraunhofer IME (Institute of Molecular Biology and Applied Ecology) Schmallenberg, Germany).
By aid of this program, the dietary burden may be calculated for the fish aquaculture species rainbow trout (*Oncorhynchus mykiss*) and common carp (*Cyprius carpio*) based on the principle of maximum reasonable balanced diets (MRBD). The mathematical basis of the calculation is the "simplex method", a procedure of linear optimization.
The composition of the fish feed is related to the lipid and protein content requirements of the fish species as well as their maximum intake limits per feed item as indicated in Table 6.4.4-1

Table 6.4.4-1: Example of feed items with protein content (CP), lipid content (CL) and the restrictions regarding the maximum intake limit of the different fish species in percentage of the diet

Feed item	CP (%)	CL (%)	Max. carp (%)	Max. trout (%)
Barley bran fractions	16.4	66	35	15
Cottonseed meal	32.9	1.7	35	15
Linseed meal	35.0	2.0	35	15
Mustard meal	42.4	1.8	10	10
Rape seed meal (toxic)	37.3	1.9	5	5
Wheat Extruded grain	13.5	1.9	15	15
Wheat bran	15.6	4.7	35	15
Wheat flour	14.3	1.7	15	15
Faba bean Treated seed	28.3	8.4	15	15
Cow pea Treated seed	25.1	4.9	15	15
Lupin (white) Treated seed	34.5	6.1	15	15
Pea Treated seed	23.7	1.7	15	15
Wheat Grain (extruded)	13.8	2.9	35	20
Wheat Grain (extruded)	13.8	2.9	35	20

Residue inputs of the respective feed items (STMR values) used for the feedburden calculations are presented in Table 6.4.4-2
Results of the feedburden calculations for rainbow trout are summarised in Table 6.4.4-3 calculations for common carp are summarized in Table 6.4.4-4

Table 6.4.4-2: Residues (STMR) of metconazole in relevant feed items used as input for fish dietary burden calculator (version 1.0.4, Feb. 2015)

Commodity	Residue (mg/kg)	Commodity	Residue (mg/kg)
Barley bran fractions	0.08	Wheat (Extruded grain)	0.04
Corn field Grain meal	0.01	Wheat (bran)	0.08
Corn field bran	0.01	Wheat (flour)	0.04
Corn field hominy meal	0.01	Wheat (germ)	0.04
Corn gluten feed	0.01	Wheat (middlings)	0.04
Corn gluten (meal)	0.01	Wheat (gluten)	0.04
Corn starch	0.01	Corn (grain)	0.01
Cottonseed (meal)	0.04	Faba bean Treated seed	0.02
Linseed meal	0.03	Lupin (white) Treated seed	0.02
Mustard meal	0.03	Pea Treated seed	0.02
Peanut (meal decorticated)	0.02	Soybean (treated seed)	0.01
Rape seed meal (toxic)	0.03	Triticale grain	0.04
Canola (meal)	0.03	Wheat Grain (extruded)	0.04
Soybean (meal decorticated)	0.01	Vegetable oil	0.01

Table 6.4.4-3: Results of Dietary Burden Calculation - Metconazole - Species: Rainbow trout [1)]

Szenario	Description	Max. Residue value (mg/kg)
1	Without PC, CC, F [2)]	0.071
2	Without PC, CC, F - "maximum reasonable balanced diet (MRBD)"	0.045
3	PC	0.071
4	PC - "maximum reasonable balanced diet (MRBD)"	0,048
5	CC used	0,071
6	CC used with - "maximum reasonable balanced diet (MRBD)"	0,045
7	F used	0,071
8	F used with - "maximum reasonable balanced diet (MRBD)"	0,045
9	PC and CC used	0,071
10	PC and CC used - "maximum reasonable balanced diet (MRBD)"	0,048
11	PC and F used	0.071
12	PC and F used - "maximum reasonable balanced diet (MRBD)"	0.048
13	CC and F used	0.071
14	CC and F used - "maximum reasonable balanced diet (MRBD)"	0.045
15	PC, CC and F used	0.071
16	PC, CC and F used - "maximum reasonable balanced diet (MRBD)"	0.048

1) Feed composition comprising 15.0% lipid and 42.0% protein contents
2) PC: protein concentrate; CC: carbohydrate concentrate; F: fat

Table 6.4.4-4: Results of Dietary Burden Calculation - Metconazole - Species: Common Carp [1)]

Szenario	Description	Max. Residue value (mg/kg)
1	Without PC, CC, F [2)]	0.076
2	Without PC, CC, F - "maximum reasonable balanced diet (MRBD)"	0.062
3	PC	0.076
4	PC - "maximum reasonable balanced diet (MRBD)"	0.062
5	CC used	0.076
6	CC used with - "maximum reasonable balanced diet (MRBD)"	0.062
7	F used	0.076
8	F used with - "maximum reasonable balanced diet (MRBD)"	0.062
9	PC and CC used	0.076
10	PC and CC used - "maximum reasonable balanced diet (MRBD)"	0.062
11	PC and F used	0.076
12	PC and F used - "maximum reasonable balanced diet (MRBD)"	0.062
13	CC and F used	0.076
14	CC and F used - "maximum reasonable balanced diet (MRBD)"	0.062
15	PC, CC and F used	0.076
16	PC, CC and F used - "maximum reasonable balanced diet (MRBD)"	0.062

1) Feed composition comprising 15.0% lipid and 42.0% protein contents
2) PC: protein concentrate; CC: carbohydrate concentrate; F: fat

II. RESULTS AND DISCUSSION

The maximum content dietary burdens ranged from 0.062-0.076 mg/kg dry feed for common carp and from 0.045-0.071 mg/kg dry feed for rainbow trout, which is well below the trigger value of 0.1 mg/kg dry feed. According to this calculation, a fish metabolism study would not be triggered.

III. CONCLUSION

With regard to its log P_{ow} (3.85) metconazole is characterized as fat soluble. However, TRR were roughly one order of magnitude higher in the excretory organs liver and kidney of goat and hen than in other animal tissue, indicating that there might be no accumulation of residues in adipose tissue (EFSA Scientific Report (2006) 64. 1-71. Conclusion on the peer review of metconazole). It should also be noted that fish bio concentration studies show very short clearance times (<1 day) (EFSA Scientific Report (2006) 64. 1-71. Conclusion on the peer review of metconazole Appendix 1 – list of endpoints). Furthermore, residues of metconazole in commodities are generally low, leading to a theoretical feed burden below 0.1 mg/kg dry feed. Thus, it can be reasonably assumed that metconazole residues in fish feed will not accumulate in edible fish tissues.

CA 6.5 Effects of Processing

CA 6.5.1 Nature of the residue

The effect of processing on the nature and the magnitude of metconazole residues was not investigated in the framework of the peer review since studies were not triggered by current data requirements due to the low chronic dietary exposure to metconazole residues (EFSA Conclusion 2006).
In EFSA Reasoned Opinion 2013 it was concluded that the nature of residues expected under standard hydrolytic conditions has to be investigated, taking into account that the crops for which MRLs were requested (barley and oats) are consumed only after processing (e.g. baking, brewing) and considering the expected exposure.

Thus, a new hydrolysis study was performed and is summarized below.

Report:	CA 6.5.1/1 Adam D., 2013a 14C-Metconazole (BAS 555 F): Simulated processing - Hydrolysis at 90°C, 100°C and 120°C 2013/1126040
Guidelines:	OECD Test Guideline 507 - Nature of the residues in processed commodities - High temperature hydrolysis, EPA 860.1520, EEC 91/414 Annex II (Part A Section 6), EEC 91/414 Annex III (Part A Section 8), EEC 7035/VI/95 rev. 5
GLP:	yes (certified by Swiss Federal Office of Public Health, Berne, Switzerland)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test item

Internal code:	BAS 555 F
Reg.No.:	4056343
Chem. name:	(1RS, 5RS;1RS,5SR)-5-(4-chlorobenzyl)-2,2-dimethyl-1-(1H-1,2,4-triazol-1-ylmethyl)cyclopentanol
Molar mass:	319.8 g/mol (unlabeled)
Label:	3,(5)-triazole-^{14}C
Batch-No.:	811-1002
Specific act.:	5.26 MBq/mg
Radiochem. Purity:	98.8% (sum of isomers); 100% as re-determined before use

2. Test system
An application solution of ^{14}C-labeled test item was prepared and suspended in aqueous buffer solutions of different pH-values, to give a final concentration of ca. 0.358 mg/L. The solvent volume did not exceed 1% of the buffer solution.

B. STUDY DESIGN

pH 4 and 90°C - pasteurization
The test solutions were pasteurized in closed high pressure stainless steel vessels using autoclave for 20 minutes at 90°C. The pH-value remained constant with 4.19 at the beginning and 4.18-4.19 at the end of the test. The test was performed in the dark with two independent samples.

pH 5 and 100°C - baking, brewing, boiling
The test solutions were treated in high pressure stainless steel vessels using autoclave at 100°C for 60 minutes. The pH-value remained constant with 5.08 at the beginning and 5.09 at the end of the test. The test was performed with two independent samples.

pH 6 and 120°C - sterilization
Sterilization of the samples was performed at about 120°C in closed high pressure stainless steel vessels using autoclave for 20 minutes. The pH-value remained constant with 6.02 at the beginning and at the end of the test. The test was performed in the dark with two independent samples.

After incubation, an aliquot of the samples was measured by LSC to check for recovery and subjected to HPLC analysis to determine the amount of ^{14}C BAS 555 F and eventual hydrolysis products. TLC of selected samples was performed as secondary analytical method.
The weight of each sample was determined before and immediately after incubation.

II. RESULTS & DISCUSSION

The recovery of applied radioactivity was 95-97% (see Table 6.5.1-1) after incubation compared to the radioactivity before incubation.

Table 6.5.1-1: Recovery after processing simulation tests with ^{14}C-BAS 555 F (sum of isomers)

Process represented	Test conditions	Recovery %
		Total radioactivity*
Pasteurization	pH 4, 90°C, 20 minutes	95
Baking/brewing/boiling	pH 5, 100°C, 60 minutes	97
Sterilization	pH 6, 120°C, 20 minutes	97

* Means of two tests

The HPLC and LC-MS results showed that no significant hydrolysis or reaction products were formed under conditions representative of pasteurization), representative of baking and representative of brewing, boiling and sterilization. Metconazole was stable during all tested conditions.
Further the isomer distribution of metconazole did not change during all tested conditions; the ratio of the isomers was stable. The isomer distribution was approximately 98.5:1.5 before and after processing.

III. CONCLUSION

^{14}C-metconazole (BAS 555 F) was hydrolytically stable in sterile buffer solution pH 4 at a temperature simulating pasteurization (90°C) after 20 minutes, in pH 5 at 100°C after 60 minutes (simulating baking/brewing/boiling) and in pH 6 at 120°C after 20 minutes simulating sterilization. The test item did not degrade under any of these test conditions.

CA 6.5.2 Distribution of the residue in inedible peel and pulp

All representative uses to be evaluated in this dossier (oilseeds and cereals) are crops with no peel or edible peel only. Therefore, studies on the distribution between peel and pulp are not required.

CA 6.5.3 Magnitude of residues in processed commodities

In the framework of the peer review, no processing studies were submitted or considered required by EFSA (EFSA Conclusion 2006) since the TMDI was below 10% ADI.
In the framework of an MRL application, a processing study in wheat was evaluated (EFSA Reasoned Opinion, EFSA Journal 2010; 8(3):1534), and EFSA determined the following:

> "Robust processing factors for enforcement purposes could be derived for white flour, whole-meal flour and bran because these processing factors are supported by 4 studies and processes are not expected to impact on the nature of residues. The processing factor for whole-meal bread was considered indicative by EFSA because the impact of baking on the nature of residues was not investigated at time of review."

Table 6.5.3-1: Overview of the available processing factors (EFSA Reasoned Opinion 2010; EFSA Reasoned Opinion 2011)

Processed commodity	Number of studies	Median PF[1]	Median CF[2]	Comments
Enforcement residue definition: metconazole				
Processing factors recommended for enforcement and risk assessment (sufficiently supported by data)				
Wheat, whole-meal flour Rye, whole-meal flour	4	0.75	1.00	Processing data on wheat; extrapolation to rye possible.
Wheat, white flour Rye, white flour	4	0.23	1.00	
Wheat, bran Rye, bran	4	2.00	1.00	
Indicative processing factors (limited data sets)				
Wheat, whole-meal bread Rye, whole-meal bread	4	0.61	1.00	Processing data on wheat; extrapolation to rye possible.

1 The median processing factor is obtained by calculating the median of the individual processing factors of each processing study.
2 The median conversion factor for enforcement to risk assessment is obtained by calculating the median of the individual conversion factors of each processing study.

As stated in the EFSA Reasoned Opinion, EFSA Journal 2011;9(10):2422) “Further processing studies were not considered as required as they were not expected to affect the outcome of the risk assessment. However, if there would be the intention to derive more robust processing factors, in particular for enforcement purposes, additional processing studies would be required.”

For the representative uses to be evaluated in this dossier (oilseed rape and cereals), the following processing procedures are relevant according to OECD guidance document 96: production of crude and refined oil, meal or cake for oilseeds; production of distillers grain/residues fresh and dried for cereals; production of pearled barley, flour, bran, brewing malt, malt sprouts, beer, dried brewer's grain and brewer's yeast for barley; production of groats/rolled oats, flour, bran, husk and dust for oats; production of bran, flour, germ, middlings, shorts, aspirated grain fraction, gluten, gluten feed meal, milled byproducts, starch, whole-meal flour, whole-grain bread for wheat. Processing studies with barley, oats and oilseed rape seed were performed and are summarized below. Since the processing study in wheat is not regarded as peer reviewed, it is also submitted and summarized in this dossier.

Wheat

Report:	CA 6.5.3/1 White M.T., Saha M., 2006b The magnitude of residues of Metconazole (BAS 555 F) and its metabolites in wheat processing commodities 2006/7007147
Guidelines:	EPA 860.1520
GLP:	yes (certified by United States Environmental Protection Agency)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	BAS 555 01 F (SL)
Lot/Batch #:	2030 (90 g/L metconazole, nominal)
CAS#:	125116-23-6

2. Test Commodity:

Crop:	Wheat
Type:	Cereals
Variety:	Ingot, Excel 201, Knudson, Millennium HRW
Botanical name:	*Triticum aestivum*
Crop parts(s) or processed Commodity:	Grain, cleaned grain, epidermis/husk, coarse bran, straight flour, fine bran, middlings, shorts, germ, low grade meal, flour type 550, whole meal flour, bread

B. STUDY DESIGN

1. Test procedure

During the 2004 and 2005 growing seasons, four field trials were conducted at representative wheat growing areas in the USA to determine the residue level of metconazole (BAS 555 F) in the raw agricultural commodity wheat and its processed fractions. BAS 555 01 F was foliar applied twice at an exaggerated target rate of 0.56 kg a.s./ha (5 x the GAP rate) to wheat with a retreatment interval of 6-7 days. The first application was made approximately four weeks prior to the harvest of mature grain. The spray volume used was 141-186 L/ha, and an adjuvant was added to the spray mixture for all applications. Wheat bulk grain RAC samples were harvested 20-22 days after the last application (DALA). The bulk wheat grain samples were processed according to simulated commercial procedures into the following samples: aspirated grain fraction (from the site in WI), husk (epidermis), cleaned grain, germ, coarse bran, fine bran, middlings, straight flour (combination of break and reduction flour), shorts or low grade meal, type 550 flour (combination of straight flour and low grade meal), whole meal flour and whole meal bread. Samples were stored frozen until analysis. The maximum storage interval from harvest until processing was 302 days.

2. Description of analytical procedures

Residues of metconazole (*cis*- and *trans*-isomer) were determined using BASF method No D0508. Residues of metconazole in wheat RAC and processed fractions samples were extracted with acetonitrile/water (70:30, v/v) using polytron homogenization. An aliquot of the extract was diluted with methanol/water (80:20, v/v), filtered and analyzed for residues of metconazole and its metabolites M21, M11 and M30 by HPLC-MS/MS. MS/MS detection in the positive ionization mode was used to monitor ion transitions from m/z 320.1 → 70.1 for metconazole (*cis*- and *trans*-isomers), m/z 336.1 → 125.1 for M21 and M11 and m/z 334.1 → 111.1 for M30. A separate aliquot of the initial extract was evaporated to dryness for the analysis of TDMs. The residues were re-dissolved in 0.1 % formic acid in water, filtered and analyzed by HPLC-MS/MS. MS/MS detection in the positive ionization mode was used to monitor ion transitions from m/z 70.1 → 43.2 for triazole (T), m/z 128.1 → 70.2 for triazole acetic acid (TAA) and m/z 157.1 → 88.1 for triazole alanine (TA). The validated limit of quantitation is 0.01 mg/kg each for parent metconazole (sum of *cis*- and *trans*-isomer at 0.005 mg/kg each) and the metabolites M21, M11 and M30 in/on wheat RAC and processed commodity samples. The validated limit of quantitation is 0.05 mg/kg each for T, TA and TAA.
Concurrent recoveries of parent metconazole (*cis*- and *trans*-isomer) were in the range of 70-117% with fortification levels of 0.005 and 0.5 mg/kg. Concurrent recoveries of the TDMs fortified in control wheat RAC and processed commodity samples at 0.05 and 5.0 mg/kg ranged between 62 and 131%. Only a few isolated recoveries of the TDMs were outside the range of 70 to 120%.

II. RESULTS AND DISCUSSION

After treatment at 2 applications at 0.56 Kg/ha, residues of metconazole (sum of both isomers) were in the range of 0.24 to 0.41 mg/kg for wheat grain sampled 20-22 days after the last application from four trial sites. The mean and median transfer factors for the different processed commodities were below 1 in various flours, meal and whole-wheat bread and above 1 in epidermis/husk, bran, middlings and shorts, which are not destined for human consumption.

Maximum residues of the triazole derivative metabolites in wheat grain were <0.05 mg/kg (LOQ), 0.31 mg/kg, and 0.10 mg/kg, respectively, for T, TA, and TAA.
Residues of the TDMs concentrated in coarse bran, fine bran and germ for TA, whereas TAA residues increase only in aspirated grain fractions.

III. CONCLUSION

Four trials in wheat were conducted with grain processed into germ, coarse bran, fine bran, middlings, straight flour (combination of break and reduction flour), shorts or low grade meal, type 550 flour (combination of straight flour and low grade meal), whole meal flour and whole meal bread. Metconazole residues concentrated in bran, middling and shorts, and declined in other commodities. Processing factors (median) are derived for flour (0.25), flour type 550 (0.25), whole-meal flour (0.7), whole-meal bread (0.6), wheat germ (1.0) and bran (2.0).
Residues of the triazole derivative metabolites concentrated in coarse bran, fine bran and germ for TA, whereas TAA residues increase only in aspirated grain fractions.

Table 6.5.3-2: Residues of metconazole (sum of isomers) in wheat processed fractions

Trial location / Trial number	Processed commodity	Application rate (kg a.s./ha)	DALA[1]	Residues found (mg/kg)			Processing factor[3] for total residue[2]
				Cis-isomer	*Trans*-isomer	Total metconazole[2]	
Pepin, WI, USA (RCN R05184) 2005	Aspirated grain fraction	2 x 0.056	21	21.190	4.474	25.664	-
	Grain (RAC)		21	0.343	0.056	**0.399**	-
	Cleaned grain			0.321	0.053	0.374	1.0
	Epidermis/husk			8.342	3.287	11.629	32
	Coarse bran			0.683	0.127	0.810	2.1
	Straight flour			0.047	0.010	0.057	0.2
	Fine bran			0.730	0.137	0.867	2.2
	Middlings			0.275	0.047	0.322	0.8
	Shorts			0.829	0.058	0.887	2.0
	Germ			0.316	0.053	0.369	1.0
	Low grade meal			0.236	0.027	0.263	0.7
	Flour type 550			0.060	0.011	0.071	0.2
	Whole meal flour			0.321	0.042	0.364	0.9
	Bread			0.209	0.041	0.250	0.6
Clinton, IL, USA (RCN R05185) 2004	**Grain (RAC)**	2 x 0.056	20	0.348	0.061	**0.408**	-
	Cleaned grain			0.393	0.070	0.463	1.2
	Epidermis/husk			19.066	3.970	23.036	53
	Coarse bran			0.556	0.097	0.652	1.7
	Straight flour			0.046	0.010	0.056	0.2
	Fine bran			0.538	0.125	0.663	1.8
	Middlings			0.387	0.078	0.465	1.1
	Shorts			0.890	0.087	0.977	2.3
	Germ			0.212	0.050	0.262	0.7
	Low grade meal			0.278	0.041	0.319	0.8
	Flour type 550			0.070	0.016	0.087	0.2
	Whole meal flour			0.204	0.042	0.246	0.6
	Bread			0.147	0.032	0.178	0.5

Table 6.5.3-2: Residues of metconazole (sum of isomers) in wheat processed fractions

Trial location / Trial number	Processed commodity	Application rate (kg a.s./ha)	DALA[1]	Residues found (mg/kg)			Processing factor[3] for total residue[2]
				Cis-isomer	*Trans*-isomer	Total metconazole[2]	
Cass, ND, USA (RCN R05186) 2005	**Grain (RAC)**	2 x 0.056	22	0.191	0.047	**0.238**	-
	Cleaned grain			0.172	0.040	0.212	0.9
	Epidermis/husk			68.748	13.941	82.689	257
	Coarse bran			0.338	0.095	0.433	2.0
	Straight flour			0.059	0.015	0.074	0.3
	Fine bran			0.427	0.081	0.508	2.1
	Middlings			1.038	0.181	1.219	3.9
	Shorts			0.475	0.054	0.529	1.7
	Germ			0.230	0.057	0.287	1.0
	Low grade meal			0.170	0.023	0.193	0.7
	Flour type 550			0.081	0.019	0.100	0.4
	Whole meal flour			0.172	0.038	0.210	0.8
	Bread			0.135	0.032	0.167	0.6
York, NE, USA (RCN R05187) 2004	**Grain (RAC)**	2 x 0.056	20	0.311	0.055	**0.366**	-
	Cleaned grain			0.276	0.055	0.331	0.9
	Epidermis/husk			16.206	3.098	19.304	47
	Coarse bran			0.642	0.122	0.764	2.2
	Straight flour			0.069	0.017	0.086	0.3
	Fine bran			0.523	0.126	0.649	2.0
	Middlings			0.293	0.065	0.358	0.9
	Shorts			0.496	0.046	0.542	1.4
	Germ			0.318	0.074	0.392	1.1
	Low grade meal			0.179	0.026	0.205	0.6
	Flour type 550			0.072	0.017	0.089	0.3
	Whole meal flour			0.105	0.039	0.144	0.5
	Bread			0.176	0.038	0.214	0.6

1 Days after last application
2 Sum of *cis*- and *trans*-isomers
3 The processing factor is calculated by dividing the residue in the processed fraction by the residue in the RAC sample; residues <LOQ were set LOQ
AGF Aspirated grain fractions

Table 6.5.3-3: Residues of triazole derivative metabolites in wheat processed fractions

Trial location / Trial number	Processed commodity	DALA[1]	Residues found[2] (mg/kg)			Processing factor		
			T	TA	TAA	T	TA	TAA
Pepin, WI, USA R05184	Grain RAC	21	<0.05	0.08	0.05	-	-	-
	Agf		<0.05	<0.05	0.08	1.0	0.6	1.6
	Cleaned grain		<0.05	0.07	<0.05	1.0	0.9	1.0
	Epidermis/husk		<0.05	0.09	<0.05	1.0	1.1	1.0
	Coarse bran		<0.05	0.15	<0.05	1.0	1.9	1.0
	Straight flour		<0.05	<0.05	<0.05	1.0	0.6	1.0
	Fine bran		<0.05	0.15	<0.05	1.0	1.9	1.0
	Middlings		<0.05	0.07	<0.05	1.0	0.9	1.0
	Shorts		<0.05	0.11	<0.05	1.0	1.4	1.0
	Germ		<0.05	0.2	0.06	1.0	2.5	1.2
	Low grade meal		<0.05	0.08	<0.05	1.0	1.0	1.0
	Flour type 550		<0.05	<0.05	<0.05	1.0	0.6	1.0
	Whole meal flour		<0.05	0.06	<0.05	1.0	0.8	1.0
	Bread		<0.05	<0.05	<0.05	1.0	0.6	1.0
Clinton, IL, USA R05185	Grain RAC	20	<0.05	0.09	0.06	-	-	-
	Cleaned grain		<0.05	0.1	0.06	1.0	1.1	1.0
	Epidermis/husk		<0.05	0.07	<0.05	1.0	0.8	0.8
	Coarse bran		<0.05	0.2	0.08	1.0	2.2	1.3
	Straight flour		<0.05	<0.05	<0.05	1.0	0.6	0.8
	Fine bran		<0.05	0.26	0.08	1.0	2.9	1.3
	Middlings		<0.05	<0.05	<0.05	1.0	0.6	0.8
	Shorts		<0.05	0.13	0.06	1.0	1.4	1.0
	Germ		<0.05	0.32	0.07	1.0	3.6	1.2
	Low grade meal		<0.05	0.12	0.06	1.0	1.3	1.0
	Flour type 550		<0.05	<0.05	<0.05	1.0	0.6	0.8
	Whole meal flour		<0.05	0.08	<0.05	1.0	0.9	0.8
	Bread		<0.05	<0.05	<0.05	1.0	0.6	0.8
Cass, ND, USA R05186	Grain RAC	22	<0.05	0.31	0.09	-	-	-
	Cleaned grain		<0.05	0.28	0.08	1.0	0.9	0.9
	Epidermis/husk		<0.05	0.09	0.09	1.0	0.3	1.0
	Coarse bran		<0.05	0.56	0.12	1.0	1.8	1.3
	Straight flour		<0.05	0.13	0.07	1.0	0.4	0.8
	Fine bran		<0.05	0.56	0.1	1.0	1.8	1.1
	Middlings		<0.05	0.18	0.08	1.0	0.6	0.9
	Shorts		<0.05	0.27	0.08	1.0	0.9	0.9
	Germ		<0.05	0.46	0.09	1.0	1.5	1.0
	Low grade meal		<0.05	0.23	0.08	1.0	0.7	0.9
	Flour type 550		<0.05	0.17	0.07	1.0	0.5	0.8
	Whole meal flour		<0.05	0.25	0.07	1.0	0.8	0.8
	Bread		<0.05	0.15	0.06	1.0	0.5	0.7

Table 6.5.3-3: Residues of triazole derivative metabolites in wheat processed fractions

Trial location / Trial number	Processed commodity	DALA[1]	Residues found[2] (mg/kg)			Processing factor		
			T	TA	TAA	T	TA	TAA
York, NE, USA R05187	Grain RAC	20	<0.05	0.2	0.1	-	-	-
	Cleaned grain		<0.05	0.19	0.1	1.0	1.0	1.0
	Epidermis/husk		<0.05	0.14	0.11	1.0	0.7	1.1
	Coarse bran		<0.05	0.6	0.11	1.0	3.0	1.1
	Straight flour		<0.05	0.07	0.09	1.0	0.4	0.9
	Fine bran		<0.05	0.58	0.12	1.0	2.9	1.2
	Middlings		<0.05	0.12	0.09	1.0	0.6	0.9
	Shorts		<0.05	0.2	0.1	1.0	1.0	1.0
	Germ		<0.05	0.44	0.1	1.0	2.2	1.0
	Low grade meal		<0.05	0.15	0.09	1.0	0.8	0.9
	Flour type 550		<0.05	0.09	0.09	1.0	0.5	0.9
	Whole meal flour		<0.05	0.19	0.09	1.0	1.0	0.9
	Bread		<0.05	0.12	0.07	1.0	0.6	0.7

1 Days after last application
2 T = 1,2,4 triazole
TAA = triazole acetic acid
TA = triazole alanine

Table 6.5.3-4: Mean and median processing factors for metconazole residues in wheat processed fractions

	Cleaned grain	Epidermis/ husk	Coarse bran	Straight flour	Fine bran	Middlings	Shorts	Germ	Low grade meal	Flour type 550	Whole meal flour	Bread
Pepin, WI, USA RCN R05184	1.0	32	2.1	0.2	2.2	0.8	2.0	1.0	0.7	0.2	0.9	0.6
Clinton, IL, USA RCN R05185	1.2	53	1.7	0.2	1.8	1.1	2.3	0.7	0.8	0.2	0.6	0.5
Cass, ND, USA RCN R05186	0.9	257	2.0	0.3	2.1	3.9	1.7	1.0	0.7	0.4	0.8	0.6
York, NE, USA RCN R05187	0.9	47	2.2.	0.3	2.0	0.9	1.4	1.1	0.6	0.3	0.5	0.6
Mean	**1.0**	**97**	**2.0**	**0.2**	**2.0**	**1.7**	**1.8**	**0.9**	**0.7**	**0.3**	**0.7**	**0.6**
Median		**50**	**2**	**0.25**	**2.1**	**1.0**	**1.9**	**1.0**	**0.7**	**0.25**	**0.7**	**0.6**

Oilseed rape

Report:	CA 6.5.3/2 Tandy R., 2013a Study on the residue behaviour of Pyraclostrobin (BAS 500 F) and Metconazole (BAS 555 F) in oilseed rape and processed fractions after treatment with BAS 556 03 F in Northern Europe during 2011 2013/1256239
Guidelines:	EEC 91/414 Annex II (Part A Section 6), EEC 91/414 Annex III (Part A Section 8), EEC 1607/VI/97 rev. 2 10.06.1999
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	BAS 556 03 F (EC)
Lot/Batch #:	380009 (80 g/L metconazole, 130 g/L pyraclostrobin, nominal)
CAS#:	125116-23-6

2. Test Commodity:

Crop:	Oilseed rape
Type:	Oilseeds
Variety:	Visby, Petrol, Artoga
Botanical name:	*Brasica napus* subsp. *napus*
Crop parts(s) or processed Commodity:	Whole plant without roots, seed, meal, crude oil, refined oil, soap stock, press cake

B. STUDY DESIGN

1. Test procedure

During the 2011 growing season, four field trials were conducted at representative oilseed rape growing areas in Germany to determine the residue level of metconazole (BAS 555 F) in the raw agricultural commodity oilseed rape and its processed fractions. BAS 556 03 F (EC) was foliar applied twice at an exaggerated target rate of 240 g metconazole/ha to oilseed rape at BBCH 65 and BBCH 71. The nominal spray volume used was 200 L/ha. At trials L110292 and L110294, whole plant w/o roots specimens were collected directly before (untreated samples) and after (treated samples) the last application. Seed specimens were collected at 76-78 days after last application (DALA). At trials L110293 and L110295, whole plant without root specimens were collected after the last application. Seed specimens were collected at 65-67 days after last application (DALA). They were processed according to simulated industrial procedures into press cake, meal (solvent extracted), crude oil, soap stock and refined oil. The maximum storage interval from harvest until extraction was 393 days.

2. Description of analytical procedures

Residues of metconazole (*cis*- and *trans*-isomer, determined separately) were determined using BASF method No 535/1. Residues of pyraclostrobin are not reported in this dossier.
The analytes were extracted with a mixture of methanol, water and hydrochloric acid. An aliquot of the extract was centrifuged and partitioned at alkaline conditions against cyclohexane for determination of the analytes by LC-MS/MS to achieve a limit of quantitation (LOQ) of 0.005 mg/kg for *cis*- and *trans*-metconazole determined separately.
T, TA, TAA and TLA were determined after extraction with methanol/water using LC-MS/MS to achieve limits of quantitation of 0.01 mg/kg per analyte except for TA in seed, meal and press cake where the blank contamination was too high.
Overall mean recoveries were in the range of 70-110%; relative standard deviations (RSD) were always ≤20%.

II. RESULTS AND DISCUSSION

After two applications at 240 g metconazole/ha, residues of metconazole (sum of both isomers) were in the range of 3.1 to 4.9 mg/kg for whole plant sampled 0 days after the last application. They were in the range of 0.040 to 0.21 mg/kg for seed sampled 65-78 days after the last application (BBCH 89). For seed sampled prior to processing, residues were 0.047-0.24 mg/kg. No residues of BAS 555 F (sum of both isomers) above the limit of quantitation (0.005 mg/kg per isomer) were detected in the untreated specimens of this study.
The seed was processed into press cake, meal (solvent extracted), crude oil, soap stock and refined oil. The mean and median transfer factors were less than 1 in meal and soap stock and greater than 1 indicating concentration of residues in crude oil, refined oil and press cake.
Residues of T were <0.01 mg/kg. The mean transfer factors were below 1 for TA, TAA and TLA in crude oil, refined oil and soapstock. Therefore it can be concluded that TA, TAA and TLA are not being accumulated or formed in these processed fractions. The mean transfer factors were only above 1 for TA, TAA and TLA in meal and press cake.

III. CONCLUSION

Four trials in oilseed rape were conducted with seed processed into press cake, meal (solvent extracted), crude oil, soap stock and refined oil. Metconazole residues concentrated in crude and refined oil and declined in meal and soap stock. Processing factors (median) are derived for press cake (1.24), soapstock (0.25), crude oil (1.61), meal (0.96) and refined oil (1.61).
Residues of T were <0.01 mg/kg. The mean transfer factors were below 1 for TA, TAA and TLA in crude oil, refined oil and soapstock. Therefore it can be concluded that TA, TAA and TLA are not being accumulated or formed in these processed fractions. The mean transfer factors were only above 1 for TA, TAA and TLA in meal and press cake.

Table 6.5.3-5: Residues of metconazole (sum of isomers) in oilseed rape processed fractions

Trial location / Trial number	Processed commodity	Application rate (kg a.s./ha)	DALA[1]	Residues found (mg/kg)	Processing factor[3] for total residue[2]
				Metconazole	
Niedersachsen, Germany (L110292) 2011	Whole plant	2 x 0.240*	0	4.8	-
	Seed		76	0.21	-
	Seed (RAC)		76	**0.23**	-
	Meal			0.21	0.91
	Crude oil			0.37	1.61
	Refined oil			0.37	1.61
	Soap stock			0.064	0.28
	Press cake			0.28	1.22
Brandenburg, Germany (L110293) 2011	Whole plant	2 x 0.240*	0	4.9	-
	Seed		67	0.040	-
	Seed (RAC)		67	**0.047**	-
	Meal			0.051	1.09
	Crude oil			0.074	1.57
	Refined oil			0.069	1.47
	Soap stock			<0.01	0.21
	Press cake			0.059	1.26
Baden-Wuerttemberg, Germany (L110294) 2011	Whole plant	2 x 0.240*	0	3.1	-
	Seed		78	0.10	-
	Seed (RAC)		78	**0.10**	-
	Meal			0.10	1.00
	Crude oil			0.16	1.60
	Refined oil			0.16	1.60
	Soap stock			0.033	0.30
	Press cake			0.13	1.30
Niedersachsen, Germany (L110295) 2011	Whole plant	2 x 0.240*	0	3.4	-
	Seed		85	0.19	-
	Seed (RAC)		85	**024**	-
	Meal			0.22	0.92
	Crude oil			0.43	1.79
	Refined oil			0.43	1.79
	Soap stock			0.052	0.21
	Press cake			0.29	1.21

1 Days after last application
2 Sum of *cis*- and *trans*-isomers
3 The processing factor is calculated by dividing the residue in the processed fraction by the residue in the RAC sample; residues <LOQ were set LOQ
* Mix formulation with pyraclostrobin

Table 6.5.3-6: Residues of TDMs in oilseed rape processed fractions

Trial location / Trial number	Processed commodity	Application rate (kg a.s./ha)	DALA[1]	Residues found[2] (mg/kg)				Processing factor[3]			
				T	TA	TAA	TLA	T	TA	TAA	TLA
Niedersachsen, Germany (L110292) 2011	Whole plant	2 x 0.240*	0	<0.01	0.043	<0.01	<0.01	-	-	-	-
	Seed		76	<0.01	0.27	<0.01	<0.01	-	-	-	-
	Seed (RAC)		76	**<0.01**	**0.34**	**<0.01**	**<0.01**	-	-	-	-
	Meal			<0.01	0.59	<0.01	0.024	N/A	1.74	N/A	<2.0
	Crude oil			<0.01	<0.01	<0.01	<0.01	N/A	<0.03	N/A	N/A
	Refined oil			<0.01	<0.01	<0.01	<0.01	N/A	<0.03	N/A	N/A
	Soap stock			<0.01	<0.01	<0.01	<0.01	N/A	<0.03	N/A	N/A
	Press cake			<0.01	0.53	<0.01	0.016	N/A	1.56	N/A	<1.6
Brandenburg, Germany (L110293) 2011	Whole plant	2 x 0.240*	0	<0.01	0.15	<0.01	<0.01	-	-	-	-
	Seed		67	<0.01	1.3	0.013	0.033	-	-	-	-
	Seed (RAC)		67	**<0.01**	**1.5**	**0.015**	**0.034**	-	-	-	-
	Meal			<0.01	3.1	0.032	0.084	N/A	2.07	2.13	2.47
	Crude oil			<0.01	<0.01	<0.01	<0.01	N/A	<0.01	<0.67	<0.29
	Refined oil			<0.01	<0.01	<0.01	<0.01	N/A	<0.01	<0.67	<0.29
	Soap stock			<0.01	<0.01	<0.01	<0.01	N/A	<0.01	<0.67	<0.29
	Press cake			<0.01	2.2	0.020	0.057	N/A	1.47	1.33	1.68
Baden-Wuerttemberg, Germany (L110294) 2011	Whole plant	2 x 0.240*	0	<0.01	0.11	<0.01	<0.01	-	-	-	-
	Seed		78	<0.01	1.0	<0.01	0.040	-	-	-	-
	Seed (RAC)		78	**<0.01**	**0.94**	**<0.01**	**0.040**	-	-	-	-
	Meal			<0.01	1.7	0.014	0.068	N/A	1.81	<1.4	1.70
	Crude oil			<0.01	<0.01	<0.01	<0.01	N/A	<0.01	N/A	<0.25
	Refined oil			<0.01	<0.01	<0.01	<0.01	N/A	<0.01	N/A	<0.25
	Soap stock			<0.01	<0.01	<0.01	<0.01	N/A	<0.01	N/A	<0.25
	Press cake			<0.01	1.4	0.011	0.061	N/A	1.49	<1.1	1.53
Niedersachsen, Germany (L110295) 2011	Whole plant	2 x 0.240*	0	<0.01	0.027	<0.01	<0.01	-	-	-	-
	Seed		85	<0.01	0.32	<0.01	<0.01	-	-	-	-
	Seed (RAC)		85	**<0.01**	**0.30**	**<0.01**	**<0.01**	-	-	-	-
	Meal			<0.01	0.67	<0.01	0.019	N/A	1.96	N/A	<2.04
	Crude oil			<0.01	<0.01	<0.01	<0.01	N/A	<0.02	N/A	N/A
	Refined oil			<0.01	<0.01	<0.01	<0.01	N/A	<0.02	N/A	N/A
	Soap stock			<0.01	<0.01	<0.01	<0.01	N/A	<0.02	N/A	N/A
	Press cake			<0.01	0.56	<0.01	0.018	N/A	1.60	N/A	<1.65

1 Days after last application
2 T = 1,2,4 triazole
TAA = triazole acetic acid
TA = triazole alanine
3 The processing factor is calculated by dividing the residue in the processed fraction by the residue in the RAC sample; residues <LOQ were set LOQ
* Mix formulation with pyraclostrobin

Table 6.5.3-7: Mean and median processing factors for metconazole residues in oilseed rape processed fractions

	Meal	Crude oil	Refined oil	Soapstock	Press cake
Niedersachsen, Germany L110292	0.91	1.61	1.61	0.28	1.22
Brandenburg, Germany L110293	1.09	1.57	1.47	0.21	1.26
Baden-Wuerttemberg, Germany L110294	1.00	1.60	1.60	0.30	1.30
Niedersachsen, Germany L110295	0.92	1.79	1.79	0.21	1.21
Mean	**0.98**	**1.64**	**1.62**	**0.25**	**1.25**
Median	**0.96**	**1.61**	**1.61**	**0.25**	**1.24**

Barley

Report:	CA 6.5.3/3 Plier S., 2015a Determination of residues of BAS 555 F (Metconazole) in barley and its processed products after two applications of BAS 555 00 F in Germany, 2012 2013/1037952
Guidelines:	OECD 509 Crop Field Trial (2009), OECD 508 Magnitude of the Pesticide Residues in Processed Commodities (2008), OECD-ENV/JM/MONO(99)22
GLP:	yes (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	BAS 555 00 F (EC)
Lot/Batch #:	0003255328 (60 g/L metconazole, nominal)
CAS#:	125116-23-6

2. Test Commodity:

Crop:	Barley
Type:	Cereals
Variety:	Marthe, Tocada, Quench, Margret
Botanical name:	*Hordeum vulgare*
Crop parts(s) or processed Commodity:	Pot barley, flour, bran, brewing malt, malt sprouts, beer, brewer's grain (dried) and brewer's yeast

B. STUDY DESIGN

1. Test procedure

During the 2012 growing season, four field trials were conducted at representative barley growing areas in Germany to determine the residue level of metconazole (BAS 555 F) in the raw agricultural commodity (RAC) barley and its processed fractions.
BAS 555 00 F was foliar applied twice at an exaggerated target rate of 0.27 kg a.s./ha to barley at BBCH 49 and 69. The spray volume used was 181-219 L/ha.
Barley RAC samples were harvested on the day of the last application (whole plants without roots) and at BBCH 89 (grain). The barley grain samples were processed according to simulated commercial procedures into the following samples: pot barley, flour, bran, brewing malt, malt sprouts, beer, brewer's grain (dried) and brewer's yeast. Samples were stored frozen until analysis. The maximum storage interval from harvest until analysis was 654 days.

2. Description of analytical procedures

Residues of metconazole (*cis*- and *trans*-isomer) were determined using BASF method No L0019/01 (550/0). Triazole derivative metabolites (TDMs) were determined using BASF method No L0170/02.
Residues of metconazole in barley RAC and processed fractions samples were extracted with methanol/water/hydrochloric acid. An aliquot of the extract was centrifuged and partitioned against dichloromethane. The final determination was performed by HPLC-MS/MS with ion transitions from m/z 320 → 70 (quantitation) and 320 → 89 or 320 → 125 (confirmation) for metconazole (*cis*- and *trans*-isomers). The validated limit of quantitation is 0.01 mg/kg for parent metconazole (sum of *cis*- and *trans*-isomer at 0.005 mg/kg each) in/on barley RAC and processed commodity samples.
Concurrent recoveries of parent metconazole (*cis*- and *trans*-isomer) were in the range of 83-100% with fortification levels of 0.005-20 mg/kg.
T, TA, TAA and TLA were extracted with methanol/water. An aliquot was filtered, concentrated and cleaned-up by a dispersive C18-SPE step. Final determination of the analytes is performed by LC-DMS/MS/MS using two different columns. The limit of quantitation is 0.01 mg/kg per analyte. Overall mean recoveries were in the range of 70-110%; relative standard deviations (RSD) were always ≤20% except for TA with 21%.

II. RESULTS AND DISCUSSION

After two treatments with BAS 555 00 F at 0.27 kg/ha, residues of metconazole (sum of both isomers) were in the range of 0.024 to 5.0 mg/kg for barley grain sampled at BBCH 89, 44-59 days after the last application. Residues were <0.01 mg/kg in all untreated samples. The processing of barley was conducted with grain specimens taken at the last sampling. The barley grain was processed into pot barley, flour, bran, brewing malt, malt sprouts, beer, brewer's grain (dried) and brewer's yeast. The mean and median transfer factors were less than 1 in beer, pot barley, brewing malt, malt sprouts, and brewer's yeast and were greater than 1, indicating concentration of metconazole residues, in flour, bran and brewer's grain (dried). Residues were <0.01 mg/kg (LOQ) in all beer samples.
The maximum storage interval from harvest until analysis was 654 days. Storage stability was established in stability studies through 26 months.
Residues of T were <0.01 mg/kg. The mean transfer factors were below 1 for TA, TAA and TLA in pot barley, beer, brewer's grain (dried) and brewer's yeast. Therefore it can be concluded that TA, TAA and TLA are not being accumulated in these processed fractions. The mean transfer factors were above 1 for TA, TAA and TLA in flour, bran, brewing malt and malt sprouts.

III. CONCLUSION

Four trials in barley were conducted with grain processed into pot barley, flour, bran, brewing malt, malt sprouts, beer, brewer's grain (dried) and brewer's yeast. Processing factors (median) are derived for items for human consumption: pot barley (0.51), flour, (2.6), and beer (<0.1) and for animal feed items: bran (3.4), brewers grain (2.2). Residues in all beer samples were <0.01 mg/kg (LOQ).
Residues of T were <0.01 mg/kg. The mean transfer factors were below 1 for TA, TAA and TLA in pot barley, beer, brewer's grain (dried) and brewer's yeast. Therefore it can be concluded that TA, TAA and TLA are not being accumulated in these processed fractions. The mean transfer factors were above 1 for TA, TAA and TLA in flour, bran, brewing malt and malt sprouts.

Table 6.5.3-8: Residues of metconazole (sum of isomers) in barley processed fractions

Trial location / Trial number	Processed commodity	Application rate (kg a.s./ha)	DALA[1]	Residues found (mg/kg)			Processing factor[3] for total residue[2]
				Cis-isomer	*Trans*-isomer	Total metconazole[2]	
Motterwitz, Germany (L120187) 2012	Whole plant[4]	2 x 0.27	0	5.3	1.1	6.4	-
	Grain		44	0.060	0.018	0.078	-
	Grain (RAC)			0.060	0.018	0.078	-
	Pot barley			0.029	0.0083	0.037	0.47
	Flour			0.096	0.025	0.12	1.54
	Bran			0.16	0.041	0.20	2.56
	Brewing malt			0.038	0.011	0.049	0.63
	Malt sprouts			0.036	0.010	0.046	0.59
	Beer			<0.005	<0.005	<0.01	<0.13
	Brewer's grain (dried)			0.14	0.032	0.17	2.18
	Brewer's yeast			0.024	<0.005	0.029	0.37
Trossin, Germany (L120188) 2012	Whole plant[4]	2 x 0.27	0	7.3	1.5	8.8	-
	Grain		50	0.11	0.030	0.14	-
	Grain (RAC)			0.11	0.027	0.14	-
	Pot barley			0.059	0.013	0.072	0.51
	Flour			0.26	0.058	0.32	2.29
	Bran			0.30	0.081	0.38	2.71
	Brewing malt			0.067	0.016	0.083	0.59
	Malt sprouts			0.040	0.011	0.051	0.36
	Beer			<0.005	<0.005	<0.01	<0.07
	Brewer's grain (dried)			0.23	0.043	0.27	1.93
	Brewer's yeast			0.023	<0.005	0.027	0.19
Neubukow, Germany (L120189) 2012	Whole plant[4]	2 x 0.27	0	5.3	1.1	6.4	-
	Grain		55	0.019*	0.0055*	0.024	-
	Grain (RAC)			0.019*	0.0054*	0.024	-
	Pot barley			0.0098	<0.005	0.013	0.54
	Flour			0.054	0.014	0.068	2.83
	Bran			0.076	0.021	0.097	4.04
	Brewing malt			0.012	<0.005	0.016	0.67
	Malt sprouts			0.014	<0.005	0.017	0.71
	Beer			<0.005	<0.005	<0.01	<0.42
	Brewer's grain (dried)			0.041	0.011	0.052	2.17
	Brewer's yeast			0.011	<0.005	0.013	0.54

Table 6.5.3-8: Residues of metconazole (sum of isomers) in barley processed fractions

Trial location / Trial number	Processed commodity	Application rate (kg a.s./ha)	DALA[1]	Residues found (mg/kg)			Processing factor[3] for total residue[2]
				Cis-isomer	*Trans*-isomer	Total metconazole[2]	
Donnersdorf, Germany (L120190) 2012	Whole plant[4]	2 x 0.27	0	8.6	1.7	10	-
	Grain		59	4.1*	0.83*	5.0	-
	Grain (RAC)			5.6*	0.76*	4.5	-
	Pot barley			0.33	0.061	0.39	0.09
	Flour			11	2.2	13	2.89
	Bran			18	3.6	22	4.89
	Brewing malt			0.57	0.13	0.70	0.16
	Malt sprouts			0.69	0.17	0.86	0.19
	Beer			<0.005	<0.005	<0.01	<0.002
	Brewer's grain (dried)			1.4	0.34	1.7	0.38
	Brewer's yeast			0.18	0.025	0.21	0.05

1 Days after last application
2 Sum of *cis*- and *trans*-isomers
3 The processing factor is calculated by dividing the residue in the processed fraction by the residue in the RAC sample; residues <LOQ were set LOQ
4 Without roots
* Mean of replicates

Table 6.5.3-9: Residues of TDMs in barley processed fractions

Trial location / Trial number	Processed commodity	Application rate (kg a.s./ha)	DALA[1]	Residues found[2] (mg/kg)				Processing factor[3]			
				T	TA	TAA	TLA	T	TA	TAA	TLA
Motterwitz, Germany (L120187) 2012	Whole plant[4]	2 x 0.27	0	<0.01	0.027	0.024	0.061	-	-	-	-
	Grain		44	<0.01	0.24	0.17	<0.01	-	-	-	-
	Grain (RAC)			**<0.01**	**0.17**	**0.14**	**<0.01**	-	-	-	-
	Pot barley			<0.01	0.14	0.11	<0.01	N/A	0.82	0.79	N/A
	Flour			<0.01	0.66	0.42	0.022	N/A	3.88	3.00	2.20
	Bran			<0.01	0.27	0.30	0.029	N/A	1.59	2.14	2.90
	Brewing malt			<0.01	0.22	0.16	<0.01	N/A	1.29	1.14	N/A
	Malt sprouts			<0.01	2.1	1.4	0.14	N/A	12.35	10.00	14.00
	Beer			<0.01	0.057	0.030	<0.01	N/A	0.34	0.21	N/A
	Brewer's grain (dried)			<0.01	0.021	0.012	<0.01	N/A	0.12	0.09	N/A
	Brewer's yeast			<0.01	0.062	0.023	<0.01	N/A	0.36	0.16	N/A
Trossin, Germany (L120188) 2012	Whole plant[4]	2 x 0.27	0	<0.01	0.27	0.23	0.40	-	-	-	-
	Grain		50	<0.01	0.55	0.27	0.012	-	-	-	-
	Grain (RAC)			**<0.01**	**0.49**	**0.29**	**0.012**	-	-	-	-
	Pot barley			<0.01	0.54	0.23	<0.01	N/A	1.10	0.79	<0.83
	Flour			<0.01	1.7	0.84	0.037	N/A	3.47	2.90	3.08
	Bran			<0.01	0.65	0.65	0.042	N/A	1.33	2.24	3.50
	Brewing malt			<0.01	0.66	0.32	0.028	N/A	1.35	1.10	<2.33
	Malt sprouts			<0.01	5.3	2.5	0.34	N/A	10.82	8.62	28.33
	Beer			<0.01	0.17	0.067	<0.01	N/A	0.35	0.23	<0.83
	Brewer's grain (dried)			<0.01	0.070	0.030	<0.01	N/A	0.14	0.10	<0.83
	Brewer's yeast			<0.01	0.16	0.051	<0.01	N/A	0.33	0.18	<0.83
Neubukow, Germany (L120189) 2012	Whole plant[4]	2 x 0.27	0	<0.01	0.010	0.018	0.047	-	-	-	-
	Grain		55	<0.01	0.095	0.089	<0.01	-	-	-	-
	Grain (RAC)			**<0.01**	**0.090**	**0.069**	**<0.01**	-	-	-	-
	Pot barley			<0.01	0.082	0.060	<0.01	N/A	0.91	0.87	N/A
	Flour			<0.01	0.45	0.30	0.010	N/A	5.00	4.35	1.00
	Bran			<0.01	0.24	0.20	<0.01	N/A	2.67	2.90	N/A
	Brewing malt			<0.01	0.088	0.073	<0.01	N/A	0.98	1.06	N/A
	Malt sprouts			<0.01	0.80	0.54	0.043	N/A	8.89	7.83	4.30
	Beer			<0.01	0.027	0.017	<0.01	N/A	0.30	0.25	N/A
	Brewer's grain (dried)			<0.01	<0.01	<0.01	<0.01	N/A	<0.11	<0.14	N/A
	Brewer's yeast			<0.01	0.033	0.013	<0.01	N/A	0.37	0.19	N/A

Table 6.5.3-9: Residues of TDMs in barley processed fractions

Trial location / Trial number	Processed commodity	Application rate (kg a.s./ha)	DALA[1]	Residues found[2] (mg/kg)				Processing factor[3]			
				T	TA	TAA	TLA	T	TA	TAA	TLA
Donnersdorf, Germany (L120190) 2012	Whole plant[4]	2 x 0.27	0	<0.01	0.15	0.057	0.19	-	-	-	-
	Grain		59	<0.01	0.36	0.21	0.012	-	-	-	-
	Grain (RAC)			**<0.01**	**0.41**	**0.19**	**<0.01**	-	-	-	-
	Pot barley			<0.01	0.42	0.16	<0.01	N/A	1.02	0.84	N/A
	Flour			<0.01	1.2	0.55	0.027	N/A	2.93	2.89	2.70
	Bran			<0.01	0.64	0.43	0.038	N/A	1.56	2.26	3.80
	Brewing malt			<0.01	0.43	0.20	0.018	N/A	1.05	1.05	1.80
	Malt sprouts			<0.01	3.2	1.3	0.18	N/A	7.80	6.84	18.00
	Beer			<0.01	0.11	0.045	<0.01	N/A	0.27	0.24	N/A
	Brewer's grain (dried)			<0.01	0.049	0.023	<0.01	N/A	0.12	0.12	N/A
	Brewer's yeast			<0.01	0.11	0.037	<0.01	N/A	0.27	0.19	N/A

1 Days after last application
2 T = 1,2,4 triazole
TAA = triazole acetic acid
TA = triazole alanine
3 The processing factor is calculated by dividing the residue in the processed fraction by the residue in the RAC sample; residues <LOQ were set LOQ
4 Without roots

Table 6.5.3-10: Mean processing factors for metconazole residues in barley processed fractions

	Pot barley	Flour	Bran	Brewing malt	Malt sprouts	Beer	Brewer's grain (dried)	Brewer's yeast
Motterwitz, Germany L120187	0.47	1.54	2.56	0.63	0.59	<0.13	2.18	0.37
Trossin, Germany L120188	0.51	2.29	2.71	0.59	0.36	<0.07	1.93	0.19
Neubukow, Germany L120189	0.54	2.83	4.04	0.67	0.71	<0.42	2.17	0.54
Donnersdorf, Germany L120190	0.09	2.89	4.89	0.16	0.19	<0.002	0.38	0.05
Mean	**0.40**	**2.39**	**3.55**	**0.51**	**0.46**	**<0.16**	**1.67[2)]**	**0.29**
Median	**0.51 [1)]**	**2.6**	**3.4**	**0.61**	**0.48**	**<0.1**	**2.2**	**0.28**

1) Three values included in the median. Transfer factor of 0.09 excluded.
2) Three values included in the median. Transfer factor of 0.38 excluded.

Oat

Report:	CA 6.5.3/4 Plier S., 2015b Determination of residues of BAS 555 F (Metconazole) in oat and its processed products after two applications of BAS 555 00 F in Germany, 2012 2013/1037951
Guidelines:	OECD 509 Crop Field Trial (2009), OECD 508 Magnitude of the Pesticide Residues in Processed Commodities (2008), EC 1107/2009 of the European Parliament and of the Council of 21 Oct 2009, EEC 7029/VI/95 rev. 5 (July 22 1997), EEC 7035/VI/95 rev. 5
GLP:	yes (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	BAS 555 00 F (EC)
Lot/Batch #:	0003255328 (60 g/L metconazole, nominal)
CAS#:	125116-23-6

2. Test Commodity:

Crop:	Oat
Type:	Cereals
Variety:	Flocke
Botanical name:	*Avena sativa*
Crop parts(s) or processed Commodity:	Flour, groats/rolled oats, husks, dust and bran

B. STUDY DESIGN

1. Test procedure

During the 2012 growing season, four field trials were conducted at representative oat growing areas in Germany to determine the residue level of metconazole (BAS 555 F) in the raw agricultural commodity (RAC) oat and its processed fractions.
BAS 555 00 F was foliar applied twice at an exaggerated target rate of 0.27 kg a.s./ha to oat at BBCH 49 and 69. The spray volume used was 182-206 L/ha.
Oat RAC samples were harvested on the day of the last application (whole plants without roots) and at BBCH 89 (grain). The oat grain samples were processed according to simulated commercial procedures into the following samples: flour, groats/rolled oats, husks, dust and bran. Samples were stored frozen until analysis. The maximum storage interval from harvest until processing was 343 days.

2. Description of analytical procedures

Residues of metconazole (*cis*- and *trans*-isomer) were determined using BASF method No L0019/01 (550/0). Triazole derivative metabolites (TDMs) were determined using BASF method No L0170/02.
Residues of metconazole in oat RAC and processed fractions samples were extracted with methanol/water/hydrochloric acid. An aliquot of the extract was centrifuged and partitioned against dichloromethane. The final determination was performed by HPLC-MS/MS with ion transitions from m/z 320 → 70 (quantitation) and 320 → 125 (confirmation) for metconazole (*cis*- and *trans*-isomers). The validated limit of quantitation is 0.01 mg/kg for parent metconazole (sum of *cis*- and *trans*-isomer at 0.005 mg/kg each) in/on oat RAC and processed commodity samples.
Concurrent recoveries of parent metconazole (*cis*- and *trans*-isomer) were in the range of 70-109% with fortification levels of 0.005-10 mg/kg.
T, TA, TAA and TLA were extracted with methanol/water. An aliquot was filtered, concentrated and cleaned-up by a dispersive C18-SPE step. Final determination of the analytes is performed by LC-DMS/MS/MS using two different columns. The limit of quantitation is 0.01 mg/kg per analyte. Overall mean recoveries were in the range of 70-110%; relative standard deviations (RSD) were always ≤20%.

II. RESULTS AND DISCUSSION

After two treatments with BAS 555 00 F at 0.27 kg/ha, residues of metconazole (sum of both isomers) were in the range of 0.063 to 0.14 mg/kg for oat grain sampled BBCH 89, 43-50 days after the last application. Residues were <0.01 mg/kg in all untreated samples. The oat grain was processed into flour, groats/rolled oats, husks, dust and bran. The mean and median transfer factors were less than 1 in flour, groats/rolled oats and bran and greater than 1, indicating concentration of metconazole residues, in husks and dust, which are not destined for human consumption.
The mean transfer factors were at or below 1 for T, TA and TAA in husks and TAA in flour. Therefore it can be concluded that T, TA and TAA are not being accumulated in these processed fractions. The mean transfer factors were above 1 for T and TA in flour, groats/rolled oats, dust and bran, for TAA in groats/rolled oats, dust and bran and for TLA in husks, dust and bran.
Residues of TA, TAA and TLA were found in untreated specimens up to 0.55 mg/kg.

III. CONCLUSION

Four trials in oat were conducted with grain processed into flour, groats/rolled oats and bran. Processing factors (median) are derived for items for human consumption: flour (0.18) and groats, rolled oats (0.13) and for animal feed item bran (0.37).
The mean transfer factors were at or below 1 for T, TA and TAA in husks and TAA in flour. Therefore it can be concluded that T, TA and TAA are not being accumulated in these processed fractions. The mean transfer factors were above 1 for T and TA in flour, groats/rolled oats, dust and bran, for TAA in groats/rolled oats, dust and bran and for TLA in husks, dust and bran.

Table 6.5.3-11: Residues of metconazole (sum of isomers) in oat processed fractions

Trial location / Trial number	Processed commodity	Application rate (kg a.s./ha)	DALA[1]	Residues found (mg/kg)			Processing factor[3] for total residue[2]
				Cis-isomer	*Trans*-isomer	Total metconazole[2]	
Motterwitz, Germany (L120183) 2012	Whole plant[4]	2 x 0.27	0	3.1	0.68	3.8	-
	Grain		50	0.11	0.031	0.14	-
	Grain (RAC)			0.11	0.030	**0.14**	-
	Flour			0.014	<0.005	0.019	0.14
	Groats/rolled oats			0.005	<0.005	0.010	0.07
	Husks			0.24	0.073	0.31	2.21
	Dust			0.16	0.063	0.23	1.64
	Bran			0.017	0.005	0.022	0.16
Breitenborn, Germany (L120184) 2012	Whole plant[4]	2 x 0.27	0	3.4	0.73	4.2	-
	Grain		48	0.049	0.015	0.064	-
	Grain (RAC)			0.049	0.014	**0.063**	-
	Flour			0.009	<0.005	0.014	0.22
	Groats/rolled oats			0.006	<0.005	0.011	0.17
	Husks			0.21	0.064	0.28	4.44
	Dust			0.22	0.074	0.29	4.60
	Bran			0.021	0.006	0.027	0.43
Tuetzpatz, Germany (L120185) 2012	Whole plant[4]	2 x 0.27	0	4.1	0.84	4.9	-
	Grain		43	0.066	0.020	0.086	-
	Grain (RAC)			0.082	0.024	**0.11**	-
	Flour			0.017	0.005	0.022	0.20
	Groats/rolled oats			0.0085	<0.005	0.013	0.12
	Husks			0.34	0.10	0.44	4.00
	Dust			0.29	0.12	0.41	3.73
	Bran			0.042	0.013	0.055	0.50
Goeppersdorf, Germany (L120186) 2012	Whole plant[4]	2 x 0.27	0	4.1	0.84	4.9	-
	Grain		48	0.057	0.016	0.073	-
	Grain (RAC)			0.053	0.014	**0.067**	-
	Flour			0.006	<0.005	0.011	0.16
	Groats/rolled oats			<0.005	<0.005	<0.01	<0.15
	Husks			0.11	0.031	0.14	2.09
	Dust			0.21	0.060	0.27	4.03
	Bran			0.017	<0.005	0.021	0.31

1 Days after last application
2 Sum of *cis*- and *trans*-isomers
3 The processing factor is calculated by dividing the residue in the processed fraction by the residue in the RAC sample; residues <LOQ were set LOQ
4 Without roots

Table 6.5.3-12: Residues of TDMs in oat processed fractions

Trial location / Trial number	Processed commodity	Application rate (kg a.s./ha)	DALA[1]	Residues found[2] (mg/kg)				Processing factor[3]			
				T	TA	TAA	TLA	T	TA	TAA	TLA
Motterwitz, Germany (L120183) 2012	Whole plant[4]	2 x 0.27	0	<0.01	0.037	0.010	<0.01	-	-	-	-
	Grain		50	<0.01	0.26	0.091	<0.01	-	-	-	-
	Grain (RAC)			**<0.01**	**0.26**	**0.093**	**<0.01**	-	-	-	-
	Flour			<0.01	0.34	0.093	<0.01	N/A	1.31	1.00	N/A
	Groats/rolled oats			<0.01	0.36	0.090	<0.01	N/A	1.38	0.97	N/A
	Husks			<0.01	0.025	0.042	<0.01	N/A	0.10	0.45	N/A
	Dust			<0.01	0.41	0.11	0.014	N/A	1.58	1.18	1.40
	Bran			<0.01	0.46	0.12	<0.01	N/A	1.77	1.29	N/A
Breitenborn, Germany (L120184) 2012	Whole plant[4]	2 x 0.27	0	<0.01	0.097	0.039	0.043	-	-	-	-
	Grain		48	<0.01	0.57	0.19	<0.01	-	-	-	-
	Grain (RAC)			**0.010**	**0.64**	**0.18**	**<0.01**	-	-	-	-
	Flour			0.014	0.61	0.17	<0.01	1.40	0.95	0.94	N/A
	Groats/rolled oats			0.017	0.67	0.22	<0.01	1.70	1.05	1.22	N/A
	Husks			<0.01	0.040	0.081	<0.01	1.00	0.06	0.45	N/A
	Dust			0.014	0.67	0.22	0.019	1.40	1.05	1.22	1.90
	Bran			0.019	0.98	0.29	<0.01	1.90	1.53	1.61	N/A
Tuetzpatz, Germany (L120185) 2012	Whole plant[4]	2 x 0.27	0	<0.01	0.075	0.025	0.037	-	-	-	-
	Grain		43	<0.01	0.49	0.13	<0.01	-	-	-	-
	Grain (RAC)			**<0.01**	**0.56**	**0.15**	**<0.01**	-	-	-	-
	Flour			0.010	0.64	0.16	<0.01	1.00	1.14	1.07	N/A
	Groats/rolled oats			0.012	0.70	0.17	<0.01	1.20	1.25	1.13	N/A
	Husks			<0.01	0.028	0.058	<0.01	N/A	0.05	0.39	N/A
	Dust			0.013	0.77	0.21	0.021	1.30	1.38	1.40	2.10
	Bran			0.014	1.0	0.25	0.011	1.40	1.79	1.67	1.10
Goeppersdorf, Germany (L120186) 2012	Whole plant[4]	2 x 0.27	0	<0.01	0.090	0.030	0.028	-	-	-	-
	Grain		48	<0.01	0.81	0.20	<0.01	-	-	-	-
	Grain (RAC)			**<0.01**	**0.66**	**0.20**	**<0.01**	-	-	-	-
	Flour			<0.01	0.68	0.17	<0.01	N/A	1.03	0.85	N/A
	Groats/rolled oats			0.011	0.73	0.22	<0.01	1.10	1.11	1.10	N/A
	Husks			<0.01	0.024	0.086	0.015	N/A	0.04	0.43	1.50
	Dust			0.012	0.71	0.23	0.031	1.20	1.08	1.15	3.10
	Bran			0.012	1.1	0.33	<0.01	1.20	1.67	1.65	N/A

1 Days after last application
2 T = 1,2,4 triazole
TAA = triazole acetic acid
TA = triazole alanine
3 The processing factor is calculated by dividing the residue in the processed fraction by the residue in the RAC sample; residues <LOQ were set LOQ
4 Without roots

Table 6.5.3-13: Mean and median processing factors for metconazole residues in oat processed fractions

	Flour	Groats/rolled oats	Husks	Dust	Bran
Motterwitz, Germany L120183	0.14	0.07	2.21	1.64	0.16
Breitenborn, Germany L120184	0.22	0.17	4.44	4.60	0.43
Tuetzpatz, Germany L120185	0.20	0.12	4.00	3.73	0.50
Goeppersdorf, Germany L120186	0.16	<0.15	2.09	4.03	0.31
Mean	**0.18**	**0.13**	**3.19**	**3.50**	**0.35**
Median	**0.18**	**0.13**	**2.6**	**3.9**	**0.37**

CA 6.6 Residues in Rotational Crops

A confined rotational crop study was submitted and evaluated during the previous Annex I inclusion process. The residue levels and the nature of residues were investigated in three different succeeding crops (lettuce, radish, wheat) at an application rate of 0.4 kg a.s./ha. In the study the ^{14}C-cyclopentyl and ^{14}C-triazolyl labelled metconazole was applied to bare soil. The study showed that the metabolic pathway in rotated crops is similar to that in the target crops, and the study is considered to be still scientifically valid and to meet the requirements included in OECD guideline 502.

For metconazole, the following conclusion was made by the EFSA in the course of the Annex I inclusion process. The relevant endpoint was copied from the EFSA Conclusion 2006 (EFSA Scientific Report (2006) 64, 1-71, Conclusion on the peer review of metconazole).

Metabolism in plants (Annex IIA, point 6.1 and 6.7, Annex IIIA, point 8.1 and 8.6)

Rotational crops	Wheat (C), lettuce (L), radish (R/T) Metabolic pathway of metconazole in succeeding crops is similar to that in the target crops

Residues in succeeding crops (Annex IIA, point 6.6, Annex IIIA, point 8.5)

The studies performed in accordance with GAP showed that the enrichment of edible plant parts of leafy vegetables, root vegetables and cereals, installed as succeeding crops, with metconazole is not sufficient to reach quantifiable levels in monitoring.

In context of the EU MRL review according to Article 12 (EFSA Journal 2011;9(10):2422) the assessment was confirmed by EFSA: The following paragraphs were taken from the relevant Reasoned Opinion.

> "Preliminary considerations
> The use of metconazole in permanent crops or in third countries is not considered relevant with regard to the potential occurrence of residues in rotational crops. Within Europe, however all crops evaluated in the framework of this MRL review might be grown in rotation with other crops. During the peer review under Directive 91/414/EEC, it was also demonstrated in several degradation studies that metconazole is persistent in soil and that DT_{90} values exceed the trigger value of 100 days (EFSA, 2006). A detailed assessment of the nature and magnitude of metconazole residues in rotational crops is therefore considered relevant.

Nature of residues

A confined rotational crop study with radio labelled metconazole was conducted to address the potential incorporation of soil residues into succeeding crops. Data are summarized in the table below. The total radioactive residues seem slightly to increase with the planting interval, and total radioactivity (as metconazole equivalents) amounted to 0.71 mg/kg in radish root, 0.20 mg/kg in lettuce foliage and 0.49 mg/kg in wheat grain in the test with triazole-^{14}C labelled material. The study indicated that unchanged metconazole was taken up from the soil, since it was present in all crops tested. Major compounds in all crops were the metabolites triazole alanine and triazole acetic acid, as well as carboxy metabolite in radish. With time, levels of metconazole appeared to be constant in the respective crop parts, and no accumulation of metconazole is expected in rotational crops.

Altogether, the study showed a comparable metabolic pattern in succeeding crops as in directly treated plants. Hence the same residue definition as for primary crops can be assumed."

Table 6.6-1: Summary of available metabolism studies in rotational crops

Crop group	Crop	Label position	Application and sampling details				
			Method, F or G[1]	Rate (kg a.s./ha)	Sowing intervals (DAT)	Harvest intervals (DAT)	Remarks
Leafy vegetables	Lettuce	^{14}C-cyclopentyl and ^{14}C-triazolyl	Bare soil application, G	0.4	30 120	Mature samples	*cis*:*trans* 78:22
Root and tuber vegetables	Radish						
Cereals	Wheat						

1 Outdoor/field application (F) or glasshouse/protected application (G)

Magnitude of residues

In addition to the confined rotational crop study, a rotational field study was evaluated in the framework of the peer review (EFSA, 2006). This study was performed on carrots, lettuce and wheat which were planted following two applications of metconazole to bare soil at a rate to 0.09 kg a.s./ha. The replanting interval was 30 days (carrots, lettuce) and 98 days (wheat). Metconazole was the residue determined in the trial. At harvest, residues in the plant samples were all below the LOQ (0.01 mg/kg; straw 0.03 mg/kg). In this study the enrichment of plant parts of leafy vegetables, root vegetables and cereals, installed as succeeding crops, with metconazole was not sufficient to reach quantifiable levels. Based on these data, no significant levels of metconazole are expected in rotational crops.

The study is considered to be still scientifically valid and to meet the requirements included in OECD guideline 504.

CA 6.6.1 Metabolism in rotational crops

CONCLUSION

The confined rotational crop study was evaluated during the previous Annex I inclusion process and was considered acceptable. The study showed a comparable metabolic pattern in succeeding crops as in directly treated plants. No further studies on rotational crops have been conducted.

CA 6.6.2 Magnitude of residues in rotational crops

CONCLUSION

The field rotational crop study was evaluated in the framework of the peer review and was considered acceptable. In this study the enrichment of plant parts of leafy vegetables, root vegetables and cereals, installed as succeeding crops, with metconazole was not sufficient to reach quantifiable levels. Based on these data, no significant levels of metconazole are expected in rotational crops.No further studies on rotational crops have been conducted.

Triazole Derivative Metabolites

The residues of triazole derivative metabolites being found in succeeding crops and the related dietary risk assessment is included in a dossier which was submitted in March 2011 by the Triazole Derivative Metabolite Group (TDMG) to UK. UK CRD is evaluating as RMS on behalf of Commission and other member states all triazole related data (CRD reference number COP 2011/00502).

CA 6.7 Proposed residue definitions and maximum residue levels

CA 6.7.1 Proposed residue definitions

The residue definitions currently established in the EU and supported in future are compiled in Table 6.7.1-1. In the subsequent section, detailed justifications for BASF's proposal are provided. The proposal is based on a careful evaluation of all studies being available at the time point of submission. Consequently it includes considerations for all those crops for which an EU MRL is or will be established. It is not limited to the representative uses in oilseed rape and cereals.

Table 6.7.1-1: Residue definition - metconazole

Endpoint	**Active substance: Metconazole**	
	EU agreed endpoints (EFSA Scientific Report (2006) 64, 1-71, Conclusion on the peer review of metconazole)	Residue definitions proposed in the context of this dossier
Residue definition in plant matrices for risk assessment	Metconazole	Metconazole
Residue definition in plant matrices for monitoring	Metconazole	Metconazole
Residue definition in animal matrices for risk assessment	Metconazole	Metconazole
Residue definition in animal matrices for monitoring	Metconazole	Metconazole
Conversion factors (monitoring to risk assessment)	None	None

Note the use of the common name 'metconazole' in the residue definitions means residues of all 4 isomers (2 diastereoisomer pairs) as defined by this ISO common name.

Residue definition plant matrices

In the peer reviewed plant metabolism studies conducted in wheat (cereal/grass crops category) and oilseed rape (pulses and oilseeds category), the parent compound, triazolyl alanine and triazolyl acetic acid were identified as the major components of the total residues. Triazole alanine and triazole acetic acid accounted for the major residue compounds in wheat grain and rape seed. In oilseed rape, the parent compound was if not the major constituent of the residue, at least a valid indicator of the level of residues in the commodity. EFSA stated the following in the EFSA conclusion (EFSA 2006):

> "In the plant metabolism studies conducted in wheat and oilseed rape, metconazole, triazolyl alanine and triazolyl acetic acid were identified as the major components of the total residues in the edible plant parts of the tested crops. Triazolyl alanine is a common metabolite of triazole fungicides, and its toxicological profile was considered as sufficiently investigated. The residue of concern in cereals and oilseed crops is therefore metconazole only."

The residue definition as stated in the EFSA conclusion (EFSA 2006) was as follows:

> **Food of plant origin**
> Definitions for risk assessment: metconazole (cereals and oilseed crops only)
> Definitions for monitoring: metconazole (cereals and oilseed crops only)

An additional study conducted in banana (fruit category) was evaluated by the RMS Belgium and EFSA in conjunction with a MRL application (EFSA 2010). The study is not considered peer reviewed and is submitted for evaluation in this dossier. In the banana metabolism, the main compound was parent metconazole; small amounts of monohydroxylated metconazole and triazole alanine were also found.

Metabolism studies in three crop groups are now submitted. Based on the three studies, EFSA concluded that the metabolism in three crop groups proceeds in a similar pathway (EFSA 2011). In all crops, the primary metabolic pathway for metconazole proceeds by oxidative hydroxylation of the benzylic methylene group, the methyl side chain on the cyclopentyl ring, and potentially the cyclopentyl ring to produce monohydroxylated metabolites of metconazole which are further conjugated through glycosidation. The presence of triazole alanine and its acetic acid derivative suggested that the methylene group between the triazole ring and the cyclopentyl ring is also susceptible to oxidative hydroxylation. In the plant metabolism studies covering three crop categories and the rotational crop studies, no metabolites, except triazole alanine and triazole acetic acid, were present at levels equal to or exceeding 10% TRR or 0.01 mg/kg in commodities consumed by humans. The toxicology of metabolites occurring in the plant metabolism studies was evaluated in M-CA 9. No metabolites were identified as toxicologically relevant. The EFSA proposal regarding the triazole-derived metabolites, as expressed in the Review of the existing MRLs for metconazole (EFSA 2011), is as follows:

> "Even though studies indicate that the main residue in cereal grain and rape seeds is triazole alanine, the peer review decided not to include it in the residue definition. Instead, the risk assessment and enforcement residue definition was set as parent metconazole. Triazole alanine and triazole acetic acid, another compound identified in cereal metabolism, are metabolites common with other active substances belonging to the triazole chemical class. Since the triazole derivative metabolites (TDMs) occur in plant commodities treated with triazoles in significant concentrations, a specific consumer exposure assessment should be performed, taking into account the different sources of TDMs. As soon as the methodology for the risk assessment of TDMs, which is currently under development, is available, the risk assessment for the TDMs has to be performed."

The EFSA proposal regarding the residue definition, as expressed in the Review of the existing MRLs for metconazole (EFSA 2011), is as follows:

> "In the meantime, EFSA proposes parent metconazole (sum of isomers) as a provisional residue definition for enforcement and risk assessment purposes in all plants."

This opinion was restated as follows in the EFSA Reasoned opinion on the modification of the existing MRLs for metconazole in barley and oats (EFSA 2013):

> "In the meantime, EFSA proposes parent metconazole (sum of isomers) as a provisional residue definition for enforcement and risk assessment purposes in all plant commodities."
>
> "The current residue definition set in Regulation (EC) No 396/2005 is identical to the residue definition for enforcement derived in the peer review."

The effect of processing on the nature of the residue was investigated in the context of this renewal of active substance approval. Test conditions simulating pasteurization, baking, brewing, boiling and sterilization were applied; the test item metconazole did not degrade under any of these conditions. The isomer distribution was also stable. Information on the residue situation in succeeding or rotational crops is available from a confined ([^{14}C] radiolabeled) and non-radiolabeled crop rotational studies conducted with metconazole. Both studies were evaluated in the context of the initial Annex I inclusion. It was found that the metabolic pathway of metconazole in succeeding crops is similar to that in primary crops and that no significant levels of metconazole are expected in rotational crops.

A completely validated method of analysis (DFG S19, see chapter M-CA 4.2) is available for the determination of residues of metconazole as *cis*- and *trans*-isomer in all crop categories (commodities with high water, high acid, high starch, high oil and high protein content).

The following regarding the triazole derivative metabolites was concluded by EFSA in the Review (EFSA 2013)

> "However, EFSA is now of the opinion that triazole alanine and triazole acetic acid as the major identified residues in cereal grain and rape seeds, have to be considered in a consumer risk assessment. Triazole alanine and triazole acetic acid are part of a group of metabolites known as triazole derivative metabolites (TDM)11 and have been identified as common metabolites of several other substances belonging to the triazole chemical class. Therefore TDMs may occur in plant commodities as a result of the use of a variety of active substances belonging to the triazole class. It is also noted that in the framework of Directive 91/414/EEC several triazole compounds are subject to the submission of confirmatory data on this matter and that a common EU approach on risk assessment of TDMs is currently under development. EFSA therefore recommends that a separate risk assessment should be performed for TDMs as soon as the confirmatory data requested for triazole compounds in the framework of Directive 91/414/EEC have been evaluated and a general methodology on the risk assessment of triazole compounds and their triazole derivative metabolites is available.
> In the meantime, EFSA proposes parent metconazole (sum of isomers) as a provisional residue definition for enforcement and risk assessment purposes in all plant commodities."

Based on the findings from all studies summarized above, the residue definition for monitoring and risk assessment in plant matrices are proposed to remain as agreed in the peer review as follows:

Metconazole (sum of *cis*- and *trans*-isomers)
Note: As defined by the ISO common name, metconazole is a racemic mixture of 4 isomers, 2 diastereoisomer pairs.

Animal matrices-Ruminants, Pigs

The metabolism studies in lactating goat evaluated during the initial Annex I inclusion process indicated an extensive degradation and rapid elimination of the parent compound. Total radioactive residues in milk, fat and muscle were < 0.007 mg/kg. Residues in liver and kidney were analyzed and identified. The parent compound was metabolized through oxidative processes, which included monohydroxylation of the cyclopentyl rings, monohydroxylation of the methyl group on the cyclopentyl ring, which is further oxidized to carboxylic acid metabolites. In liver, metconazole was the major component (33-42% TRR), while in kidney, the metabolites M1 (M555F001), M12 (M555F012) were identified at 12-14% TRR and 13-20% TRR, respectively. Metconazole was present in kidney at 1-2% TRR and could thus serve as a valid indicator of the level of residues in the commodity.

The residue definition proposed in the EFSA conclusion (EFSA 2006) was as follows:

> "Based on the information gained from the goat studies the residue definition in animal matrices for risk assessment and for compliance with MRLs is proposed as metconazole."

The list of endpoints in EFSA conclusion Appendix 1.4 re the residue definition was as follows:

Animals covered	Goats, hens
Animal residue definition for monitoring	Metconazole
Animal residue definition for risk assessment	metconazole
Conversion factor (monitoring to risk assessment)	None
Metabolism in rat and ruminant similar (yes/no)	Yes
Fat soluble residue (yes/no)	Yes

In the EFSA opinion (EFSA 2010) the residue definition for animals was confirmed and applied to pigs as follows:

> "Metabolism in lactating ruminants was sufficiently investigated and findings can be extrapolated to pigs as well. The relevant residue definition for both enforcement and risk assessment in pigs and ruminants was therefore defined as metconazole. Available studies also demonstrated that residues of metconazole are not expected in significant amounts and MRLs in pigs and ruminants can be therefore set at the LOQ."

All the metabolites identified in goat tissues were identical or structurally similar to those recovered in the rat. The toxicology of metabolites occurring in the goat metabolism studies was evaluated in M-CA 6.9. No metabolites were identified as toxicologically relevant.

Animal matrices-Poultry

For poultry, the dietary burden does not exceed the trigger of 0.1 mg/kg feed when calculated using the agreed European methodology (EFSA EC, 1996). However the dietary burden does exceed the trigger for layer hens when calculated using the OECD feed items for poultry in the OECD Livestock Guidance document dated September 4, 2013. Based on the initial metabolism studies on laying hens, no degradation pathway could be established since no characterization of the metabolites was done due to the low residue levels in the matrices. Thus, a new poultry metabolism study is submitted in this dossier.

Parent metconazole was metabolized through various oxidative processes, sulfate ester formation and cleavage to release triazole. Using the quantitation based on the cyclopentyl label, total metconazole (sum of *cis*- and *trans*-) was a major residue in fat and skin with fat (20-35% TRR, 0.03-0.05 mg/kg). Total metconazole comprised 4% TRR (0.03 mg/kg in liver) while the major residues were M-1 (M555F001) at 16% TRR (0.13 mg/kg) and M-12 (M555F012) at 12% TRR (0.09 mg/kg). Total metconazole was present in muscle at 3% TRR (0.005 mg/kg) while M-1 comprised 16-20% TRR (0.006-0.008 mg/kg). Total metconazole was present in egg samples (9% TRR, 0.006 mg/kg) while M-1 comprised 17% TRR (0.01 mg/kg).

Metabolite 1,2,4-triazole was a major residue in the triazole label samples. This metabolite is common with other active substances belonging to the triazole chemical class. It is proposed that the same rationale applied to the TDMs in plants is applied to the triazole derivative metabolite in poultry, i.e. a specific consumer exposure assessment should be performed, taking into account the different sources of 1,2,4-triazole. It is proposed that the residue definition for monitoring or risk assessment should not include 1,2,4-triazole at this time.

It is proposed that the metabolism in laying hens was sufficiently investigated and the relevant residue definition for both enforcement and risk assessment in poultry is defined as metconazole, the sum of the *cis*- and trans-isomers.

A multi-residue method of analysis (DFG S19) for the determination of residues of metconazole as its *cis*-and *trans*-isomers in milk, egg, tissues and fat is available.

Based on these findings from all studies summarized above, the following residue definitions for monitoring and risk assessment in animal matrices are proposed to be maintained:

Animals covered	Ruminants, pigs, poultry
Animal residue definition for monitoring	Metconazole (sum of *cis*- and *trans*-isomers)
Animal residue definition for risk assessment	Metconazole (sum of *cis*- and *trans*-isomers)
Conversion factor (monitoring to risk assessment	none

CA 6.7.2 Proposed maximum residue levels (MRLs) and justification of the acceptability of the levels proposed

The following table shows the current EU MRLs for metconazole (mg/kg) as of October 7, 2014 (Commission Regulation (EU) No 737/2014) (source: European Commission website http://ec.europa.eu/sanco_pesticides/public/index.cfm?event=activesubstance.detail&language=EN&selectedID=1561).

Table 6.7.2-1: EU MRLs set for the uses of metconazole (BAS 555 F)

Code number	Groups and examples of individual products to which the MRLs apply	Metconazole (sum of isomers)
100000	. FRUITS, FRESH or FROZEN; TREE NUTS	-
110000	. Citrus fruits	0.02*
120000	. Tree nuts	0.05*
130000	. Pome fruits	0.02*
140000	. Stone fruits	-
140010	. Apricots	0.1
140020	. Cherries (sweet)	0.2
140030	. Peaches	0.1
140040	. Plums	0.02*
140990	. Others (2)	0.02*
150000	. Berries and small fruits	0.02*
160000	. Miscellaneous fruits with	-
161000	. (a) edible peel	0.02*
162000	. (b) inedible peel, small	0.02*
163000	. (c) inedible peel, large	-
163010	. Avocados	0.02*
163020	. Bananas	0.1
163030	. Mangoes	0.02*
163040	. Papayas	0.02*
163050	. Granate apples/pomegranates	0.02*
163060	. Cherimoyas	0.02*
163070	. Guavas	0.02*
163080	. Pineapples	0.02*
163090	. Breadfruits	0.02*
163100	. Durians	0.02*
163110	. Soursops/guanabanas	0.02*
163990	. Others (2)	0.02*
200000	. VEGETABLES, FRESH or FROZEN	-
210000	. Root and tuber vegetables	0.02*
220000	. Bulb vegetables	0.02*
230000	. Fruiting vegetables	-
231000	. (a) solanacea	0.02*
232000	. (b) cucurbits with edible peel	0.02*
233000	. (c) cucurbits with inedible peel	-
233010	. Melons	0.05 (ft)
233020	. Pumpkins	0.02*
233030	. Watermelons	0.02*
233990	. Others (2)	0.02*

Table 6.7.2-1: EU MRLs set for the uses of metconazole (BAS 555 F)

Code number	Groups and examples of individual products to which the MRLs apply	Metconazole (sum of isomers)
234000	. (d) sweet corn	0.02*
239000	. (e) other fruiting vegetables	0.02*
240000	. Brassica vegetables (excluding brassica roots and brassica baby leaf crops)	0.02*
250000	. Leaf vegetables, herbs and edible flowers	-
251000	. (a) lettuces and salad plants	0.02*
252000	. (b) spinaches and similar leaves	0.02*
253000	. (c) grape leaves and similar species	0.02*
254000	. (d) watercresses	0.02*
255000	. (e) witloofs/Belgian endives	0.02*
256000	. (f) herbs and edible flowers	0.05*
260000	. Legume vegetables	0.02*
270000	. Stem vegetables	0.02*
280000	. Fungi, mosses and lichens	0.02*
290000	. Algae and prokaryotes organisms	0.02*
300000	. PULSES	-
300010	. Beans	0.05
300020	. Lentils	0.02*
300030	. Peas	0.05
300040	. Lupins/lupini beans	0.05
300990	. Others (2)	0.02*
400000	. OILSEEDS AND OIL FRUITS	-
401000	. Oilseeds	-
401010	. Linseeds	0.2
401020	. Peanuts/groundnuts	0.05*
401030	. Poppy seeds	0.15
401040	. Sesame seeds	0.05*
401050	. Sunflower seeds	0.05*
401060	. Rapeseeds/canola seeds	0.2
401070	. Soyabeans	0.05*
401080	. Mustard seeds	0.2
401090	. Cotton seeds	0.3
401100	. Pumpkin seeds	0.05*
401110	. Safflower seeds	0.05*
401120	. Borage seeds	0.05*
401130	. Gold of pleasure seeds	0.05*
401140	. Hemp seeds	0.05*
401150	. Castor beans	0.05*
401990	. Others (2)	0.05*
402000	. Oil fruits	0.05*

Table 6.7.2-1: EU MRLs set for the uses of metconazole (BAS 555 F)

Code number	Groups and examples of individual products to which the MRLs apply	Metconazole (sum of isomers)
500000	. CEREALS	-
500010	. Barley	0.4
500020	. Buckwheat and other pseudo-cereals	0.02*
500030	. Maize/corn	0.1
500040	. Common millet/proso millet	0.02*
500050	. Oat	0.4
500060	. Rice	0.02*
500070	. Rye	0.06
500080	. Sorghum	0.02*
500090	. Wheat	0.15
500990	. Others (2)	0.02*
600000	. TEAS, COFFEE, HERBAL INFUSIONS, COCOA AND CAROBS	0.1*
700000	. HOPS	0.1*
800000	. SPICES	-
810000	. Seed spices	0.1*
820000	. Fruit spices	0.1*
830000	. Bark spices	0.1*
840000	. Root and rhizome spices	-
840010	. Liquorice	0.1*
840020	. Ginger	0.1*
840030	. Turmeric/curcuma	0.1*
840040	. Horseradish	(ft)
840990	. Others (2)	0.1*
850000	. Bud spices	0.1*
860000	. Flower pistil spices	0.1*
870000	. Aril spices	0.1*
900000	. SUGAR PLANTS	-
900010	. Sugar beet roots	0.06
900020	. Sugar canes	0.02*
900030	. Chicory roots	0.02*
900990	. Others (2)	0.02*
1000000	. PRODUCTS OF ANIMAL ORIGIN -TERRESTRIAL ANIMALS	-

Table 6.7.2-1: EU MRLs set for the uses of metconazole (BAS 555 F)

Code number	Groups and examples of individual products to which the MRLs apply	Metconazole (sum of isomers)
1010000	. Meat, preparations of meat, offals, blood, animal fats fresh chilled or frozen, salted, in brine, dried or smoked or processed as flours or meals; other processed products such as sausages and food preparations based on these	0.02*
1020000	. Milk	0.02*
1030000	. Birds eggs	0.02*
1040000	. Honey and other apiculture products	0.05*
1050000	. Amphibians and Reptiles	0.02*
1060000	. Terrestrial invertebrate animals	0.02*
1070000	. Wild terrestrial vertebrate animals	0.02*

* Indicates lower limit of analytical determination

0233010 Melons: The European Food Safety Authority identified some information on residue trials as unavailable. When reviewing the MRL, the Commission will take into account the information referred to in the first sentence, if it is submitted by 17 August 2015, or, if that information is not submitted by that date, the lack of it.

0840040 Horseradish: The applicable maximum residue level for horseradish (*Armoracia rusticana*) in the spice group (code 0840040) is the one set for horseradish (*Armoracia rusticana*) in the vegetables category, root and tuber vegetables group (code 0213040) taking into account changes in the levels by processing (drying) according to Art. 20 (1) of Regulation (EC) No 396/2005.

Plant Matrices

BASF proposes to change the representative formulation from BAS 555 00 F submitted in Annex I to BAS 555 01 F. The formulations have only minor differences in the antifoaming agent and solvent as discussed in Document J of the formulated product dossier, so the change should have no impact on residue levels in treated crops. The amount of applied active ingredient is the same. In this dossier, residue studies with BAS 555 00 F and other formulations are submitted for wheat, barley and oilseed rape using a cGAP consistent with that used in the Annex I submission to demonstrate that metconazole has been tested in a large number of field trials over many seasons. The data is submitted to provide a weight of the evidence approach that no additional residue studies with BAS 555 01 F are needed to support the designation of BAS 555 01 F as the representative formulation in place of BAS 555 00 F. Within Chapter MCA 6.3 and this chapter, BASF presents data which demonstrates that the level of residues for cereals and oilseed rape using the new representative formulation are covered by the current approved MRLs. In the section below, the new data are evaluated using the OECD MRL calculator in combination with the data being included in the DAR and the EFSA Conclusion 2006 to confirm that current MRLs are sufficient and that no new MRLs need be proposed.

Wheat, triticale and rye

Multiple residue trials on wheat were performed for the previous active substance approval procedure and were evaluated in the DAR (Belgium, 2004) and by EFSA (EFSA Conclusion 2006). A total number of 16, 1 and 1 supervised residue trials were considered respectively for winter wheat, triticale and winter rye covering Northern and Southern Europe. For most of the available trials, samples of whole plants were taken at different growth stages (PHIs) up to harvest and grain were sampled at maturity. All the submitted studies were carried out under GLP conditions. Samples of whole green plants at day 0 as well as samples of ears, plants with ears removed, straw and grains at different PHIs up to normal harvest time were analyzed for parent compound.

The following residue values were cited in the EFSA Conclusion (EFSA 2006):

Winter wheat
North n = 8 (85-90 g a.s./ha, 2 applications – BBCH: 32-39; 65-79, PHI: 35 days)
- Grain: <0.002, <0.01 (4x), <0.02 (2x), 0.04 mg/kg
- Straw: 0.25, 0.44, 0.53, 0.57, 0.64, 0.75, 0.76, 0.87 mg/kg

South n = 8 (82-95 g a.s./ha, 2 applications – BBCH: 33-39; 65-77, PHI: 35 days:
- Grain: <0.02 (6x), 0.03, 0.05 mg/kg
- Straw: 0.16, 0.23 (3x), 0.27, 0.30 (2x), 0.57 mg/kg

Winter rye
North (trial performed in accordance with the critical GAP)
- Grain: <0.01 mg/kg
- Straw: 0.37 mg/kg

South: no residue trials provided.

Triticale
North (trial performed in accordance with the critical GAP)
- Grain: <0.01 mg/kg
- Straw: 0.16 mg/kg

South: no residue trials provided.

Additional trials matching the critical GAP were performed since the Annex I approval. In the growing seasons 2007-2010, in total 20 field trials were performed in Northern and Southern Europe. The trials were conducted with formulations BAS 627 00 F, BAS 627 02 F and BAS 555 00 F as bridging trials according to the critical GAP (± 25%; 2 x 0.090 kg a.s./ha, PHI 35 or harvest at maturity).
The following parent compound residues were found in grain and straw samples at a PHI of 35 days or thereafter (if higher residues occurred or maturity was not reached):

Northern Europe grain (n=12):

	Grain	Straw
2007/1050102		
BAS 555 00 F:	4x (<0.01	1.29, 0.97, 0.12, 0.56
BAS 627 00 F:	3x (<0.01), 0.01	2.83, 1.15, 0.2, 0.49
Mean residue from replicate trial[1)]	3x (<0.01), 0.01	2.1, 1.1, 0.16, 0.53
2010/1075867		
BAS 555 00 F:	0.016, <0.01, <0.01, <0.01, <0.01, 0.013	1.4, 0.86, 2.8, 1.4, 0.44, 3.5
BAS 627 00 F:	0.025, 0.01, 0.011, 0.012, 0.012, 0.023	2.7, 1.4, 2.2, 2.1, 0.71, 2.5
Mean residue from replicate trial[1)]	0.021, 0.01, 0.011, 0.012, 0.012, 0.018	2.1, 1.1, 2.5, 1.8, 0.58, 3.0
2010/1144333		
BAS 627 00 F:	0.011, 0.016	1.7. 5.1
BAS 627 02 F:	0.01, 0.014	1.8, 7.3
Mean residue from replicate trial[1)]	0.011, 0.015	1.8, 5.7

Southern Europe (n=8):

	Grain	Straw
2007/1050102		
BAS 555 00 F:	<0.01, 0.01	0.34, 0.46
BAS 627 00 F:	<0.01, 0.01	0.36, 0.55
Mean residue from replicate trial[1)]	<0.01, 0.01	0.35, 0.51
2010/1075867		
BAS 555 00 F:	3x (<0.01), 0.016	1.2, 1.4, 4.4, 2.1
BAS 627 00 F:	4x (<0.01)	1.2, 1.4, 8.4, 3.0
Mean residue from replicate trial[1)]	3x (<0.01), <0.013	1.2, 1.4, 6.4, 2.6
2010/1144333		
BAS 627 00 F:	0.010, <0.01	1.4, 2.9
BAS 627 02 F:	0.012, 0.01	1.0, 4.0
Mean residue from replicate trial[1)]	0.01, 0.011	1.2, 3.5

1) For residues <LOQ, the LOQ was used in the calculation of the mean.

Northern Europe (n=12): <0.01 (3x), 0.01 (2x), 0.011 (2x), 0.012 (2x), 0.015, 0.018, 0.021 mg/kg (grain)
0.16, 0.53, 0.58 1.1, 1.1, 1.8, 1.8, 2.1, 2.1, 2.5, 3.0, 5.7 mg/kg (straw)
For wheat straw the median residue is 0.815 mg/kg

Southern Europe (n=8): <0.01 (5x), 0.01 (2x), 0.012 mg/kg (grain)
0.35, 0.51, 1.2, 1.2, 1.4, 2.6, 3.5, 6.4 mg/kg (straw)
For wheat straw the median residue is 0.430 mg/kg

In order to derive the most robust MRL, wheat trials submitted in Annex 1 evaluation and those performed since and submitted with this dossier were considered. The side by side trials performed in the same study and submitted with this dossier were considered replicate trials. Following the manner in which the replicate trials were considered in the application for a modified MRL in barley (EFSA 2013), the mean of the replicate residues were calculated for each trial. The mean value was used in the calculation of the MRL. For MRL calculation the current version of the OECD MRL calculator was used.

Table 6.7.2-2: MRL calculation for wheat grain in the EU for metconazole based on parent residues (mg/kg)

	Metconazole [mg/kg]		
	North (n=20)	**South (n=16)**	**North + South (n=36)**
STMR	0.011	0.016	0.011
HR	0.04	0.05	0.05
Calculated MRL	**0.05**	**0.06**	**0.06**

The data show that the calculated MRL for metconazole in wheat is covered by the current MRL of 0.15 mg/kg, based on import tolerance from United States. Therefore BASF proposes to keep the current EU MRL for metconazole at 0.15 mg/kg for wheat and at 0.06 mg/kg for rye.

Code number 500090 (wheat): 0.15 mg/kg
Code number 500070 (rye): 0.06 mg/kg

Barley and oat

Multiple residue trials on barley were performed for the previous active substance approval procedure and were evaluated in the DAR (Belgium, 2004) and by EFSA (EFSA Conclusion 2006). A total number of 16 supervised residue trials were considered for winter and spring barley covering Northern Europe and 4 trials covering Southern Europe. For most of the available trials, samples of whole plants were taken at different growth stages (PHIs) up to harvest and grain were sampled at maturity. All the submitted studies were carried out under GLP conditions. Samples of whole green plants at day 0 as well as samples of ears, plants with ears removed, straw and grains at different PHIs up to normal harvest time were analyzed for parent compound.

Winter and spring barley

North (89-96 g a.s./ha, 2 applications BBCH 33; 71-75, PHI: 35-37 days)
- Grain: <0.01 (4x), 0.01 (3x), 0.02, 0.03 (5x), 0.05 (2x), 0.09 mg/kg
- Straw: 0.03, 0.13, 0.15, 0.18, 0.22, 0.23, 0.32, 0.37, 0.73, 0.74, 0.82, 0.99, 1.06, 1.61, 1.33, 1.37 mg/kg

South (target dose of 90 g a.s./ha, 2 applications (BBCH 51-83), PHI: 35 days)
-Grain: 0.03 0.03 0.04, 0.05 mg/kg
-Straw 1.3, 1.4, 1.5, 2.6 mg/kg

The four additional trials in barley matching the critical GAP to complete the requirement for Southern Europe were performed in 2007 and are submitted in this dossier. The trials were performed with BAS 555 01 F (90 g a.s./L, EC formulation).
Additional trials matching the critical GAP were performed since and are submitted in this dossier to demonstrate that metconazole has been tested in a large number of field trials over many seasons. The data is submitted to provide a weight of the evidence approach to support the BASF proposal that no additional residue studies with BAS 555 01 F are needed to support the designation of BAS 555 01 F as the representative formulation in place of BAS 555 00 F. In the growing seasons 2007-2010, in total 24 field trials were performed in Northern and Southern Europe. The trials were conducted with representative solo formulation BAS 555 01 F or with formulations BAS 627 00 F, BAS 627 02 F and BAS 555 00 F as bridging trials according to the critical GAP (± 25%; 2 x 0.090 kg a.s./ha, PHI 35 or harvest at maturity).
The following parent compound residues were found in grain and straw samples at a PHI of 35 days or thereafter (if higher residues occurred or maturity was not reached):

Northern Europe grain (n=12):

	Grain	**Straw**
2007/1050101		
BAS 555 00 F:	0.01, 0.06, 0.01, 0.02	0.45, 0.07, 0.15, 0.20
BAS 627 00 F:	0.01, 0.05, 0.01, 0.02	1.0, 0.09, 0.33, 0.27
Mean residue from replicate trial[1)]	0.01.0.06, 0.01, 0.02	0.73, 0.08, 0.24, 0.24
2010/1110643		
BAS 555 00 F:	0.077, 0.021, 0.019, 0.03, 0.055, 0.079	0.6, 0.65, 0.20, 0.76, 0.45, 0.59
BAS 627 00 F:	0.20, 0.013, 0.017, 0.056, 0.065, 0.087	1.6, 0.41, 0.28, 1.3, 0.24, 0.34
Mean residue from replicate trial[1)]	0.14, 0.017, 0.018, 0.043, 0.060, 083	1.1, 0.53, 0.24, 1.0, 0.35, 0.47
2010/1144334		
BAS 627 00 F:	0.023, 0.087	2.2, 1.6
BAS 627 02 F:	0.021, 0.056	3.1, 2.5
Mean residue from replicate trial[1)]	0.022, 0.067	2.7, 2.1

Southern Europe (n=12):

	Grain	**Straw**
20008/1009268		
BAS 555 01 F	0.02, 0.01, 0.05, 0.02	0.39, 0.34, 0.18, 0.08
2007/1050101		
BAS 555 00 F:	0.03, 0.16	0.29, 1.08
BAS 627 00 F:	0.03, 0.19	0.14, 0.79
Mean residue from replicate trial[1)]	0.03, 0.18	0.22, 0.94
2010/11110643		
BAS 555 00 F:	0.011, 0.041, 0.037, 0.035	0.12, 1.2, 2.5, 2.0
BAS 627 00 F:	0.02, 0.04, 0.025, 0.067	0.76, 2.5, 2.2, 1.2
Mean residue from replicate trial[1)]	0.02, 0.041, 03, 0.05	0.44, 1.9, 2.4, 1.6
2010/1144334		
BAS 627 00 F:	0.036, 0.19	0.38, 5.6
BAS 627 02 F:	0.035, 0.18	0.58, 6.7
Mean residue from replicate trial[1)]	0.036, 0.19	0.48, 6.15

1) For residues <LOQ, the LOQ was used in the calculation of the mean.

Northern Europe (n=12): 0.01 (2x), 0.017, 0.018, 0.02, 0.022, 0.043, 0.06 (2x),.067, 0.083, 0.14, , mg/kg (grain)
0.08, 0.24, 0.24, 0.24, 0.35, 0.47, 0.53, 0.73, 1.0, 1.1, 2.1, 2.7 mg/kg (straw)
For barley straw the median residue is 0.50 mg/kg

Southern Europe (n=12): 0.01, 0.02 (3x), 0.03 (2x), 0.036, 0.041, 0.05(2x), 0.18, 0.19 mg/kg (grain)
0.08, 0.18, 0.22, 0.34, 0.39, 0.44, 0.48, 0.94, 1.6, 1.9, 2.4, 6.15 mg/kg (straw)
For barley straw the median residue is 1.07 mg/kg

In order to derive the most robust MRL, barley trials submitted in Annex 1 evaluation and those performed since and submitted with this dossier were considered. The side by side trials performed in the same study and submitted with this dossier were considered replicate trials. Following the manner in which the replicate trials were considered in the application for a modified MRL in barley (EFSA 2013), the mean of the replicate residues were calculated for each trial. The mean value was used in the calculation of the MRL. For MRL calculation the current version of the OECD MRL calculator was used.

Table 6.7.2-3: MRL calculation for barley grain in the EU for metconazole based on parent residues (mg/kg)

	Metconazole [mg/kg]		
	North (n=28)	**South (n=16)**	**North + South (n=44)**
STMR	0.026	0.033	0.030
HR	0.14	0.19	0.19
Calculated MRL	**0.2**	**0.3**	**0.2**

The data show that the calculated MRL for metconazole in barley is covered by the current MRL of 0.4 mg/kg. Therefore BASF proposes to keep the current EU MRL for metconazole at 0.4 mg/kg for barley and oat.

Code number 500010 (barley) and 500050 (oat): 0.4 mg/kg

Oilseed rape

Multiple residue trials on oilseed rape were performed for the previous active substance approval procedure and were evaluated in the DAR (Belgium, 2004) and by EFSA (EFSA Conclusion 2006). A total number of 17 supervised residue trials, 8 in the north of Europe and 9 in the south, were considered for oilseed rape covering Northern and Southern Europe. For most of the available trials, samples of whole plants were taken at different growth stages (PHIs) up to harvest and seed were sampled at maturity. All the submitted studies were carried out under GLP conditions. The results as reported in the EFSA Conclusion (EFSA 2006) are shown below.

Oilseed rape

North (90 g a.s./ha, 2 applications – BBCH: 63-67; 69-71 , PHI: 56-70 days)
- Seed: <0.01 (5x), 0.04, 0.06, 0.07 mg/kg
South (79-90 g a.s./ha, 2 applications – BBCH: 65; 69-75, PHI: 56-63 days)
- Seed: <0.01, 0.02 (3x), 0.03, 0.04, 0.05 (2x), 0.11 mg/kg

Additional trials matching the critical GAP were performed since and are submitted in this dossier to demonstrate that metconazole has been tested in a large number of field trials over many seasons. The data is submitted to provide a weight of the evidence approach to support the BASF proposal that no additional residue studies with BAS 555 01 F are needed to support the designation of BAS 555 01 F as the representative formulation in place of BAS 555 00 F. In the growing season 2011, in total 8 field trials were performed: 4 in Northern and 4 in Southern Europe. The trials were conducted with formulation BAS 556 03 F according to the critical GAP (± 25%; 2 x 0.072 kg a.s./ha, PHI 56 or harvest at maturity).
The following parent compound residues were found in seed samples at a PHI of 56 days or at maturity:

Northern Europe (n=4): 0.014, 0.025, 0.046, 0.072 mg/kg (seed)

Southern Europe (n=4): 0.015, 0.016, 0.037, 0.051 mg/kg (seed)

In order to derive the most robust MRL, barley trials submitted in Annex 1 evaluation and those performed since and submitted with this dossier were considered For MRL calculation the current version of the OECD MRL calculator was used.

Table 6.7.2-4: MRL calculation for oilseed rape in the EU for metconazole based on parent residues (mg/kg)

	Metconazole [mg/kg]		
	North (n=12)	**South (n=13)**	**North + South (n=25)**
STMR	0.020	0.030	0.025
HR	0.072	0.11	0.11
Calculated MRL	**0.15**	**0.15**	**0.15**

The data show that the calculated MRL for metconazole in oilseed rape is covered by the current MRL of 0.2 mg/kg. Therefore BASF proposes to keep the current EU MRL for metconazole at 0.2 mg/kg for oilseed rape with extrapolation to linseed and mustard seed and at 0.15 mg/kg for poppy seed.

Code number 401060 (rapeseeds), 401010 (linseed) and 401080 (mustard seed): 0.2 mg/kg
Code number 401030 (poppy seed): 0.15 mg/kg

Animal matrices

In the EFSA Conclusion (EFSA 2006) the determination of the animal feed burden given in Appendix 1.4 demonstrated intakes for poultry and pig that would not trigger feeding studies. Based on the poultry metabolism studies and the ruminant metabolism study applied to pigs, no significant exposure to residues in poultry and pig matrices can be expected. MRLs in poultry and pig matrices were not required.

Calculation of the animal feed burden for ruminant EFSA addendum Jan. 2006) indicated possible residues in liver based on the goat metabolism studies, and EFSA proposed a provisional MRL of 0.05 mg/kg in liver (not peer reviewed).

During the review of the MRLs including import tolerances, new feed burdens were calculated to take into account the imported commodities. Accordingly, a feeding study with ruminants was required, and a feeding study in lactating cows was submitted in conjunction with the MRL dossier (2009). The feeding study was evaluated and accepted as documented in the EFSA Reasoned Opinion (2010):

> "Metabolism studies and a new feeding study demonstrated that no measurable metconazole residues above the LOQ are expected in swine and ruminant food commodities at the calculated maximum dietary burdens"

The study is submitted and summarized in this dossier (M-CA 6.4) since it is not considered peer-reviewed, and the results are reviewed in this section in conjunction with a recalculation of the animal feed burden.

For estimating the feed burden, the procedure of EFSA (Profile file) has been applied. A worst case diet was derived for different livestock species according to the table in Appendix G (Lundehn document 7031/VI/95 rev. 4. July 1996). It is assumed that from each group of crops/commodities the item with the highest potential residue contribution on a dry matter basis is chosen. Then, the total diet is composed beginning with the group representing the highest contribution and filling the rest with feed from the other groups in descending order.
The evaluation is based on the following formula:

$$\text{Uptake [mg/kg bw/day]} = \frac{\text{Total intake of dry matter [kg/animal/day]} \times \text{\% of diet} \times \text{Residue in feed item [mg/kg]}}{\text{Dry matter content of feed item [\%]} \times \text{Bodyweight [kg]}}$$

The input values for the representative uses for calculation of median and maximum dietary burden and the results are shown in the tables below. For barley, wheat grain and rape seed, the STMR is taken for the southern zone which has the higher STMR. The process factors are the median process factors (PF) presented in M-CA 6.5. The inputs for a dietary feed burden calculation covering representative uses and other registered crops and imported crops are shown in Table 6.7.2-6.

Table 6.7.2-5: Input values for representative uses for the dietary burden calculation (EU methodology)

Commodity	Median dietary burden		Maximum dietary burden	
	Input value (mg/kg)	Comment	Input value (mg/kg)	Comment
Wheat grain	0.016	STMR	0.016	STMR
Barley grain	0.033	STMR	0.033	STMR
Rye grain	0.02	STMR	0.02	STMR
Wheat bran	0.032	STMR x PF 2	0.032	STMR x PF 2
Rye bran	0.04	STMR x PF 2	0.04	STMR x PF 2
Wheat straw	0.815	STMR (N EU)	6.4	HR (S EU)
Barley straw	1.075	STMR (S EU)	6.15	HR (S EU)
Rye straw	0.815	STMR	6.4	HR
Oat straw	1.075	STMR	6.15	HR
Oilseed rape seed	0.03	STMR	0.03	STMR
Oilseed rape seed meal	0.03	STMR x PF 1	0.03	STMR x PF 1

Table 6.7.2-6: Input values for the dietary burden calculation for representative uses, other registered uses and import tolerances (EU methodology)

Commodity	Median dietary burden		Maximum dietary burden	
	Input value (mg/kg)	Comment	Input value (mg/kg)	Comment
Wheat grain	0.016	STMR	0.016	STMR
Barley grain	0.033	STMR	0.033	STMR
Rye grain	0.02	STMR	0.02	STMR
Wheat bran	0.032	STMR x PF 2	0.032	STMR x PF 2
Rye bran	0.04	STMR x PF 2	0.04	STMR x PF 2
Wheat straw	0.815	STMR	6.4	HR
Barley straw	1.07	STMR	6.15	HR
Rye straw	0.815	STMR	6.4	HR
Oat straw	1.07	STMR	6.15	HR
Sugar beet	0.02	STMR	0.04	HR
Oilseed rape seed	0.03	STMR	0.03	STMR
Oilseed rape seed meal	0.03	STMR x PF 1	0.03	STMR x PF 1
Cotton seed meal	0.03	STMR x PF 1	0.03	STMR x PF 1
Linseed meal	0.03	STMR x PF 1	0.03	STMR x PF 1
Peanut meal	0.04	STMR x default PF 2	0.04	STMR x default PF 2

Table 6.7.2-7: Results of the dietary burden calculation for representative uses (EU methodology)

	Maximum dietary burden (mg/kg bw/d)	Median dietary burden (mg/kg bw/d)	Highest contributing commodity	Max dietary burden (mg/kg DM)	Trigger exceeded (Y/N)
Risk assessment residue definition: metconazole					
Dairy ruminants	0.055	0.010	Barley straw	1.51	Y
Meat ruminants	0.160	0.028	Barley straw	3.74	Y
Poultry	0.002	0.002	Barley grain	0.030	N
Pigs	0.0015	0.0015	Barley grain	0.037	N

Table 6.7.2-8: Results of the dietary burden calculation using inputs listed in Table 6.7.2-6 (representative uses, other registered uses and import tolerances, EU methodology)

	Maximum dietary burden (mg/kg bw/d)	Median dietary burden (mg/kg bw/d)	Highest contributing commodity	Max dietary burden (mg/kg DM)	Trigger exceeded (Y/N)
Risk assessment residue definition: metconazole					
Dairy ruminants	0.057	0.010	Wheat straw	1.57	Y
Meat ruminants	0.164	0.029	Wheat straw	3.82	Y
Poultry	0.0045	0.0033	Barley grain	0.071	N
Pigs	0.005	0.003	Sugar beet	0.137	Y

Additionally, the most recent version of the OECD feed burden calculation (using the OECD methodology) was applied. The information is provided as supplemental information. The following input values were used for calculation of the results for Reasonable Worst Case Feeding Levels (RWCFL):

Table 6.7.2-9: Input values for the dietary burden calculation (OECD methodology)

Commodity	RWCFL EU	
	Input value (mg/kg)	Comment
Forages		
Barley, straw	6.15	HR
Rye, straw	6.4	HR
Wheat, straw	6.4	HR
Cereal Grain/Crops Seeds		
Barley, grain	0.033	STMR
Rye, grain	0.02	STMR
Wheat, grain	0.016	STMR
By-Products		
Barley, bran fractions	0.119	STMR x PF 3.6
Brewer's grain, dried	0.056	STMR x PF 1.7
Canola, meal	0.03	STMR x PF 1.0
Wheat, milled byproducts	0.032	STMR x 2 (bran PF)

Table 6.7.2-10: Summary of EU Results for Reasonable Worst Case Feeding Levels (RWCFL) for Representative Uses

	Cattle Beef	Cattle Dairy	Ram/ewe	lamb	Swine Breeding	Swine Finishing	Poultry Broiler	Poultry Layer	Poultry Turkey
Body weight (kg)	500	650	75	40	260	100	1.7	1.9	7
Daily intake (kg DM)	12	25	2.5	1.7	6	3	0.12	0.13	0.5
Med Dietary burden (mg/kg bw)	0.009	0.015	0.025	0.031	0.0015	0.001	0.002	0.009	0.002
Med Feed burden (mg/kg DM)	0.389	0.388	0.747	0.740	0.037	0.037	0.034	0.126	0.034
Max Dietary burden (mg/kg bw)	0.050	0.081	0.139	0.177	0.0015	0.001	0.002	0.052	0.002
Max Feed burden (mg/kg DM)	2.099	2.099	4.168	4.161	0.037	0.037	0.034	0.761	0.026
	Barley straw	Barley straw	Barley straw	Barley straw	Barley grain	Barley grain	Barley grain	Wheat straw	Barley grain

Calculation of overdosing factors based on EFSA PROFile

Since the animal feed burden levels using the EFSA PROFile increased for both dairy and beef cattle compared to those determined in the Annex I evaluation, the overdosing factors were recalculated for all species based on the animal feed burdens derived using the EU methodology in this dossier.

Table 6.7.2-11: Calculation of the overdosing factors based on the animal feed burden for representative uses as determined using the EFSA PROFile

Species	**Dose level metabolism study (mg/kg feed)**	**Dose level metabolism study (mg/kg bw/d)**	**Max. dietary feed burden (mg/kg feed)**	**Max. dietary feed burden (mg/kg bw/d)**	**Overdosing factor [1]**
Dairy cattle (lactating goat - study 1992	25	0.91	1.51	0.055	16.6
Beef cattle (lactating goat - study 1992	25	1.07	3.74	0.160	6.7
Pigs [2]	25	1.0	0.037	0.0015	676
Dairy cattle (lactating goat -study 1993)	10.6	0.39	1.51	0.055	7.0
Beef cattle (lactating goat - study 1993)	10.6	0.45	3.74	0.160	2.8
Poultry (laying hen - study 1991)	10 0	0.63	0.030	0.002	333
Poultry (laying hen cyclopentyl label study 2006 [3])	14	0.88	0.030	0.002	467
Poultry (laying hen triazole label study 2006 [3])	12.6	0.80	0.030	0.002	420
Species	**Dose level metabolism study (mg/kg feed)**	**Dose level metabolism study (mg/kg bw/d)**	**Med. dietary feed burden (mg/kg feed)**	**Med dietary feed burden (mg/kg bw/d)**	**Overdosing factor [1]**

Species	Dose level metabolism study (mg/kg feed)	Dose level metabolism study (mg/kg bw/d)	Max. dietary feed burden (mg/kg feed)	Max. dietary feed burden (mg/kg bw/d)	Overdosing factor [1]
Dairy cattle (lactating goat - study 1992[1)]	25	0.477	0.276	0.010	91
Beef cattle (lactating goat - study 1992 [1)]	25	0.477	0.644	0.028	39
Pigs [2]	25	0.477	0.037	0.0015	676
Dairy cattle (lactating goat -study 1993)	10.6	0.64	0.276	0.010	38
Beef cattle (lactating goat - study 1993)	10.6	0.64	0.644	0.028	16
Poultry (laying hen - study 1991)	10 0	0.63	0.030	0.002	333
Poultry (laying hen cyclopentyl label study 2006 [3])	14	0.88	0.030	0.002	467
Poultry (laying hen triazole label study 2006 [3])	12.6	0.80	0.030	0.002	420

1 Overdosing factor calculated using mg/kg feed; slight differences occur if using mg/kg bw/d.
2 Extrapolated from goat since metabolism similar in rat and goat
3 Based on actual dose

The overdosing factors were then used to determine the expected residues in animal matrices.

Table 6.7.2-12: Calculation of the expected residues in ruminant matrices based on levels measured in metabolism in lactating goat (1992)

Commodity	TRR in matrices goat 2 (Mg/kg)	Extrapolated TRR in goat matrices[1] (mg/kg)	Conc. of metconazole in matrix in mg/kg (%TRR)	Extrapolated metconazole residue from actual intake (mg/kg)
Milk	0.004	0.00024	Not relevant	
Meat	0.005	0.00075	Not relevant	
Fat	0.003	0.00045	Not relevant	
Liver	0.559	0.0834	0.236 (42.2%)	0.0352
Kidney	0.276	0.0412	0.003 (1%)	0.00045

1) Based on overdosing factor = 15.6 for milk and = 6.6 for tissue

Table 6.7.2-13: Calculation of the expected residues in ruminant matrices based on levels measured in metabolism of lactating goat (1993)

Commodity	TRR in matrices (Mg/kg)	Extrapolated TRR in goat matrices[1] (mg/kg)	Conc. of metconazole in matrix in mg/kg (%TRR)	Extrapolated metconazole residue from actual intake (mg/kg)
Milk	<0.002	Not relevant	Not relevant	
Meat	0.004-0.007	Not relevant	Not relevant	
Fat	0.006	Not relevant	Not relevant	
Liver	0.317	0.113	0.105 (33.12%)	0.038
Kidney	0.148	0.053	0.003 (2.02%)	0.001

1) Based on overdosing factor = 2.9 for tissue

Table 6.7.2-14: Calculation of the expected residues in relevant pig matrices based on levels measured in metabolism of lactating goat (1992)

Commodity	TRR in matrices (Mg/kg)	Extrapolated TRR in goat matrices[1] (mg/kg)	Conc. of metconazole in matrix in mg/kg (%TRR)	Extrapolated metconazole residue from actual intake (mg/kg)
Liver	0.559	0.0008	Not relevant	
Kidney	0.276	0.0004	Not relevant	

1) Based on overdosing factor = 182.5

Since the values extrapolated from the metabolism studies show possible measurable residues in ruminant liver, the livestock feeding study was used to give a more precise measurement of anticipated residues at the expected maximum and median feed burdens. The residue definition for monitoring and risk assessment is proposed as metconazole so the feeding study results are the same for both monitoring and risk assessment. No correction factor is proposed.

Table 6.7.2-15: Overview of the residues in liver found in the cow livestock study and consideration of possible residues in sheep

Commodity	Feed burden mg/kg bw		Cow feeding Study [a]	Feeding study results in cow (mg/kg)		Median residue	Highest residue	MRL proposal
	Median	Maximum	Dose level (mg/kg bw)	Mean [c)]	maximum [c)]	(mg/kg)	(mg/kg)	(mg/kg)
Ram liver [d)]	0.025	0.139	0.2	<0.02	<0.02			
			0.57	<0.02	<0.02			
			1.66	<0.02	<0.03 [b)]			
Lamb liver[d)]	0.031	0.177	0.2	<0.02	<0.02			
			0.57	<0.02	<0.02	0.02	0.02	0.02*
			1.66	<0.02	<0.03 [b)]			
Ruminant liver	0.028	0.160	0.2	<0.02	<0.02			
			0.57	<0.02	<0.02			
			1.66	<0.02	<0.03 [b)]			

(*): Indicates that the MRL is set at the limit of analytical quantification.
a): The feeding studies were carried out with ruminants, according to the metabolism pathway, an extrapolation between ruminant and sheep is acceptable
b): Residue above the LOD but below the lowest validated LOQ of 0.04 mg/kg in the study
c): The residue definition for monitoring and risk assessment is proposed as metconazole so the feeding study results are metconazole. No correction factor is proposed.
d) The feed burden was determined with the OECD consumption levels for the EU.

Based on the calculated dietary burdens, the metabolism and livestock feeding studies, no measurable metconazole residues above the LOQ are expected in swine and ruminant food commodities. MRLs for ruminant matrices including milk and pig matrices should be set at the limit of analytical quantitation and no changes are proposed in the current MRLs.

Code number 1010000 (meat): 0.02* mg/kg
Code number 1020000 (milk and cream) 0.02* mg/kg

* Indicates lower limit of analytical determination

Poultry

A new metabolism study in laying hens is submitted in this dossier (M-CA 6.2) performed with metconazole labeled in the cyclopenyl ring and in the triazole ring. The expected residues in hen matrices were calculated using the overdosing factors (see Table 6.7.2-16) and the results from the new metabolism study.

Table 6.7.2-16: Calculation of the expected metconazole residues in poultry matrices based on levels measured in metabolism of laying hen (2006) and the dietary burden calculated using EFSA PROFile

Commodity	TRR 2006 study (Mg/kg)	Extrapolated total residue in hen (mg/kg)	Conc. of metconazole in matrix mg/kg (%TRR)	Extrapolated metconazole residue from actual intake (mg/kg)
Egg white C[a)]	0.047	0.00010	0.003 (7.3)	0.00001
Egg white T[b)]	0.186	0.00044	0.005 (2.5)	0.00001
Egg yolk C	0.088	0.00019	0.010 (11.4)	0.00002
Egg yolk T	0.156	0.00037	0.01 (6.6)	0.00002
Thigh Muscle C	0.049	0.00010	0.005 (9.8)	0.00001
Thigh Muscle T	0.152	0.00036	0.005 (3.3)	0.00001
Fat C	0.091	0.00019	0.033 (36.7)	0.00007
Fat T	0.141	0.00034	0.050 (35.3)	0.00012
Skin with fat	0.075	0.00016	0.021 (27.8)	0.00005
Skin with fat	0.137	0.00033	0.027(19.7)	0.00006
Liver C	0.790	0.00169	0.033 (4.1)	0.00007
Liver T	0.972	0.00231	0.017(1.7)	0.00004

a) Cyclopentyl label: N = 467
b) Triazole label: N = 420

The results with the new study confirm that no residue above the limit of quantitation are anticipated in poultry matrices when the dietary burden is calculated using the EFSA PROFile.

Since the maximum dietary burden determined using the OECD animal feed items indicated a feed burden of 0.761 mg/kg feed (0.052 mg/kg bw/d)for laying hens, driven almost totally by the impact of wheat straw, the anticipated residues in hen matrices were recalculated using the overdosing factors derived from the higher feed burden. The feeding levels in the 2006 metabolism study were 14 mg/kg feed and 12.6 mg/kg feed for the cyclopentyl and triazole labels, respectively. The overdosing factors are 18 and 16.6 for the cyclopentyl and triazole labels, respectively. The expected residues of metconazole in matrices is below the limit of quantitation (see table below).

A hen feeding study (MCA 6.4) was conducted at feeding levels of 2 mg/kg (0.15 mg/kg bw/d), 6 mg/kg (0.45 mg/kg bw/d) and 20 mg/kg dry feed (1.55 mg/kg bw/d). Residues of metconazole in eggs at the relevant feeding level of 2 mg/kg feed (0.15 mg/kg bw/d) and at the next higher feeding level of 6 mg/kg (0.45 mg/kg bw/d) were < LOD of 0.1 mg/kg. Residues of metconazole were <LOD in muscle, liver and fat at all feeding levels, confirming that residues of metconazole are not anticipated in poultry matrices when the dietary burden is calculated with the OECD consumption data. Residues of the metabolite M12 in liver were <LOD of 0.01 mg/kg at the highest feeding level. Residues of the metabolite M1 were <LOD of 0.01 mg/kg in muscle at the highest feeding level. Residues of M1 were <LOD in liver at the 2 mg/kg feeding level, a feeding level that is ~3x higher than the feeding level calculated with the OECD.

The results of the hen feeding study confirm that residues of metconazole including metabolites are not anticipated in poultry matrices, even assuming consumption of a maximum amount of wheat straw.

Table 6.7.2-17: Calculation of the expected residues in poultry matrices based on levels measured in metabolism of laying hen (2006) and the dietary burden calculated using OECD spreadsheet

Commodity	TRR 2006 study (Mg/kg)	Extrapolated total residue in hen (mg/kg)	Conc. of metconazole in matrix mg/kg (%TRR)	Extrapolated metconazole residue from actual intake (mg/kg)
Egg white C [a)]	0.047	0.00255	0.003 (7.3)	0.00016
Egg white T [b)]	0.186	0.01120	0.005 (2.5)	0.00030
Egg yolk C	0.088	0.00478	0.010 (11.4)	0.00054
Egg yolk T	0.156	0.00940	0.01 (6.6)	0.00060
Thigh Muscle C	0.049	0.00266	0.005 (9.8)	0.00027
Thigh Muscle T	0.152	0.00916	0.005 (3.3)	0.00030
Fat C	0.091	0.00495	0.033 (36.7)	0.00179
Fat T	0.141	0.00849	0.050 (35.3)	0.00301
Skin with fat	0.075	0.00408	0.021 (27.8)	0.00114
Skin with fat	0.137	0.00825	0.027 (19.7)	0.00163
Liver C	0.790	0.04293	0.033 (4.1)	0.00179
Liver T	0.972	0.05855	0.017 (1.7)	0.00102

a) Cyclopentyl label: N = 18.4
b) Triazole label: N = 16.6

No change to the current MRLs in poultry or eggs are proposed.

Code number 1016000 (poultry): 0.02* mg/kg
Code number 1030000 (birds' eggs) 0.02* mg/kg

* Indicates lower limit of analytical determination

CA 6.7.3 Proposed maximum residue levels (MRLs) and justification of the acceptability of the levels proposed for imported products (import tolerance)

The MRLs listed in M-CA 6.7.2 include domestic uses, but also values for imported crops. Prior to final approval, the import tolerances have been carefully evaluated by the RMS Belgium and by EFSA (EFSA reasoned Opinion 2010, EFSA review of the MRLS, (EFSA 2011). The MRLs are approved by the European Commission. No Codex MRLs (CXLs) exist for metconazole; it has not been assessed by JMPR.

CA 6.8 Proposed safety intervals

Pre-harvest interval
For oilseed rape the application is intended to meet a pre-harvest interval of 56 days. For cereals the application is intended to meet a pre-harvest minimum interval of 35 days or is based on the interval from the latest application at BBCH growth stage 69 to harvest at crop maturity.

Re-entry period for livestock to areas to be grazed
Because metconazole is not intended to be used in areas to be grazed, no re-entry period for livestock has to be defined.

Re-entry period for man to treated crops
Re-entry assessments are given for the representative uses in the supplemental product dossiers (M-CP 7.2). Re-entry is possible after the spray deposits on the crops have dried given the worker is wearing adequate work clothing (but no PPE).

Withholding period for animal feed stuffs
The withholding period for animal feeding stuff is given by the intended use (last application at BBCH growth stage 71 or 69 for oilseed rape and cereals, respectively). In order to avoid residues in products of animal origin above the MRLs, a withholding period of at least 56 or 35 days after the application for seeds or grains and for other plant parts to be used as feedstuffs is recommended, respectively.

Waiting period between application and crop sowing or planting the crop to be protected
No waiting period is necessary since metconazole is not intended in a pre-emergence use.

Waiting period between application and handling treated produce
This is not relevant here since a post-harvest treatment is not intended for oilseed rape and cereals.

Waiting period between last application and sowing or planting succeeding crops
Waiting periods before sowing or planting succeeding crops do not need to be defined at this point in time. No residues above the LOQ of the method can be expected in succeeding crops (see chapter M-CA 6.6).

CA 6.9 Estimation of the potential and actual exposure through diet and other sources

Assessments of the potential chronic and acute dietary consumer risk resulting from exposure to residues of metconazole (BAS 555 F) were performed using the EFSA calculation model for acute and chronic consumer exposure (rev. 2.0). The EFSA model was used since it considers all the different diets and all consumer groups in the EU.

The ADI and ARfD values for the active substance are summarized in the table below.

Table 6.9-1: Toxicological endpoints - metconazole

Endpoint	Value	Study	Safety factor	Reference
Acceptable Daily Intake (ADI)	0.01 mg/kg bw/d	Developmental rabbit	400	EFSA Conclusion (2006)
Acute Reference Dose (ARfD)	0.01 mg/kg bw	Developmental rabbit	400	EFSA Conclusion (2006)

The toxicological relevance of metconazole metabolites excluding the triazole derivative metabolites (TDMs) is discussed in M-CA 6.9.4. The exposure to the triazole derivative metabolites after treatment with metconazole containing products is discussed in M-CA 6.9.5.

TMDI calculations

No new MRLs are proposed for metconazole. The calculation of the TMDI was performed taking into account the representative crops. In addition, the calculation of the TMDI was performed taking into account all the crops to which metconazole may be applied including import tolerances and the default MRLs. The respective MRLs are indicated in Table 6.9-2. The consumer risk assessments were performed with revision 2 of the EFSA_Acute_Chronic RA Pesticide Residues Intake Model (PRIMo).

Table 6.9-2: EU MRLs set for the uses of metconazole (BAS 555 F)

Code number	Groups and examples of individual products to which the MRLs apply	Metconazole (sum of isomers)
100000	. FRUITS, FRESH or FROZEN; TREE NUTS	-
110000	. Citrus fruits	0.02*
120000	. Tree nuts	0.05*
130000	. Pome fruits	0.02*
140000	. Stone fruits	-
140010	. Apricots	0.1
140020	. Cherries (sweet)	0.2
140030	. Peaches	0.1
140040	. Plums	0.02*
140990	. Others (2)	0.02*
150000	. Berries and small fruits	0.02*
160000	. Miscellaneous fruits with	-
161000	. (a) edible peel	0.02*
162000	. (b) inedible peel, small	0.02*
163000	. (c) inedible peel, large	-
163010	. Avocados	0.02*
163020	. Bananas	0.1
163030	. Mangoes	0.02*
163040	. Papayas	0.02*
163050	. Granate apples/pomegranates	0.02*
163060	. Cherimoyas	0.02*
163070	. Guavas	0.02*
163080	. Pineapples	0.02*
163090	. Breadfruits	0.02*
163100	. Durians	0.02*
163110	. Soursops/guanabanas	0.02*
163990	. Others (2)	0.02*
200000	. VEGETABLES, FRESH or FROZEN	-
210000	. Root and tuber vegetables	0.02*
220000	. Bulb vegetables	0.02*

Table 6.9-2: EU MRLs set for the uses of metconazole (BAS 555 F)

Code number	Groups and examples of individual products to which the MRLs apply	Metconazole (sum of isomers)
230000	. Fruiting vegetables	-
231000	. (a) solanacea	0.02*
232000	. (b) cucurbits with edible peel	0.02*
233000	. (c) cucurbits with inedible peel	-
233010	. Melons	0.05 (ft)
233020	. Pumpkins	0.02*
233030	. Watermelons	0.02*
233990	. Others (2)	0.02*
234000	. (d) sweet corn	0.02*
239000	. (e) other fruiting vegetables	0.02*
240000	. Brassica vegetables (excluding brassica roots and brassica baby leaf crops)	0.02*
250000	. Leaf vegetables, herbs and edible flowers	-
251000	. (a) lettuces and salad plants	0.02*
252000	. (b) spinaches and similar leaves	0.02*
253000	. (c) grape leaves and similar species	0.02*
254000	. (d) watercresses	0.02*
255000	. (e) witloofs/Belgian endives	0.02*
256000	. (f) herbs and edible flowers	0.05*
260000	. Legume vegetables	0.02*
270000	. Stem vegetables	0.02*
280000	. Fungi, mosses and lichens	0.02*
290000	. Algae and prokaryotes organisms	0.02*
300000	. PULSES	-
300010	. Beans	0.05
300020	. Lentils	0.02*
300030	. Peas	0.05
300040	. Lupins/lupini beans	0.05
300990	. Others (2)	0.02*
400000	. OILSEEDS AND OIL FRUITS	-
401000	. Oilseeds	-
401010	. Linseeds	0.2
401020	. Peanuts/groundnuts	0.05*
401030	. Poppy seeds	0.15
401040	. Sesame seeds	0.05*
401050	. Sunflower seeds	0.05*
401060	. Rapeseeds/canola seeds	0.2
401070	. Soyabeans	0.05*
401080	. Mustard seeds	0.2
401090	. Cotton seeds	0.3
401100	. Pumpkin seeds	0.05*
401110	. Safflower seeds	0.05*
401120	. Borage seeds	0.05*
401130	. Gold of pleasure seeds	0.05*
401140	. Hemp seeds	0.05*
401150	. Castor beans	0.05*
401990	. Others (2)	0.05*
402000	. Oil fruits	0.05*

Table 6.9-2: EU MRLs set for the uses of metconazole (BAS 555 F)

Code number	Groups and examples of individual products to which the MRLs apply	Metconazole (sum of isomers)
500000	. CEREALS	-
500010	. Barley	0.4
500020	. Buckwheat and other pseudo-cereals	0.02*
500030	. Maize/corn	0.1
500040	. Common millet/proso millet	0.02*
500050	. Oat	0.4
500060	. Rice	0.02*
500070	. Rye	0.06
500080	. Sorghum	0.02*
500090	. Wheat	0.15
500990	. Others (2)	0.02*
600000	. TEAS, COFFEE, HERBAL INFUSIONS, COCOA AND CAROBS	0.1*
700000	. HOPS	0.1*
800000	. SPICES	-
810000	. Seed spices	0.1*
820000	. Fruit spices	0.1*
830000	. Bark spices	0.1*
840000	. Root and rhizome spices	-
840010	. Liquorice	0.1*
840020	. Ginger	0.1*
840030	. Turmeric/curcuma	0.1*
840040	. Horseradish	(ft)
840990	. Others (2)	0.1*
850000	. Bud spices	0.1*
860000	. Flower pistil spices	0.1*
870000	. Aril spices	0.1*
900000	. SUGAR PLANTS	-
900010	. Sugar beet roots	0.06
900020	. Sugar canes	0.02*
900030	. Chicory roots	0.02*
900990	. Others (2)	0.02*
1000000	. PRODUCTS OF ANIMAL ORIGIN -TERRESTRIAL ANIMALS	-
1010000	. Tissues from	0.02*
1020000	. Milk	0.02*
1030000	. Birds eggs	0.02*
1040000	. Honey and other apiculture products	0.05*
1050000	. Amphibians and Reptiles	0.02*
1060000	. Terrestrial invertebrate animals	0.02*
1070000	. Wild terrestrial vertebrate animals	0.02*

* Indicates lower limit of analytical determination

0233010 Melons: The European Food Safety Authority identified some information on residue trials as unavailable. When reviewing the MRL, the Commission will take into account the information referred to in the first sentence, if it is submitted by 17 August 2015, or, if that information is not submitted by that date, the lack of it.

0840040 Horseradish: The applicable maximum residue level for horseradish (Armoracia rusticana) in the spice group (code 0840040) is the one set for horseradish (Armoracia rusticana) in the Vegetables category, root and tuber vegetables group (code 0213040) taking into account changes in the levels by processing (drying) according to Art. 20 (1) of Regulation (EC) No 396/2005.

The summary report of each assessment result using the EFSA model is presented in Appendix 1A. With the current EFSA model the TMDI for the representative crops and defaults MRLs for all animal commodities ranges from 0.0 to 15.8% use of the ADI. The diet with the highest TMDI is "Denmark child" with 15.8% of the ADI. For this diet, the highest contributor is wheat with 8.3% of the ADI. The diet with the next highest TMDI is "WHO Cluster diet B" with 15.5% of the ADI where wheat is the major contributor with 12.8% of the ADI.
For all registered crops including default MRLS the TMDI ranges from 2 to 28.6% of the ADI. The diet with the highest TMDI is "UK Toddler" with 28.6% of the ADI. For this diet, the highest contributor is sugar beet (root) with 13.7% of the ADI. The diet with the next highest TMDI is "WHO Cluster diet B" with 26.2% of the ADI where wheat is the major contributor with 12.8% of the ADI.

NEDI calculations

For all models included in the EFSA model, the use of STMR or $STMR_P$ values in the estimation of the chronic dietary consumer risk is up to this point in time not necessary since the crude overestimated TMDI of metconazole was well below 100% of the ADI.

IESTI calculations

The acute dietary risk assessment was performed according to the EFSA PRIMo using EU MRLs (Table 6.9-2). The acute risk assessment showed no exceedance of the ARfD for adults and children for either IESTI calculation with unprocessed and processed commodities. The commodity with the highest use of the ARfD is banana for children (UK 4-6 year) at 83.6% ARfD and barley for Netherlands adult at 29% use of the ARfD. The use of the ARfD for the representative crops are shown below. The report is shown in Appendix 1A.

	children			adult	
	Highest use (% ARfD)	Population	Commodity	Highest use (% ARfD)	Population
banana	83.6	UK 4-6 year	barley	29	Netherlands

		children		adult
Commodity	Highest use (% ARfD)	Population	Highest use (% ARfD)	Population
Barley	7.1	UK 4-6 year	29	Netherlands
Oat	15.9	German	5.7	LT
Rye	3.8	UK infant	2.9	LT
wheat	21.7	UK 4-6 year	11.7	UK vegetarian
Oilseed rape seed	2.2	German	0	-
Wheat flour	17.7	German		
Wheat bread/pizza			6.6	Italian adult

Toxicological Relevance of Metabolites

Report: CA 6.9/1
Panek M., 2015a
Metconazole (BAS 555 F): Toxicological relevance of metabolites
2014/7000203
Guidelines: None
GLP: no

Executive Summary

The EFSA Scientific Opinion on Evaluation of the Toxicological Relevance of Pesticide Metabolites for Dietary Risk Assessment proposed options to assess toxicological relevance and the need for further toxicological testing of pesticide metabolites. In this discussion paper the Threshold of Toxicological Concern (TTC) approach described in the Scientific Opinion is used to evaluate possible required testing of metconazole metabolites found in plant and animal matrices. Using the exposure threshold levels of 0.0000025 mg/kg bw day for genotoxicological testing and 0.0003 mg/kg bw/day specified in the EFSA Scientific Opinion for further testing of metabolites, the need for possible further testing of metconazole metabolites is assessed. The level of consumer exposure to all identified metabolites relevant in plant or animal was assessed for the populations in the EFSA acute_chronic_RA PRIMo model with inputs of residue levels of metabolites measured in residue trials or feeding studies and extrapolation to metabolites not measured. The consumer exposure to a calculated residue level of total parent and metabolites was compared to the current metconazole chronic and acute reference doses.

The consumer exposure to the triazole derivative metabolites (TDMs) will not be considered as the assessment of these metabolites will be based on the outcome of the ongoing UK assessment.

I. MATERIAL AND METHODS

A. MATERIALS

Not relevant.

B. STUDY DESIGN AND METHODS: Part 1

1. Test procedure

Generally all metabolites, for which there is a defined structure and which are not per se excluded from the TTC concept, have to be taken into consideration

- Rat metabolites
- Plant metabolites
- Rotational crop metabolites
- Livestock metabolites

The metabolites identified in two goat metabolism studies submitted and evaluated in the peer review process and a hen metabolism study submitted with the dossier for renewal of the active substance (M-CA 6.2) and plant metabolism studies in wheat, oilseed rape (peer reviewed) and a banana study submitted with the dossier for renewal of the active substance (M-CA 6.2) were reviewed. Metconazole was not degraded during processing based on the high temperature hydrolysis study. Levels of metabolites from rotational crop were not considered relevant since levels of metconazole found in the field rotational crop were below the LOQ of 0.01 mg/kg as cited in the following excerpt from the EFSA Conclusion (EFSA 2006), and the levels of metabolites found in the confined rotational crop study were always significantly less than the level of parent metconazole. A fish metabolism study was not performed since the anticipated metconazole residues in fish feed items were less than the trigger specified in the draft guidance. The identified metabolites were compared to the metabolites identified in the two rat metabolism studies evaluated in the peer review process

Note that metabolites are derived from *cis*-metconazole unless otherwise noted since the formulated product contains at least 80% *cis*-isomer, and thus the metabolism studies were conducted with 80:20 or 85:15 cis:trans metconazole. The short metabolite codes, such as M1 or M30, used in this discussion are consistent with the codes used in the metabolism studies. The formal BASF codes, BASF Reg. No. (signify a synthesized metabolite) and the CL number used in early metabolism studies are included in the metabolite table below with structures to allow easy cross-reference.

Table 6.9-3: Metconazole metabolites and codes

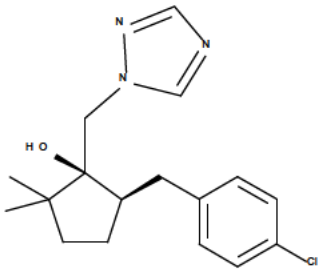
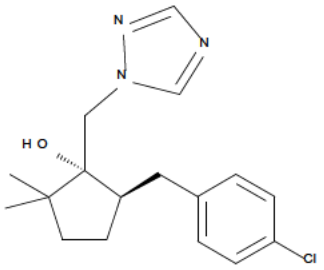
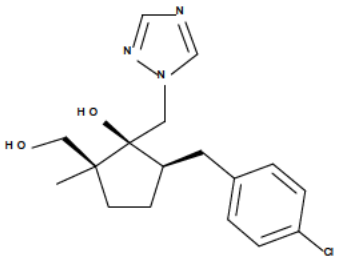
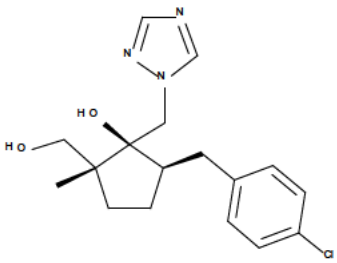
Metabolite designation		Chemical Name	CAS-No.	CL No.	Structure
Code	Other code (Reg. No.)	IUPAC Name			
M555F000 cis	4079468	cis-1-(1H-1,2,4-triazole-1-ylmethyl)-2,2-dimethyl-5-(4-chlorobenzyl)cyclopentanol	115850-27-6	CL 354801	
M555F000 trans	4079654	trans-1-(1H-1,2,4-triazole-1-ylmethyl)-2,2-dimethyl-5-(4-chlorobenzyl)cyclopentanol	115850-28-7	none	
M555F001 cis	M1 (4111795)	(1SR,2SR,5RS)-5-(4-chlorobenzyl)-2-(hydroxymethyl)-2-methyl-1-(1H-1,2,4-triazol-1-ylmethyl)cyclopentanol	155413-23-3	CL 359451	
M555F002 cis	M2 (4111882)	(1SR,2RS,5RS)-5-(4-chlorobenzyl)-2-(hydroxymethyl)-2-methyl-1-(1H-1,2,4-triazol-1-ylmethyl)cyclopentanol	155488-30-5	CL 359452	

Table 6.9-3: Metconazole metabolites and codes

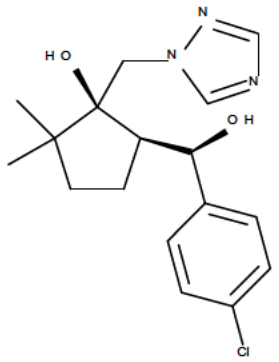
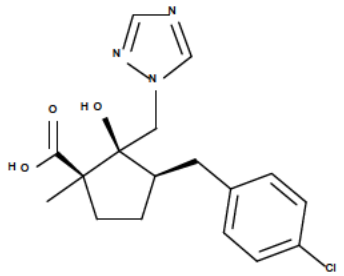
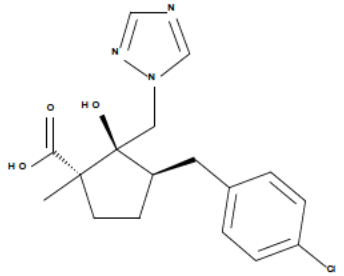
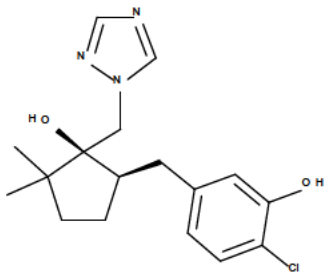
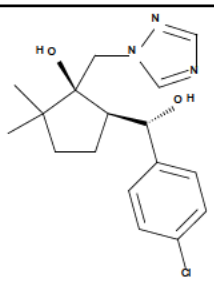
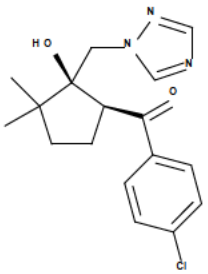
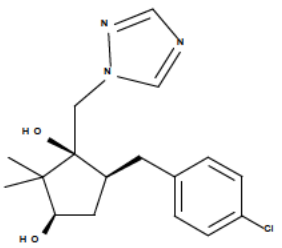
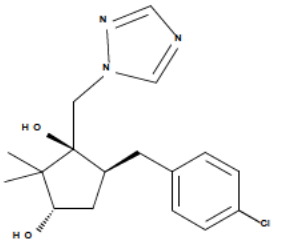
Metabolite designation		Chemical Name	CAS-No.	CL No.	Structure
Code	Other code (Reg. No.)	IUPAC Name			
M555F011 cis	M11 (4111112)	(1RS,5SR)5-[(SR)-(4-chlorophenyl)(hydroxy)methyl]-2,2-dimethyl-1-(1H-1,2,4-triazol-1-ylmethyl)cyclopentanol	153322-43-1	CL 382390	
M555F012 cis	M12 (4543815)	(1RS,2SR,3RS)-3-(4-chlorobenzyl)-2-hydroxy-1-methyl-2-(1H-1,2,4-triazol-1-ylmethyl)cyclopentanecarboxylic acid	none	CL 359138	
M555F013 cis	M13 (4543816)	(1SR,2SR,3RS)-3-(4-chlorobenzyl)-2-hydroxy-1-methyl-2-(1H-1,2,4-triazol-1-ylmethyl)cyclopentanecarboxylic acid	none	CL 359139	
M555F015 cis	M15	(1RS,5SR)-5-(4-chloro-3-hydroxybenzyl)-2,2-dimethyl-1-(1H-1,2,4-triazol-1-ylmethyl)cyclopentanol	171438-41-8	CL 359453	

Table 6.9-3: Metconazole metabolites and codes

Metabolite designation		Chemical Name	CAS-No.	CL No.	Structure
Code	Other code (Reg. No.)	IUPAC Name			
M555F021 cis	4558878	(1RS,5SR)-5-[(RS)-(4-chlorophenyl)(hydroxy)methyl]-2,2-dimethyl-1-(1H-1,2,4-triazol-1-ylmethyl)cyclopentanol	153208-75-4	CL 382391	
M555F030 cis	M30 (4110625)	(1RS,5SR)-5-(4-chlorobenzoyl)-2,2-dimethyl-1-(1H-1,2,4-triazol-1-ylmethyl)cyclopentanol	153208-73-2	CL 382389	
M555F031 cis	5968488	(1RS,3SR,5RS)-5-(4-chlorobenzyl)-2,2-dimethyl-1-(1H-1,2,4-triazol-1-ylmethyl)cyclopentane-1,3-diol			
M555F032 cis	5968479	(1RS,3RS,5RS)-5-(4-chlorobenzyl)-2,2-dimethyl-1-(1H-1,2,4-triazol-1-ylmethyl)cyclopentane-1,3-diol			

Metabolites to consider in plant matrices

Based on metabolism studies in wheat, oilseed rape and banana, the metabolites that might occur in plant commodities consumed by humans are M1 and M11 (Table 6.9-4). Metabolites that might occur in animal feed items are M11, M21 and M30 (Table 6.9-5). M1, as a conjugate, was detected in the rat metabolism study in urine of male and female rats. M11, M21 and M30 metabolites were not detected in rat urine. The bile was not analyzed for metabolites.

Metabolites to consider in animal matrices

Based on metabolism studies in ruminant the metabolites that might occur through degradation of metconazole in ruminant commodities are M1 and M1 conjugates, M12, M31 and M31conjugates and M32 and M32 conjugates (Table 6.9-6). Identified metabolites that might occur through degradation of metconazole in hen commodities are M1, M2, M31, M32 and M32 conjugates. Metabolites in hen commodities that were characterized but not identified are CM-2, dihydroxy metconazole (DHM) sulfate, monohydroxy metconazole (MHM) sulfate and metconazole dicarboxylic acid (DCM) (Table 6.9-7). Metabolites M1 and M12 were present in rat urine at low levels, M1 as a conjugate in male and female rats; M12 at 2% AD in male and 7% AD in female rats.

Table 6.9-4: Metabolites found in in plant metabolism studies in items for human consumption

	Wheat grain [1)]		Oilseed rape seed [1)]		Banana Pulp [1)]		Banana peel [1)]		Whole banana [1)]		Rat urine
Component	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	% AD
Metconazole	nd na	nd na	**0.57** *0.72*	**24** *39*	**0.52** *0.70*	**86** *89*	**1.4** *2.2*	**97** *87*	**1.2** *0.8*	87 86	nd
M1	nd na	nd na	nd	nd	**0.014** *0.014*	**2.3** *1.7*	**0.014** *0.022*	**0.87** *0.87*	**0.017** *0.01*	**1.2** *0.06*	as conjugates [2)]
M2	nd na	nd na	nd	nd	nd	nd	nd	nd	nd	nd	nd
M11	nd na	nd na	**0.1** *0.15*	**4.3** *8.3*	**0.01** *0.016*	**1.7** *2.1*	**0.023** 0.04	**1.4** *1.56*	**0.017** 0.016	**1.3** *1.7*	nd
M21	nd na	nd na	nd	nd	nd	nd	nd	nd	nd	nd	nd
M30	nd na	nd na	nd	nd	nd	nd	nd	nd	nd	nd	nd
1,2,4-triazole	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	5 (m)
Triazolyl alanine	**0.46**	**69**	**0.96**	**40**	**0.02**	**3.5**	**0.01**	**0.64**	**0.017**	**1.2**	nd
Triazolyl acetic acid	**0.16**	**25**	nd	nd	nd	nd	nd	nd	nd	nd	nd

nd = not detected; na = not analyzed

1) **Bold face:** study done with ^{14}C- triazole label; Italic *font:* study done with ^{14}C- chlorophenyl label; Normal font: study done with ^{14}C- cyclopentyl label

2) Mixture not resolved after acid treatment of urine (MK-440-009)

Table 6.9-5: Metabolites found in in plant metabolism studies in animal feed items

	Wheat straw [1)]		Oilseed rape foliage [1)] 0 - 42 DALA		Oilseed rape pod[1)]		Rat urine
Component	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	% AD
Metconazole	**1.2** 2.1	**19** 35	**4.8-11.5** 3.6 -12.8	**55-93** 61-96	**3.3** 8.30	**16** 40	nd
M1	nd	nd	nd	nd	nd	nd	conjugates[2)]
M2	**<0.25**	**<4**					
M11	0.59	10	**0-0.4** 0.05-0.16	**0-2.0** 0.5-1.4	**0.33** 0.32	**1.7** 1.5	nd
M21	0.59	10	nd	nd	nd	nd	nd
M30	**0.1** <0.21	**1.7** <3.5	nd	nd	nd	nd	nd
1,2,4-triazole	nd	nd	nd	nd	nd	nd	5(m)
Triazolyl alanine	nd	nd	1.15	5.6	1.3	**6.7**	nd
Triazolyl acetic acid	nd	nd	nd	nd	nd	nd	nd

nd = not detected; na = not analyzed
1) **Bold face:** study done with ^{14}C- triazole label; *Italic font:* study done with ^{14}C- chlorophenyl label; Normal font: study done with ^{14}C- cyclopentyl label
2) Mixture not resolved after acid treatment of urine (MK-440-009)
< indicates a mixture of metabolites

Table 6.9-6: Metconazole metabolites identified in goat metabolism studies[1)]

	Liver [2)]		Kidney [2)]		Rat urine
Component	mg/kg	% TRR	mg/kg	% TRR	% AD
Metconazole	0.11-0.21	33-37.5	0.003	1.0-1.7	nd
M1	0.22	7	0.02-0.04	12-13.7	as conjugates[3)]
M2	0.004	1.2	0.004-.01	3-3.6	as conjugates[3)]
M12	nd	nd	0.02-0.055	13.4-20	2(m), 7(f)
M13	nd	nd	0.003	1.0	1 (m)
M15	nd	nd	0.004	3,0	as conjugates[3)]
M31[4)]	0.06	20.1	0.02	15.2	nd
M32[4)]	0.013	4.1	0.011	7.7	nd

1) composite of two studies with ^{14}C-cyclopentyl label
2) Residues in fat, muscle and milk were <0.01 mg/kg at feed burdens of 11-25 mg/kg dry feed and the matrices were not analyzed further
3) Mixture not resolved after acid treatment of urine (MK-440-009)
4) Free and conjugate sulfate and glucuronide conjugates of hydoxylated metconazole

Table 6.9-7: Metconazole metabolites identified in laying hen metabolism study [1)]

	Liver		Muscle		Egg		Rat urine
Component	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	% AD
Metconazole	0.034	4.3	0.005	9.8	0.006	8.6	nd
M1[3)]	0.128	16.2	0.003	10	0.005	8.3	as conjugates[2)]
M2	nd	nd	nd	nd	nd	nd	as conjugates[2)]
M12	0.091	11.5	nd	nd	nd	nd	2(m), 7(f)
M13	nd	nd	nd	nd	nd	nd	1 (m)
M15	nd	nd	nd	nd	nd	nd	as conjugates[2)]
M31[3)]	0.064	8.1	0.003	10	0.005	8.3	nd
M32	0.047	5.9	nd	nd	nd	nd	nd
M32 sulfate	0.031	3.9	0.003	10.3	0.008	13.9	nd
CM-2 [4)]	nd	nd	0.003	8.5	0.007	13.7	
DHM sulfate [4)]	0.015	1.9	0.003	9.9	0.001	0.9	
MHM sulfate [4)]	0.012	1.5	0.006	18.0	0.009	15.3	
DCM [4)]	0.073	9.3	nd	nd	nd	nd	
1,2-4 triazole			0.112	77.2			5 (m)

1) Levels are based on cyclopentyl label since slightly higher residues were found with this label
2) Mixture not resolved after acid treatment of urine (MK-440-009)
3) M1 and M31 coeluted; the peak was considered to be 50:50 M1:M31
4) Characterized

2. Description of analytical procedures

Determination of residue levels of M1, M11, M21 and M30 in plant commodities

In residue field trials performed with metconazole in a wide range of areas in the United States and Canada, the commodities of cereals, soybean, sugar beet and cotton were analyzed for metconazole, (cis- and trans- isomers), and metabolites M11, M21 and M30. The U.S. trials were the only source of actual measured residues of metabolites. Though the trials were performed in the U.S., the residue levels for the metabolites were taken as representative of expected metabolite residues in cereals treated in the EU since the U.S. cGAP was just slightly higher than the cGAP for the EU. The metabolite residues measured in the U.S. soybean trials with a similar cGAP were extrapolated to oilseed and dry pea and bean commodities in the EU. The U.S. residue studies supporting this assessment were submitted and evaluated in requests for import tolerances as reviewed by the Rapporteur Member State Belgium and EFSA (EFSA 2010) and MRL Review (EFSA 2011). Since these studies are considered as not peer reviewed, the residue studies are submitted with this dossier and are summarized in M-CA 6.10.

The U.S. commodities of cereals, soybean, sugar beet and cotton were analyzed for metconazole, (cis- and trans- isomers), and metabolites M11, M21 and M30 using BASF Analytical Method D0508. The method limit of quantitation (LOQ) was 0.005 mg/kg for each metconazole parent isomer and 0.01 mg/kg for each metabolite analyte, and the method limit of detection (LOD) was 0.002 mg/kg. Within each U.S. study, the details of the analysis are reported in the analytical detail tables including measurements reported as not detected (ND). In the final report summary tables, the level of an analyte measured as ND is reported as <0.01 mg/kg, the LOQ. Within this assessment, for commodities in which the measured level of metabolite residues of M11, M21 or M30 was >LOD but <LOQ of 0.01 mg/kg, the level of metabolite residue was set to 0.01 mg/kg. For commodities in which the measured level of metabolite residue was <LOD of 0.002 mg/kg, the residue level was set to 0.002 mg/kg. For commodities which are not blended, the highest measured residue level for the metabolite was used. For blended commodities the median measured residue level of the metabolite was used. For commodities in which the level of metabolites M11, M21 and M30 were not analyzed, the metabolite residue level was extrapolated from that measured in related US crops, taking into account the most closely related metabolism study in wheat, oilseed rape or banana.

The metabolite M1 was not measured in any residue trials. Since M1 was identified in the banana metabolism, the metabolite M1 level was estimated for the imported commodities of peach, apricot, cherry, banana and melon. A level was estimated in based on the ratio of %TRR of M1 to % TRR parent in the banana pulp. The resulting conversion factor was set as M1:metconazole = 2.3:86 = 0.0267. This conversion factor was applied to the measured high residue for each fruit commodity.

The levels and extrapolations in plant commodities are explained in detailed comments in Table 6.9-10.

Determination of residue levels of metabolites M1, M12, M31 & M32 in animal commodities

Ruminant

A cow feeding study which measured the levels of M1 and M12 in liver and kidney was submitted and evaluated in EFSA Reasoned Opinion (EFSA 2010). Since the study is considered as not peer reviewed, the study is submitted with this dossier (M-CA 6.4). The feeding study, performed with metconazole (80:20 cis:trans), used feeding levels of 5 mg/kg dry feed (0.2 mg/kg bw/d), 15 mg/kg dry feed (0.57 mg/kg bw/d) and 50 mg/kg dry feed (1.66 mg/kg bw/d).

In order to compare the expected metabolite residues at actual feeding levels, the anticipated maximum feed burden for parent metconazole was determined using the EU methodology. In the recent EFSA Scientific Opinion on modification of existing MRLs for barley, the animal feed burden was calculated as dairy cattle 1.5 mg/kg dry feed and beef cattle 3.67 mg/kg dry feed. In the dossier for renewal of the active substance (M-CA 6.7) the animal feed burden was calculated using only the representative crops. However this assessment is based on all possible feed items including imported sugar beet. The feed burden was determined for dairy cattle 1.6 mg/kg dry feed (0.057 mg/kg bw/d) and beef cattle 3.82 mg/kg dry feed (0.164 mg/kg bw d). The inputs and the results of the feed burden calculation are summarized (M-CA 6.7).

Ruminant meat, fat, milk

In the cow feeding study, residues in muscle, fat and milk were very low and residues of metconazole were <0.01 mg/kg at the 50 mg/kg feed level. The metabolites in the matrices of meat, fat or milk were not quantitated in the metabolism studies due to total radioactive residues (TRR) measured at <0.01 mg/kg. Since parent levels were <0.01 mg/kg at the highest cow feeding level of 50 mg/kg, the levels of all metabolites M1, M12, M31 or M32 were considered to be zero mg/kg level in these matrices.

Ruminant kidney and liver

Within the cow feeding study, the levels of metabolites M1 and M12 were measured in liver and kidney (MCA 6.4), the matrices in which they were found in the metabolism studies. The analysis method included acid hydrolysis to convert the conjugates to the free M1. In the cow feeding study, residues of M1 and M12 in liver were <0.01 mg/kg at the 5 and 15 mg/kg feeding level. The maximum dietary burden determined in this dossier is 3.8 mg/kg bw/day. Therefore the metabolite level of M1 or M12 was considered to be at zero mg/kg in liver. In kidney, the residues of M1 and M12 were <0.01 mg/kg at the 5 mg/kg feeding level (0.2 mg/kg bw/d). Since the expected maximum feed burden is 3.8 mg/kg (0.16 mg/kg bw d), the levels for M1 and M12 in kidney and edible offal were set to 0.01 mg/kg for all ruminants.

Residues of M31 and the M31 conjugates and M32 and the M32 conjugates were found in the goat metabolism in kidney and liver, but residues of M31 or M32 were not measured in the cow feeding study. To determine the expected exposure to M31 and conjugates, the ^{14}C-residues of M31 can be compared to total ^{14}C-residues of M1 and M1 conjugates based on results found in the goat metabolism study (Table 6.9-8). Based on the metabolism study the amount of M31 (includes conjugates) is 2.9 times the level of M1 in the liver. As stated above, the maximum anticipated dietary burden for beef cattle is 3.8 mg/kg dry feed. The lowest feeding level used in the cow feeding study was 5 mg/kg dry feed. A review of the levels of M1 found in the cow feeding study showed that residues of M1 were <0.01 mg/kg at both the 5 mg/kg and 15 mg/kg feeding levels. Residues of M31 were set to 0.01 mg/kg for liver in the dietary evaluation since the level of M31 was 2.9 times the M1 level. In the cow feeding study, residues of M1 in kidney were <0.01 mg/kg at the 5 mg/kg feeding level; residues of M31, 1.3 times the M1 level in kidney, were set to 0.01 mg/kg in kidney. In the metabolism study, residues of M32 were 0.7 and 0.6 the level of M1 in liver and kidney, respectively. Residues of M32 were set to zero mg/kg in liver and kidney based on the comparison to levels of M1.

Table 6.9-8: Comparison of levels of metabolites M1, M31 and M32 found in goat metabolism study

Metabolite	Liver		Kidney	
	% TRR	mg/kg	% TRR	mg/kg
Metconazole Total	32.9	0.098	1.7	0.003
Total M1 (includes conjugates)	6.9	0.022	12.1	0.018
M-31 (includes conjugates)	20.1	0.063	15.2	0.022
Ratio of M31 to total M1	2.9		1.3	
M-32 (includes conjugates)	4.1	0.013	7.7	0.011
Ratio of M32 to total M1	0.59		0.64	

Hen

A hen feeding study is not submitted, but a hen metabolism study is available in the dossier for renewal of the active substance (M-CA 6.2) which used feeding levels of 14 mg/kg feed (cyclopentyl label) and 12.6 (triazole label). The maximum anticipated animal feed burden is calculated for poultry as 0.071 mg/kg dry feed (0.0045 mg/kg bw/d) (M-CA 6.7). The possible residues in all poultry matrices were extrapolated from the residues in the metabolism studies based on an overdosing factor of 200 and 180 (shown in Table 6.9-9), respectively. The highest residue level was 0.0006 mg/kg for metabolite M1 in liver, and all other levels were lower with levels in muscle and egg lower than 8 x 10^{-5} mg/kg. Therefore all metabolite residues in poultry matrices were considered as zero when determining exposure to each metabolite.

Swine

The animal feed burden was calculated for swine as 0.137 mg/kg dry feed (0.005 mg/kg bw/d) (M-CA 6.7). The metabolism of metconazole in ruminant and rat is consistent and can be extended to swine. All metabolite residues in swine matrices were considered as zero when determining exposure to each metabolite due to the extremely low maximum feed burden.

Table 6.9-9: Calculated metabolite levels in hen matrices at the maximum anticipated feed burden (0.071 mg/kg dry feed)

	Liver	Breast	Thigh	Fat	Skin	Egg white	Egg yolk	Whole egg
	Mg/kg							
M-1	6.4E-04							
M-1 (+ M-31)		3.33E-05	4.0E-05	0.000125	9.0E-05	6.11E-05	0.000111	7.78E-05
M-12 (+ CM-2/3)	4.50E-04							
M-31	3.20E-04							
M-32 (+ CM-1)	2.35E-04							
M-32 sulfate	1.55E-04	1.67E-05	3.33E-05	7.78E-05	2.22E-05	3.89E-05	2.78E-05	3.89E-05
CM-2		1.11E-05	1.67E-05	3.89E-05	2.22E-05	0.00005	2.22E-05	4.44E-05
DHM sulfate	7.50E-05	1.67E-05	1.11E-05	3.33E-05	1.00E-05		1.11E-05	5.56E-06
MHM sulfate	6.00E-05	2.78E-05	2.22E-05	0.00005	1.67E-05	5.56E-05	7.22E-05	6.11E-05
MHM-4		1.11E-05	1.11E-05	2.22E-05	1.67E-05	2.78E-05		
MHM (1-2 isomers)							3.89E-05	2.78E-05

Determination of residues of M11 in animal commodities

Since the plant metabolites M11 and M21 were found in straw in the wheat metabolism study, the maximum anticipated animal feed burden for M11 was determined based on the expected residue levels of M11 in animal feed items. The inputs for the animal feed burden calculation were estimated considering residues in cereal straw and canola seed. In the metabolism study, the level of the metabolite M21 was equal to the level of metabolite M11 in wheat straw, so the calculations for M11 can also be used for M21. The calculation of the feed burden was performed using EFSA Profile. For beef cattle the M11 feed burden corresponded to a feeding level of 0.645 mg//kg dry feed. For dairy cattle the M11 feed burden corresponded to a feeding level of 0.27 mg//kg dry feed. The polarity and structure of M11 is similar to the parent compound so the reasonable expectation is that the ruminant will metabolize M11 analogous to the metabolism of metconazole. The feeding study performed with metconazole used feeding levels of 5 mg/kg, 15 mg/kg and 50 mg/kg of dry feed. The expected feed burden for M11 is about 10% the lowest feeding level used in the study. Therefore, for all animal matrices, the level of M11 or M21 due to intake of these metabolites in crops were considered as zero when determining exposure to each metabolite.

For poultry the exposure to M11 could occur in oilseed and oilseed process fractions. However the level of exposure to total residues in hen matrices is extremely low; therefore residues from poultry were considered as zero when determining exposure to M11.

The levels and extrapolations in animal commodities are explained in detailed comments in Table 6.9-11.

II. RESULTS AND DISCUSSION

Estimation of exposure through diet to metconazole metabolite residues

The metabolite residue values summarized in Table 6.9-10 for plant and Table 6.9-11 for animal matrices were used as input levels for the exposure assessment for each metabolite using the EFSA acute chronic_RA_PRIMo, (Rev. 2.00). The same input values were used in the chronic and acute assessments. Within the EFSA model, the acceptable daily intake was first set at the genotoxic threshold of 0.0000025 mg/kg bw/day. If the genotoxic threshold was exceeded, a second analysis was performed with the acceptable daily intake (ADI) and acute reference dose set at the threshold of 0.0003 mg/kg bw/day.

Results of exposure assessments of M11, M21, M30, M1, M12 and M31 and evaluation of the need for further toxicological testing

Dietary exposure to metabolite M11

In the rat metabolism studies, metabolite M11 was not detected in rat urine and the bile was not analyzed for metabolites. The structure of M11 is similar to that of parent. As calculated using the EFSA_acute_chronic_PRIMO model, (Rev. 2), the exposure to metabolite M11 exceeded the genotoxic threshold of 0.0000025 mg/kg bw/day for most populations except PL general population (Appendix Table 1A-4). The chronic exposure to metabolite M11 was then assessed against the threshold of 0.0003 mg/kg bw/day, and the chronic exposure was acceptable (Appendix Table 1A-5). The maximum chronic exposure was for the Denmark child diet at 37.6% of the reference dose set at 0.0003 mg/kg bw/day. The residue inputs for the chronic and acute assessments for metabolites were the same. The acute exposure assessed against a threshold dose of 0.0003 mg/kg bw/day indicated acceptable utilization for all commodities with the highest use of the dose for melon at 51% (Appendix Table 1A-6).

Dietary exposure to metabolite M21 or M30

In the rat metabolism studies, metabolite M21 or M30 was not detected in rat urine, and the bile was not analyzed for metabolites. The structures of M21 and M30 are similar to that of parent. Though the levels of M21 and M30 were found to be lower in both the metabolism studies and in the matrices measured in the field, the results for M11 were extrapolated to M21 and M30. Exposure to each metabolite was judged as exceeding the genotoxic threshold of 0.0000025 mg/kg bw/day and falling below the chronic and acute exposure of 0.0003 mg/kg.

Dietary exposure to metabolite M1

Conjugates of the metabolite M1 were present in urine in male at 5% administered dose (AD) and in female at 14% AD. The structures of M1 is similar to that of parent.

Metabolite M1 was found in the banana metabolism and goat metabolism in liver and kidney. The metabolite M1 was not analyzed in residue studies performed in whole banana and pulp or in other fruit matrices, so the residue level was extrapolated from measured parent residues in banana. For banana, the total residues of parent were <0.1 mg/kg (method LOQ = 0.05 mg/kg for each analyte). Levels of parent in pulp in the metabolism study were about 60% of the parent residue levels in whole banana. Levels of the metabolite M1 were 2.3% TRR compared to 86% TRR for total parent. Therefore the expected level of M1 in banana was calculated as follows:

M1 = 2.3/86 x 0.05 mg/kg (parent in pulp) =0.0013 in banana, melon pulp

Residues were entered for peach, apricot, cherry and melon based on similar extrapolations using the highest total parent residue measured in these commodities.

M1 = 2.3/86 x 0.09 mg/kg (parent HR) =0.0024 in peach, apricot

M1 = 2.3/86 x 0.16 mg/kg (parent HR) =0.004 M1 in cherry

Residues of M1 were set to zero mg/kg for ruminant liver and 0.01 mg/kg in kidney and inedible offal in the dietary evaluation as discussed in Section 1.3. The level of 0.01 mg/g in kidney and offal is set at a conservative level since the measured residues in kidney in the cow feeding study were <0.01 mg/kg at a comparable feeding level.

The chronic exposure exceeded the genotoxic threshold of 0.0000025 mg/kg bw/day for ten subpopulations (Appendix Table 1A-7). The chronic exposure did not exceed 0.0003 mg/kg bw/day (Appendix Table 1A-8). The acute exposure was acceptable for all commodities at the reference dose of 0.0003 mg/kg bw/day.

The metabolite M1 exceeds the genotoxic threshold for a small number of populations. Considering that this metabolite was present in rat urine and that the assessment used conservative estimates in levels in animal matrices based on maximum feed burdens, testing would not seem justified based on the exceedance of the very low genotoxic threshold for a small number of populations using a conservative risk assessment.

Dietary exposure to metabolite M12

Metabolite M12 were present in urine in male at 2% administered dose (AD) and in female at 7% AD. The structure of M12 is similar to that of parent.

The metabolite M12 was not found in any plant metabolism studies. Levels of M12 in the cow feeding study (MCA 6.4) were <0.01 mg/kg in liver at all feeding levels. Therefore residues of M12 were considered zero for liver in the dietary evaluation. Residues of M12 in kidney were <0.01 mg/kg at the 5 mg/kg feeding level; residues of M12 were set to 0.01 mg/kg in ruminant kidney and inedible offal in the dietary evaluation.

Chronic exposure to metabolite M12 was about 99% of the genotoxic threshold of 0.0000025 mg/kg bw/day, and acute exposure was well below the threshold of 0.0003 mg/kg for the limited commodities of animal kidney and offal (Appendix Table 1A-9).

Dietary exposure to metabolite M31 and M31 conjugates

Metabolite 31 was not present in rat urine and the bile was not analyzed for metabolites. The structure of M31 is similar to that of parent.

The metabolite M31 was not found in any plant metabolism study. Total residues of M31 and the M31 conjugates were set at 0.01 mg/kg in ruminant liver and zero in ruminant kidney as discussed above. The exposure exceeds the genotox trigger for one population, the WHO diet, as shown below and in Appendix Table 1A-10. Chronic exposure is less than the threshold of 0.0003 mg/kg bw/day.

Highest calculated TMDI values in % of ADI	MS Diet	Highest contributor to MS diet (in % of ADI)	Commodity / group of commodities	2nd contributor to MS diet (in % of ADI)	Commodity / group of commodities	3rd contributor to MS diet (in % of ADI)	Commodity / group of commodities
128.7	WHO Cluster diet B	58.7	Bovine: Edible offal	29.3	Bovine: Liver	29.3	Bovine: Liver

Acute exposure was below the threshold of 0.0003 mg/kg with the highest utiliztion for bovine liver at 27%.

Dietary exposure to metabolite M32 and M32 conjugates

Metabolite 32 was not present in rat urine and the bile was not analyzed for metabolites. The structure of M32 is similar to that of parent.

The metabolite M32 was not identified in any plant metabolism study. Residues of M32 and the M32 conjugates were found in the goat metabolism in kidney and liver at levels significantly less than M31 (Table 6.9-11). Since the level of M31 exposure just exceeded the threshold for genotoxic testing for one population, by comparison the exposure to residues of M32 would be less than the genotoxic threshold of 0.0000025 mg/kg bw/day and acute exposure is acceptable in all matrices.

III. CONCLUSION

Conclusion on required toxicological testing for metabolites

According to evaluation of metconazole metabolites using the threshold values proposed in the Scientific Opinion on the toxicological relevance of metabolites, in-vitro genotox and minimum in vivo testing should be performed for the metconazole metabolites M11, M21, M30 and M31.

The metabolite M1 exceeds the genotoxic threshold for a small number of populations. Considering that this metabolite was present in rat urine in male at 5% administered dose (AD) and in female at 14% AD, testing would not seem justified based on the exceedence of the very low genotoxic threshold for a small number of populations. Exposure to M12 and M32 does not exceed the genotoxic threshold.

Acute exposure was acceptable in all commodities for all metabolites.

B. STUDY DESIGN AND METHODS Part 2

For the assessment of dietary risk to the consumer that takes into account exposure to the total residues of parent and metabolites found in plant and animal matrices, the total residues of parent metconazole and metabolites for each commodity was calculated and the total residue value for each commodity was entered into the EFSA_acute_chronic_RA_ model (Rev 2.00). The exposure for the range of populations included in the model was compared to the ADI and ARfD of parent metconazole. The EFSA model was used since it considers all the different diets and all consumer groups in the EU.

1. Test procedure

Determination of the total residue level for each commodity

The MRL was used for the parent level in all plant and animal matrices rather than the measured high or median residue to provide a conservative assessment. The MRLs are those adopted by the European Commission for commodities with registrations or import tolerances. Default MRLs for plant commodities with no registrations or import tolerances were not used. Default MRLs for animal commodities were used and adjusted as needed to take account of metabolite levels.

The strategy described in Part 1 above was used to estimate the expected level of each metabolite in a crop or animal commodity. The levels and extrapolations used for the input of the exposure calculations are summarized, and the total of parent and metabolite residues are calculated for plant (Table 6.9-10) and for animal commodities (Table 6.9-11). Though metabolite M30 is not a metabolite detected in commodities consumed by humans, the metabolite residue level of 0.002 mg/kg was included in the total residue for those commodities measured in U.S. trials. The same input values were used in the chronic and acute assessments.

II. RESULTS AND DISCUSSION

Acceptable Daily Intake (ADI) and Dietary Exposure Calculation

Using the current EFSA model, the chronic risk assessment ranges from 0.5% ADI for the general PL population to 28.6% of ADI for the "UK Toddler" (Appendix Table 1A-11). For the UK toddler diet, the highest contributor is sugar beet (root) with 15.1% of ADI. The diet with the second highest use of the ADI is that of the “DK child” with 24.4% of ADI where wheat is the major contributor (9.9% of ADI).

A TMDI assessment was also performed with the MRLs (no default MRLs for plant commodities with no registrations or import tolerances) to compare results to the exposure that takes into account the metabolites. The chronic risk assessment ranges from 0.5% ADI for the general PL population to 27.3% of ADI for the "UK Toddler". For the UK toddler diet, the highest contributor is sugar beet (root) with 15.1% of ADI. The diet with the second highest use of the ADI is that of the “UK infant” with 22.1% of ADI where milk and cream is the major contributor (7.7% of ADI).

Acute Reference Dose (ARfD) and Dietary Exposure Calculation

In an acute assessment taking into account total residue of parent and metabolites, the commodity with the highest use of the acute reference dose is banana at 85.3% of the ARfD (Appendix Table 1A-12). The second highest is melon at 78.9% ARfD. In an acute assessment using only parent MRL, the highest use of the acute reference dose is banana at 83.6% of the ARfD; the second highest is melon at 75.8% ARfD.

Residue input into PRIMo	Highest use % ADI	2nd highest use % ADI	Highest % ARfD
Input of total residues of parent & metabolites	UK toddler 28.6	DK child 24.4	Banana 85.3
Input of residues of parent	UK toddler 27.3	UK infant 22.1	Banana 83.6

III. CONCLUSION

Conclusion on dietary exposure to total metconazole residue

According to the presented chronic assessment, a long-term intake of metconazole residues, considering exposure to a total of metconazole and metconazole metabolites, is unlikely to present a public health concern. Dietary assessment using the current residue definition of metconazole is sufficient to determine consumer safety.

According to the presented acute assessment, a short term intake of metconazole residues, considering exposure to a total of metconazole and metconazole metabolites, is unlikely to present a public health concern. Dietary assessment using the current residue definition of metconazole is sufficient to determine consumer safety.

Table 6.9-10: Residue levels for Metconazole Metabolites M1, M11, M21 and M30 in plant matrices

Code no.	Commodity	Total	Parent MRL	M11	M21 or M1[1]	M30	Comment on derivation of metabolite residue level
		mg/kg					
120000	Tree nuts	0.05	0.05	0.002	0.002	0.002	NAFTA Application rate: 2 x 280 g/ha, 14 d PHI Measured: all samples cis met. <0.01 trans met.<0.01 Metabolites not measured
140000	Stone fruit						Metabolites not measured in NAFTA studies Extrapolate from banana metabolism; no M21 or M30 in banana metabolism ratio of TRR for M11/parent = (1.7 / 86) ratio of TRR for M1/parent = (2.3 / 86)
140010	Apricots	0.104	0.1	0.002	**0.0024**	0	Extrapolate M11 and M1 levels from peaches
140020	Cherries	0.207	0.2	0.003	**0.004**	0	cherries (sweet and sour) PHI of 14 days Parent HR = 0.16 mg/kg (STMR = 0.05 mg/kg) M11 = 0.16 x 1.7/86 = 0.003 mg/kg M1 = 0.16 x 2.3/86 = 0.004
140030	Peaches	0.104	0.1	0.002	**0.0024**	0	For peaches PHI of 14 days Parent HR = 0.09 mg/kg (STMR = 0.03 mg/kg) M11 = 0.09 x 1.7/86 = 0.0018 M1 = 0.09 x 2.3/86 = 0.0024
140040	Plums						No import tolerance
163000	Miscellaneous fruit (inedible peel, large)						Residue study in banana: method LOQ = 0.05 mg/kg; metabolites not measured Use banana metabolism study TRR pulp = 0.6 mg/kg, TRR whole banana is 1.3 mg/kg Level of M11 is 1.7% TRR compared to parent =86% TRR ratio of TRR for M11/parent = 1.7 / 86 Level of M1 in pulp is 2.3% compared to 86% TRR ratio of TRR for M1/parent = 2.3 / 86 no M21 or M30 in banana
163020	Bananas	0.102	0.1	0.001	**0.0013**	0	Residue study in banana method LOQ = 0.05 mg/kg & metabolites not measured Cis met. <0.05 trans met. <0.05 mg/kg; <0.1 mg/kg for pulp or whole banana. Based on metabolism: parent residue in pulp = 0.6/1.3 x <0.1 mg/kg =<0.05 mg/kg Pulp: M11 = 1.7/86 x 0.05 = 0.001 mg/kg ; M1 = 2.3/86 x 0.05 =0.0013

Table 6.9-10: Residue levels for Metconazole Metabolites M1, M11, M21 and M30 in plant matrices

Code no.	Commodity	Total	Parent MRL	M11	M21 or M1[1]	M30	Comment on derivation of metabolite residue level
		mg/kg					
233000	Cucurbits - inedible peel						
233010	Melons	**0.052**	0.05	0.001	**0.0013**	0	Residue study in melon Method LOQ = 0.05 mg/kg & metabolites not measured Parent residue = <0.05 mg/kg; use banana ratio of TRR for M11 and M1 M11 = 1.7/86 x 0.05 = 0.001 M1 is 2.3/86*0.05 =0.0013
234000	Sweet corn	0.026	0.02	0.002	0.002	0.002	Extrapolate from maize; all measured metabolites are ND
260000	Legume vegetables (fresh)						
260040	Peas (wo pods)	**0.026**	0.02	0.002	0.002	0.002	EU cGAP 2 x 72 g/ha day, 14 d PHI Compare to soybean: NAFTA GAP 2 x 90 g/ha,30 d PHI Extrapolate from soya seed; all metabolite results are ND (<0.002 mg/kg)
300000	Pulses, dry						
300010	Beans	**0.056**	0.05	0.002	0.002	0.002	Extrapolate from dry pea
300030	Peas	**0.056**	0.05	0.002	0.002	0.002	Dry pea: EU cGAP 3 x 72 g/ha day, 14 d PHI Compare to soybean: Application 2 x 90 g/ha,30 d PHI Extrapolate from soya seed; all metabolite results are ND (<0.002 mg/kg)
300040	Lupins	**0.056**	0.05	0.002	0.002	0.002	Extrapolate from dry pea
401000	Oilseeds						
401010	Linseed	**0.206**	0.20	0.002	0.002	0.002	Extrapolate from soybean
401020	Peanuts	**0.056**	0.05	0.002	0.002	0.002	NAFTA GAP 2 x 280 g/ha, 14 d PHI; MRL of 0.02 mg/kg proposed by EFSA for peanut; current MRL = 0.05 mg/kg Extrapolate metabolite levels from soybean
401030	Poppy seed	**0.156**	0.15	0.002	0.002	0.002	Rape metabolism M11, M36 (like M21); no M30 or M1 Rape: application 2 x 90 g/ha 56 d PHI Compare to soybean: Application 2 x 90 g/ha, 30 d PHI Compare to cotton: Application 3x110 g/ha, 30 d PHI checked Soybean: Metabolites M11, M21, M 30 measured & all ND (<0.002 mg/kg) Cotton: Metabolites M11, M21, M 30 measured & all are 0 - 0.003 mg/kg. Will not use cotton due to higher application rate; will use 0.002 mg/kg for oilseed metabolites based on soybean. Use 0.002 mg/kg for M11, M21, M30 based on soybean
401040	Sesame seed	**0.056**	0.05	0.002	0.002	0.002	
401050	Sunflower seed	**0.056**	0.05	0.002	0.002	0.002	
401060	Rape seed	**0.206**	0.20	0.002	0.002	0.002	
401070	Soya bean	**0.056**	0.05	0.002	0.002	0.002	Soybean: Metabolites M11, M21, M 30 measured; all ND (<0.002 mg/kg)

Table 6.9-10: Residue levels for Metconazole Metabolites M1, M11, M21 and M30 in plant matrices

Code no.	Commodity	Total	Parent MRL	M11	M21 or M1[1]	M30	Comment on derivation of metabolite residue level
		mg/kg					
401080	Mustard seed	**0.206**	0.20	0.002	0.002	0.002	Extrapolate from soybean
401090	Cotton seed	**0.306**	0.30	0.002	0.002	0.002	Cotton: Metabolites M11, M21, M 30 measured; all are 0.0 - 0.003 mg/kg.
401100 - 401990	Other oilseeds	**0.056**	0.05	0.002	0.002	0.002	Extrapolate from soybean
500000	CEREALS						Wheat & barley: U.S. GAP: 2 x 100-110 g/ha, 21 d PHI EU Application rate= 2 x 90 g/ha, 35 d PHI Maize U.S. GAP: 4x110 g/ha, 21 d PHI Metabolites M11, M21 & M30 measured in all U.S. studies
500010	Barley	**0.45**	0.40	0.03	0.01	0.01	M11: Median residue in barley grain = 0.03 mg/kg M21: Median residue in barley grain = 0.01 mg/kg M30: Median residue in barley grain = 0.01 mg/kg
500030	Maize	**0.026**	0.02	0.002	0.002	0.002	Metabolites M11, M21, M 30 measured & all ND (<0.002 mg/kg)
500050	Oats	**0.45**	0.40	0.03	0.01	0.01	Extrapolate from barley
500070	Rye	**0.09**	0.06	0.01	0.01	0.01	Extrapolate metabolite level from wheat
500090	Wheat	**0.18**	0.15	0.01	0.01	0.01	Median residue of M11, M21, M30 in wheat grain = 0.01
900010	Sugar beet (root)	**0.066**	0.06	0.002	0.002	0.002	Metabolites M11, M21, M 30 measured in U.S. study & all ND (<0.002 mg/kg)

1) Boldface indicates that residue is M1 not M21

Table 6.9-11: Residue levels for Metconazole Metabolites M1, M12, M31 and M32 in ruminant matrices

1000000	**Products of animal origin -terrestrial animals**	**Total**	**Parent MRL**	**M11**	**M1**	**M12**	**M31**	**M32**	**Dosing in cow feeding study: 1 x = 5 mg/kg, 3x = 15 mg/kg, 10x = 50 mg/kg dry feed Parent feed burden determined using Profile (MCA 6.7) dairy = 1.5 mg/kg feed, meat = 3.8 mg/kg feed, hen = 0.07 mg/kg feed swine = 0.14 mg/kg feed**
		mg/kg							
1011000	Swine	**0.02**	0.02	0	0	0	0	0	Due to low feed burden, no residues of metabolites expected in swine
1012010	Bovine: Meat	**0.02**	0.02	0	0	0	0	0	Meat: Cow feeding study Residues of metconazole parent at 50 mg/kg dose level <0.01 mg/kg; therefore no possible residues of metabolites in these matrices
1012020	Bovine: Fat	**0.02**	0.02	0	0	0	0	0	Fat: Cow feeding study Residues of metconazole parent at 50 mg/kg dose level <0.01 mg/kg; therefore no possible residues of metabolites in these matrices
1012030	Bovine: Liver	**0.02**	0.02	0	0	0	0	0	Residues of M1, M12 < 0.01 (limit of detection) at 5 mg/kg or 15 mg/kg feeding level; therefore consider residues of metabolites in liver to be 0.0
1012040	Bovine: Kidney	**0.05**	0.02	0	0.01	0.01	0.01	0	Residues of M1, M12 < 0.01 (limit of detection) at 5 mg/kg feeding level; Feed burden = to feed level so M1 = 0.01, M12 = 0.01 mg/kg
1012050	Bovine: Edible offal	**0.05**	0.02	0	0.01	0.01	0.01		Same as bovine kidney
1013000	Sheep, goat, horse meat, fat, liver	**0.02**	0.02	0	0	0	0	0	Same as bovine matrices
	Sheep, goat, horse kidney	**0.05**	0.02	0	0.01	0.01	0.01	0	Same as bovine kidney
	Sheep, goat, horse edible offal	**0.05**	0.02	0	0.01	0.01	0.01	0	Same as bovine kidney
1016000	Poultry	**0.02**	0.02	0	0	0	0	0	Due to low feed burden, no residues of metabolites expected
1020000	Milk and cream	**0.02**	0.02	0	0	0	0	0	Milk: Cow feeding study Residues of metconazole parent at 50 mg/kg dose level <0.01 mg/kg; therefore no possible residues of metabolites in milk
1030000	Birds' eggs	**0.02**	0.02	0	0	0	0	0	Due to low feed burden, no residues expected in eggs
1040000	Honey	**0.05**	0.05	0	0	0	0	0	

Exposure to Triazole Derivative Metabolites

Residues of 1,2,4-triazole (T), triazoyl alanine (TA), triazoyl acetic acid (TAA) and triazoyl lactic acid (TLA) were measured in wheat (CA 6.3.1/4), barley (CA 6.3.2/6), and oilseed rape matrices (CA 6.3.3/1) after treatment with BAS 555 00 F and BAS 506 03 F (a mixture of metconazole and pyraclostrobin) at the cGAP. Residues of T were less than the LOQ of 0.01 mg/kg in all matrices for all crops. For wheat and barley the residues were measured after treatment with BAS 555 00 F or BAS 556 03 F in side by side plots. The plots were treated as replicate samples and the average value is reported in the table below. The STMR for residue levels of TA, TAA and TLA in wheat and barley grain and rape seed were determined and compared to the STMR for each commodity compiled by the TDMG task force (Table 6.9-12). The high residue (HR) for TA, TAA and TLA in wheat and barley straw were determined and compared to the HR for each commodity compiled by the TDMG task force (Table 6.9-13). The values measured after treatment of metconazole are significantly less than those reported by the task force. Therefore the exposure is covered by the risk assessment performed by the task force.

Residues in cereal whole plant and rape plant and pod were measured but are not summarized here since these matrices are not part of any dietary risk assessment or animal feed burden.

Table 6.9-12: Comparison of TDM residues with the worst case STMRs reported by the TDM DG

	TA	TAA	TLA
Wheat grain			
Germany	0.30	0.098	< 0.01
United Kingdom	0.37	0.18	< 0.01
France	0.086	0.037	< 0.01
Italy	0.19	0.078	< 0.01
STMR (metconazole)	**0.24**	**0.088**	**< 0.01**
TDMG STMR	**0.621**	**0.231**	**0.011**
Barley grain			
Germany	0.24	0.042	< 0.01
United Kingdom	0.059	0.046	< 0.01
France	0.033	0.0245	< 0.01
Italy	0.089	0.045	< 0.01
STMR (metconazole)	**0.074**	**0.043**	**< 0.01**
TDMG STMR	**0.57**	**0.79**	**0.02**
Oilseed rape seed			
Germany	1.24	< 0.01	**0.024**
United Kingdom	0.65	< 0.01	0.017
France	0.44	< 0.01	0.012
Denmark	0.11	< 0.01	< 0.01
France	0.37	< 0.01	< 0.01
Greece	0.23	< 0.01	< 0.01
Italy	0.35	< 0.01	< 0.01
Spain	0.11	< 0.01	< 0.01
STMR (metconazole)	**0.358**	**< 0.01**	**< 0.01**
TDMG STMR	**1.039**	**0.028**	**0.04**

Table 6.9-13: Comparison of TDM residues in straw with the worst case HRs reported by the TDM DG

	TA	TAA	TLA
Wheat straw			
Germany	**0.0175**	0.048	0.029
United Kingdom	0.015	**0.18**	**0.16**
France	0.01	0.028	0.021
Italy	0.015	0.074	0.052
TDMG high residue	**0.65**	**0.45**	**0.57**
Barley straw			
Germany	**0.0305**	**0.0435**	0.034
United Kingdom	0.023	0.022	0.015
France	<0.01	0.0135	0.015
Italy	0.0105	0.029	**0.051**
TDMG high residue	**0.38**	**0.78**	**1.1**

CA 6.10 Other studies

Evaluation of metabolism on cis:trans isomer ratio and enantiomeric ratio and the impact on consumer dietary exposure

Report: CA 6.10/1
Panek M., 2015b
Metconazole (BAS 555 F): Evaluation of metabolism on cis:trans isomer isomer and enantiomeric ratio and the impact on consumer dietary exposure
2014/7000201
Guidelines: None
GLP: no

Executive Summary

To ensure consumer dietary safety is acceptable, the question of whether the experimental toxicology adequately covers the isomeric ratio of metconazole in the commodities for human and livestock consumption should be addressed. If the isomeric ratio in the commodities consumed generally matches the isomeric ratio in the test substance evaluated in toxicology studies, the safe use can be considered to be covered by already assessed evaluations. Alternately, the dietary exposure can be determined which takes account of changes in isomeric content.

The ratio of *cis*-metconazole: *trans*-metconazole was not measured in the rat or plant metabolism studies. The levels of cis- and trans-metconazole were measured separately in the great majority of residue trials. Data from selected wheat, barley and oilseed rape trials treated with metconazole-containing products at the cGAP in northern and southern Europe were reviewed to determine the cis:trans ratio at application of the product and at harvest of the mature matrices of barley grain, wheat and barley straw and rape seed. In a range of EU residue trials performed with metconazole, the cis:trans isomer ratio changed slightly from that in the applied formulation, range of 82-87% cis:18-13% trans, to that found in mature crops due to a slight preferential metabolism of the cis-isomer. For wheat grain and rape seed, analysis showed that the cis:trans ratio in the residues in wheat grain and oilseed rape seed were consistent with the 85% cis:15% trans tested in the toxicology studies. Residues in barley grain had, on average, a cis:trans ratio of 78% cis: 22% trans slightly different than 85% cis:15% trans tested in the toxicology studies.

The goat and hen metabolism studies were performed with ^{14}C-metconazole with a ratio of 80:20 or 85:15 cis:trans. The cis:trans ratio was measured in liver in the goat metabolism study and in all matrices in the hen metabolism. The levels in the matrices reflected the varying extents of preferential metabolism of the cis-isomer with a maximum change to about 35% cis: 65% trans seen in goat liver, poultry muscle and egg yolk.

The impact of the isomeric shift on the dietary exposure of consumers to barley, oat and animal commodities was calculated using the EFSA acute chronic PRIMo model. The acute and chronic dietary calculations support the conclusion that the slight excess of *trans*-metconazole in barley and oat commodities over that tested in the toxicology studies and the extremely low overall exposure to animal commodities result in extremely low chronic dietary exposure attributed to the result of preferential metabolism of the cis-isomer. In conclusion preferential metabolism of the cis-isomer is of no toxicological significance regarding dietary exposure.

Toxicology studies performed using a racemic test substance, generally 85:15 cis:trans-metconazole, were used to set the reference doses for both chronic and acute exposure. Given a ratio of 85:15 cis:trans, the composition in the racemic metconazole test substance or product would be 42.5% (-)cis,42.5% (+)cis, 7.5% (-)trans and 7.5% (+)trans. Chiral analysis of samples from recent selected field trials showed a very slight preferential metabolism of (+) cis-metconazole enantiomer in wheat grain and rape seed but not in barley grain, barley or wheat straw or rape process fractions. Due to the low residues in wheat grain and rape seed, the exposure of the consumer to the slight increase in the (–) cis-enantiomer (average 8.5% change in wheat, 10% change in rape seed) over the 42.5% (–) cis-enantiomer tested in the toxicology studies would have little impact on consumer dietary safety.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: metconazole

B. STUDY DESIGN Part 1
Evaluation of metabolism on cis:trans isomer ratio and the impact on consumer dietary exposure

1. Test procedure

Plant
The levels of *cis*- and *trans*-metconazole were not measured in the plant metabolism studies. The levels of cis- and trans-metconazole were measured separately in the great majority of residue trials. Data from selected wheat, barley and oilseed rape trials treated with metconazole-containing products at the cGAP in northern and southern Europe were reviewed to determine the cis:trans ratio at application of the product and at harvest of the mature matrices of barley grain, wheat and barley straw and rape seed. The data included a range of formulations. The data from trials included in the analysis are from reports submitted during the Annex I approval process or residue studies not previously peer reviewed and submitted with this dossier. The studies are summarized in M-CA 6.3 and the Tier 1 tables with the reported levels of *cis* and *trans*-metconazole are included in the Appendix to M-CA Chapter 6.

Data from processing studies with wheat and oilseed rape treated with metconazole-containing products were reviewed to provide information on the cis:trans levels in wheat grain and rape seed which are usually <LOQ when treated at the cGAP and also document the cis:trans ratio in the processed commodities. The data from trials included in the analysis are from residue studies not previously peer reviewed and submitted with this dossier. The studies are summarized in M-CA 6.5 and the Tier 1 tables with the reported levels of cis and trans-metconazole are included in the Appendix of M-CA Chapter 6.

A table of the studies included in the plant residue review is shown below.

Table 6.10-1: Residue studies used for analysis of ratio of cis:trans metconazole in plant

Crop	Matrix	Formulation (all EC)	BASF Reg. Doc. No.	Study submitted
wheat	straw	BAS 555 00 F (batch 1072), BAS 627 00 F (Batch 1568) Grain residues too low for cis:trans analysis	2007/1050102	MCA 6.3
wheat	straw	BAS 627 02 F, (batch 241238), BAS 627 00 F (Batch 1568) Grain residues too low for cis:trans analysis	2010/1144333	MCA 6.3
barley	grain, straw	BAS 555 01 F (batch 200001)	2008/1009268	MCA 6.3
barley	grain, straw	BAS 555 00 F (batch 1072), BAS 627 00 F (batch 1568),	2007/1050101	MCA 6.3
barley	grain, straw	BAS 627 02 F(batch 241238), BAS 627 00 F(batch 1615)	2010/1144334	MCA 6.3
rape	seed	seed residues too low for cis:trans analysis	MK-750-003	DAR
rape	seed	seed residues too low for cis:trans analysis	MK-750-005	DAR
rape	seed	seed residues too low for cis:trans analysis	MK-750-006	DAR
rape	seed	seed residues too low for cis:trans analysis	MK-750-007	DAR
rape	seed	seed residues too low for cis:trans analysis	2004/1015942	MCA 6.10
rape	seed	seed residues too low for cis:trans analysis	2004/1015943	MCA 6.10
rape	seed	seed residues too low for cis:trans analysis	2006/1026865	MCA 6.10
rape	seed	seed residues too low for cis:trans analysis	2007/1057852	MCA 6.10
rape	seed	BAS 556 03 F (batch 380009)	2012/1255033	MCA 6.3
wheat	grain, PF	BAS 555 01 F (batch 2030)	2006/7007147	MCA 6.5
rape	seed, PF	BAS 556 03 F(batch 380009)	2013/1256239	MCA 6.5
barley	grain, PF	BAS 555 00 F	2013/1037952	MCA 6.5
oat	grain, PF	BAS 555 00 F	2013/1037951	MCA 6.5

2. Description of analytical procedures

The residues in the studies were measured using a variety of validated residue methods that separately quantitate *cis*- and *trans*-metconazole, generally with a LOQ of 0.005 for each isomer. The methods are documented in the studies summarized in M-CA 6.3 and M-CA 6.5.

Analysis

The ratio of cis:trans isomer in the applied formulation product was determined in the whole plant sampled at the last day of application. For example the formulation BAS 555 00 F (batch 1072) was applied in six trials in barley, 4 north and 2 south, as reported in study BASF Reg. Doc. No. 2007/1050101 (Chapter 6.3.2/3). The cis:trans ratio in the whole plant at 0 DALA for the six trials was 6.72, 7.65, 7.73, 5.93, 5.50, 6.19 for an average of 6.62:1 equivalent to 87% cis:13% trans (BAS 555 00 F column, Table 6.10-15).

The ratio of cis:trans isomer was determined in the mature raw agricultural commodity. For example, the mature barley grain and barley straw in the six trials were sampled at the relevant harvest times of 35, 42 and 49 DALA for each trial. The average cis:trans ratio of the six trials was determined for each sampling time (Table 6.10-15) expressed as a percentage of cis:trans.

II. RESULTS AND DISCUSSION

In a range of EU residue trials performed with metconazole, the cis:trans isomer ratio changed slightly from that in the applied formulation, range of 82-87% cis:18-13% trans, to that found in mature crops due to a slight preferential metabolism of the cis-isomer. For wheat grain and rape seed, the residues were often too low to determine a ratio, but studies with exaggerated rates (used to generate process fractions) showed that the cis:trans ratio in the residues in wheat grain and oilseed rape seed were consistent with the 85% cis:15% trans tested in the toxicology studies (Table 6.10-4, Table 6.10-5). Residues in barley grain had, on average, a cis:trans ratio of 78% cis: 22% trans slightly different than 85% cis:15% trans tested in the toxicology studies as shown in Table 6.10-2.

Within the Reasoned Opinion on modification of the barley MRL (EFSA 2013), EFSA had some notes relating to isomer ratio as follows:

> "EFSA notes that the metabolism studies did not investigate the possible impact of plant metabolism on the isomer ratio of metconazole. However, in 6 replicate residue trials included in the current submission of the application to modify the MRL for barley and oats, the ratio of metconazole *cis*- and *trans*-isomers was determined in the different barley commodities and at various pre-harvest intervals. Based on the specific analysis of a total of 84 samples of cereal matrices it can be concluded that in cereals the ratio of metconazole *cis*- and *trans*- isomers did not significantly change over a period of up to 50 days, i.e. from application until harvest of the fully mature crop."

Table 6.10-2: Ratio of cis:trans metconazole in barley matrices

		2007/ 1050101			2008/ 1009268	2010/ 1144334	
Matrix	DALA	BAS 555 00 F	BAS 627 00 F	BAS 627 02 F	BAS 555 01 F	BAS 627 02 F	BAS 627 00 F
		Cis:trans ratio (%)					
Whole plant	0	87/13	84/16	84/16	84/16	85/15	86/14
Grain	35	82/18	79/21	79/21	78/22	77/23	80/20
Straw	35	82/18	80/20	80/20	79/21	82/18	83/17
Grain	42	83/17	76/24	76/24	80/20	78/22	79/21
Straw	42	82/18	80/20	80/20	82/18	80/20	82/18
Grain	49	79/21	76/24	76/24	78/23	78/22	78/22
Straw	49	83/17	80/20	80/20	83/17	82/18	82/18

Table 6.10-3: Ratio of cis:trans metconazole in wheat straw

		2007/ 1050102		2010/ 1144333	2008/ 1009268
	DALA	BAS 555 00 F	BAS 627 00 F	BAS 627 02 F	BAS 627 00 F
		Cis:trans ratio (%)			
Whole plant	0	86/14	83/17	82/18	82/18
Straw	35	79/21	76/24	80/20	80/20
Straw	42	80/20	79/21	79/21	79/21
Straw	49	81/19	78/22	79/21	79/21
Straw	54, 65	79/21	71/29		

Table 6.10-4: Ratio of cis:trans metconazole in oilseed rape seed after treatment with BAS 556 03 F (M-CA 6.3.3/5)

Matrix	DE	UK	North FR	Den-mark	South FR	Greece	IT	ES	DALA	Cis/ trans Mean[1)]	Cis/ trans (%) [2)]
Whole plant	4.83	4.91	5.59	5.14	4.28	5.03	5.05	5.22	0	5.01	83/17
Seed	na	na	na	na	Na	na	6.41	7.39	41	6.90	87/13
Seed	4.06	5.10	6.37	6.02	5.91	8.17	na	na	56	5.94	86/14
Seed	6.45	4.80	na	na	na	na	na	na	67	5.63	85/15

1) The cis/trans ratio is obtained by dividing the *cis*-metconazole (mg/kg) by trans-metconazole (mg/kg). The mean is the mean across trials for that site.
2) The ratio is converted to a % of the whole to allow an easy comparison across trials using the formula:
% cis = mean (100)/(1+mean). % trans = 100 - % cis.
na = not applicable since no sampling at this interval
(Reg. Doc. No. 2012/1255033)

Table 6.10-5: Ratio of cis:trans metconazole in wheat grain and processed fractions after treatment with BAS 555 01 F[1)]

		Cis-metconazole	Trans-metconazole	Total	Cis: trans (%)
Pepin WI	Grain	0.343	0.056	0.399	**86/14**
	Straight Flour	0.047	0.01	0.057	**82/18**
	Flour Type 550	0.06	0.011	0.071	**85/15**
	Whole meal flour	0.321	0.042	0.364	**88/12**
	Bread	0.209	0.041	0.25	**84/16**
Clinton IL	Grain	0.348	0.061	0.408	**85/15**
	Straight Flour	0.046	0.01	0.056	**82/18**
	Flour Type 550	0.07	0.016	0.087	**81/19**
	Whole meal flour	0.204	0.042	0.246	**83/17**
	Bread	0.147	0.032	0.178	**82/18**
Cass ND	Grain	0.191	0.047	0.238	**80/20**
	Straight Flour	0.059	0.015	0.074	**80/20**
	Flour Type 550	0.081	0.019	0.1	**81/19**
	Whole meal flour	0.172	0.038	0.21	**82/18**
	Bread	0.135	0.032	0.167	**81/19**
York NE	Grain	0.311	0.055	0.366	**85/15**
	Straight Flour	0.069	0.017	0.086	**80/20**
	Flour Type 550	0.072	0.017	0.089	**81/19**
	Whole meal flour	0.105	0.039	0.144	**73/27**
	Bread	0.176	0.038	0.214	**82.18**

1) Reg. Doc. No. 2006/7007147, M-CA 6.5.

Table 6.10-6: Ratio of cis:trans metconazole in rape seed and processed fractions after treatment with BAS 556 03 F[1]

	Matrix	DALA	Cis-metconazole	Trans-metconazole	Total	Cis/ trans (%)
L110292/ Germany	Whole plant	0	4.0	0.76	4.76	**84/16**
	Seed	76	0.14	0.016	0.23	**91/9**
	Meal	n.a.	0.20	0.020	0.21	**90/10**
	Refined Oil	n.a.	0.42	0.044	0.37	**90/10**
L110293/ Germany	Whole plant	0	4.1	0.770	4.87	**84/16**
	Seed	67	0.03	0.002	0.05	**89/11**
	Meal	n.a.	0.05	0.003	0.05	**90/10**
	Refined Oil	n.a.	0.07	0.008	0.07	**91/9**
L110294/ Germany	Whole plant	0	2.600	0.480	3.08	**84/16**
	Seed	78	0.093	0.011	0.10	**89/11**
	Meal	n.a.	0.093	0.01	0.10	**90/10**
	Refined Oil	n.a.	0.140	0.017	0.16	**89/11**
L110295/ Germany	Whole plant	0	2.900	0.52	3.42	**84/16**
	Seed	65	0.210	0.027	0.24	**89/11**
	Meal	n.a.	0.20	.024	0.22	**83/17**
	Refined Oil	n.a.	0.390	0.043	0.43	**90/10**

n.a.= not applicable
1) Reg. Doc. No. 2013/1256239, M-CA 6.5

Results of evaluation of cis:trans isomers in animal matrices:

The goat and hen metabolism studies were performed with ^{14}C-metconazole with a ratio of 80:20 or 85:15 cis:trans. The cis:trans ratio was measured in liver in the goat metabolism study and in all matrices in the hen metabolism. The levels in the matrices reflected the varying extents of preferential metabolism of the cis-isomer with a maximum change to about 35% cis: 65% trans seen in goat liver, poultry muscle and egg yolk (M-CA 6.2).

Table 6.10-7: Ratio of cis:trans metconazole in hen matrices in metabolism study

Components	Liver (pre-hydrolysis)		Liver (post-hydrolysis)		Breast muscle		Thigh muscle	
	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]
Cis-metconazole	0.031	3.9	-	-	0.001	1.8	0.002	3.7
Trans-metconazole	0.003	0.4	-	-	0.001	3.6	0.003	6.0
	0.034	4.3	0.033	4.1	0.002	5.4	0.005	9.8
Cis:trans ratio	10.3:1	9.8:1			1:1	0.5:1	0.7:1	0.6:1
Cis:trans (%)		91/9				33/67		38/62

	Abdominal fat		Skin with fat		Egg white[1]		Egg yolk[1]		Whole egg[1]	
Components	**[mg/kg]**	**[% TRR]**	**[mg/kg]**	**[% TRR]**	**[mg/kg]**	**[% TRR]**	**[mg/kg]**	**[% TRR]**	**[mg/kg]**	**[% TRR]**
Cis-metconazole	0.017	19.0	0.011	14.4	0.001	2.1	0.004	4.4	0.002	2.8
Trans-metconazole	0.016	17.7	0.010	13.4	0.002	5.2	0.006	7.1	0.004	5.8
Total metconazole	0.033	36.7	0.021	27.8	0.003	7.3	0.010	11.4	0.006	8.6
Cis:trans ratio	1.1:1	1.1:1	1.1:1	1.1:1	0.5:1	0.4:1	0.7:1	0.6:1	0.5:1	0.5:1
Cis:trans (%)		52/48		52/48		29/71		38/62		33/67

1. Analysis was done with day 4 and day 5 samples**.**

IMPACT OF PREFERENTIAL METABOLISM OF CIS ISOMER ON DIETARY RISK ASSESSMENT

Consumer exposure to metconazole through plant commodities of the representative crops is mainly consumption of processed commodities of cereal grain, rape seed, especially flour, bread, rolled oats and oil. The residues in wheat grain and oil seed rape after treatment with metconazole are generally low, often less than the limit of quantitation, leading to minimal exposure. While the data on the cis:trans ratio in wheat grain and rape seed is limited due to the generally low residues, the ratio of cis:trans metconazole was calculated in rape seed and indicated no increase of the trans-metconazole isomer over time. The same results for seed were seen in the trials in rape performed to generate processed fractions. For wheat grain from trials treated at an exaggerated rate in a study to generate process fractions, the cis:trans ratio in grain and process fractions of flour and bread could be measured and was approximately 85:15 cis:trans. Thus the exposure of consumers is consistent with the exposure of animals in the toxicology studies dosed with metconazole with a ratio of 85% cis: 15% trans.

For barley grain, the data from trials with a range of metconazole-containing formulations indicated a slight preferential metabolism of the cis isomer so that consumers would be exposed to metconazole residues with a cis:trans ratio of, on average, 78:22 cis:trans. The amount of trans-metconazole in the barley or oat (extrapolated from barley grain) would be on average 8% higher (22% - 15%) than the metconazole dosed in the toxicology studies. A study performed in oat to generate process fractions (data not shown) indicated a cis:trans ratio in the grain of 78/22 cis:trans consistent with the extrapolation of barley.

The data from the hen metabolism study indicated a change in the cis:trans ratio in matrices to various extents in hen matrices with the maximum change of 33% cis: 67% trans in whole egg and breast muscle. A ratio of 34% cis: 66% trans was measured in the goat liver. For assessing the impact, consumers are assumed to be exposed to metconazole residues in animal commodities with a ratio of 35% cis: 65% trans, approximating the maximum change. The amount of trans-metconazole, on average, would thus be about 40% higher (65% - 15%) than the metconazole dosed in the toxicology studies.

Dietary assessment considering impact of preferential metabolism of *cis*-metconazole in barley, oat and animal matrices

The impact of the isomeric shift on the dietary exposure of consumers to barley, oat and animal commodities was calculated using the EFSA acute chronic PRIMo model and the inputs shown in the tables below.

- The residue level for barley grain was set at the STMR 0.030 mg/kg multiplied by the processing factor of 2.4 for barley flour. The residue level was then multiplied by a factor of 10 % to reflect the amount of excess trans-isomer.
- The residue level for oat grain was set at the STMR 0.030 mg/kg multiplied by the processing factor of 1.0 since all processing factors are <1. The residue level was then multiplied by a factor of 10 % to reflect the amount of excess trans-isomer.
- The residue levels for milk, ruminant and swine meat and fat commodities were based on TRR measured in the metabolism studies adjusted by an overdosing factor based on the actual feed burden. The residue levels for liver and kidney were based on the metconazole concentration measured in the metabolism studies adjusted by an overdosing factor based on the actual feed burden. The residue levels for egg and poultry muscle were based on the metconazole concentration measured in the metabolism study adjusted by an overdosing factor based on the actual feed burden (see M-CA 6.7 for calculations). The residue levels were then multiplied by a factor of 40% to reflect the amount of excess trans-isomer in animal commodities.

Table 6.10-8: Inputs and acute dietary exposure related to *trans*-metconazole in excess of the 15% used in the toxicology dosing

Matrix	STMR-p or calc. residue in animal matrix	Adjust ment factor	Input (mg/kg)	Population child	Pop. exposure (mg/kg bw/day	Pop. exposu re % ARfD	Population adult	Pop exposure (mg/kg bw/day	Pop exposure % ARfD
barley grain	0.030x2.4	10%	0.007	UK child	0.0000124	0.12	NL	0.000051	0.51
oat grain	0.030x2.4	10%	0.007	DE child	0.0000119	0.12	LT	0.000004	0.04
milk	2.56E-04	40%	1.03E-04	UK Infant	0.0000124	0.12	NL	0.0000017	0.02
meat	7.58E-04	40%	3.03E-04	DE	0.0000038	0.04	NL	0.0000018	0.02
fat	4.55E-04	40%	1.82E-04	UK Infant	0.0000004	0.00	UK Adult	0.0000001	0.00
liver	3.58E-02	40%	1.44E-02	UK Infant	0.0001130	1.13	UK Adult	0.0000377	0.38
kidney	4.55E-04	40%	4.00E-04	UK Infant	0.000102	1.02	UK Adult	0.0000007	0.01
edible offal	3.58E-02	40%	1.44E-02	UK Tod.	0.0000015	0.02	UK Adult	0.0000414	0.41
poultry	2.78E-05	40%	1.11E-05	DE	0.0000001	0.001	UK Veg.	0.0000001	0.001
eggs	5.00E-05	40%	2.22E-05	UK Infant	0.0000003	0.003	UK Veg.	0.0000001	0.001

Table 6.10-9: Chronic dietary exposure related to trans-metconazole in excess of the 15% used in the toxicology dosing

Commodity	Inputs	IE adult		WHO cluster E		WHO cluster F		UK infant	
	(mg/kg)	Mg/kg bw/day	% ADI	Mg/kg bw/day	% ADI	Mg/kg bw/day	% ADI	Mg/kg bw/day	% ADI
Oat & barley	0.003, 0.007	9.23E-06	0.092	5.96E-06	0.060	4.70E-06	0.047	7.60E-07	0.008
Animal	See table	5.17E-06	0.052	2.14E-06	0.021	1.90E-06	0.019	5.20E-06	0.052
Total		1.440E-05	0.144	8.10E-06	0.081	6.60E-06	0.066	7.00E-06	0.060

III. CONCLUSION

The acute and chronic dietary calculations support the conclusion that the slight excess of *trans*-metconazole in barley and oat commodities over that tested in the toxicology studies and the extremely low overall exposure to animal commodities result in extremely low chronic dietary exposure attributed to the result of preferential metabolism of the cis-isomer. In conclusion preferential metabolism of the cis-isomer is of no toxicological significance regarding dietary exposure.

B. STUDY DESIGN Part 2
Evaluation of metabolism on enantiomeric ratio and the impact on consumer dietary exposure

As defined by the ISO common name, metconazole is a racemic mixture of 4 isomers, 2 diastereoisomer pairs).

IUPAC Name	Description	Description	BASF Reg. No.
(1S,5R)-5-(4-chlorobenzyl)-2,2-dimethyl-1-(1H-1,2,4,triazol-1-ylmethyl)cyclopentanol	(-) cis-metconazole	1S,5R enantiomer	4677200
(1R,5S)-5-(4-chlorobenzyl)-2,2-dimethyl-1-(1H-1,2,4,triazol-1-ylmethyl)cyclopentanol	(+) cis-metconazole	1R,5S-enantiomer	5836046
(1S,5S)-5-(4-chlorobenzyl)-2,2-dimethyl-1-(1H-1,2,4,triazol-1-ylmethyl)cyclopentanol	(-) trans-metconazole	1S,5S-enatiomer	5836047
(1R,5R)-5-(4-chlorobenzyl)-2,2-dimethyl-1-(1H-1,2,4,triazol-1-ylmethyl)cyclopentanol	(+) trans-metconazole	1R,5R-enantiomer	5836048

Toxicology studies performed using a racemic test substance, generally 85:15 cis:trans-metconazole, were used to set the reference doses for both chronic and acute exposure. Given a ratio of 85:15 cis:trans, the composition in the racemic metconazole test substance or product would be 42.5% (-)cis,42.5% (+)cis, 7.5% (-)trans and 7.5% (+)trans.

1. Test procedure
Samples of wheat, barley, oilseed rape and rape process fractions from recent EU field trials treated with metconazole-containing product were extracted and analyzed using a chiral method, BASF Method Number L0961/01, to determine the enantiomeric ratio at application and at harvest of the mature crops. The chiral analysis of commodities at treatment and harvest would show any shift in the enantiomeric proportion in the commodity treated with metconazole.

Table 6.10-10: Residue studies used for chiral analysis of metconazole residues in plant

Crop	**matrix**	**Formulation (all EC)**	BASF Reg. Doc. No.	**Study submitted**
wheat	straw	BAS 627 02 F, (batch 241238), BAS 627 00 F (batch 1568) Grain residues too low for cis:trans analysis	2010/1144333	MCA 6.3
barley	grain, straw	BAS 627 02 F(batch 241238), BAS 627 00 F(batch 1615)	2010/1144334	MCA 6.3
rape	seed	BAS 556 03 F (batch 380009)	2012/1255033	MCA 6.3
rape	Seed, PF	BAS 556 03 F(batch 380009)	2013/1256239	MCA 6.5

2. Description of analytical procedures

In brief the method described in technical procedure L0961 is as follows. The enantiomers are extracted using methanol. An aliquot of the extract is portioned against dichloromethane. The final determination of the enantiomers from BAS 555F is performed by HPLC-MS/MS. The limit of quantitation (LOQ) for all enantiomers (Reg. No. 4677200, Reg.No.5836046, Reg. No. 5836047 and Reg. No. 5836046) is 0.01 mg/kg, sum of all enantiomers, which are determined separately. The enantiomers of metconazole could be clearly separated using a Chiralpak AD-H column. Prior to the analysis of barley and wheat, the recovery of each enantiomer was validated at levels of 0.010, 0.1 and 1 mg/kg (n=5 at each level) in whole plant, grain and straw. For the analysis of rape seed and process fractions the quantitation each enantiomer was validated in seed at levels of 0.0025, 0.025 and 0.25 mg/kg and in whole plant, meal, and refined oil at 0.025 and 0.25 mg/kg. The fortification results indicate satisfactory recoveries and standard deviation for all enantiomers in all matrices.

Table 6.10-11: Fortification results for analysis of metconazole enantiomers using TP L0196/01 in cereal and rape matrices

Enantiomer	Mean [%]	SD [±]	RSD [%]	n
Cereal whole plant, grain and straw				
4677200 (-) cis	92.0	6	7	15
5836046 (+) cis	96.5	8	8	15
5836047 (-) trans	90.3	9	10	15
5836048 (+) trans	93.4	9	9	15
Rape whole plant, seed, meal, refined oil				
4677200 (-) cis	93.8	14	15	13
5836046 (+) cis	93.8	16	17	13
5836047 (-) trans	95.2	11	11	13
5836048 (+) trans	95.8	7	7	3

II. RESULTS AND DISCUSSION

The samples were extracted and analysed using BASF Method L0961/01 within 26 months of sampling, within the proven storage interval for metconazole in plant matrices.

Based on the chiral analysis, the residues in barley and wheat whole plant samples taken immediately after application of metconazole exhibit the expected enantiomeric ratio of about 42.5% (-)cis,42.5% (+)cis, 7.5% (-) trans and 7.5% (+)trans. Enantiomeric ratio changes seen in barley grain do not appear to be consistent or significant. For wheat grain the shift in enantiomeric ratio appears more consistent, but the measured residues in wheat grain are very low with levels of 0.001 mg/kg to 0.007 mg/kg. The tables (see 6.10.2-12 , 6.10.2-14) show a decrease of >10% in the (+) cis-metconazole enantiomer at three of four trial sites while the (-) cis enantiomer shows a slight increase indicating a slight preferential metabolism for the (+) cis enantiomer. The (-) cis enantiomer at levels of 45-51% of the total varies at maximum from the test substance level tested in toxicology studies of 51-42.5 = 8.5% and average difference of 6%.

Based on chiral analysis, the residues in rape whole plant samples taken immediately after application of metconazole exhibit the expected enantiomeric ratio of about 42.5% (-)cis,42.5% (+)cis, 7.5% (-)trans and 7.5% (+)trans. For rape seed a >10% decrease in the (+) cis-metconazole enantiomer from the initial 42.5% is evident in five of eight trial sites. In conjunction, the (-) cis metconazole ratio increases from the starting point of 42.5% to 44-62% for a maximum increase over the level used in toxicology studies of 62-42.5 ≅ 20% and an average increase of ~10% (see 6.10.2-13, 6.10.2-15).

Impact on dietary assessment

Considering the generally low residues in wheat grain with a standard median trial residue of 0.01 mg/kg for north EU, 0.02 mg/kg for south EU or 0.04 mg/kg for imported grain (EFSA 2011), the possible increase in exposure due to an average 6% increase of the (-) cis enantiomer over the level tested in the toxicology studies would not have a significant impact on exposure or the resulting safety evaluation.

Considering the generally low residues in rape seed with a standard median trial residue of 0.03 mg/kg (EFSA 2011, MCA 6.7), the possible impact on dietary safety due to a 10% increase of the proportion of (-)cis enantiomer over the proportion tested in toxicology studies does not seem significant.

III. CONCLUSION

Conclusion on chiral evaluation:

Based on chiral analysis of samples from recently conducted residue trials, changes in the enantiomeric ratios were very small in barley and wheat straw and barley grain. In wheat grain, the (-) cis metconazole proportion increases an average of 6% over the initial value of 42.5% due to a preferential metabolism of the (+) cis-enantiomer. Considering the generally low residues in wheat grain, the possible 6% increase of the (-) cis enantiomer would not have a significant impact on the resulting safety evaluation of dietary exposure of humans. Likewise, considering the generally low residues in rape seed with a standard median trial residue of 0.03 mg/kg, the possible impact on dietary safety due to a 10% increase of the proportion of (-)cis enantiomer over the proportion tested in toxicology studies does not seem significant. Chronic and acute dietary assessments using the EFSA model show a large margin of safety for long term use of metconazole and low use of the acute reference dose for the blended commodities of wheat and barley grain and rape seed and the processed commodities thereof.

Due to the low levels of trans-isomer in the product and matrices, the impact of any enantiomeric shift would have little to no impact on consumer exposure or safety.

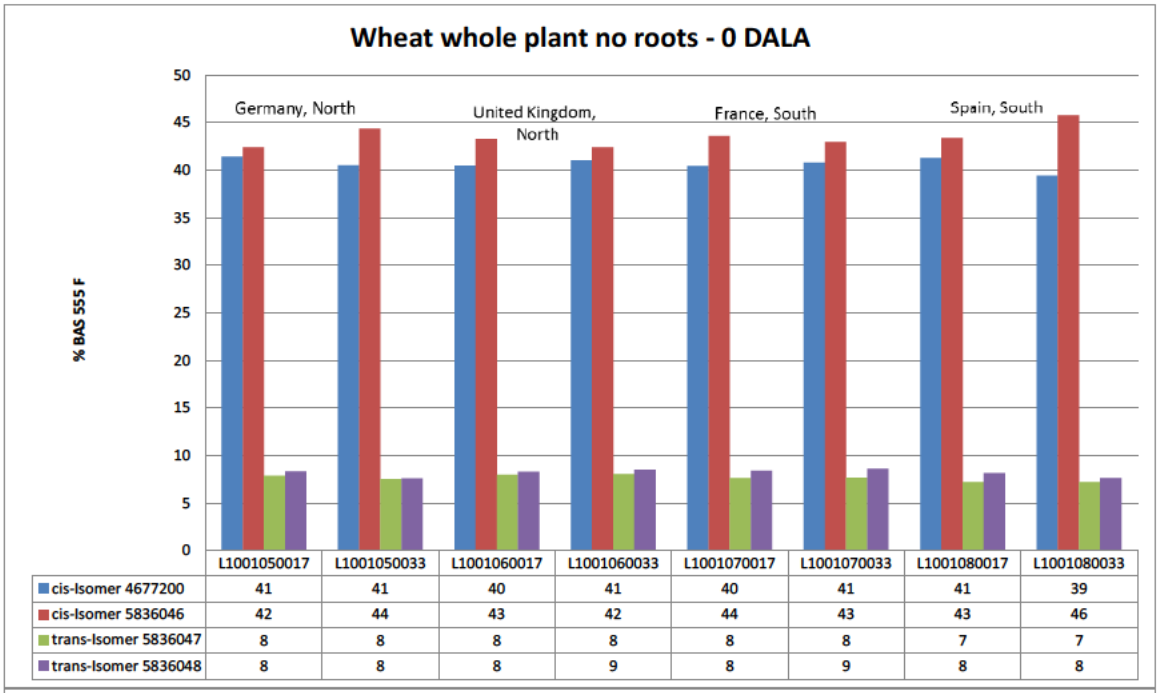
Table 6.10-12: Metconazole enantiomer residues in wheat after treatment at the cGAP with BAS 627 00 F (plot 2) or BAS 627 02 F (plot 3) [expressed as percent of each enantiomer]

Trial No.	plot	Matrix	DALA	Growth	Sample	AP	BAS 555 F (%)			
Country				stage	number	No.	(-) cis	(+)cis	(-) trans	(+) trans
		WHEAT					4677200	5836046	5836047	5836048
L100105 (Germany, North)	2	Whole Plant[3)]	0	69	L1001050017	L004	41	42	8	8
	2	Grain	34	89	L1001050028	L006	48	25	12	15
	2	Straw	34	87	L1001050021	L005	47	32	11	10
	3	Whole Plant[3)]	0	69	L1001050033	L004	41	44	8	8
	3	Grain	34	89	L1001050044	L006	51	25	12	13
	3	Straw	34	87	L1001050037	L005	47	32	10	10
L100106 (United Kingdom, North)	2	Whole Plant[3)]	0	69	L1001060017	L004	40	43	8	8
	2	Grain	35	89	L1001060028	L006	48	31	10	11
	2	Straw	35	87	L1001060021	L005	44	36	10	9
	3	Whole Plant[3)]	0	69	L1001060033	L004	41	42	8	9
	3	Grain	35	89	L1001060044	L006	45	33	11	11
	3	Straw	35	87	L1001060037	L005	46	35	10	9
L100107 (France, South)	2	Whole Plant[3)]	0	69	L1001070017	L004	40	44	8	8
	2	Grain	35	89	L1001070028	L006	50	26	12	12
	2	Straw	35	87-89	L1001070025	L005	44	35	11	11
	3	Whole Plant[3)]	0	69	L1001070033	L004	41	43	8	9
	3	Grain	35	87-89	L1001070044	L006	49	27	12	13
	3	Straw	35	87-89	L1001070041	L005	44	35	10	10
L100108 (Spain, South)	2	Whole Plant[3)]	0	69	L1001080017	L004	41	43	7	8
	2	Grain	34	89	L1001080028	L006	39	36	8	16
	2	Straw	34	89	L1001080021	L005	43	40	8	9
	3	Whole Plant[3)]	0	69	L1001080033	L004	39	46	7	8
	3	Grain	34	89	L1001080044	L006	32	40	8	19
	3	Straw	34	89	L1001080037	L005	43	41	8	8

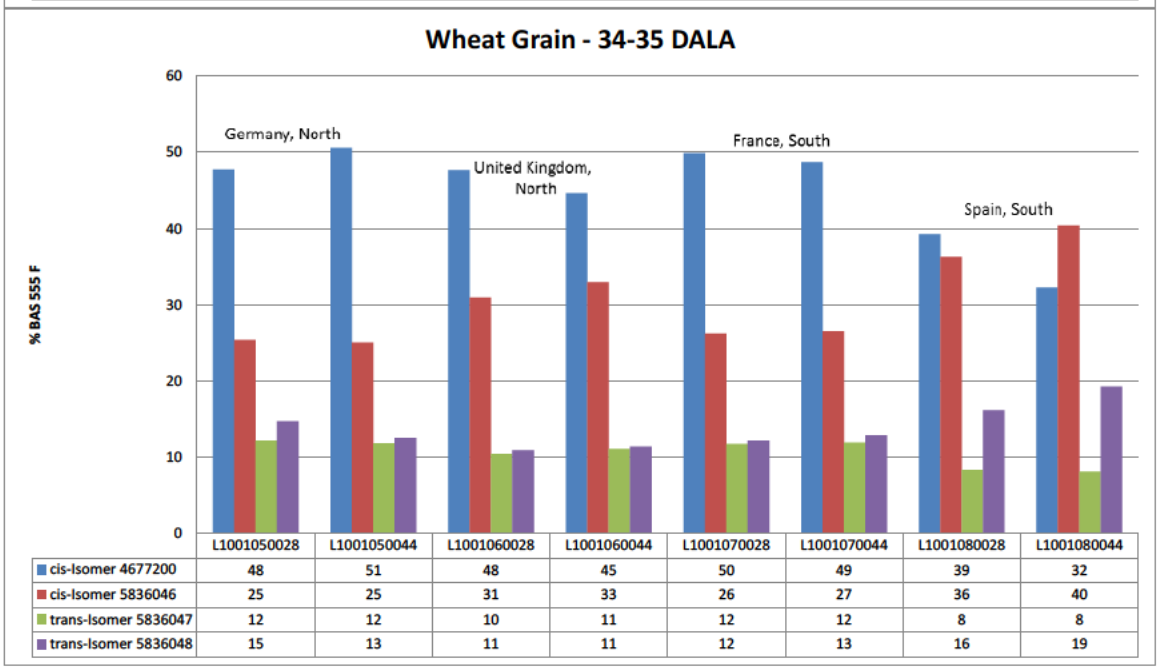
Table 6.10-13: Metconazole enantiomer residues in rape after treatment at the cGAP with BAS 556 03 F [expressed as percent of each enantiomer]

Trial No.	Matrix	DALA	BBCH	Sample	BAS 555 F (%)			
Country				number	(-) cis	(+)cis	(-) trans	(+) trans
					4677200	5836046	5836047	5836048
L110180 (Germany)	Whole Plant[3)]	0	71	1101800007	43	40	9	9
	Seed	56	89	1101800008	59	21	9	10
	Seed	67	89	1101800011	62	25	6	8
L110181 (United Kingdom)	Whole Plant[3)]	0	71	1101810007	44	39	8	9
	Seed	56	87	1101810008	51	32	6	10
	Seed	69	89	1101810011	58	25	8	10
L110182 (Northern France)	Whole Plant[3)]	0	71	1101820007	44	41	7	9
	Seed	57	89	1101820008	44	43	6	7
L110183 (Denmark	Whole Plant[3)]	0	71	1101830007	43	41	8	9
	Seed	56	89	1101830008	48	38	6	8
L110184 (South France)	Whole Plant[3)]	0	71	1101840007	42	39	9	10
	Seed	57	89	1101840008	54	32	5	9
L110185 (Greece)	Whole Plant[3)]	0	71	1101850007	43	40	8	9
	Seed	55	89	1101850008	41	48	4	7
L110186 (Italy)	Whole Plant[3)]	0	71	1101860007	44	40	8	9
	Seed	43	89	1101860011	51	36	5	9
L110187 (Spain)	Whole Plant[3)]	0	75	1101870007	45	39	7	9
	Seed	41	89	1101870011	57	31	6	6

Figure 6.10-1: Metconazole enantiomer residues in wheat after treatment at the cGAP with BAS 627 00 F (plot 2) or BAS 627 02 F (plot 3) [residues expressed as percent of each enantiomer]



	L1001050017	L1001050033	L1001060017	L1001060033	L1001070017	L1001070033	L1001080017	L1001080033
cis-Isomer 4677200	41	41	40	41	40	41	41	39
cis-Isomer 5836046	42	44	43	42	44	43	43	46
trans-Isomer 5836047	8	8	8	8	8	8	7	7
trans-Isomer 5836048	8	8	8	9	8	9	8	8

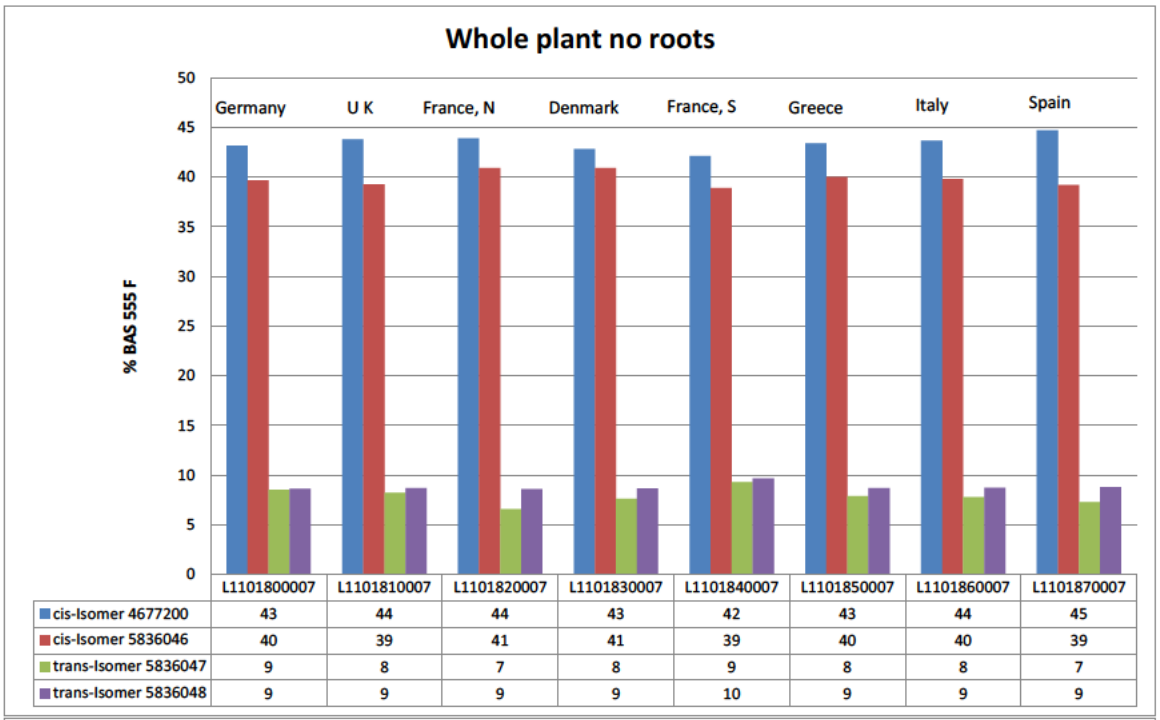


	L1001050028	L1001050044	L1001060028	L1001060044	L1001070028	L1001070044	L1001080028	L1001080044
cis-Isomer 4677200	48	51	48	45	50	49	39	32
cis-Isomer 5836046	25	25	31	33	26	27	36	40
trans-Isomer 5836047	12	12	10	11	12	12	8	8
trans-Isomer 5836048	15	13	11	11	12	13	16	19

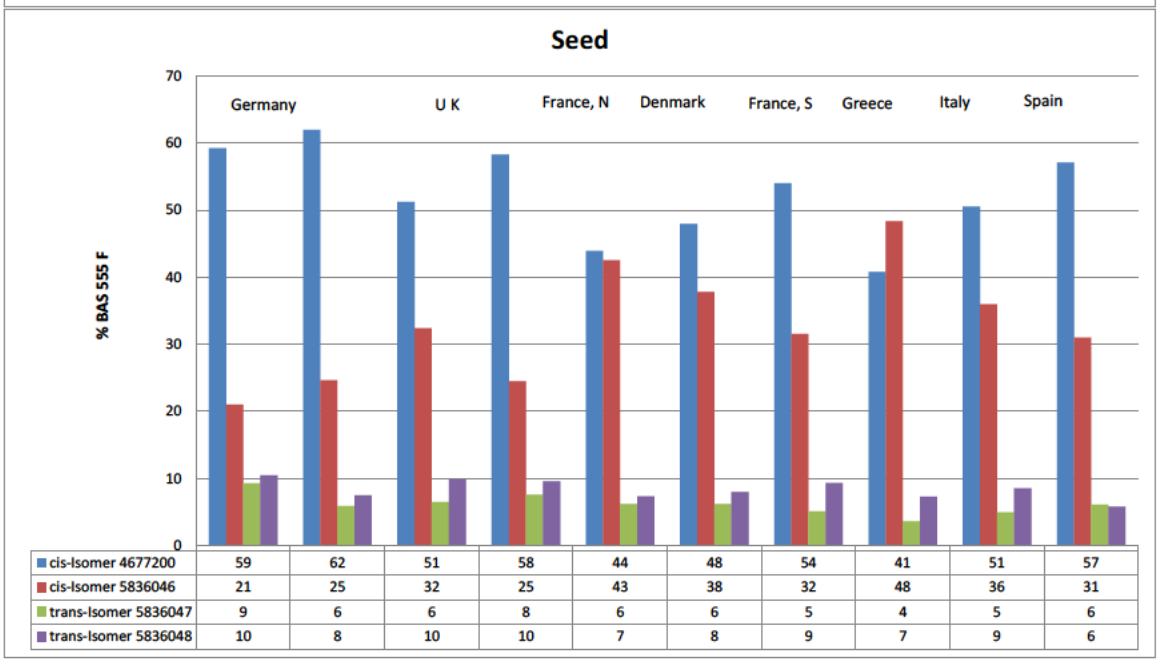
For each location, the first results are from Plot 2 treated with BAS 627 00 F; the second results are from Plot 3 treated with BAS 627 02 F.

Oilseed rape

Figure 6.10-2: Metconazole enantiomer residues in rape after treatment at the cGAP with BAS 556 03 F [residues expressed as percent of each enantiomer]



	L1101800007	L1101810007	L1101820007	L1101830007	L1101840007	L1101850007	L1101860007	L1101870007
cis-Isomer 4677200	43	44	44	43	42	43	44	45
cis-Isomer 5836046	40	39	41	41	39	40	40	39
trans-Isomer 5836047	9	8	7	8	9	8	8	7
trans-Isomer 5836048	9	9	9	9	10	9	9	9



cis-Isomer 4677200	59	62	51	58	44	48	54	41	51	57
cis-Isomer 5836046	21	25	32	25	43	38	32	48	36	31
trans-Isomer 5836047	9	6	6	8	6	6	5	4	5	6
trans-Isomer 5836048	10	8	10	10	7	8	9	7	9	6

Residue studies supporting data used for 6.10.2/1 Evaluation of metabolism on cis:trans isomer ratio and enantiomeric ratio and the impact on consumer dietary exposure

Data from the following residue studies, 6.10/3, 6.10/4, 6.10/5 and 6.10/6 were mentioned in the discussion paper in the previous section. The studies are not included in M-CA 6.3 since they are not performed at the critical GAP. The studies are submitted to demonstrate that residues in rape seed are generally too low to provide accurate data on the cis:trans metconazole ratio. The studies are not used in the dossier for any other purpose. Studies in rape already evaluated in the peer review (MK-750-003, MK-750-005, MK-750-006 and MK-750-007) also demonstrate that residues in rape seed are generally too low to provide accurate data on the cis:trans metconazole ratio.

Report:	CA 6.10/2 Schulz H., 2005a Study on the residue behaviour of BAS 083 W and BAS 555 F in oilseed rape after application of BAS 134 00 W under field conditions in France (North), Germany and the United Kingdom, 2003 2004/1015942
Guidelines:	EEC 91/414 (1607/VI/97), EEC 7029/VI/95 rev. 5, EEC 7525/VI/95 rev. 7, EEC 96/46, SANCO/825/00 rev. 7 (17 March 2004)
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Laendlichen Raum und Verbraucherschutz)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	BAS 134 00 W (SL)	
Lot/Batch #:	84409, mepiquat chloride: 210.0 g/L nominal; metconazole: 30.0 g/L nominal	
Purity:	Not relevant	
CAS#:	Mepiquat chloride:	24307-26-4
	Metconazole:	125116-23-6
Development code:		
Spiking levels:	Mepiquat chloride:	0.05-100 mg/kg
	Metconazole:	0.0016-1.7 mg/kg

2. Test Commodity:

Crop:	Oilseed rape
Type:	Oilseeds
Variety:	Expreß, Hearthy, Standing, Winner
Botanical name:	*Brassica napus* L.
Crop parts(s) or processed Commodity:	Whole plant without roots shoots without pods, pods, seeds
Sample size:	12 plants (min.1.0 kg); pods, 0.5 kg; seeds, 0.5 kg

B. STUDY DESIGN

1. Test procedure

During the 2003/2004 growing season, 5 decline field trials were conducted with oilseed rape in France (North), Germany, and in United Kingdom to determine the residue level of metconazole (BAS 555 F) and mepiquat chloride (BAS 083 W) in or on raw agricultural commodities after application of BAS 134 00 W.

BAS 134 00 W, a SL formulation of mepiquat chloride (210 g/L) and metconazole (30 g/L) was applied twice at a rate of 1.4 L/ha of formulated product equivalent to an application rate of 0.294 kg/ha of BAS 083 W and 0.042 kg/ha of BAS 555 F, with a spray rate of 300 L/ha. The applications took place at BBCH 14-18 and BBCH 55-59. Specimens of whole plant without roots were taken immediately after the last application (0 DALA) at BBCH 55-59, as well as after 26-48 DALA (BBCH 69) and at 54-56 DALA (BBCH 75-79). Seed samples were taken at 82-109 DALA (BBCH 89).

Table 6.10-14: Target application rates and timings for BAS 134 02 W in oilseed rape

Year	No of trials	No. of appl.	F P or G	Test item	Active substance	Appl. rate (kg a.s./ha)	Water volume (L/ha)	Target date/ Timing
2003/2004	5	2	F	BAS 134 00 W (SL)	Mepiquat chloride Metconazole	0.294 0.042	300	BBCH: 1st 13-20 2nd 51-59

2. Description of analytical procedures

The specimens were analyzed for mepiquat chloride (BAS 083 W) and metconazole (BAS 555 F). Mepiquat chloride residues were analyzed using the BASF Method No. 505/0. Residues were extracted from plant matrices using a mixture of methanol, water and hydrochloric acid. For clean-up, an SPE cartridge filled up with Al_2O_3 is used. The final determination of mepiquat chloride is performed by HPLC-MS/MS. For all matrices of oilseed rape, the limit of quantitation (LOQ) for mepiquat chloride is 0.05 mg/kg.

Metconazole was analyzed using the BASF Method No. 550/0. Residues are extracted using a mixture of methanol, water and hydrochloric acid. An aliquot of the extract is centrifuged and partitioned against dichloromethane. The final determination of metconazole is performed by HPLC-MS/MS. The limit of quantitation (LOQ) is 0.01 mg/kg (sum of *cis* and *trans* isomer, which are determined separately; 0.005 mg/kg for each isomer).

The methods were validated concurrently with fortified untreated specimens. Validation acceptance criteria were fully met for mepiquat chloride and for metconazole fortified at LOQ and 10 x LOQ.

The results of procedural recovery experiments are summarized in the following table:

Table 6.10-15: Summary of recoveries for mepiquat chloride and metconazole in oilseed rape

Matrix	Fortification level (mg/kg)	Summary recoveries		
		n	Mean (%)	RSD (%)
BASF method No 505/0		**BAS 083 W**		
whole plant w/o roots	0.05/5/100	5	94	5
Dried seeds	0.05/0.5/5	3	96	3
Pods	0.05/5.0	2	94	n.a.
Shoots w/o pods	0.05/5.0	2	104	n.a.
BASF method No 550/0		**BAS 555 F (*cis*-metconazole)**		
whole plant w/o roots	0.008/0.08/1.7	4	97	1
Dried seeds	0.008/0.08	2	92	n.a.
Pods	0.008	1	84	n.a.
Shoots w/o pods	0.8	1	99	n.a.
		BAS 555 F (*trans*-metconazole)		
whole plant w/o roots	0.0016/0.016/0.32	4	96	2
Dried seeds	0.0016/0.016	2	101	n.a.
Pods	0.0016	1	86	n.a.
Shoots w/o pods	0.16	1	95	n.a.

n.a. = Not applicable

II. RESULTS AND DISCUSSION

A summary of residues is presented in the table below. Details are shown in Table 6.10-17.
Immediately after application, the residues of mepiquat chloride in whole plant without roots specimens ranged from 4.48 to 10.29 mg/kg. They decreased sharply to 0.90-1.76 mg/kg at 26-48 DALA (BBCH 69), and further to <0.05-1.09 mg/kg at 54-56 DALA (BBCH 75-79). The mepiquat chloride residues in the shoots without pods and pods specimens at 56 DALA (BBCH 77) were 1.90 mg/kg and 0.93 mg/kg, respectively. In the seed specimens at 82-109 DALA (BBCH 89), the mepiquat chloride residues ranged from 0.76 to 1.88 mg/kg.
Immediately after application, the residues of total metconazole (sum of *cis* and *trans* isomer) in whole plant without roots specimens ranged from 0.56 to 1.18 mg/kg. They decreased sharply to 0.02-0.10 mg/kg at 26-48 DALA (BBCH 69), and further to <0.01-0.01 mg/kg at 54-56 DALA (BBCH 75-79). The metconazole residues in the shoots without pods and pods specimens at 56 DALA (BBCH 77) were 0.02 mg/kg and <0.01 mg/kg, respectively. In the seed specimens at 82-109 DALA (BBCH 89), the metconazole residues were <0.01 mg/kg.

Table 6.10-16: Summary of residues in oilseed rape after application of BAS 134 00 W

Crop	Year	Application (kg a.s./ha)	DALA[1]	Residues found (mg/kg)		
				Matrix	BAS 083 W	BAS 555 F[2]
Rape	2003/2004 (EU North)	BAS 134 00 W: BAS 083 W 2 x 0.294 BAS 555 F 2 x 0.042	0	Whole plant w/o roots	4.48-10.29	0.56-1.18
			26-48	Whole plant w/o roots	0.90-1.76	0.02-0.10
			54-56	Whole plant w/o roots	<0.05-1.09	<0.01-0.01
			56	Shoots w/o pods	1.90	0.02
			56	Pods	0.93	<0.01
			82-109	Seed	0.76-1.88	<0.01

1 Days after last application
2 Sum of *cis* and *trans* isomers

III. CONCLUSION

Dried seeds were collected at harvest at BBCH 89 from each plot at all locations, 82 to 109 DALA. In the seed specimens taken at harvest, the mepiquat chloride concentrations ranged from 0.76 to 1.88 mg/kg. In all the seed specimens taken at harvest, total metconazole residues were <0.01 mg/kg (<LOQ).

Table 6.10-17: Residues of mepiquat chloride (BAS 083 W) and metconazole (BAS 555 F) in oilseed rape in Northern Europe

Study details		Crop	Country	Formulation Application rate (kg a.s./ha)	GS[1] BBCH	DALA[2]	Residues found (mg/kg)		
							Matrix	BAS 083 W	BAS 555 F[3]
Study code:	144055	Oilseed rape	Germany	BAS 134 00 W: BAS 083 W 2 x 0.294 BAS 555 F 2 x 0.042	55	0	Whole plant*	4.48	0.56
Doc ID:	2004/1015942					28	Whole plant*	1.19	0.04
Trial No.:	DU4/08/03					54	Whole plant*	0.92	0.01
GLP:	yes					89	Dried seeds	1.88	<0.01
Year:	2004								
Study code:	144055	Oilseed rape	France	BAS 134 00 W: BAS 083 W 2 x 0.294 BAS 555 F 2 x 0.042	59	0	Whole plant*	5.11	0.67
Doc ID:	2004/1015942					26	Whole plant*	1.55	0.10
Trial No.:	FAN/06/03					56	Shoots**	1.90	0.02
GLP:	yes					56	Pods	0.93	<0.01
Year:	2004					82	Dried seeds	1.14	<0.01
Study code:	144055	Oilseed rape	France	BAS 134 00 W: BAS 083 W 2 x 0.294 BAS 555 F 2 x 0.042	59	0	Whole plant*	7.62	0.87
Doc ID:	2004/1015942					38	Whole plant*	1.76	0.07
Trial No.:	FBM/03/03					54	Whole plant*	1.09	0.01
GLP:	yes					102	Dried seeds	1.38	<0.01
Year:	2004								
Study code:	144055	Oilseed rape	UK	BAS 134 00 W: BAS 083 W 2 x 0.294 BAS 555 F 2 x 0.042	55	0	Whole plant*	7.55	1.07
Doc ID:	2004/1015942					48	Whole plant*	0.90	0.02
Trial No.:	OAT/03/03					56	Whole plant*	<0.05	<0.01
GLP:	yes					109	Dried seeds	0.97	<0.01
Year:	2004								
Study code:	144055	Oilseed rape	UK	BAS 134 00 W: BAS 083 W 2 x 0.294 BAS 555 F 2 x 0.042	55	0	Whole plant*	10.29	1.18
Doc ID:	2004/1015942					43	Whole plant*	1.14	0.02
Trial No.:	OAT/04/03					55	Whole plant*	<0.05	<0.01
GLP:	yes					108	Dried seeds	0.76	<0.01
Year:	2004								

1 Growth stage at last treatment
2 Days after the last application
3 Sum of *cis* and *trans* isomer
* Without roots
** Without pods

Report: CA 6.10/3
Raunft E. et al., 2005a
Study on the residue behaviour of Mepiquat chloride and Metconazole in winter oil seed rape after application of BAS 134 XO W under field conditions in France (N), Germany and the United Kingdom, 2002-2003
2004/1015943
Guidelines: EEC 91/414, EEC 1607/VI/97 rev. 2 10.06.1999, EEC 7029/VI/95 rev. 5, EEC 7525/VI/95 rev. 7
GLP: yes
(certified by Landesamt fuer Umweltschutz und Gewerbeaufsicht, Mainz, Germany)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	BAS 134 00 W (SL)	
Lot/Batch #:	84316, mepiquat chloride: 210.0 g/L nominal; metconazole: 30.0 g/L nominal	
Purity:	Not relevant	
CAS#:	Mepiquat chloride :	24307-26-4
	Metconazole:	125116-23-6
Development code:		
Spiking levels:	Mepiquat chloride:	0.05-0.5 mg/kg
	Metconazole:	0.005-0.05 mg/kg

2. Test Commodity:

Crop:	Oilseed rape
Type:	Oilseeds
Variety:	Smart, Expreß, Pollen, Winner
Botanical name:	*Brassica napus* L.
Crop parts(s) or processed Commodity:	Whole plant without roots shoots without pods, pods, seeds
Sample size:	12 plants (min.1.0 kg); seeds 0.5 kg

B. STUDY DESIGN

1. Test procedure

During the 2002/2003 growing season, 4 decline field trials were conducted with oilseed rape in France (North), Germany, and in United Kingdom to determine the residue level of metconazole (BAS 555 F) and mepiquat chloride (BAS 083 W) in or on raw agricultural commodities after application of BAS 134 00 W.

BAS 134 00 W, a SL formulation of mepiquat chloride (210 g/L) and metconazole (30 g/L) was applied twice at a rate of 1.4 L/ha of formulated product equivalent to an application rate of 0.294 kg/ha of BAS 083 W and 0.042 kg/ha of BAS 555 F, with a spray rate of 300 L/ha. The target applications took place at BBCH 13-20 and BBCH 51-59. Specimens of whole plant without roots were taken immediately after the last application (0 DALA) at BBCH 53-59, as well as after 30-48 DALA (BBCH 69) and one sample at 56 DALA. Seed samples were taken at 79-113 DALA (BBCH 89).

Table 6.10-18: Target application rates and timings for BAS 134 02 W in oilseed rape

Year	No of trials	No. of appl.	F P or G	Test item	Active substance	Appl. rate (kg a.s./ha)	Water volume (L/ha)	Target date/ Timing
2002/2003	4	2	F	BAS 134 00 W (SL)	Mepiquat chloride Metconazole	0.294 0.042	300	BBCH: 1st 13-20 2nd 51-59

2. Description of analytical procedures

The specimens were analyzed for mepiquat chloride (BAS 083 W) and metconazole (BAS 555 F). Mepiquat chloride residues were analyzed using the BASF Method No. 505/0. Residues were extracted from plant matrices using a mixture of methanol, water and hydrochloric acid. For clean-up, an SPE cartridge filled up with Al_2O_3 is used. The final determination of mepiquat chloride is performed by HPLC-MS/MS. For all matrices of oilseed rape, the limit of quantitation (LOQ) for mepiquat chloride is 0.05 mg/kg.

Metconazole was analyzed using the BASF Method No. 550/0. Residues are extracted using a mixture of methanol, water and hydrochloric acid. An aliquot of the extract is centrifuged and partitioned against dichloromethane. The final determination of metconazole is performed by HPLC-MS/MS. The limit of quantitation (LOQ) is 0.01 mg/kg (sum of *cis* and *trans* isomer, which are determined separately; 0.005 mg/kg for each isomer).

The methods were validated concurrently with fortified untreated specimens. Validation acceptance criteria were fully met for mepiquat chloride and for metconazole fortified at LOQ and 10 x LOQ.

The results of procedural recovery experiments are summarized in the following table:

Table 6.10-19: Summary of recoveries for mepiquat chloride and metconazole in oilseed rape

Matrix	Fortification level (mg/kg)	Summary recoveries		
		n	Mean (%)	RSD (%)
BASF Method No. 505/0		**BAS 083 W**		
Whole plant w/o roots	0.05/0.5	4	103	7
Seeds	0.05/0.5	4	103	4
BASF Method No. 550/0		**BAS 555 F (*cis*-metconazole)**		
Whole plant w/o roots	0.005/0.05	2	95	n.a.
Seeds	0.005/0.05	4	96	2
BAS 555 F (*trans*-metconazole)				
Whole plant w/o roots	0.005/0.05	2	94	n.a.
Seeds	0.005/0.05	4	94	2

n.a. = Not applicable

II. RESULTS AND DISCUSSION

A summary of residues is presented in the table below. Details are shown in Table 6.10-21.
Directly after the last application application, the residues of mepiquat chioride ranged between 7.52 and 11.66 mg/kg in oilseed plant. The residues degraded to a range of 0.81 to 2.24 mg/kg after 30 to 48 days, the one plant sample taken at 56 DALA contained 0.94 mg/kg of mepiquat chloride. In oilseed rape seed taken between 79 and 113 DALA, mepiquat chioride was found between 0.21 and 0.61 mg/kg.
At 0 DALA, total metconazole residues were found between 0.87 and 1.27 mg/kg which degraded to a level of 0.02 to 0.04 mg/kg after 30 to 48 days. The one plant sample taken at 56 DALA as well as all seed samples did not contain metconazole above the limit of quantitation (<0.01 mg/kg).

Table 6.10-20: Summary of residues in oilseed rape after application of BAS 134 00 W

Crop	Year	Application (kg a.s./ha)	DALA[1]	Residues found (mg/kg)		
				Matrix	BAS 083 W	BAS 555 F[2]
Oilseed rape	2003/2004 (EU North)	BAS 134 00 W: BAS 083 W 2 x 0.294 BAS 555 F 2 x 0.042	0	Whole plant w/o roots	7.52-11.66	0.87-1.27
			30-48	Whole plant w/o roots	0.81-2.24	0.02-0.04
			56	Whole plant w/o roots	0.94	<0.01
			79-113	Seeds	0.21-0.61	<0.01

1 Days after last application
2 Sum of *cis* and *trans* isomers

III. CONCLUSION

Dried seeds were collected at harvest at BBCH 89 from each plot at all locations, 79 to 113 DALA. In the seed specimens taken at harvest, the mepiquat chloride concentrations ranged from 0.21 to 0.61 mg/kg. In all the seed specimens taken at harvest, total metconazole residues were <0.01 mg/kg (<LOQ).

Table 6.10-21: Residues of mepiquat chloride (BAS 083 W) and metconazole (BAS 555 F) in oilseed rape in Northern Europe

Study details		Crop	Country	Formulation Application rate (kg a.s./ha)	GS[1] BBCH	DALA[2]	Residues found (mg/kg)		
							Matrix	BAS 083 W	BAS 555 F[3]
Study code:	133933	Oilseed rape	Germany	BAS 134 00 W: BAS 083 W 2 x 0.294 BAS 555 F 2 x 0.042	59	0	Whole plant*	11.66	1.27
Doc ID:	2004/1015943					46	Whole plant*	0.81	0.02
Trial No.:	DU2/14/02					87	Seeds	0.61	<0.01
GLP:	Yes								
Year:	2003								
Study code:	133933	Oilseed rape	Germany	BAS 134 00 W: BAS 083 W 2 x 0.294 BAS 555 F 2 x 0.042	59	0	Whole plant*	8.84	1.18
Doc ID:	2004/1015943					44	Whole plant*	1.15	0.02
Trial No.:	DU4/15/02					89	Seeds	0.47	<0.01
GLP:	Yes								
Year:	2003								
Study code:	133933	Oilseed rape	France	BAS 134 00 W: BAS 083 W 2 x 0.294 BAS 555 F 2 x 0.042	55	0	Whole plant*	7.52	0.87
Doc ID:	2004/1015943					30	Whole plant*	0.97	0.04
Trial No.:	FAN/14/02					56	Whole plant*	0.94	<0.01
GLP:	Yes					79	Seeds	0.21	<0.01
Year:	2003								
Study code:	133933	Oilseed rape	UK	BAS 134 00 W: BAS 083 W 2 x 0.294 BAS 555 F 2 x 0.042	53	0	Whole plant*	9.74	1.08
Doc ID:	2004/1015943					48	Whole plant*	2.24	0.04
Trial No.:	OAT/20/02					113	Seeds	0.61	<0.01
GLP:	Yes								
Year:	2003								

1 Growth stage at last treatment
2 Days after the last application
3 Sum of *cis* and *trans* isomer
* Without roots

Report: CA 6.10/4
North L., 2007a
Study on the residue behaviour of Mepiquat-Chloride and Metconazole in oilseed rape after treatment with BAS 134 00 W under field conditions in Southern Europe during 2005 and 2006
2006/1026865
Guidelines: None
GLP: yes
(certified by Groupe Interministeriel des Produits Chimiques, France)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	BAS 134 00 W (SL)	
Lot/Batch #:	1022, mepiquat chloride: 210.0 g/L nominal; metconazole: 30.0 g/L nominal	
Purity:	Not relevant	
CAS#:	Mepiquat chloride :	24307-26-4
	Metconazole:	125116-23-6
Development code:		
Spiking levels:	Mepiquat chloride:	0.05-5.0 mg/kg
	Metconazole:	0.005-0.5 mg/kg

2. Test Commodity:

Crop:	Oilseed rape
Type:	Oilseeds
Variety:	Expreß, Hearthy, Standing, Winner
Botanical name:	*Brassica napus* L.
Crop parts(s) or processed Commodity:	Whole plant without roots shoots without pods, pods, seeds
Sample size:	Plant: 1.0 kg; pods: 0.5 kg; seeds: 0.5 kg

B. STUDY DESIGN

1. Test procedure

During the 2005/2006 growing season, 2 decline field trials were conducted with oilseed rape in France (South) to determine the residue level of metconazole (BAS 555 F) and mepiquat chloride (BAS 083 W) in or on raw agricultural commodities after application of BAS 134 00 W.
BAS 134 00 W, a SL formulation of mepiquat chloride (210 g/L) and metconazole (30 g/L) was applied twice at a rate of 1.4 L/ha of formulated product equivalent to an application rate of 0.294 kg/ha of BAS 083 W and 0.042 kg/ha of BAS 555 F, with a spray rate of 300 L/ha. The target applications took place at BBCH 13-20 and BBCH 51-59. Specimens of whole plant without roots were taken immediately after the last application (0 DALA) at BBCH 53-57, as well as at 28-33 DALA (BBCH 69) and 55-56 days after the last application (BBCH 75-80). Seed samples were taken at 78-83 DALA (BBCH 89).

Table 6.10-22: Target application rates and timings for BAS 134 02 W in oilseed rape

Year	No of trials	No. of appl.	F P or G	Test item	Active substance	Appl. rate (kg a.s./ha)	Water volume (L/ha)	Target date/ Timing
2005/2006	2	2	F	BAS 134 00 W (SL)	Mepiquat chloride Metconazole	0.294 0.042	300	BBCH: 1st 13-20 2nd 51-59

2. Description of analytical procedures

The specimens were analyzed for mepiquat chloride (BAS 083 W) and metconazole (BAS 555 F). Mepiquat chloride residues were analyzed using the BASF Method No. 505/0. Residues were extracted from plant matrices using a mixture of methanol, water and hydrochloric acid. For clean-up, an SPE cartridge filled up with Al_2O_3 is used. The final determination of mepiquat chloride is performed by HPLC-MS/MS. For all matrices of oilseed rape, the limit of quantitation (LOQ) for mepiquat chloride is 0.05 mg/kg.

Metconazole was analyzed using the BASF Method No. 550/0. Residues are extracted using a mixture of methanol, water and hydrochloric acid. An aliquot of the extract is centrifuged and partitioned against dichloromethane. The final determination of metconazole is performed by HPLC-MS/MS. The limit of quantitation (LOQ) is 0.01 mg/kg (sum of *cis* and *trans* isomer, which are determined separately; 0.005 mg/kg for each isomer).

The methods were validated concurrently with fortified untreated specimens. Validation acceptance criteria were fully met for mepiquat chloride and for metconazole fortified at LOQ and 10 x LOQ.

The results of procedural recovery experiments are summarized in the following table:

Table 6.10-23: Summary of recoveries for mepiquat chloride and metconazole in oilseed rape

Matrix	Fortification level (mg/kg)	Summary recoveries		
		n	Mean (%)	RSD (%)
BASF Method No. 505/0		**BAS 083 W**		
Whole plant w/o roots	0.05/0.5/5.0	6	106	8
Seeds	0.05/0.5	5	102	6
BASF Method No. 550/0		**BAS 555 F (*cis*-metconazole)**		
Whole plant w/o roots	0.005/0.05/0.5	5	94	3
Seeds	0.005/0.05	5	96	9
		BAS 555 F (*trans*-metconazole)		
Whole plant w/o roots	0.005/0.05/0.5	5	92	6
Seeds	0.005/0.05	5	99	8

II. RESULTS AND DISCUSSION

A summary of residues is presented in the table below. Details are shown in Table 6.10-25.
Directly after the last application, the residues of mepiquat chlioride were 5.0 mg/kg in oilseed plant. These residues degraded to a range of 0.62 to 2.4 mg/kg after 28 to 33 days and ranged between 1.0 to 1.1 mg/kg at 55-56 DALA. In treated seed specimens collected at 78-83 DALA (89 BBCH), residues in the range of 1.2-1.3 mg/kg were observed.
At 0 DALA, total metconazole residues were found between 0.51 and 0.61 mg/kg which degraded to a level of 0.016 to 0.08 mg/kg after 28 to 33 days. The plant samples taken at 55-56 DALA as well as all seed samples did not contain metconazole above the limit of quantitation (<0.01 mg/kg).

Table 6.10-24: Summary of residues in oilseed rape after application of BAS 134 00 W

Crop	Year	Application (kg a.s./ha)	DALA[1]	Residues found (mg/kg)		
				Matrix	BAS 083 W	BAS 555 F[2]
Rape	2005/2006 (EU South)	BAS 134 00 W: BAS 083 W 2 x 0.294 BAS 555 F 2 x 0.042	0	Whole plant w/o roots	5.0	0.51-0.61
			28-33	Whole plant w/o roots	0.62-2.4	0.016-0.08
			55-56	Whole plant w/o roots	1.0-1.1	<0.01
			78-83	Seeds	1.2-1.3	<0.01

1 Days after last application
2 Sum of *cis* and *trans* isomers

III. CONCLUSION

Dried seeds were collected at harvest at BBCH 89 from each plot at all locations, 78 to 83 DALA. In the seed specimens taken at harvest, the mepiquat chloride residues ranged from 1.2 to 1.3 mg/kg. In all the seed specimens taken at harvest, total metconazole residues were <0.01 mg/kg (<LOQ).

Table 6.10-25: Residues of mepiquat chloride (BAS 083 W) and metconazole (BAS 555 F) in oilseed rape in Southern Europe

Study details		Crop	Country	Formulation Application rate (kg a.s./ha)	GS[1] BBCH	DALA[2]	Residues found (mg/kg)		
							Matrix	BAS 083 W	BAS 555 F[3]
Study code:	182053	Oilseed rape	France	BAS 134 00 W: BAS 083 W 2 x 0.294 BAS 555 F 2 x 0.042	57	0	Whole plant*	5.0	0.61
Doc ID:	2006/1026865					28	Whole plant*	2.4	0.08
Trial No.:	AF/10166/BA/1					55	Whole plant*	1.0	<0.01
GLP:	Yes					78	Seeds	1.3	<0.01
Year:	2006								
Study code:	182053	Oilseed rape	France	BAS 134 00 W: BAS 083 W 2 x 0.294 BAS 555 F 2 x 0.042	53	0	Whole plant*	5.0	0.51
Doc ID:	2006/1026865					33	Whole plant*	0.62	0.016
Trial No.:	AF/10166/BA/2					56	Whole plant*	1.1	<0.01
GLP:	Yes					83	Seeds	1.2	<0.01
Year:	2006								

1 Growth stage at last treatment
2 Days after the last application
3 Sum of *cis* and *trans* isomer
* Without roots

Report: CA 6.10/5
Klaas P., 2007a
Study on the residue behaviour of Mepiquat Chloride and Metconazole in oilseed rape after treatment with BAS 134 00 W under field conditions in Southern France, 2006/2007
2007/1057852

Guidelines: EEC 91/414 Annex II (Part A Section 6), EEC 91/414 Annex III (Part A Section 8), EEC 1607/VI/97 rev. 2 10.06.1999, EEC 7029/VI/95 rev. 5, EEC 7525/VI/95 rev. 7, SANCO/825/00 rev. 7 (17 March 2004), EEC 96/46

GLP: yes
(certified by Hessisches Ministerium fuer Umwelt, Laendlichen Raum und Verbraucherschutz)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description: BAS 134 00 W (SL)
Lot/Batch #: 1022, mepiquat chloride: 210.0 g/L nominal; metconazole: 30.0 g/L nominal
Purity: Not relevant
CAS#: Mepiquat chloride : 24307-26-4
Metconazole: 125116-23-6
Development code:
Spiking levels: Mepiquat chloride: 0.05-5.0 mg/kg
Metconazole: 0.005-0.5 mg/kg

2. Test Commodity:

Crop: Oilseed rape
Type: Oilseeds
Variety: Mendel, Aviso
Botanical name: *Brassica napus* L.
Crop parts(s) or processed
Commodity: Whole plant without roots shoots without pods, pods, seeds
Sample size: Plant: 1.0 kg; pods: 0.5 kg; seeds: 0.5 kg

B. STUDY DESIGN

1. Test procedure

During the 2006/2007 growing season, 2 decline field trials were conducted with oilseed rape in France (South) to determine the residue level of metconazole (BAS 555 F) and mepiquat chloride (BAS 083 W) in or on raw agricultural commodities after application of BAS 134 00 W.
BAS 134 00 W, a SL formulation of mepiquat chloride (210 g/L) and metconazole (30 g/L) was applied twice at a rate of 1.4 L/ha of formulated product equivalent to an application rate of 0.294 kg/ha of BAS 083 W and 0.042 kg/ha of BAS 555 F, with a spray rate of 300 L/ha. The target applications took place at BBCH 13-20 and BBCH 51-59. Specimens of whole plant without roots were taken immediately after the last application (0 DALA) at BBCH 59. Shoots without pods and pods were taken at 55-56 DALA. Seed samples were taken at maturity 84-92 DALA (BBCH 89).

Table 6.10-26: Target application rates and timings for BAS 134 02 W in oilseed rape

Year	No of trials	No. of appl.	F P or G	Test item	Active substance	Appl. rate (kg a.s./ha)	Water volume (L/ha)	Target date/ Timing
2006/2007	2	2	F	BAS 134 00 W (SL)	Mepiquat chloride Metconazole	0.294 0.042	300	BBCH: 1st 13-20 2nd 51-59

2. Description of analytical procedures

The specimens were analyzed for mepiquat chloride (BAS 083 W) and metconazole (BAS 555 F). Mepiquat chloride residues were analyzed using the BASF Method No. 505/0. Residues were extracted from plant matrices using a mixture of methanol, water and hydrochloric acid. For clean-up, an SPE cartridge filled up with Al_2O_3 is used. The final determination of mepiquat chloride is performed by HPLC-MS/MS. For all matrices of oilseed rape, the limit of quantitation (LOQ) for mepiquat chloride is 0.05 mg/kg.

Metconazole was analyzed using the BASF Method No. 550/0. Residues are extracted using a mixture of methanol, water and hydrochloric acid. An aliquot of the extract is centrifuged and partitioned against dichloromethane. The final determination of metconazole is performed by HPLC-MS/MS. The limit of quantitation (LOQ) is 0.01 mg/kg (sum of *cis* and *trans* isomer, which are determined separately; 0.005 mg/kg for each isomer).

The methods were validated concurrently with fortified untreated specimens. Validation acceptance criteria were fully met for mepiquat chloride and for metconazole fortified at LOQ and 10 x LOQ.

The results of procedural recovery experiments are summarized in the following table:

Table 6.10-27: Summary of recoveries for mepiquat chloride and metconazole in oilseed rape

Matrix	Fortification level (mg/kg)	Summary recoveries		
		n	Mean (%)	RSD (%)
BASF Method No. 505/0		**BAS 083 W**		
Whole plant w/o roots/shoots	0.05/0.5/5.0	6	107	3
Seeds/pods	0.05/0.5	6	104	4
BASF Method No. 550/0		**BAS 555 F (*cis*-metconazole)**		
Whole plant w/o roots/shoots	0.005/0.05/0.5	6	89	12
Seeds/pods	0.005/0.05	6	91	13
		BAS 555 F (*trans*-metconazole)		
Whole plant w/o roots/shoots	0.005/0.05/0.5	6	84	11
Seeds/pods	0.005/0.05	6	94	14

II. RESULTS AND DISCUSSION

A summary of residues is presented in the table below. Details are shown in Table 6.10-29.
Directly after the last application, residues of BAS 083 W ranged between 7.0 mg/kg and 7.6 mg/kg in plants without roots. At 31-39 DALA (BBCH 69) they had decreased to 1.3-1.4 mg/kg. Residues in seeds sampled at BBCH 89 ranged from 1.10 to 1.50 mg/kg. Directly after the last application, residues of BAS 555 F ranged between 0.51 mg/kg and 0.55 mg/kg in plants without roots. At growth stage BBCH 69 they had decreased to 0.02-0.04 mg/kg. Residues in seeds sampled at BBCH 89 were at or below the limit of quantitation (<0.01 mg/kg).

Table 6.10-28: Summary of residues in oilseed rape after application of BAS 134 00 W

Crop	Year	Application (kg a.s./ha)	DALA[1]	Residues found (mg/kg)		
				Matrix	BAS 083 W	BAS 555 F[2]
Oilseed rape	2006/2007 (EU South)	BAS 134 00 W: BAS 083 W 2 x 0.294 BAS 555 F 2 x 0.042	0	Whole plant w/o roots	7.00-7.60	0.51-0.55
			31-39	Whole plant w/o roots	1.30-1.40	0.02-0.04
			55-56	Pods	0.88-1.10	<0.01
			55-56	Shoots without pods	1.10-1.20	<0.01-0.01
			84-92	Seeds	1.10-1.50	<0.01

1 Days after last application
2 Sum of *cis* and *trans* isomers

III. CONCLUSION

Dried seeds were collected at harvest at BBCH 89 from each plot at all locations, 84 to 92 DALA. In the seed specimens taken at harvest, the mepiquat chloride residues ranged from 1.10 to 1.50 mg/kg. In all the seed specimens taken at harvest, total metconazole residues were <0.01 mg/kg (<LOQ).

Table 6.10-29: Residues of mepiquat chloride (BAS 083 W) and metconazole (BAS 555 F) in oilseed rape in Southern Europe

Study details		Crop	Country	Formulation Application rate (kg a.s./ha)	GS[1] BBCH	DALA[2]	Residues found (mg/kg)		
							Matrix	BAS 083 W	BAS 555 F[3]
Study code:	240079	Oilseed rape	France	BAS 134 00 W: BAS 083 W 2 x 0.294 BAS 555 F 2 x 0.042	59	0	Whole plant*	7.60	0.51
Doc ID:	2007/1057852					31	Whole plant*	1.30	0.04
Trial No.:	L06901					55	Pods	1.10	<0.01
GLP:	Yes					55	Shoots**	1.20	<0.01
Year:	2007					84	Seeds	1.10	<0.01
Study code:	240079	Oilseed rape	France	BAS 134 00 W: BAS 083 W 2 x 0.294 BAS 555 F 2 x 0.042	59	0	Whole plant*	7.00	0.55
Doc ID:	2007/1057852					39	Whole plant*	1.40	0.02
Trial No.:	L06902					56	Pods	0.88	<0.01
GLP:	Yes					56	Shoots**	1.10	<0.01
Year:	2007					92	Seeds	1.50	<0.01

1 Growth stage at last treatment
2 Days after the last application
3 Sum of *cis* and *trans* isomer
* Without roots
** Without pods

Residue studies providing supporting data for the discussion paper Toxicological relevance of metabolites (M-CA 6.9.4)

The following residue studies conducted in the United States provide supporting data for the discussion paper on the Toxicological Relevance of Metabolites (M-CA 6.9.4). The U. S. residue studies supporting this assessment were submitted and evaluated in requests for import tolerances as reviewed by the Rapporteur Member State Belgium and EFSA (EFSA 2010) and MRL Review (EFSA 2011). Since these studies are considered as not peer reviewed, the studies are submitted with this dossier and are summarized in this chaper.

In residue field trials performed with metconazole in a wide range of areas in the United States and Canada, the commodities of cereals, soybean, sugar beet and cotton were analyzed for metconazole, (cis- and trans- isomers), and metabolites M11, M21 and M30. The U. S. trials were the only source of actual measured residues of metabolites in crops. Though the trials were performed in the U. S., the residue levels for the metabolites were taken as representative of expected metabolite residues in cereal treated in the EU since the U. S. cGAP was just slightly higher than the cGAP for the EU. The metabolite residues measured in the U.S. soybean trials with a similar cGAP were extrapolated to oilseed and dry pea and bean commodities in the EU.

Report: CA 6.10/6
White M.T.,Saha M., 2006d
The magnitude of residues of Metconazole (BAS 555 F) and its metabolites in soybeans
2006/7006995
Guidelines: EPA 860.1500
GLP: yes
(certified by United States Environmental Protection Agency)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description: BAS 555 01 F (SL)
Lot/Batch #: 2030 (90 g/L metconazole, nominal)
Purity: 87.9 g/L
CAS#: 125116-23-6
Development code: Not reported
Spiking levels: 0.005-0.5 mg/kg

2. Test Commodity:

Crop: Soya bean
Type: Oilseeds
Variety: Dekalb 3451, H7242; Asgrow AG1603, AG1903; Pioneer 92M80, 93B87-G4, 93M11; DKB38-52, NK 43-B1, Dyna-Gro 37B28RR, Mycogen 5140 RR, 26-02R RR
Botanical name: *Glycine max*
Crop parts(s) or processed commodity: Forage, hay, seed
Sample size: ≥0.5 kg, seed ≥1 kg

B. STUDY DESIGN

1. Test procedure

During the 2005 growing season 15 field trials in soya bean were conducted in different representative growing areas in the USA to determine the residue level of metconazole in or on raw agricultural commodities (RAC). BAS 555 01 F was applied twice at individual rates equivalent to 0.080 kg metconazole/ha at a 9-11 day retreatment interval in a spray volume of 131-299 L/ha. An adjuvant was added to the spray mixture for all applications. One untreated plot of each trial served as control. The first application targeted 40 days prior to harvest of mature (dry) seed. Duplicate samples of forage and hay were taken 6-8 days after the last application (DALA) and seed samples were harvested 28-32 DALA. Samples were generally stored frozen at or below -10°C for a maximum of 6.4 months until analysis.

Table 6.10-30: Target application rates and timings for soya bean

Year	No of trials	No of appl.	F P or G	Test item	Active substance	Appl. rate (kg a.s./ha)	Water volume (L/ha)	Target date/ Timing
2005	15	2	F	BAS 555 01 F (SL)	BAS 555 F	0.080	131-299	40 DBH 30 DBH

DBH Days before harvest

2. Description of analytical procedures

Soybean RAC samples were analyzed for residues of metconazole using an HPLC/ MS/MS method, BASF Analytical Method Number D0508, the data collection method for plant commodities. Briefly, residues of metconazole in soybean RAC (forage, hay and seed) samples were extracted with acetonitrile:water (70:30, v/v) using polytron homogenization. For seed, residues of metconazole (cis and trans), M11, M21 and M30 in the initial extract were diluted with methanol:water (80:20, v/v), filtered (0.45 µ), and analyzed. For forage and hay, an aliquot of the initial extract was concentrated, diluted with water, and partitioned with hexane:ethyl acetate (9:1, v/v). Residues of metconazole (cis and trans), M11, M21 and M30 in the organic phase were evaporated to dryness, re-dissolved in methanol:water (80:20, v/v), and filtered prior to analysis. The specimens were analyzed for metconazole (*cis*- and *trans*-isomer determined separately) with BASF method No 550/0 quantifying the analytes with a limit of quantitation (LOQ) of 0.005 mg/kg. Residues of metconazole in soya bean samples were extracted with methanol/water/HCl using Polytron homogenization. A portion of the extract was centrifuged, and an aliquot of the supernatant was diluted with water and subjected to liquid/liquid partitioning with dichloromethane (DCM). An aliquot of the DCM phase was collected and evaporated to dryness. The metconazole residues were then re-dissolved in methanol/water for analysis by HPLC-MS/MS.

The limit of quantitation (LOQ) is defined as the lowest fortification level successfully tested. During this study, a limit of quantitation of 0.005 mg/kg for BAS 555 F (each isomer, *cis* and *trans*) and a limit of quantitation of 0.01 mg/kg for M555F021, M555F011 and M555F030 was confirmed in different plant matrices. The limit of detection (LOD) was set at 20% of the limit of quantitation, corresponding to the lowest calibration level used. The LOD was equivalent to 0.002 mg/kg

Table 6.10-31: Summary of procedural recovery data for metconazole and metabolites

Matrix	Fortification level (mg/kg)	Summary recoveries					
		n	Mean (%)	RSD (%)	n	Mean (%)	RSD (%)
BASF method No D0508; LOQ = 0.005 mg/kg		***Cis*-isomer**			***Trans*-isomer**		
Forage	0.005, 0.5	6	101	12	6	90	5
Hay	0.005, 0.5	6	100	9	6	85	8
Seed	0.005, 0.5	8	100	14	8	100	15
		M11			**M21**		
Forage	0.01, 1	6	93	14	6	85	15
Hay	0.01, 1	6	85	10	6	96	13
Seed	0.01, 1	8	87	17	8	94	11
		M30					
Forage	0.01, 1	6	95	12			
Hay	0.01, 1	6	100	8			
Seed	0.01, 1	8	94	11			

II. RESULTS AND DISCUSSION

The residue ranges for the different trials are shown in Table 6.10-32, detailed residue levels are shown in Table 6.10-33.
Mean residues of metconazole (sum of both isomers) analyzed in forage specimens taken 6-8 DALA ranged between 1.18 and 2.17 mg/kg; in hay, residues were 1.38-3.29 mg/kg. At 28-32 DALA residues in seed specimens ranged between <0.01 and 0.03 mg/kg.
No residues of metconazole at or above the limit of quantitation (0.01 mg/kg) were found in any of the analyzed untreated specimens. Residues of metabolites are reported in the summary tables as <0.01 mg/kg but the residues are reported in the analytical detail tables as ND equivalent to <LOD or < 0.002 mg/kg.

Table 6.10-32: Summary of residues in soya bean treated with BAS 555 01 F

Region	Year	DALA[1]	Growth stage[2] (BBCH)	Range of residues (mg/kg)			
				Matrix	*Cis*-isomer	*Trans*-isomer	Total metconazole
North America	2005	6-8	n.r.	Forage	0.40-1.93	0.07-0.41	0.47-2.34
		6-8	n.r.	Hay	1.00-3.93	0.17-0.81	1.17-4.74
		28-32	n.r.	Seed	<0.005-0.025	<0.005-0.006	<0.01-0.031
					M11	**M21**	**M30**
		6-8	n.r.	Forage	<0.01-0.09	<0.01-0.03	<0.01-0.01
		6-8	n.r.	Hay	0.02-0.20	0.01-0.08	<0.01-0.04
		28-32	n.r.	Seed	<0.002 [3]	<0.002 [3]	<0.002 [3]

1 Days after last application
2 At sampling
3 Residues are reported in summary tables as < LOQ but are shown as ND in the analytical report detail tables.
n r. Not reported

III. CONCLUSION

Residues of metconazole in treated soya bean seed specimens collected at 28-32 DALA ranged from <0.01 to 0.031 mg/kg. Residues of metabolites M11, M21 and M30 were not detected (< LOD of 0.002 mg/kg) in soya bean seed.

Table 6.10-33: Residues of metconazole in soya bean after two applications of BAS 555 01 F in North America

Trial details		Crop	Country	Formulation, Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1)]	Residues found[3] (mg/kg)			
							Matrix	*Cis*-isomer	*Trans*-isomer	Total metconazole
Study code:	137726	Soya bean	USA	BAS 555 01 F 2 x 0.080	n r.	30	Seed	<0.005	<0.005	<0.01
Doc ID:	2006/7006995									
Trial No.:	R05111									
GLP:	yes									
Year:	2005									
Study code:	137726	Soya bean	USA	BAS 555 01 F 2 x 0.080	n r.	30	Seed	<0.005	<0.005	<0.01
Doc ID:	2006/7006995									
Trial No.:	R05112									
GLP:	yes									
Year:	2005									
Study code:	137726	Soya bean	USA	BAS 555 01 F 2 x 0.080	n r.	30	Seed	<0.005	<0.005	<0.01
Doc ID:	2006/7006995									
Trial No.:	R05113									
GLP:	yes									
Year:	2005									
Study code:	137726	Soya bean	USA	BAS 555 01 F 2 x 0.080	n r.	30	Seed	0.006 (0.007, 0.005)	<0.005	0.011 (0.012, 0.010)
Doc ID:	2006/7006995									
Trial No.:	R05114									
GLP:	yes									
Year:	2005									
Study code:	137726	Soya bean	USA	BAS 555 01 F 2 x 0.080	n r.	30	Seed	<0.005	<0.005	<0.01
Doc ID:	2006/7006995									
Trial No.:	R05115									
GLP:	yes									
Year:	2005									
Study code:	137726	Soya bean	USA	BAS 555 01 F 2 x 0.080	n r.	30	Seed	<0.005	<0.005	<0.01
Doc ID:	2006/7006995									
Trial No.:	R05116									
GLP:	yes									
Year:	2005									
Study code:	137726	Soya bean	USA	BAS 555 01 F 2 x 0.080	n r.	29	Seed	<0.005	<0.005	<0.01
Doc ID:	2006/7006995									
Trial No.:	R05117									
GLP:	yes									
Year:	2005									
Study code:	137726	Soya bean	USA	BAS 555 01 F 2 x 0.080	n r.	23	Seed	0.005 (<0.005, 0.006)	<0.005	0.01 (<0.01, 0.011)
Doc ID:	2006/7006995									
Trial No.:	R05118					30	Seed	<0.005	<0.005	<0.01
GLP:	yes					36	Seed	<0.005	<0.005	<0.01
Year:	2005					43	Seed	0.005 (0.005, <0.005)	<0.005	0.01 (0.010, <0.01)
Study code:	137726	Soya bean	USA	BAS 555 01 F 2 x 0.080	n r.	30	Seed	<0.005	<0.005	<0.01
Doc ID:	2006/7006995									
Trial No.:	R05119									
GLP:	yes									
Year:	2005									
Study code:	137726	Soya bean	Canada	BAS 555 01 F 2 x 0.080	n r.	32	Seed	0.024 (0.023, 0.025)	0.006	0.030 (0.029, 0.031)
Doc ID:	2006/7006995									
Trial No.:	R05120									
GLP:	yes									
Year:	2005									
Study code:	137726	Soya bean	USA	BAS 555 01 F 2 x 0.080	n r.	31	Seed	0.007 (0.006, 0.007)	<0.005	0.012 (0.011, 0.012)
Doc ID:	2006/7006995									
Trial No.:	R05121									
GLP:	yes									
Year:	2005									

Table 6.10-33: Residues of metconazole in soya bean after two applications of BAS 555 01 F in North America

Trial details		Crop	Country	Formulation, Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1)]	Residues found[3] (mg/kg)			
							Matrix	*Cis*-isomer	*Trans*-isomer	Total metconazole
Study code:	137726	Soya bean	USA	BAS 555 01 F 2 x 0.080	n r.	23	Seed	<0.005	<0.005	<0.01
Doc ID:	2006/7006995					30	Seed	<0.005	<0.005	<0.01
Trial No.:	R05122					37	Seed	<0.005	<0.005	<0.01
GLP:	yes					44	Seed	<0.005	<0.005	<0.01
Year:	2005									
Study code:	137726	Soya bean	USA	BAS 555 01 F 2 x 0.080	n r.	30	Seed	<0.005	<0.005	<0.01
Doc ID:	2006/7006995									
Trial No.:	R05123									
GLP:	yes									
Year:	2005									
Study code:	137726	Soya bean	USA	BAS 555 01 F 2 x 0.080	n r.	28	Seed	<0.005	<0.005	<0.01
Doc ID:	2006/7006995									
Trial No.:	R05124									
GLP:	yes									
Year:	2005									
Study code:	137726	Soya bean	Canada	BAS 555 01 F 2 x 0.080	n r.	30	Seed	<0.005	<0.005	<0.01
Doc ID:	2006/7006995									
Trial No.:	R05125									
GLP:	yes									
Year:	2005									

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
3 Mean of two samples; individual values are given in parentheses
n r. Not reported

Cotton

Report:	CA 6.10/7 Carringer S.J., 2007a Magnitude of the residue of Metconazole and its metabolites in or on cotton raw agricultural and processed commodities following applications of BAS 555 01 F 2007/7001663
Guidelines:	EPA 860.1500, EPA 860.1520
GLP:	yes (certified by United States Environmental Protection Agency)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	BAS 555 01 F (SL)
Lot/Batch #:	2030 (90 g/L metconazole, nominal)
Purity:	Not reported
CAS#:	125116-23-6
Development code:	Not reported
Spiking levels:	0.005-5.0 mg/kg

2. Test Commodity:

Crop:	Cotton
Type:	Oilseeds
Variety:	PHY 480 WR, DP444 BG/RR, PHY 485 WRF, DP444BR, DPL 444, FM9063 B2F, Fiber Max 960 BG II, DG 2242 B2RF, FM 960 B2R, Sierra RR, Roundup Ready, 45001 G
Botanical name:	*Gossypium* L.
Crop parts(s) or processed commodity:	Seed, undelinted seed, gin byproducts, hulls, meal, refined oil
Sample size:	≥0.9 kg / ≥12 units

B. STUDY DESIGN

1. Test procedure

During the 2006 growing season 12 field trials in cotton were conducted in different representative growing areas in the USA to determine the residue level of metconazole in or on raw agricultural commodities (RAC). BAS 555 01 F was applied three times at individual rates equivalent to 0.11 kg metconazole/ha at a 6-8 day retreatment interval in a spray volume of 94-243 L/ha. An adjuvant was added to the spray mixture for all applications. One untreated plot of each trial served as control. At one test site, an additional plot was established for the processing phase. This plot received three applications at 0.55 kg a.s./ha. The first application was performed approximately 44 days prior to the harvest. Duplicate samples of seed were taken 29-32 days after the last application (DALA) at crop maturity. At six sites, gin byproducts were collected. Seed samples were ginned simulating commercial practices for the collection of ginned undelinted cotton seed. Undelinted seed samples destined for processing were processed into hulls, meal and refined oil using simulated commercial procedures. Samples were generally stored deep-frozen at or below -10°C for a maximum of 192 days until analysis.

Table 6.10-34: Target application rates and timings for cotton

Year	No of trials	No of appl.	F P or G	Test item	Active substance	Appl. rate (kg a.s./ha)	Water volume (L/ha)	Target date/ Timing
2006	12	3	F	BAS 555 01 F (SL)	BAS 555 F	0.11*	n r.	44 DBH 37 DBH 30 DBH

n r. Not reported
DBH Days before harvest
* A second plot in one trial received 0.55 kg a.s./ha

2. Description of analytical procedures

The specimens were analyzed for metconazole (*cis*- and *trans*-isomer determined separately) with BASF method No D0604 quantifying the analytes with a limit of quantitation (LOQ) of 0.005 mg/kg. Residues of metconazole in cotton RAC and processed commodity samples (except oil) were extracted with acetonitrile/water using Polytron homogenization. An aliquot of the extract was diluted with water, concentrated to an aqueous remainder, and cleaned-up by partitioning with hexane/ethyl acetate. An aliquot of the organic phase was evaporated to dryness, and the residues were redissolved in methanol/water. The residues were then filtered and analyzed for residues of metconazole by HPLC/MS/MS. Residues in oil samples were extracted with acetonitrile/formic acid in water and hexane by liquid/liquid partitioning. Residues in the aqueous phase were evaporated to dryness, redissolved in methanol/water and analyzed by HPLC-MS/MS.

Table 6.10-35: Summary of procedural recovery data for metconazole and metabolites

Matrix	Fortification level (mg/kg)	Summary recoveries					
		n	Mean (%)	RSD (%)	n	Mean (%)	RSD (%)
BASF method No D0604; LOQ = 0.005 mg/kg		***Cis*-isomer**			***Trans*-isomer**		
Undelinted seed	0.005, 0.5	5	92	16	5	85	16
Gin byproducts	0.005, 0.5, 5.0	6	86	8.7	6	86	12
Refined oil	0.005, 0.5	2	103	N/A	2	107	N/A
		M11			**M21**		
Undelinted seed	0.01, 1	5	72	11	5	77	11
Gin byproducts	0.01, 1	4	77	5.5	4	83	9.0
Refined oil	0.01, 1	2	91	N/A	2	92	N/A
		M30					
Undelinted seed	0.01, 1	5	82	11			
Gin byproducts	0.01, 1	4	81	7.7			
Refined oil	0.01, 1	2	99	N/A			

II. RESULTS AND DISCUSSION

The residue ranges for the different trials are shown in Table 6.10-36, detailed residue levels are shown in Table 6.10-37.
Mean residues of metconazole (sum of both isomers) analyzed in undelinted seed specimens taken 29-32 DALA ranged between 0.020 and 0.232 mg/kg; in gin byproducts, residues were 0.17-4.15 mg/kg.
Residues declined in all processed commodities.
No residues of metconazole at or above the limit of quantitation (0.01 mg/kg) were found in any of the analyzed untreated specimens.

Table 6.10-36: Summary of mean residues in cotton treated with BAS 555 01 F

Region	Year	DALA[1]	Growth stage[2]	Range of residues (mg/kg)			
				Matrix	*Cis*-isomer	*Trans*-isomer	Total metconazole
North America	2006	29-32	Maturity	Undelinted seed	0.015-0.188	<0.005-0.044	0.020-0.232
		30-32	Maturity	Gin byproducts	0.139-3.368	0.032-0.780	0.17-4.15
					M11[3, 4]	**M21[3, 4]**	**M30[3, 4]**
		29-32	Maturity	Undelinted seed	<0.002-0.006	<0.002-0.004	<0.002-0.002
		30-32	Maturity	Gin byproducts	0.01-0.14	<0.01-0.04	<0.01-0.02

1 Days after last application
2 At sampling
3 Refers to single residues (no means)
4 Residues are reported as <0.01 mg/kg in summary tables; the calculated value in the analytical detail tables is reported here.

III. CONCLUSION

Average residues of metconazole in treated cotton undelinted seed specimens collected at 29-32 DALA ranged from 0.020 to 0.232 mg/kg. Residues of metabolites M11, M21 and M30 were below the LOQ of 0.01 mg/kg.

Table 6.10-37: Residues of metconazole in cotton after three applications of BAS 555 01 F in North America

Trial details	Crop	Country	Formulation, Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1)]	Residues found[3] (mg/kg)			
						Matrix	*Cis*-isomer	*Trans*-isomer	Total metconazole
Study code: 254317 **Doc ID:** 2007/7001663 **Trial No.:** RCN R06450 **GLP:** yes **Year:** 2006	Cotton	USA (GA)	BAS 555 01 F 3 x 0.11	50% open bolls	30	Seed[4]	0.055 (0.062, 0.048)	0.013 (0.015, 0.011)	0.068 (0.077, 0.059)
Study code: 254317 **Doc ID:** 2007/7001663 **Trial No.:** RCN R06451 **GLP:** yes **Year:** 2006	Cotton	USA (AR)	BAS 555 01 F 3 x 0.11	81	30 30	Seed[4] Gin bpdts[5]	0.019 (0.017, 0.020) 2.012 (1.945, 2.078)	0.006 (0.005, 0.006) 0.499 (0.492, 0.505)	0.024 (0.022, 0.026) 2.52 (2.44, 2.58)
Study code: 254317 **Doc ID:** 2007/7001663 **Trial No.:** RCN R06452 **GLP:** yes **Year:** 2006	Cotton	USA (LA)	BAS 555 01 F 3 x 0.11	81	29	Seed[4]	0.021 (0.020, 0.021)	0.006 (0.006, 0.006)	0.027 (0.026, 0.027)
Study code: 254317 **Doc ID:** 2007/7001663 **Trial No.:** RCN R06453 **GLP:** yes **Year:** 2006	Cotton	USA (AR)	BAS 555 01 F 3 x 0.11	15% open bolls	30 30	Seed[4] Gin bpdts[5]	0.015 (0.017, 0.012) 0.142 (0.136, 0.148)	0.005 (0.006, <0.005) 0.035 (0.034, 0.036)	0.020 (0.023, 0.017) 0.18 (0.17, 0.18)
Study code: 254317 **Doc ID:** 2007/7001663 **Trial No.:** RCN R06454 **GLP:** yes **Year:** 2006	Cotton	USA (TX)	BAS 555 01 F 3 x 0.11	81	31 31	Seed[4] Gin bpdts[5]	0.017 (0.011, 0.023) 0.139 (0.116, 0.161)	<0.005 (<0.005, 0.005) 0.032 (0.027, 0.037)	0.022 (0.016, 0.028) 0.17 (0.14, 0.20)
			BAS 555 01 3 x 0.55		31	Seed Meal Hulls Refined oil	0.106 (0.116, 0.097) <0.005 0.011 (0.007, 0.014) 0.011 (0.011, 0.011)	0.026 (0.029, 0.023) <0.005 <0.005 (<0.005,<0.005) <0.005 (<0.005,<0.005)	0.132 (0.144, 0.120) <0.01 0.016 (0.012, 0.019) 0.016 (0.016, 0.016)
Study code: 254317 **Doc ID:** 2007/7001663 **Trial No.:** RCN R06455 **GLP:** yes **Year:** 2006	Cotton	USA (TX)	BAS 555 01 F 3 x 0.11	79-80	30 30	Seed[4] Gin bpdts[5]	0.033 (0.023, 0.042) 3.368 (2.952, 3.783)	0.009 (0.006, 0.011) 0.780 (0.703, 0.856)	0.042 (0.030, 0.053) 4.15 (3.65, 4.64)
Study code: 254317 **Doc ID:** 2007/7001663 **Trial No.:** RCN R06456 **GLP:** yes **Year:** 2006	Cotton	USA (TX)	BAS 555 01 F 3 x 0.11	79	30 30	Seed[4] Gin bpdts[5]	0.058 (0.065, 0.050) 3.155 (3.203, 3.106)	0.014 (0.015, 0.012) 0.516 (0.529, 0.502)	0.071 (0.080, 0.062) 3.67 (3.73, 3.61)
Study code: 254317 **Doc ID:** 2007/7001663 **Trial No.:** RCN R06457 **GLP:** yes **Year:** 2006	Cotton	USA (OK)	BAS 555 01 F 3 x 0.11	87	32 32	Seed[4] Gin bpdts[5]	0.061 (0.063, 0.058) 2.288 (2.098, 2.478)	0.015 (0.016, 0.014) 0.530 (0.512, 0.547)	0.075 (0.078, 0.071) 2.82 (2.61, 3.02)

Table 6.10-37: Residues of metconazole in cotton after three applications of BAS 555 01 F in North America

Trial details		Crop	Country	Formulation, Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1)]	Residues found[3] (mg/kg)			
							Matrix	*Cis*-isomer	*Trans*-isomer	Total metconazole
Study code: **Doc ID:** **Trial No.:** **GLP:** **Year:**	254317 2007/7001663 RCN R06458 yes 2006	Cotton	USA (OK)	BAS 555 01 F 3 x 0.11	5% open bolls	31	Seed[4]	0.015 (0.016, 0.014)	0.005 (0.006, <0.005)	0.021 (0.022, 0.019)
Study code: **Doc ID:** **Trial No.:** **GLP:** **Year:**	254317 2007/7001663 RCN R06459 yes 2006	Cotton	USA (CA)	BAS 555 01 F 3 x 0.11	76	30	Seed[4]	0.041 (0.028, 0.053)	0.010 (0.007, 0.012)	0.051 (0.036, 0.065)
Study code: **Doc ID:** **Trial No.:** **GLP:** **Year:**	254317 2007/7001663 RCN R06460 yes 2006	Cotton	USA (CA)	BAS 555 01 F 3 x 0.11	76	30	Seed[4]	0.018 (0.017, 0.019)	0.005 (0.005, 0.005)	0.024 (0.023, 0.025)
Study code: **Doc ID:** **Trial No.:** **GLP:** **Year:**	254317 2007/7001663 RCN R06461 yes 2006	Cotton	USA (AZ)	BAS 555 01 F 3 x 0.11	50% open bolls	29	Seed[4]	0.188 (0.177, 0.199)	0.044 (0.041, 0.047)	0.232 (0.218, 0.246)

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
3 Mean of two samples; individual values are given in parentheses
4 Undelinted
5 Gin byproducts
_ Underlined values were used for MRL calculation

Table 6.10-38: Residues of metconazole metabolites in cotton after three applications of BAS 555 01 F in North America

Trial details		Crop	Country	Formulation, Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1)]	Residues found[3] (mg/kg)			
							Matrix	M11	M21	M30
Study code: **Doc ID:** **Trial No.:** **GLP:** **Year:**	254317 2007/7001663 RCN R06450 yes 2006	Cotton	USA (GA)	BAS 555 01 F 3 x 0.11	50% open bolls	30	Seed[4]	(0.003, 0.003)	(0.003, 0.003)	(<0.01, <0.01)
Study code: **Doc ID:** **Trial No.:** **GLP:** **Year:**	254317 2007/7001663 RCN R06451 yes 2006	Cotton	USA (AR)	BAS 555 01 F 3 x 0.11	81	30 30	Seed[4] Gin bpdts[5]	(0.003, 0.004) 0.13 (0.12, 0.14)	(<0.002, <0.002) 0.04 (0.04, 0.04)	(<0.002, <0.002 0.02 (0.02, 0.02)
Study code: **Doc ID:** **Trial No.:** **GLP:** **Year:**	254317 2007/7001663 RCN R06452 yes 2006	Cotton	USA (LA)	BAS 555 01 F 3 x 0.11	81	29	Seed[4]	(0.003, 0.003)	(<0.002, <0.002	(<0.01, <0.01)
Study code: **Doc ID:** **Trial No.:** **GLP:** **Year:**	254317 2007/7001663 RCN R06453 yes 2006	Cotton	USA (AR)	BAS 555 01 F 3 x 0.11	15% open bolls	30 30	Seed[4] Gin bpdts[5]	(0.003, 0.003 0.02 (0.02, 0.02)	(<0.002, <0.002 <0.01 (<0.01, <0.01)	(<0.002, <0.002) <0.01 (<0.01, <0.01)
Study code: **Doc ID:** **Trial No.:** **GLP:** **Year:**	254317 2007/7001663 RCN R06454 yes 2006	Cotton	USA (TX)	BAS 555 01 F 3 x 0.11	81	31 31	Seed[4] Gin bpdts[5]	(0.004, 0.003) 0.02 (0.01, 0.02)	(<0.002,0.0003) 0.03 (0.02, 0.03)	(<0.002, <0.002 <0.01 (<0.01, <0.01)
				BAS 555 01 3 x 0.55		31	Seed Meal Hulls Refined oil	(<0.01, <0.01) <0.01 <0.01 (<0.01, <0.01) <0.01 (<0.01, <0.01)	(<0.01, <0.01) <0.01 <0.01 (<0.01, <0.01) <0.01 (<0.01, <0.01)	(<0.01, <0.01) <0.01 <0.01 (<0.01, <0.01) <0.01 (<0.01, <0.01)
Study code: **Doc ID:** **Trial No.:** **GLP:** **Year:**	254317 2007/7001663 RCN R06455 yes 2006	Cotton	USA (TX)	BAS 555 01 F 3 x 0.11	79-80	30 30	Seed[4] Gin bpdts[5]	(0.003, 0.003) 0.07 (0.06, 0.08)	(<0.002, 0.003 0.04 (0.04, 0.04)	(<0.002, <0.002 0.01 (0.01, 0.01)
Study code: **Doc ID:** **Trial No.:** **GLP:** **Year:**	254317 2007/7001663 RCN R06456 yes 2006	Cotton	USA (TX)	BAS 555 01 F 3 x 0.11	79	30 30	Seed[4] Gin bpdts[5]	(0.004, 0.003) 0.07 (0.06, 0.07)	(0.003, 0.003) 0.04 (0.04, 0.04)	(<0.002, <0.002 0.01 (0.01, 0.01)
Study code: **Doc ID:** **Trial No.:** **GLP:** **Year:**	254317 2007/7001663 RCN R06457 yes 2006	Cotton	USA (OK)	BAS 555 01 F 3 x 0.11	87	32 32	Seed[4] Gin bpdts[5]	(0.006, 0.006) 0.12 (0.11, 0.13)	(0.003, <0.002) 0.03 (0.03, 0.03)	(0.002, 0.002) 0.02 (0.02, 0.02)
Study code: **Doc ID:** **Trial No.:** **GLP:** **Year:**	254317 2007/7001663 RCN R06458 yes 2006	Cotton	USA (OK)	BAS 555 01 F 3 x 0.11	5% open bolls	31	Seed[4]	(<0.002, <0.002)	(<0.002, <0.002)	(<0.002, <0.002)

Table 6.10-38: Residues of metconazole metabolites in cotton after three applications of BAS 555 01 F in North America

Trial details		Crop	Country	Formulation, Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1)]	Residues found[3] (mg/kg)			
							Matrix	M11	M21	M30
Study code: **Doc ID:** **Trial No.:** **GLP:** **Year:**	254317 2007/7001663 RCN R06459 yes 2006	Cotton	USA (CA)	BAS 555 01 F 3 x 0.11	76	30	Seed[4]	(0.003, 0.004)	(<0.002, <0.002)	(<0.002, <0.002)
Study code: **Doc ID:** **Trial No.:** **GLP:** **Year:**	254317 2007/7001663 RCN R06460 yes 2006	Cotton	USA (CA)	BAS 555 01 F 3 x 0.11	76	30	Seed[4]	(0.003, 0.003	(<0.002, <0.002)	(<0.002, <0.002
Study code: **Doc ID:** **Trial No.:** **GLP:** **Year:**	254317 2007/7001663 RCN R06461 yes 2006	Cotton	USA (AZ)	BAS 555 01 F 3 x 0.11	50% open bolls	29	Seed[4]	(0.003, 0.003)	(0.005, 0.005)	(0.002, 0.002

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
3 Mean of two samples; individual values are given in parentheses; expressed as parent equivalents
4 Undelinted
5 Gin byproducts

Maize / sweet corn

Report: CA 6.10/8
Carringer S.J., 2007b
Magnitude of the residue of Metconazole and its metabolites in or on field corn and sweet corn raw agricultural commodities and field corn processed commodities following applications of BAS 555 01 F
2006/7012839
Guidelines: EPA 860.1500, EPA 860.1520
GLP: yes
(certified by United States Environmental Protection Agency)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	BAS 555 01 F (SL)
Lot/Batch #:	2030 (90 g/L metconazole, nominal)
Purity:	Not reported
CAS#:	125116-23-6
Development code:	Not reported
Spiking levels:	0.005-0.5 mg/kg

2. Test Commodity:

Crop:	Maize; sweet corn
Type:	Cereals; fruiting vegetables
Variety:	Speedy Sweet, TA5750, Garst 8377, 8102 R Bicolor, Crows 7R154 Crows 5151, BT6516RR2Y G, Burrus 644 RWR, Wyffels W5531, Golden Harvest HX 9323, Pioneer 34A16, Middlekoop 2212, 33P65, DKC52-40 (RR2/YGPL), Pioneer 38H66, Pioneer 34N45 RR/YG, NK N73-F7 RR/LL/YG, NK N73-F7, DK C48-53 AF2, Bodacious, Golden Jubilee, Super Sweet Jubilee Plus
Botanical name:	*Zea mays*; *Zea mays* convar. *saccharata*
Crop parts(s) or processed commodity:	Kernels plus cobs with husks removed, forage, grain, stover; grits, meal, flour, refined oil, starch
Sample size:	≥1 kg / 12 units (with few exceptions)

B. STUDY DESIGN

1. Test procedure

During the 2006 growing season 20 field trials in maize and 5 trials in sweet corn were conducted in different representative growing areas in the USA to determine the residue level of metconazole in or on raw agricultural commodities (RAC). BAS 555 01 F was applied four times at individual rates equivalent to 0.11 kg metconazole/ha at a 5-9 day retreatment interval in a spray volume of 120-290 L/ha. The applications were initiated approximately 4 weeks prior to the harvest of sweet corn forage and kernel plus cob with husk removed (K+CWHR) samples and field corn forage samples (one plot) or approximately 6 weeks prior to harvest of mature grain and stover samples (other plot). An adjuvant was added to the spray mixture for all applications. At one test site, an additional plot was established for the processing phase. This plot received four applications at about 0.55 kg a.s./ha starting approximately 6 weeks prior to harvest of mature grain. One untreated plot of each trial served as control.

Duplicate K+CWHR samples were harvested 6-7 days after the last application (DALA) when the crop was at the milk stage. Forage samples were harvested 6-7 DALA when the crop was at the milk stage at the sweet corn trials and the seven maize trials also collecting sweet corn samples and at the late dough/early dent stage at the 13 maize only trials. Grain and stover samples were harvested at 20-22 DALA at the 20 maize trials. In addition, aspirated grain fraction samples, grits, meal, flour, starch and refined oil (dry milling and wet milling) were generated, according to simulated commercial practices, from bulk grain samples collected from one trial. Samples were generally stored deep-frozen at or below -10°C for a maximum of 170 days until analysis.

Table 6.10-39: Target application rates and timings for maize and sweet corn

Year	No of trials	No of appl.	F P or G	Test item	Active substance	Appl. rate (kg a.s./ha)	Water volume (L/ha)	Target date/ Timing
2006	25	4	F	BAS 555 01 F (SL)	BAS 555 F	0.11*	n r.	28 /42 DBH 21 /35 DBH 14 /28 DBH 7 /21 DBH

n r. Not reported
DBH Days before harvest
* A second plot in one trial received about 0.55 kg a.s./ha

2. Description of analytical procedures

The specimens were analyzed for metconazole (*cis*- and *trans*-isomer determined separately) with BASF method No D0604 quantifying the analytes with a limit of quantitation (LOQ) of 0.005 mg/kg. Residues of metconazole in maize and sweet corn samples were extracted with acetonitrile/water using polytron homogenization. The initial extract was cleaned-up by liquid/liquid partitioning and residues diluted with methanol/water, filtered and analyzed. Residues in oil samples were extracted with acetonitrile/formic acid in water and hexane by liquid-liquid partitioning. Residues in the aqueous phase were re-dissolved in methanol/water and analyzed. Residues of metconazole in the final extracts were determined by HPLC-MS/MS.

Table 6.10-40: Summary of procedural recovery data for metconazole

Matrix	Fortification level (mg/kg)	Summary recoveries					
		n	Mean (%)	RSD (%)	n	Mean (%)	RSD (%)
BASF method No D0604; LOQ = 0.005 mg/kg		***Cis*-isomer**			***Trans*-isomer**		
Forage	0.005, 0.5	8	92	8	8	94	18
Grain	0.005, 0.5	10	104	3	10	98	8
K+CWHR	0.005, 0.5	3	104	4	3	95	12
Stover	0.005, 0.5	4	91	5	4	84	14
Refined oil (dry milling)	0.005	1	93	N/A	1	84	N/A
Starch	0.005, 0.5	2	107	N/A	2	84	N/A
		M11			**M21**		
Forage	0.01, 1	8	78	11	8	89	18
Grain	0.01, 1	10	78	15	10	96	6
K+CWHR	0.01, 1	3	74	8	3	101	4
Stover	0.01, 1	4	78	17	4	95	10
Refined oil (dry milling)	0.01	1	130	N/A	1	123	N/A
Starch	0.01, 1	2	84	N/A	2	94	N/A
		M30					
Forage	0.01, 1	8	90	8			
Grain	0.01, 1	10	90	11			
K+CWHR	0.01, 1	3	88	18			
Stover	0.01, 1	4	78	11			
Refined oil (dry milling)	0.01	1	100	N/A			
Starch	0.01, 1	2	89	N/A			

II. RESULTS AND DISCUSSION

The residue ranges for the different trials are shown in Table 6.10-41, detailed residue levels are shown in Table 6.10-42.
Mean residues of metconazole (sum of both isomers) analyzed in maize and sweet corn forage specimens taken 6-7 DALA ranged between 0.04 and 2.66 mg/kg; in kernel plus cob with husk removed, mean residues were <0.01-0.010 mg/kg. In maize grain, mean residues ranged from <0.01 to 0.015 mg/kg at 20-22 DALA; in stover, mean residues were between 0.14 and 3.23 mg/kg.
No residues of *cis*- and *trans*-metconazole at or above the limit of quantitation (0.005 mg/kg) were found in any of the analyzed untreated specimens with the exception of two samples which bore *cis*-metconazole residues at 0.007 mg/kg (R06428, K+CWHR and R06432, stover). Apparent residues of metconazole were also below the method LOQ in all untreated control corn processed commodity samples including grits, meal, flour, refined oil (dry and wet milling), and starch.

Table 6.10-41: Summary of mean residues in maize and sweet corn treated with BAS 555 01 F

Region	Year	DALA[1]	Growth stage[2]	Range of residues (mg/kg)			
				Matrix	*Cis*-isomer	*Trans*-isomer	Total metconazole
North America	2006	6-7	Milk stage or late dough/ early dent stage	Forage	0.01-2.16	0.01-1.01	0.04-2.66
		6-7	Milk stage	K+CWHR	<0.005-0.005	<0.005	<0.01-0.010
		20-22	Maturity	Grains	<0.005-0.010	<0.005	<0.01-0.015
		20-22		Stover	0.11-2.59	0.03-0.62	0.14-3.23
					M11[3]	**M21[3]**	**M30[3]**
		6-7	Milk stage or late dough/ early dent stage	Forage	<0.01-0.040	0.01-0.06	<0.01
		6-7	Milk stage	K+CWHR	0.02-0.20	0.01-0.05	<0.01-0.03
		20-22	Maturity	Grains	<0.002	<0.002	<0.002
		20-22		Stover	<0.01	<0.01	<0.01

1 Days after last application
2 At sampling
3 Residues are reported as < L)Q of 0.01 mg/kg; measured concentrations in the analytical report detail tables are 0.0 equivalent to < LOD of 0.002 mg/kg.
K+CWHR Kernel plus cob with husk removed

III. CONCLUSION

Average residues of metconazole in treated maize grain specimens collected at 20-22 DALA ranged from <0.01 to 0.015 mg/kg; in maize or sweet corn kernel plus cob with husk removed, residues were at or below the LOQ of 0.01 mg/kg at 6-7 DALA. Residues of metabolites M11, M21 and M30 in maize grains were below the LOQ of 0.01 mg/kg. In maize or sweet corn kernel plus cob with husk removed, residues of up to 0.20, 0.05 and 0.03 mg/kg were found for M11, M21 and M30, respectively.

Table 6.10-42: Residues of metconazole in maize and sweet corn after four applications of BAS 555 01 F in North America

Trial details		Crop	Country	Formulation, Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1)]	Residues found[3] (mg/kg)			
							Matrix	*Cis*-isomer	*Trans*-isomer	Total metconazole
Study code:	254314	Sweet corn	USA (NY)	BAS 555 01 F 4 x 0.11	59-71	7	Forage	0.03 (0.02, 0.03)	0.01 (0.01, 0.01)	0.04 (0.03, 0.04)
Doc ID:	2006/7012839					7	K+CWHR	0.005 (<0.005, 0.006)	<0.005 (<0.005,<0.005)	0.010 (<0.01,0.011)
Trial No.:	RCN R06425									
GLP:	yes									
Year:	2006									
Study code:	254314	Maize	USA (PA)	BAS 555 01 F 4 x 0.11	Milk stage	7	Forage	0.69 (0.71, 0.66)	0.14 (0.13, 0.14)	0.82 (0.83, 0.80)
Doc ID:	2006/7012839					7	K+CWHR	<0.005	<0.005	<0.01
Trial No.:	RCN R06426				87	21	Grain	<0.005	<0.005	<0.01
GLP:	yes					21	Stover	0.88 (0.74, 1.01)	0.27 (0.18, 0.36)	1.15 (0.93, 1.37)
Year:	2006									
Study code:	254314	Maize	USA (NC)	BAS 555 01 F 4 x 0.11	67	7	Forage	1.42 (1.48, 1.36)	0.45 (0.44, 0.45)	1.87 (1.92, 1.81)
Doc ID:	2006/7012839					7	K+CWHR	<0.005	<0.005	<0.01
Trial No.:	RCN R06427				87	21	Grain	<0.005	<0.005	<0.01
GLP:	yes					21	Stover	0.11	0.03	0.14
Year:	2006									
Study code:	254314	Sweet corn	USA (FL)	BAS 555 01 F 4 x 0.11	75	7	Forage	0.95 (0.89, 1.00)	0.20 (0.19, 0.21)	1.15 (1.08, 1.21)
Doc ID:	2006/7012839					7	K+CWHR	<0.005	<0.005	<0.01
Trial No.:	RCN R06428									
GLP:	yes									
Year:	2006									
Study code:	254314	Maize	USA (OH)	BAS 555 01 F 4 x 0.11	73	7	Forage	0.01	0.09	0.10
Doc ID:	2006/7012839					7	K+CWHR	<0.005	<0.005	<0.01
Trial No.:	RCN R06429				87	20	Grain	<0.005	<0.005	<0.01
GLP:	yes					20	Stover	1.66 (1.66, 1.65)	0.37 (0.34, 0.39)	2.02 (2.00, 2.04)
Year:	2006									
Study code:	254314	Maize	USA (OH)	BAS 555 01 F 4 x 0.11	85	7	Forage	1.21 (1.21, 1.21)	0.29 (0.30, 0.27)	1.49 (1.51, 1.47)
Doc ID:	2006/7012839									
Trial No.:	RCN R06430				87	20	Grain	<0.005	<0.005	<0.01
GLP:	yes					20	Stover	1.33 (1.21, 1.45)	0.28 (0.21, 0.35)	1.61 (1.41, 1.80)
Year:	2006									

Table 6.10-42: Residues of metconazole in maize and sweet corn after four applications of BAS 555 01 F in North America

Trial details		Crop	Country	Formulation, Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1)]	Residues found[3] (mg/kg)			
							Matrix	*Cis*-isomer	*Trans*-isomer	Total metconazole
Study code:	254314	Maize	USA	BAS 555 01 F	R3-R4	7	Forage	0.36	0.17	0.53
Doc ID:	2006/7012839		(IL)	4 x 0.11						
Trial No.:	RCN R06431				R6	21	Grain	<0.005	<0.005	<0.01
GLP:	yes					21	Stover	2.23	0.46	2.69
Year:	2006							(2.59, 1.87)	(0.54, 0.37)	(3.13, 2.24)
						21	Grain	<0.005	<0.005	<0.01
							AGF	0.032	0.007	0.04
								(0.027, 0.037)	(0.006, 0.008)	(0.03, 0.04)
				BAS 555 01 F	R6	21	Grain	<0.005	<0.005	<0.01
				4 x 0.55			Grits	<0.005	<0.005	<0.01
							Meal	<0.005	<0.005	<0.01
							Flour	<0.005	<0.005	<0.01
							Ref. oil[4]	<0.005	<0.005	<0.01
							Starch	<0.005	<0.005	<0.01
							Ref. oil[5]	0.006	<0.005	0.011
								(0.007, 0.006)	(<0.005,<0.005)	(0.012, 0.011)
Study code:	254314	Maize	USA	BAS 555 01 F	85	7	Forage	0.25	1.01	1.25
Doc ID:	2006/7012839		(IL)	4 x 0.11				(0.20, 0.29)	(0.96, 1.05)	(1.16, 1.34)
Trial No.:	RCN R06432									
GLP:	yes				87-89	21	Grain	<0.005	<0.005	<0.01
Year:	2006					21	Stover	1.16	0.22	1.37
								(1.17, 1.14)	(0.22, 0.21)	(1.39, 1.35)
Study code:	254314	Maize	USA	BAS 555 01 F	R3	6	Forage	0.02	0.15	0.17
Doc ID:	2006/7012839		(IL)	4 x 0.11				(0.02, 0.01)	(0.17, 0.13)	(0.19, 0.14)
Trial No.:	RCN R06433					6	K+CWHR	<0.005	<0.005	<0.01
GLP:	yes				R5	22	Grain	<0.005	<0.005	<0.01
Year:	2006					22	Stover	1.69	0.40	2.10
								(1.58, 1.80)	(0.37, 0.43)	(1.96, 2.23)
Study code:	254314	Maize	USA	BAS 555 01 F	83	7	Forage	1.02	0.15	0.17
Doc ID:	2006/7012839		(IN)	4 x 0.11				(1.16, 0.88)	(0.13, 0.17)	(1.29, 1.05)
Trial No.:	RCN R06434				87-89	21	Grain	<0.005	<0.005	<0.01
GLP:	yes					21	Stover	1.50	0.30	1.81
Year:	2006							(1.09, 1.91)	(0.21, 0.39)	(1.31, 2.30)
Study code:	254314	Maize	USA	BAS 555 01 F	71-73	7	Forage	0.83	0.08	0.91
Doc ID:	2006/7012839		(IN)	4 x 0.11				(0.90, 0.76)	(0.06, 0.09)	(0.96, 0.85)
Trial No.:	RCN R06435					7	K+CWHR	<0.005	<0.005	<0.01
GLP:	yes				87	21	Grain	<0.005	<0.005	<0.01
Year:	2006					21	Stover	2.61	0.62	3.23
								(2.84, 2.38)	(0.69, 0.54)	(3.53, 2.93)
Study code:	254314	Maize	USA	BAS 555 01 F	R4	7	Forage	0.38	0.09	0.48
Doc ID:	2006/7012839		(IA)	4 x 0.11				(0.31, 0.45)	(0.07, 0.11)	(0.38, 0.57)
Trial No.:	RCN R06436				R5	21	Grain	<0.005	<0.005	<0.01
GLP:	yes					21	Stover	1.14	0.32	1.46
Year:	2006							(1.13, 1.15)	(0.32, 0.32)	(1.45, 1.47)
Study code:	254314	Maize	USA	BAS 555 01 F	R4	7	Forage	0.39	0.14	0.52
Doc ID:	2006/7012839		(IA)	4 x 0.11				(0.45, 0.32)	(0.11, 0.16)	(0.56, 0.48)
Trial No.:	RCN R06437				R5	21	Grain	<0.005	<0.005	<0.01
GLP:	yes					21	Stover	1.50	0.27	1.77
Year:	2006							(1.33, 1.66)	(0.19, 0.35)	(1.52, 2.01)
Study code:	254314	Maize	USA	BAS 555 01 F	R2.5	7	Forage	0.81	0.17	0.97
Doc ID:	2006/7012839		(IA)	4 x 0.11				(0.95, 0.66)	(0.14, 0.19)	(1.09, 0.85)
Trial No.:	RCN R06438					7	K+CWHR	<0.005	<0.005	<0.01
GLP:	yes				R5	21	Grain	<0.005	<0.005	<0.01
Year:	2006					21	Stover	1.91	0.39	2.30
								(2.16, 1.66)	(0.44, 0.34)	(2.60, 2.00)

Table 6.10-42: Residues of metconazole in maize and sweet corn after four applications of BAS 555 01 F in North America

Trial details		Crop	Country	Formulation, Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1)]	Residues found[3] (mg/kg)			
							Matrix	*Cis*-isomer	*Trans*-isomer	Total metconazole
Study code:	254314	Maize	USA	BAS 555 01 F	75	7	Forage	0.69	0.11	0.80
Doc ID:	2006/7012839		(IA)	4 x 0.11				(0.73, 0.65)	(0.10, 0.11)	(0.83, 0.76)
Trial No.:	RCN R06439				85	21	Grain	0.010	<0.005	0.015
GLP:	yes							(0.013, 0.006)	(<0.005,<0.005)	(0.018, 0.011)
Year:	2006					21	Stover	1.61	0.30	1.91
								(1.87, 1.35)	(0.37, 0.22)	(2.25, 1.57)
Study code:	254314	Maize	USA	BAS 555 01 F	R3	7	Forage	0.97	0.18	1.15
Doc ID:	2006/7012839		(WI)	4 x 0.11				(1.02, 0.92)	(0.19, 0.17)	(1.21, 1.08)
Trial No.:	RCN R06440					7	K+CWHR	<0.005	<0.005	<0.01
GLP:	yes				R5	21	Grain	<0.005	<0.005	<0.01
Year:	2006					21	Stover	1.49	0.28	1.76
								(1.19, 1.78)	(0.22, 0.33)	(1.41, 2.11)
Study code:	254314	Maize	USA	BAS 555 01 F	R4	7	Forage	1.02	0.24	1.26
Doc ID:	2006/7012839		(MN)	4 x 0.11				(0.96, 1.07)	(0.18, 0.30)	(1.14, 1.38)
Trial No.:	RCN R06441				R5/R6	21	Grain	<0.005	<0.005	<0.01
GLP:	yes					21	Stover	2.25	0.48	2.72
Year:	2006							(2.14, 2.36)	(0.47, 0.48)	(2.61, 2.83)
Study code:	254314	Maize	USA	BAS 555 01 F	R4	7	Forage	0.88	0.17	1.05
Doc ID:	2006/7012839		(MN)	4 x 0.11				(0.92, 0.84)	(0.18, 0.16)	(1.10, 1.00)
Trial No.:	RCN R06442				R5/R6	21	Grain	<0.005	<0.005	<0.01
GLP:	yes					21	Stover	1.59	0.35	1.94
Year:	2006							(1.84, 1.34)	(0.41, 0.28)	(2.25, 1.63)
Study code:	254314	Maize	USA	BAS 555 01 F	85	7	Forage	0.01	0.10	0.11
Doc ID:	2006/7012839		(NE)	4 x 0.11				(0.01, 0.01)	(0.09, 0.10)	(0.10, 0.11)
Trial No.:	RCN R06443				87	21	Grain	<0.005	<0.005	<0.01
GLP:	yes					21	Stover	1.30	0.21	1.51
Year:	2006							(1.38, 1.22)	(0.22, 0.20)	(1.60, 1.42)
Study code:	254314	Maize	USA	BAS 555 01 F	85	7	Forage	0.01	0.09	0.10
Doc ID:	2006/7012839		(NE)	4 x 0.11				(0.01, 0.01)	(0.09, 0.08)	(0.10, 0.09)
Trial No.:	RCN R06444				87	21	Grain	<0.005	<0.005	<0.01
GLP:	yes					21	Stover	0.79	0.15	0.94
Year:	2006							(0.84, 0.73)	(0.16, 0.14)	(1.00, 0.88)
Study code:	254314	Maize	USA	BAS 555 01 F	85	7	Forage	0.01	0.08	0.09
Doc ID:	2006/7012839		(NE)	4 x 0.11				(0.01, 0.01)	(0.08, 0.07)	(0.09, 0.08)
Trial No.:	RCN R06445				87	21	Grain	<0.005	<0.005	<0.01
GLP:	yes					21	Stover	1.70	0.33	2.03
Year:	2006							(1.60, 1.79)	(0.32, 0.34)	(1.92, 2.13)
Study code:	254314	Maize	USA	BAS 555 01 F	83	7	Forage	1.62	0.15	1.77
Doc ID:	2006/7012839		(OK)	4 x 0.11				(1.79, 1.45)	(0.13, 0.16)	(1.92, 1.61)
Trial No.:	RCN R06446				87	21	Grain	0.005	<0.005	0.01
GLP:	yes					21	Stover	2.05	0.40	2.45
Year:	2006							(1.72, 2.37)	(0.35, 0.45)	(2.07, 2.82)
Study code:	254314	Sweet	USA	BAS 555 01 F	78	7	Forage	2.16	0.50	2.66
Doc ID:	2006/7012839	corn	(CA)	4 x 0.11				(2.15, 2.17)	(0.46, 0.54)	(2.61, 2.71)
Trial No.:	RCN R06447					7	K+CWHR	<0.005	<0.005	<0.01
GLP:	yes									
Year:	2006									

Table 6.10-42: Residues of metconazole in maize and sweet corn after four applications of BAS 555 01 F in North America

Trial details		Crop	Country	Formulation, Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1)]	Residues found[3] (mg/kg)			
							Matrix	*Cis*-isomer	*Trans*-isomer	Total metconazole
Study code:	254314	Sweet corn	USA (WA)	BAS 555 01 F 4 x 0.11	73	7	Forage	0.02 (0.02, 0.02)	0.17 (0.14, 0.20)	0.19 (0.16, 0.22)
Doc ID:	2006/7012839									
Trial No.:	RCN R06448					7	K+CWHR	<0.005	<0.005	<0.01
GLP:	yes									
Year:	2006									
Study code:	254314	Sweet corn	USA (OR)	BAS 555 01 F 4 x 0.11	73	7	Forage	0.96 (1.01, 0.91)	0.17 (0.17, 0.16)	1.13 (1.19, 1.06)
Doc ID:	2006/7012839									
Trial No.:	RCN R06449					7	K+CWHR	<0.005	<0.005	<0.01
GLP:	yes									
Year:	2006									

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
3 Mean of two samples; individual values are given in parentheses
4 Dry milling
5 Wet milling
K+CWHR Kernel plus cob with husk removed
_ Underlined values were used for MRL calculation

Table 6.10-43: Residues of metconazole metabolites in maize and sweet corn after four applications of BAS 555 01 F in North America

Trial details		Crop	Country	Formulation, Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1)]	Residues found[3, 6] (mg/kg)			
							Matrix	M11	M21	M30
Study code:	254314	Sweet corn	USA (NY)	BAS 555 01 F 4 x 0.11	59-71	7	Forage	<0.01 (<0.01, <0.01)	0.03 (0.02, 0.03)	<0.01 (<0.01, <0.01)
Doc ID:	2006/7012839					7	K+CWHR	<0.01 (<0.01, <0.01)	<0.01 (<0.01, <0.01)	<0.01 (<0.01, <0.01)
Trial No.:	RCN R06425									
GLP:	yes									
Year:	2006									
Study code:	254314	Maize	USA (PA)	BAS 555 01 F 4 x 0.11	Milk stage	7	Forage	0.01 (<0.01, 0.02)	0.05 (0.04, 0.06)	<0.01 (<0.01, <0.01)
Doc ID:	2006/7012839					7	K+CWHR	<0.01	<0.01	<0.01
Trial No.:	RCN R06426				87	21	Grain	<0.01	<0.01	<0.01
GLP:	yes					21	Stover	0.08 (0.09, 0.07)	0.04 (0.03, 0.04)	0.01 (0.01, 0.01)
Year:	2006									
Study code:	254314	Maize	USA (NC)	BAS 555 01 F 4 x 0.11	67	7	Forage	0.03 (0.02, 0.03)	0.04 (0.04, 0.04)	<0.01 (<0.01, <0.01)
Doc ID:	2006/7012839					7	K+CWHR	<0.01	<0.01	<0.01
Trial No.:	RCN R06427				87	21	Grain	<0.01	<0.01	<0.01
GLP:	yes					21	Stover	0.11 (0.10, 0.11)	0.03 (0.03, 0.03)	0.05 (0.05, 0.04)
Year:	2006									
Study code:	254314	Sweet corn	USA (FL)	BAS 555 01 F 4 x 0.11	75	7	Forage	0.02 (0.02, 0.02)	0.04 (0.04, 0.04)	<0.01 (<0.01, <0.01)
Doc ID:	2006/7012839					7	K+CWHR	<0.01 (<0.01, <0.01)	<0.01 (<0.01, <0.01)	<0.01 (<0.01, <0.01)
Trial No.:	RCN R06428									
GLP:	yes									
Year:	2006									
Study code:	254314	Maize	USA (OH)	BAS 555 01 F 4 x 0.11	73	7	Forage	0.03 (0.02, 0.03)	0.02 (0.02, 0.02)	<0.01 (<0.01, <0.01)
Doc ID:	2006/7012839					7	K+CWHR	<0.01	<0.01	<0.01
Trial No.:	RCN R06429				87	20	Grain	<0.01	<0.01	<0.01
GLP:	yes					20	Stover	0.48 (0.48, 0.47)	0.14 0.14, 0.13)	0.07 (0.07, 0.06)
Year:	2006									
Study code:	254314	Maize	USA (OH)	BAS 555 01 F 4 x 0.11	85	7	Forage	0.20 (0.20, 0.19)	0.05 (0.05, 0.05)	0.03 (0.03, 0.03)
Doc ID:	2006/7012839									
Trial No.:	RCN R06430				87	20	Grain	<0.01	<0.01	<0.01
GLP:	yes					20	Stover	0.46 (0.43, 0.49)	0.08 (0.07, 0.08)	0.06 (0.05, 0.06)
Year:	2006									
Study code:	254314	Maize	USA (IL)	BAS 555 01 F 4 x 0.11	R3-R4	7	Forage	0.05 (0.04, 0.05)	0.03 (0.02, 0.03)	<0.01 (<0.01, <0.01)
Doc ID:	2006/7012839									
Trial No.:	RCN R06431				R6	21	Grain	<0.01	<0.01	<0.01
GLP:	yes					21	Stover	2.00 (2.41, 1.58)	0.37 (0.40, 0.34)	0.29 (0.30, 0.27)
Year:	2006					21	Grain AGF			
				BAS 555 01 F 4 x 0.55	R6	21	Grain Grits Meal Flour Ref. oil[4] Starch Ref. oil[5]			
Study code:	254314	Maize	USA (IL)	BAS 555 01 F 4 x 0.11	85	7	Forage	0.12 (0.13, 0.11)	0.04 (0.04, 0.04)	0.02 (0.02, 0.02)
Doc ID:	2006/7012839									
Trial No.:	RCN R06432				87-89	21	Grain	<0.01	<0.01	<0.01
GLP:	yes					21	Stover	0.30 (0.26, 0.34)	0.10 (0.09, 0.11)	0.06 (0.05, 0.06)
Year:	2006									

Table 6.10-43: Residues of metconazole metabolites in maize and sweet corn after four applications of BAS 555 01 F in North America

Trial details		Crop	Country	Formulation, Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1)]	Residues found[3, 6] (mg/kg)			
							Matrix	M11	M21	M30
Study code:	254314	Maize	USA	BAS 555 01 F	R3	6	Forage	0.04	0.04	<0.01
Doc ID:	2006/7012839		(IL)	4 x 0.11				(0.04, 0.04)	(0.04, 0.03)	(<0.01, <0.01)
Trial No.:	RCN R06433					6	K+CWHR	<0.01	<0.01	<0.01
GLP:	yes				R5	22	Grain	<0.002	<0.002	<0.002
Year:	2006					22	Stover	0.17	0.05	0.03
								(0.16, 0.17)	(0.05, 0.05)	(0.03, 0.03)
Study code:	254314	Maize	USA	BAS 555 01 F	83	7	Forage	0.04	0.03	<0.01
Doc ID:	2006/7012839		(IN)	4 x 0.11				(0.03, 0.04)	(0.03, 0.03)	(<0.01, <0.01)
Trial No.:	RCN R06434				87-89	21	Grain	<0.002	<0.002	<0.002
GLP:	yes					21	Stover	0.41	0.18	0.08
Year:	2006							(0.32, 0.50)	(0.14, 0.21)	(0.07, 0.08)
Study code:	254314	Maize	USA	BAS 555 01 F	71-73	7	Forage	0.02	0.04	<0.01
Doc ID:	2006/7012839		(IN)	4 x 0.11				(0.02, 0.02)	(0.03, 0.04)	(<0.01, <0.01)
Trial No.:	RCN R06435					7	K+CWHR	<0.01	<0.01	<0.01
GLP:	yes				87	21	Grain	<0.002	<0.002	<0.002
Year:	2006					21	Stover	0.43	0.11	0.06
								(0.47, 0.38)	(0.13, 0.09)	(0.06, 0.05)
Study code:	254314	Maize	USA	BAS 555 01 F	R4	7	Forage	0.04	0.02	<0.01
Doc ID:	2006/7012839		(IA)	4 x 0.11				(0.03, 0.05)	(0.02, 0.02)	(<0.01, <0.01)
Trial No.:	RCN R06436				R5	21	Grain	<0.002	<0.002	<0.002
GLP:	yes					21	Stover	0.34	0.06	0.05
Year:	2006							(0.36, 0.32)	(0.05, 0.07)	(0.04, 0.05)
Study code:	254314	Maize	USA	BAS 555 01 F	R4	7	Forage	0.05	0.02	<0.01
Doc ID:	2006/7012839		(IA)	4 x 0.11				(0.04, 0.06)	(0.01, 0.02)	(<0.01, 0.01)
Trial No.:	RCN R06437				R5	21	Grain	<0.002	<0.002	<0.002
GLP:	yes					21	Stover	0.20	0.08	0.04
Year:	2006							(0.14, 0.26)	(0.07, 0.09)	(0.03, 0.04)
Study code:	254314	Maize	USA	BAS 555 01 F	R2.5	7	Forage	0.02	0.04	<0.01
Doc ID:	2006/7012839		(IA)	4 x 0.11				(0.02, 0.02)	(0.03, 0.04)	(<0.01, <0.01)
Trial No.:	RCN R06438					7	K+CWHR	<0.01	<0.01	<0.01
GLP:	yes				R5	21	Grain	<0.002	<0.002	<0.002
Year:	2006					21	Stover	0.13	0.09	0.03
								(0.14, 0.12)	(0.09, 0.08)	(0.03, 0.03)
Study code:	254314	Maize	USA	BAS 555 01 F	75	7	Forage	0.02	0.03	<0.01
Doc ID:	2006/7012839		(IA)	4 x 0.11				(0.02, 0.02)	(0.03, 0.02)	(<0.01, <0.01)
Trial No.:	RCN R06439				85	21	Grain	<0.002	<0.002	<0.002
GLP:	yes							(<0.01, <0.01)	(<0.01, <0.01)	(<0.01, <0.01)
Year:	2006					21	Stover			
Study code:	254314	Maize	USA	BAS 555 01 F	R3	7	Forage	0.04	0.04	<0.01
Doc ID:	2006/7012839		(WI)	4 x 0.11				(0.03, 0.04)	(0.04, 0.03)	(<0.01, <0.01)
Trial No.:	RCN R06440					7	K+CWHR	<0.01	<0.01	<0.01
GLP:	yes				R5	21	Grain	<0.002	<0.002	<0.002
Year:	2006					21	Stover			
Study code:	254314	Maize	USA	BAS 555 01 F	R4	7	Forage	0.06	0.03	0.01
Doc ID:	2006/7012839		(MN)	4 x 0.11				(0.05, 0.07)	(0.03, 0.03)	(0.01, 0.01)
Trial No.:	RCN R06441				R5/R6	21	Grain	<0.002	<0.002	<0.002
GLP:	yes					21	Stover			
Year:	2006									
Study code:	254314	Maize	USA	BAS 555 01 F	R4	7	Forage	0.05	0.02	0.02
Doc ID:	2006/7012839		(MN)	4 x 0.11				(0.04, 0.05)	(0.02, 0.02)	(0.01, 0.02)
Trial No.:	RCN R06442				R5/R6	21	Grain	<0.002	<0.002	<0.002
GLP:	yes					21	Stover			
Year:	2006									

Table 6.10-43: Residues of metconazole metabolites in maize and sweet corn after four applications of BAS 555 01 F in North America

Trial details		Crop	Country	Formulation, Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1)]	Residues found[3, 6] (mg/kg)			
							Matrix	M11	M21	M30
Study code:	254314	Maize	USA	BAS 555 01 F	85	7	Forage	0.06	0.02	0.02
Doc ID:	2006/7012839		(NE)	4 x 0.11				(0.05, 0.06)	(0.02, 0.02)	(0.01, 0.02)
Trial No.:	RCN R06443				87	21	Grain	<0.002	<0.002	<0.002
GLP:	yes					21	Stover			
Year:	2006									
Study code:	254314	Maize	USA	BAS 555 01 F	85	7	Forage	0.05	0.02	0.01
Doc ID:	2006/7012839		(NE)	4 x 0.11				(0.05, 0.05)	(0.02, 0.01)	(0.01, 0.01)
Trial No.:	RCN R06444				87	21	Grain	<0.002	<0.002	<0.002
GLP:	yes					21	Stover			
Year:	2006									
Study code:	254314	Maize	USA	BAS 555 01 F	85	7	Forage	0.05	<0.01	0.02
Doc ID:	2006/7012839		(NE)	4 x 0.11				(0.06, 0.04)	(0.01, <0.01)	(0.02, 0.01)
Trial No.:	RCN R06445				87	21	Grain	<0.002	<0.002	<0.002
GLP:	yes					21	Stover			
Year:	2006									
Study code:	254314	Maize	USA	BAS 555 01 F	83	7	Forage	0.09	0.02	0.02
Doc ID:	2006/7012839		(OK)	4 x 0.11				(0.08, 0.09)	(0.02, 0.02)	(0.02, 0.02)
Trial No.:	RCN R06446				87	21	Grain	<0.002	<0.002	<0.002
GLP:	yes					21	Stover			
Year:	2006									
Study code:	254314	Sweet	USA	BAS 555 01 F	78	7	Forage	0.02	0.04	<0.01
Doc ID:	2006/7012839	corn	(CA)	4 x 0.11				(0.01, 0.02)	(0.04, 0.04)	(<0.01, <0.01)
Trial No.:	RCN R06447					7	K+CWHR	<0.01	<0.01	<0.01
GLP:	yes							(<0.01, <0.01)	(<0.01, <0.01)	(<0.01, <0.01)
Year:	2006									
Study code:	254314	Sweet	USA	BAS 555 01 F	73	7	Forage	<0.01	0.02	<0.01
Doc ID:	2006/7012839	corn	(WA)	4 x 0.11				(<0.01, <0.01)	(0.01, 0.02)	(<0.01, <0.01)
Trial No.:	RCN R06448					7	K+CWHR	<0.01	<0.01	<0.01
GLP:	yes							(<0.01, <0.01)	(<0.01, <0.01)	(<0.01, <0.01)
Year:	2006									
Study code:	254314	Sweet	USA	BAS 555 01 F	73	7	Forage	0.03	0.02	<0.01
Doc ID:	2006/7012839	corn	(OR)	4 x 0.11				(0.02, 0.03)	(0.02, 0.02)	(<0.01, <0.01)
Trial No.:	RCN R06449					7	K+CWHR	<0.01	<0.01	<0.01
GLP:	yes							(<0.01, <0.01)	(<0.01, <0.01)	(<0.01, <0.01)
Year:	2006									

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
3 Mean of two samples; individual values are given in parentheses
4 Dry milling
5 Wet milling
6 Residues of M11, M21 and M30 are reported as < LOQ in summary tables; concentrations in analytical detail tables are reported as 0.0 mg/kg equivalent to < LOD of 0.002 mg/kg.

K+CWHR Kernel plus cob with husk removed

Wheat

Report: CA 6.10/9
White M.T.,Saha M., 2006a
The magnitude of residues of Metconazole (BAS 555 F) and its metabolites in wheat
2006/7006723
Guidelines: EPA 860.1500
GLP: yes
(certified by United States Environmental Protection Agency)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	BAS 555 01 F (SL)
Lot/Batch #:	2030 or 2003 (90 g/L metconazole, nominal)
Purity:	Not reported
CAS#:	125116-23-6
Development code:	Not reported
Spiking levels:	0.005-0.5 mg/kg

2. Test Commodity:

Crop:	Wheat
Type:	Cereals
Variety:	Pioneer 26R24, Genesis R033, Millennium, Excel 201, Jagalene HRW, Dapps, Knudson, Jagger, TAM 200, Penawawa, Bounty, AC Barrie, AC Intrepid, 5700PR
Botanical name:	*Triticum aestivum*
Crop parts(s) or processed commodity:	Grain, hay, straw
Sample size:	≥0.5 kg

B. STUDY DESIGN

1. Test procedure

During the 2004/2005 growing seasons 15 field trials in winter and spring wheat were conducted in different representative growing areas in the USA and Canada to determine the residue level of metconazole in or on raw agricultural commodities (RAC). BAS 555 01 F was applied twice at individual rates equivalent to 0.11 kg metconazole/ha at a 6-8 day retreatment interval in a spray volume of 105-284 L/ha. The applications were initiated approximately 4 weeks prior to the harvest of mature grain (one plot) or approximately 2 weeks prior to the cutting of hay (other plot). An adjuvant was added to the spray mixture for all applications. One untreated plot of each trial served as control.

Duplicate hay samples were harvested 6-8 days after the last application (DALA), and grain and straw samples were harvested at 20-22 DALA. At two sites hay samples were harvested at 0, 7-8, and 14 DALA and grain and straw samples were harvested at 14, 21-22, 28, and 35-36 DALA to examine residue decline. Wheat hay samples were allowed to dry for up to 14 days before collection. Samples were generally stored deep-frozen at or below -10°C for a maximum of 250 days until analysis.

Table 6.10-44: Target application rates and timings for wheat

Year	No of trials	No of appl.	F P or G	Test item	Active substance	Appl. rate (kg a.s./ha)	Water volume (L/ha)	Target date/ Timing
2004/2005	15	2	F	BAS 555 01 F (SL)	BAS 555 F	0.11	n r.	14 /28 DBH 7 /21 DBH

n r. Not reported
DBH Days before harvest

2. Description of analytical procedures

The specimens were analyzed for metconazole (*cis*- and *trans*-isomer determined separately) with BASF method No D0508 quantifying the analytes with a limit of quantitation (LOQ) of 0.005 mg/kg. Residues of metconazole in maize and sweet corn samples were extracted with acetonitrile/water using polytron homogenization. The initial extract was diluted with methanol/water, filtered and analyzed. Residues of metconazole in the final extracts were determined by HPLC-MS/MS.

Table 6.10-45: Summary of procedural recovery data for metconazole

Matrix	Fortification level (mg/kg)	Summary recoveries					
		n	Mean (%)	RSD (%)	n	Mean (%)	RSD (%)
BASF method No D0508; LOQ = 0.005 mg/kg		***Cis*-isomer**			***Trans*-isomer**		
Grain	0.005, 0.5	6	93	12	6	98	16
Hay	0.005, 0.5	6	84	13	6	98	13
Straw	0.005, 0.5	6	87	19	6	96	18
		M11			**M21**		
Grain	0.01, 1	6	97	8	6	100	10
Hay	0.01, 1	6	85	15	6	98	9
Straw	0.01, 1	6	93	28	6	91	23
		M30					
Grain	0.01, 1	6	103	9			
Hay	0.01, 1	6	93	10			
Straw	0.01, 1	6	90	22			

II. RESULTS AND DISCUSSION

The residue ranges for the different trials are shown in Table 6.10-46, detailed residue levels are shown in Table 6.10-48.

Mean residues of metconazole (sum of both isomers) analyzed in wheat hay specimens taken 6-8 DALA ranged between 1.357 and 12.580 mg/kg. In grain, mean residues ranged from 0.011 to 0.096 mg/kg at 20-22 DALA; in straw, mean residues were between 0.420 and 9.729 mg/kg. The data from the residue decline experiments indicate that residues of metconazole decrease in wheat commodities with increasing pre-harvest intervals.

No residues of metconazole at or above the limit of quantitation (0.01 mg/kg) were found in any of the analyzed untreated specimens.

Table 6.10-46: Summary of mean residues in wheat treated with BAS 555 01 F

Region	Year	DALA[1]	Growth stage[2] (BBCH)	Range of residues (mg/kg)			
				Matrix	*Cis*-isomer	*Trans*-isomer	Total metconazole
North America	2004/2005	0	n.r.	Hay	9.430-10.160	1.504-1.682	10.934-11.842
		6-8	Commercial maturity (early flower (boot) to soft dough stage)	Hay	1.184-10.940	0.173-1.682	1.357-12.580
		14	n.r.	Hay	3.324-4.380	0.616-0.822	3.940-5.202
		14	Maturity	Grain	0.015-0.034	<0.005-0.006	0.020-0.040
		20-22	Maturity	Grain	0.006-0.080	<0.005-0.016	0.015-0.096
		28	Maturity	Grain	0.008-0.044	<0.005-0.007	0.013-0.011
		35-36	Maturity	Grain	0.008-0.035	<0.005-0.007	0.013-0.042
		14	Maturity	Straw	1.108-2.793	0.174-0.547	1.282-3.339
		20-22	Maturity	Straw	0.157-8.285	0.059-4.132	0.420-9.729
		28	Maturity	Straw	1.038-3.955	0.151-0.796	1.189-4.751
		35-36	Maturity	Straw	0.760-3.315	0.108-0.645	0.868-3.960
					M11[3]	**M21[3]**	**M30[3]**
		0	n.r.	Hay	0.03-0.04	0.05-0.07	<0.01-0.01
		6-8	Commercial maturity (early flower (boot) to soft dough stage)	Hay	<0.01-0.48	0.03-0.19	<0.01-0.06
		14	n.r.	Hay	0.05-0.10	0.07-0.08	0.01-0.02
		14	Maturity	Grain	<0.01-0.02	<0.01	<0.01
		20-22	Maturity	Grain	<0.01-0.03	<0.01-0.01	<0.01-0.01
		28	Maturity	Grain	<0.01-0.02	<0.01	<0.01
		35-36	Maturity	Grain	0.01-0.04	<0.01	<0.01
		14	Maturity	Straw	0.12-0.50	0.06-0.16	0.02-0.07
		20-22	Maturity	Straw	0.03-1.29	0.04-0.45	0.01-0.14
		28	Maturity	Straw	0.17-1.24	0.07-0.44	0.04-0.18
		35-36	Maturity	Straw	<0.01-1.64	0.05-0.47	0.03-0.23

1 Days after last application
2 At sampling
3 Refers to single residues (no means)
n r. Not reported

III. CONCLUSION

Average residues of metconazole in treated wheat grain specimens collected at 20-22 DALA ranged from 0.011 to 0.096 mg/kg. Residues of metabolites M11, M21 and M30 in wheat grain at 20-22 DALA were in the range of <0.01-0.03 mg/kg.

Table 6.10-47: Residues of metconazole in wheat after two applications of BAS 555 01 F in North America

Trial details		Crop	Country	Formulation, Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1)]	Residues found[3] (mg/kg)			
							Matrix	*Cis*-isomer	*Trans*-isomer	Total metconazole
Study code:	137711	Wheat	USA	BAS 555 01 F	Flag leaf	7	Hay	3.480	0.682	4.162
Doc ID:	2006/7006723	(winter)	(GA)	2 x 0.11	stage			(3.160, 3.800)	(0.628, 0.736)	(3.788, 4.536)
Trial No.:	RCN R05044				Soft	21	Grain	0.029	0.007	0.036
GLP:	yes				dough			(0.030, 0.027)	(0.007, 0.007)	(0.037, 0.035)
Year:	2004/2005					21	Straw	1.415	0.309	1.724
								(1.200, 1.630)	(0.269, 0.349)	(1.469, 1.979)
Study code:	137711	Wheat	USA	BAS 555 01 F	Full	7	Hay	5.280	0.904	6.184
Doc ID:	2006/7006723	(winter)	(AR)	2 x 0.11	flowering			(5.140, 5.420)	(0.868, 0.940)	(6.008, 6.360)
Trial No.:	RCN R05045				Medium	21	Grain	0.080	0.014	0.094
GLP:	yes				milk			(0.076, 0.083)	(0.011, 0.017)	(0.087, 0.100)
Year:	2004/2005					21	Straw	6.650	1.205	7.855
								(6.500, 6.800)	(1.130, 1.280)	(7.630, 8.080)
Study code:	137711	Wheat	USA	BAS 555 01 F	End of	8	Hay	1.184	0.173	1.357
Doc ID:	2006/7006723	(winter)	(NE)	2 x 0.11	flowering			(1.164, 1.204)	(0.168, 0.178)	(1.332, 1.382)
Trial No.:	RCN R05046				Early	20	Grain	0.014	0.005	0.019
GLP:	yes				dough			(0.012, 0.016)	(0.005,<0.005)	(0.017, 0.021
Year:	2004/2005					20	Straw	4.525	0.776	5.301
								(3.300, 5.750)	(0.553, 0.999)	(3.853, 6.749)
Study code:	137711	Wheat	USA	BAS 555 01 F	Full	0	Hay	9.430	1.504	10.934
Doc ID:	2006/7006723	(winter)	(IL)	2 x 0.11	flowering			(9.000, 9.860)	(1.544, 1.464)	(10.544, 11.324)
Trial No.:	RCN R05047					7	Hay	6.190	1.094	7.284
GLP:	yes							(6.740, 5.640)	(1.176, 1.012)	(7.916, 6.652)
Year:	2004/2005					14	Hay	4.380	0.822	5.202
								(4.940, 3.820)	(0.820, 0.824)	(5.760, 4.644)
					Early	14	Grain	0.034	0.006	0.040
					dough			(0.028, 0.040)	(0.007,<0.005)	(0.034, 0.045
							Straw	2.793	0.547	3.339
								(2.510, 3.075)	(0.515, 0.578)	(3.025, 3.653)
						21	Grain	0.058	0.011	0.070
								(0.065, 0.051)	(0.011, 0.010)	(0.077, 0.062)
(							Straw	3.833	0.779	4.611
								(3.860, 3.805)	(0.816, 0.741)	(4.676, 4.546)
						28	Grain	0.044	0.007	0.051
								(0.042, 0.046)	(0.008,<0.005)	(0.051, 0.051)
							Straw	3.955	0.796	4.751
								(3.660, 4.250)	(0.743, 0.849)	(4.403, 5.099)
						35	Grain	0.035	0.007	0.042
								(0.031, 0.039)	(<0.005,0.009)	(0.036, 0.048)
							Straw	3.315	0.645	3.960
								(3.435, 3.195)	(0.661, 0.628)	(4.096, 3.823)
Study code:	137711	Wheat	USA	BAS 555 01 F	End of	7	Hay	1.672	0.334	2.006
Doc ID:	2006/7006723	(winter)	(NE)	2 x 0.11	flowering			(1.824, 1.520)	(0.362, 0.306)	(2.186, 1.826)
Trial No.:	RCN R05048				Late	22	Grain	0.010	<0.005	0.015
GLP:	yes				milk			(0.012, 0.008)	(<0.005,<0.005)	(0.017, 0.013)
Year:	2004/2005						Straw	1.875	0.422	2.297
								(1.410, 2.340)	(0.305, 0.538)	(1.715, 2.878)

Table 6.10-47: Residues of metconazole in wheat after two applications of BAS 555 01 F in North America

Trial details	Crop	Country	Formulation, Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1)]	Residues found[3] (mg/kg)			
						Matrix	*Cis*-isomer	*Trans*-isomer	Total metconazole
Study code: 137711 **Doc ID:** 2006/7006723 **Trial No.:** RCN R05049 **GLP:** yes **Year:** 2005	Wheat (spring)	USA (ND)	BAS 555 01 F 2 x 0.11	Full flowering	8	Hay	5.000 (4.080, 5.920)	0.916 (0.740, 1.092)	5.916 (4.820, 7.012)
				Medium milk	20	Grain	0.032 (0.031, 0.032)	0.009 (0.009, 0.009)	0.040 (0.039, 0.040)
						Straw	2.108 (2.004, 2.212)	0.380 (0.347, 0.412)	2.488 (2.351, 2.624)
Study code: 137711 **Doc ID:** 2006/7006723 **Trial No.:** RCN R05050 **GLP:** yes **Year:** 2005	Wheat (spring)	USA (ND)	BAS 555 01 F 2 x 0.11	Early milk	0	Hay	10.160 (10.000, 10.320)	1.682 (1.764, 1.600)	11.842 (11.764, 11.920)
					8	Hay	5.130 (5.080, 5.180)	0.904 (0.868, 0.940)	6.034 (5.948, 6.120)
					14	Hay	3.324 (3.180, 3.468)	0.616 (0.592, 0.640)	3.940 (3.772, 4.108)
				Late milk	14	Grain	0.015 (0.013, 0.017)	<0.005 (<0.005,<0.005)	0.020 (0.018, 0.022)
						Straw	1.108 (1.108, 1.108)	0.174 (0.177, 0.170)	1.282 (1.285, 1.278)
					22	Grain	0.010 (0.009, 0.011)	<0.005 (<0.005,<0.005)	0.015 (0.014, 0.016)
						Straw	1.098 (1.260, 0.936)	0.144 (0.161, 0.126)	1.242 (1.421, 1.062)
					28	Grain	0.008	<0.005	0.013
						Straw	1.038 (1.044, 1.032)	0.151 (0.154, 0.147)	1.189 (1.198, 1.179)
					36	Grain	0.008 (0.007, 0.008)	<0.005 (<0.005,<0.005)	0.013 (0.012, 0.013)
						Straw	0.760 (0.660, 0.860)	0.108 (0.097, 0.118)	0.868 (0.757, 0.978)
Study code: 137711 **Doc ID:** 2006/7006723 **Trial No.:** RCN R05051 **GLP:** yes **Year:** 2004/2005	Wheat (winter)	USA (OK)	BAS 555 01 F 2 x 0.11	First grains half final size	7	Hay	6.220 (6.720, 5.720)	1.050 (1.136, 0.964)	7.270 (7.856, 6.684)
				Soft dough	22	Grain	0.006	<0.005	0.011
					22	Straw	0.361 (0.218, 0.504)	0.059 (0.048, 0.070)	0.420 (0.266, 0.574)
Study code: 137711 **Doc ID:** 2006/7006723 **Trial No.:** RCN R05052 **GLP:** yes **Year:** 2004/2005	Wheat (winter)	USA (OK)	BAS 555 01 F 2 x 0.11	Late milk	6	Hay	8.330 (8.540, 8.120)	1.396 (1.400, 1.392)	9.726 (9.940, 9.512)
				Soft dough	21	Grain	0.080 (0.097, 0.063)	0.016 (0.019, 0.013)	0.096 (0.116, 0.075)
					21	Straw	8.285 (8.180, 8.390)	1.444 (1.436, 1.452)	9.729 (9.616, 9.842)
Study code: 137711 **Doc ID:** 2006/7006723 **Trial No.:** RCN R05053 **GLP:** yes **Year:** 2004/2005	Wheat (winter)	USA (TX)	BAS 555 01 F 2 x 0.11	End of heading	7	Hay	8.970 (8.440, 9.500)	1.682 (1.644, 1.720)	10.652 (10.084, 11.220)
				Hard dough	21	Grain	0.029 (0.026, 0.031)	0.006 (0.005, 0.006)	0.035 (0.032, 0.037)
					21	Straw	3.466 (3.080, 3.852)	0.662 (0.564, 0.760)	4.138 (3.664, 4.612)
Study code: 137711 **Doc ID:** 2006/7006723 **Trial No.:** RCN R05054 **GLP:** yes **Year:** 2005	Wheat (spring)	USA (ID)	BAS 555 01 F 2 x 0.11	Full flowering	7	Hay	5.080 (4.860, 5.300)	0.828 (0.916, 0.740)	5.908 (5.776, 6.040)
				Hard dough	21	Grain	0.041 (0.043, 0.038)	0.008 (0.008, 0.007)	0.048 (0.051, 0.045)
					21	Straw	0.772 (0.632, 0.912)	4.132 (3.544, 4.720)	4.904 (4.176, 5.632)

Table 6.10-47: Residues of metconazole in wheat after two applications of BAS 555 01 F in North America

Trial details		Crop	Country	Formulation, Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1)]	Residues found[3] (mg/kg)			
							Matrix	*Cis*-isomer	*Trans*-isomer	Total metconazole
Study code:	137711	Wheat	Canada	BAS 555 01 F	Full	7	Hay	10.940	1.640	12.580
Doc ID:	2006/7006723	(spring)	(SK)	2 x 0.11	flowering			(10.980, 10.900)	(1.684, 1.596)	(12.664, 12.496)
Trial No.:	RCN R05055				Soft	21	Grain	0.018	<0.005	0.023
GLP:	yes				dough			(0.019, 0.017)	(<0.005,<0.005)	(0.024, 0.022)
Year:	2005					21	Straw	0.157	1.032	1.189
								(0.153, 0.160)	(1.044, 1.020)	(1.197, 1.180)
Study code:	137711	Wheat	Canada	BAS 555 01 F	First	7	Hay	5.380	0.886	6.266
Doc ID:	2006/7006723	(spring)	(MB)	2 x 0.11	grains			(5.520, 5.240)	(0.956, 0.816)	(6.476, 6.056)
Trial No.:	RCN R05056				half					
GLP:	yes				final size					
Year:	2005				Soft	21	Grain	0.022	<0.005	0.027
					dough			(0.020, 0.023)	(<0.005,<0.005)	(0.025, 0.028)
						21	Straw	0.387	2.118	2.505
								(0.416, 0.358)	(2.040, 2.196)	(2.456, 2.554)
Study code:	137711	Wheat	Canada	BAS 555 01 F	Begin of	7	Hay	4.720	0.790	5.510
Doc ID:	2006/7006723	(spring)	(AB)	2 x 0.11	flowering			(4.780, 4.660)	(0.744, 0.836)	(5.524, 5.496)
Trial No.:	RCN R05057				Soft	21	Grain	0.022	0.005	0.028
GLP:	yes				dough			(0.022, 0.022)	(0.005, 0.005)	(0.028, 0.027)
Year:	2005					21	Straw	0.379	2.090	2.469
								(0.353, 0.404)	(2.068, 2.112)	(2.421, 2.516)
Study code:	137711	Wheat	Canada	BAS 555 01 F	Full	7	Hay	5.740	0.918	6.658
Doc ID:	2006/7006723	(spring)	(AB)	2 x 0.11	flowering			(5.500, 5.980)	(1.012, 0.824)	(6.512, 6.804)
Trial No.:	RCN R05058				Soft	21	Grain	0.044	0.010	0.054
GLP:	yes				dough	21	Straw	0.554	2.944	3.488
Year:	2005							(0.554, 0.554)	(2.820, 3.068)	(3.364, 3.612)

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
3 Mean of two samples; individual values are given in parentheses

Table 6.10-48: Residues of metconazole metabolites in wheat after two applications of BAS 555 01 F in North America

Trial details	Crop	Country	Formulation, Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1)]	Residues found[3] (mg/kg)			
						Matrix	M11	M21	M30
Study code: 137711 **Doc ID:** 2006/7006723 **Trial No.:** RCN R05044 **GLP:** yes **Year:** 2004/2005	Wheat (winter)	USA (GA)	BAS 555 01 F 2 x 0.11	Flag leaf stage	7	Hay	0.02	0.05 (0.04, 0.05)	<0,01
				Soft dough	21	Grain	0.02	<0.01	<0.01
					21	Straw	1.08 (0.86, 1.29)	0.11 (0.09, 0.12)	0.10 (0.08, 0.11)
Study code: 137711 **Doc ID:** 2006/7006723 **Trial No.:** RCN R05045 **GLP:** yes **Year:** 2004/2005	Wheat (winter)	USA (AR)	BAS 555 01 F 2 x 0.11	Full flowering	7	Hay	0.05	0.06	0.01
				Medium milk	21	Grain	<0.01	<0.01	<0.01
					21	Straw	0.39 (0.38, 0.39)	0.20 (0.19, 0.21)	0.05
Study code: 137711 **Doc ID:** 2006/7006723 **Trial No.:** RCN R05046 **GLP:** yes **Year:** 2004/2005	Wheat (winter)	USA (NE)	BAS 555 01 F 2 x 0.11	End of flowering	8	Hay	0.13	0.09 (0.08, 0.09)	0.04 (0.03, 0.04)
				Early dough	20	Grain	<0.01	<0.01	<0.01
					20	Straw	0.66 (0.56, 0.76)	0.27 (0.21, 0.32)	0.11 (0.10, 0.12)
Study code: 137711 **Doc ID:** 2006/7006723 **Trial No.:** RCN R05047 **GLP:** yes **Year:** 2004/2005	Wheat (winter)	USA (IL)	BAS 555 01 F 2 x 0.11	Full flowering	0	Hay	0.03	0.06 (0.05, 0.06)	<0.01
					7	Hay	0.04 (0.03, 0.05)	0.07 (0.06, 0.07)	0.01
					14	Hay	0.06 (0.05, 0.06)	0.07	0.02 (0.01, 0.02)
				Early dough	14	Grain	0.02 (0.01, 0.02)	<0.01	<0.01
						Straw	0.49 (0.48, 0.50)	0.15 (0.14, 0.15)	0.06 (0.05, 0.07)
					21	Grain	0.03	<0.01	<0.01
						Straw	0.86 (0.78, 0.93)	0.31 (0.23, 0.38)	0.14 (0.11, 0.17)
					28	Grain	0.02	<0.01	<0.01
						Straw	1.23 (1.22, 1.24)	0.41 (0.38, 0.44)	0.18 (0.1, 0.18)
					35	Grain	0.04	<0.01	<0.01
						Straw	1.58 (1.51, 1.64)	0.42 (0.37, 0.47)	0.23 (0.22, 0.23)
Study code: 137711 **Doc ID:** 2006/7006723 **Trial No.:** RCN R05048 **GLP:** yes **Year:** 2004/2005	Wheat (winter)	USA (NE)	BAS 555 01 F 2 x 0.11	End of flowering	7	Hay	0.02	0.03	<0.01
				Late milk	22	Grain	<0.01	<0.01	<0.01
						Straw	0.52 (0.45, 0.59)	0.17 (0.14, 0.20)	0.10 (0.08, 0.12)
Study code: 137711 **Doc ID:** 2006/7006723 **Trial No.:** RCN R05049 **GLP:** yes **Year:** 2005	Wheat (spring)	USA (ND)	BAS 555 01 F 2 x 0.11	Full flowering	8	Hay	<0.01 (<0.01, 0.01)	0.05 (0.04, 0.05)	<0.01
				Medium milk	20	Grain	<0.01	<0.01	<0.01
						Straw	0.07	0.09 (0.08, 0.09)	0.02

Table 6.10-48: Residues of metconazole metabolites in wheat after two applications of BAS 555 01 F in North America

Trial details		Crop	Country	Formulation, Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1)]	Residues found[3] (mg/kg)			
							Matrix	M11	M21	M30
Study code:	137711	Wheat	USA	BAS 555 01 F	Early milk	0	Hay	0.04	0.07 (0.06, 0.07)	0.01
Doc ID:	2006/7006723	(spring)	(ND)	2 x 0.11		8	Hay	0.37 (0.36, 0.37)	0.13	0.05 (0.04, 0.05)
Trial No.:	RCN R05050					14	Hay	0.1 (0.09, 0.1)	0.08	0.02
GLP:	yes				Late milk	14	Grain	<0.01	<0.01	<0.01
Year:	2005						Straw	0.12	0.06	0.02
						22	Grain	<0.01	<0.01	<0.01
							Straw	0.16 (0.14, 0.17)	0.07	0.03
						28	Grain	<0.01 (<0.01, 0.01)	<0.01	<0.01
							Straw	0.18 (0.17, 0.18)	0.07	0.04
						36	Grain	0.01	<0.01	<0.01
							Straw	0.07 (<0.01, 0.14)	0.06 (0.05, 0.06)	0.04 (0.03, 0.04)
Study code:	137711	Wheat	USA	BAS 555 01 F	First grains half final size	7	Hay	0.14 (0.13, 0.14)	0.17	0.04
Doc ID:	2006/7006723	(winter)	(OK)	2 x 0.11						
Trial No.:	RCN R05051									
GLP:	yes				Soft dough	22	Grain	<0.01	<0.01	<0.01
Year:	2004/2005					22	Straw	0.10 (0.08, 0.11)	0.05 (0.04, 0.06)	0.03 (0.02, 0.03)
Study code:	137711	Wheat	USA	BAS 555 01 F	Late milk	6	Hay	0.09 (0.08, 0.09)	0.12 (0.11, 0.12)	0.02
Doc ID:	2006/7006723	(winter)	(OK)	2 x 0.11	Soft dough	21	Grain	0.02 (0.01, 0.02)	<0.01	<0.01
Trial No.:	RCN R05052									
GLP:	yes					21	Straw	0.89 (0.86, 0.91)	0.45	0.12
Year:	2004/2005									
Study code:	137711	Wheat	USA	BAS 555 01 F	End of heading	7	Hay	0.07	0.08 (0.07, 0.08)	0.02
Doc ID:	2006/7006723	(winter)	(TX)	2 x 0.11						
Trial No.:	RCN R05053				Hard dough	21	Grain	<0.01	<0.01	<0.01
GLP:	yes									
Year:	2004/2005					21	Straw	0.29 (0.18, 0.40)	0.06 (0.05, 0.08)	0.05 (0.04, 0.05)
Study code:	137711	Wheat	USA	BAS 555 01 F	Full flowering	7	Hay	0.03	0.08	0.01
Doc ID:	2006/7006723	(spring)	(ID)	2 x 0.11						
Trial No.:	RCN R05054				Hard dough	21	Grain	<0.01	<0.01	<0.01
GLP:	yes									
Year:	2005					21	Straw	0.04 (0.03, 0.04)	0.06 (0.05, 0.06)	0.01
Study code:	137711	Wheat	Canada	BAS 555 01 F	Full flowering	7	Hay	0.03	0.08	0.02
Doc ID:	2006/7006723	(spring)	(SK)	2 x 0.11						
Trial No.:	RCN R05055				Soft dough	21	Grain	<0.01	<0.01	<0.01
GLP:	yes									
Year:	2005					21	Straw	0.07 (0.06, 0.07)	0.07	0.01

Table 6.10-48: Residues of metconazole metabolites in wheat after two applications of BAS 555 01 F in North America

Trial details		Crop	Country	Formulation, Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1)]	Residues found[3] (mg/kg)			
							Matrix	M11	M21	M30
Study code:	137711	Wheat (spring)	Canada (MB)	BAS 555 01 F 2 x 0.11	First grains half final size	7	Hay	0.06 (0.05, 0.06)	0.08	0.02
Doc ID:	2006/7006723									
Trial No.:	RCN R05056									
GLP:	yes									
Year:	2005				Soft dough	21	Grain	0.02	<0.01	<0.01
						21	Straw	0.14	0.13 (0.12, 0.13)	0.03
Study code:	137711	Wheat (spring)	Canada (AB)	BAS 555 01 F 2 x 0.11	Begin of flowering	7	Hay	0.12 (0.11, 0.13)	0.13	0.03
Doc ID:	2006/7006723				Soft dough	21	Grain	<0.01	<0.01	<0.01
Trial No.:	RCN R05057									
GLP:	yes									
Year:	2005					21	Straw	0.08 (0.07, 0.08)	0.09 (0.08, 0.09)	0.01
Study code:	137711	Wheat (spring)	Canada (AB)	BAS 555 01 F 2 x 0.11	Full flowering	7	Hay	0.34 (0.19, 0.48)	0.18 (0.16, 0.19)	0.05 (0.04, 0.06)
Doc ID:	2006/7006723				Soft dough	21	Grain	0.02	<0.01	<0.01
Trial No.:	RCN R05058					21	Straw	0.44 (0.43, 0.45)	0.21 (0.20, 0.21)	0.04
GLP:	yes									
Year:	2005									

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
3 Mean of two samples; individual values are given in parentheses

Sugar beet

Report: CA 6.10/10
Jordan J.M.,Saha M., 2006a
The magnitude of residues of Metconazole (BAS 555 F) and its metabolites in sugar beet
2006/7006726
Guidelines: EPA 860.1500
GLP: yes
(certified by United States Environmental Protection Agency)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	BAS 555 01 F (SL)
Lot/Batch #:	2030 (90 g/L metconazole, nominal)
Purity:	Not reported
CAS#:	125116-23-6
Development code:	Not reported
Spiking levels:	0.005-0.5 mg/kg

2. Test Commodity:

Crop:	Sugar beet
Type:	Sugar plants
Variety:	VDH66556 8232 Medium, Vanderhave VDH 66556, 66453 (Vanderhave), Eagle R, Treasure, HM WS91, Beta 8422
Botanical name:	*Beta vulgaris*
Crop parts(s) or processed commodity:	Tops, roots
Sample size:	≥12 units

B. STUDY DESIGN

1. Test procedure

During the 2005 growing season 12 field trials in sugar beet were conducted in different representative growing areas in the USA to determine the residue level of metconazole in or on raw agricultural commodities (RAC). BAS 555 01 F was applied twice at individual rates equivalent to 0.11 kg metconazole/ha at a 13-15 day retreatment interval in a spray volume of 168-286 L/ha. On a second plot, the test item was applied twice at individual rates equivalent to 0.17 kg metconazole/ha at a 13-15 day retreatment interval in a spray volume of 156-278 L/ha. An adjuvant was added to the spray mixture for all applications. One untreated plot of each trial served as control. Duplicate commercially mature sugar beet tops (leaves) and root RAC samples were harvested 13-15 days after the last application (DALA). At two sites, tops and root samples were harvested at 1, 7, 14-15, 21, and 27-28 DALA to examine residue decline. Samples were generally stored deep-frozen at or below -10°C for a maximum of 197 days until analysis.

Table 6.10-49: Target application rates and timings for sugar beet

Year	No of trials	No of appl.	F P or G	Test item	Active substance	Appl. rate (kg a.s./ha)	Water volume (L/ha)	Target date/ Timing
2005	12	2	F	BAS 555 01 F (SL)	BAS 555 F	0.11 0.17	n r.	28 DBH 14 DBH

n r. Not reported
DBH Days before harvest

2. Description of analytical procedures

The specimens were analyzed for metconazole (*cis*- and *trans*-isomer determined separately) with BASF method No D0508 quantifying the analytes with a limit of quantitation (LOQ) of 0.005 mg/kg. Residues of metconazole in sugar beet RAC samples were extracted with acetonitrile/water using polytron homogenization. The initial extract was diluted with methanol/water, filtered and analyzed. Residues of metconazole in the final extracts were determined by HPLC-MS/MS.

Table 6.10-50: Summary of procedural recovery data for metconazole

Matrix	Fortification level (mg/kg)	Summary recoveries					
		n	Mean (%)	RSD (%)	n	Mean (%)	RSD (%)
BASF method No D0508; LOQ = 0.005 mg/kg		***Cis*-isomer**			***Trans*-isomer**		
Roots	0.005, 0.5	12	93	12	12	99	20
Tops	0.005, 0.5	12	90	13	12	82	18
		M11			**M21**		
Roots	0.01, 1.0	12	93	13	12	96	14
Tops	0.01, 1.0	12	82	11	12	97	14
		M30					
Roots	0.01, 1.0	12	93	10			
Tops	0.01, 1.0	12	93	14			

II. RESULTS AND DISCUSSION

The residue ranges for the different trials are shown in Table 6.10-51, detailed residue levels are shown in Table 6.10-53.
After treatment of 2 x 0.11 kg a.s./ha, mean residues of metconazole (sum of both isomers) analyzed in top specimens taken 13-15 DALA ranged between 0.039 and 1.210 mg/kg; in roots, residues were <0.01-0.044 mg/kg. After treatment of 2 x 0.17 kg a.s./ha, mean residues in top specimens taken 13-15 DALA ranged between 0.051 and 2.249 mg/kg; in roots, residues were <0.01-0.083 mg/kg. The data from the residue decline experiments indicate that residues of metconazole decrease in sugar beet commodities (tops and root) with increasing pre-harvest intervals.
No residues of metconazole at or above the limit of quantitation (0.01 mg/kg) were found in any of the analyzed untreated specimens.

Table 6.10-51: Summary of mean residues in sugar beet treated with BAS 555 01 F

Region	Year	DALA[1]	Growth stage[2] (BBCH)	Range of residues (mg/kg)			
				Matrix	*Cis*-isomer	*Trans*-isomer	Total metconazole
North America	2005 (2 x 0.11 kg a.s./ha)	1	n.r.	Tops	0.027-2.078	<0.005-0.362	0.032-2.440
		7	n.r.	Tops	0.021-1.363	0.176-0.248	0.197-1.661
		13-15	Maturity	Tops	0.010-1.047	0.006-0.173	0.039-1.210
		21	n.r.	Tops	0.010-0.765	0.056-0.110	0.066-0.885
		27-28	n.r.	Tops	0.009-0.661	0.078-0.082	0.087-0.742
		1	n.r.	Roots	<0.005-0.008	<0.005	<0.01-0.013
		7	n.r.	Roots	<0.005-0.019	<0.005	<0.01-0.024
		13-15	Maturity	Roots	<0.005-0.036	<0.005-0.013	<0.01-0.044
		21	n.r.	Roots	<0.005-0.005	<0.005	<0.01-0.010
		27-28	n.r.	Roots	<0.005-0.007	<0.005	<0.01-0.012
	2005 (2 x 0.17 kg a.s./ha)	1	n.r.	Tops	0.035-2.671	0.006-0.492	0.041-3.163
		7	n.r.	Tops	0.028-2.567	0.103-0.449	0.131-3.016
		13-15	Maturity	Tops	0.016-1.865	0.008-0.384	0.051-2.249
		21	n.r.	Tops	0.019-1.363	0.153-0.170	0.172-1.533
		27-28	n.r.	Tops	0.015-0.938	0.114-0.130	0.129-1.067
		1	n.r.	Roots	<0.005-0.009	<0.005	<0.01-0.014
		7	n.r.	Roots	<0.005-0.020	<0.005	<0.01-0.025
		13-15	Maturity	Roots	<0.005-0.069	<0.005-0.015	<0.01-0.083
		21	n.r.	Roots	<0.005-0.011	<0.005	<0.01-0.016
		27-28	n.r.	Roots	<0.005-0.009	<0.005	<0.01-0.014
					M11[3, 4]	**M21[3, 4]**	**M30[3, 4]**
	2005 (2 x 0.11 kg a.s./ha)	1	n.r.	Tops	<0.01	<0.01-0.01	<0.01
		7	n.r.	Tops	<0.01	<0.01-0.01	<0.01
		13-15	Maturity	Tops	<0.01-0.02	<0.01-0.02	<0.01
		21	n.r.	Tops	<0.01	<0.01-0.01	<0.01
		27-28	n.r.	Tops	<0.01	<0.01-0.01	<0.01
		1	n.r.	Roots	<0.002	<0.002	<0.002
		7	n.r.	Roots	<0.002	<0.002	<0.002
		13-15	Maturity	Roots	<0.002	<0.002	<0.002
		21	n.r.	Roots	<0.002	<0.002	<0.002
		27-28	n.r.	Roots	<0.002	<0.002	<0.002
	2005 (2 x 0.17 kg a.s./ha)	1	n.r.	Tops	<0.01	<0.01-0.01	<0.01
		7	n.r.	Tops	<0.01	<0.01-0.02	<0.01
		13-15	Maturity	Tops	<0.01-0.03	<0.01-0.02	<0.01
		21	n.r.	Tops	<0.01	<0.01-0.01	<0.01
		27-28	n.r.	Tops	<0.01	<0.01-0.01	<0.01
		1	n.r.	Roots	<0.002	<0.002	<0.002
		7	n.r.	Roots	<0.002	<0.002	<0.002
		13-15	Maturity	Roots	<0.002	<0.002	<0.002
		21	n.r.	Roots	<0.002	<0.002	<0.002
		27-28	n.r.	Roots	<0.002	<0.002	<0.002

1 Days after last application
2 At sampling
3 Refers to single residues (no means)
4 Residues are reported as < LOQ of 0.01; calculated residues in analytical detail tables are reported as 0.0 mg/kg, equivalent to < LOD or < 0.002 mg/kg.
n r. Not reported

III. CONCLUSION

Average residues of metconazole in treated sugar beet root specimens collected at 13-15 DALA ranged from <0.01 to 0.044 mg/kg after application of 2 x 0.11 kg a.s./ha and from <0.01 to 0.083 mg/kg after application of 2 x 0.17 kg a.s./ha. Residues of metabolites in sugar beet roots were <0.01 mg/kg, regardless of the treatment rate applied.

Table 6.10-52: Residues of metconazole in sugar beet after two applications of BAS 555 01 F in North America

Trial details		Crop	Country	Formulation, Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1)]	Residues found[3] (mg/kg)			
							Matrix	*Cis*-isomer	*Trans*-isomer	Total metconazole
Study code:	137729	Sugar beet	USA (MN)	BAS 555 01 F 2 x 0.11	Crop cover complete	14	Tops	1.047 (0.997, 1.077)	0.173 (0.167, 0.178)	1.210 (1.163, 1.256)
							Roots	0.005 (<0.005,0.006)	<0.005 (<0.005,<0.005)	0.010 (0.011,<0.01)
Doc ID:	2006/7006726									
Trial No.:	RCN R05085									
GLP:	yes									
Year:	2005			BAS 555 01 F 2 x 0.17		14	Tops	1.865 (1.662, 2.067)	0.384 (0.342, 0.426)	2.249 (2.005, 2.493)
							Roots	0.010 (0.007, 0.012)	<0.005 (<0.005,<0.005)	0.015 (0.012, 0.017)
Study code:	137729	Sugar beet	USA (MN)	BAS 555 01 F 2 x 0.11	Crop cover complete	14	Tops	0.898 (0.870, 0.926)	0.145 (0.145, 0.145)	1.043 (1.015, 1.070)
							Roots	0.005 (<0.005,0.005)	<0.005 (<0.005,<0.005)	0.010 (<0.01,0.010)
Doc ID:	2006/7006726									
Trial No.:	RCN R05086									
GLP:	yes									
Year:	2005			BAS 555 01 F 2 x 0.17		14	Tops	0.871 (0.811, 0.931)	0.140 (0.131, 0.148)	1.011 (0.942, 1.079)
							Roots	0.010 (0.011, 0.008)	<0.005 (<0.005,<0.005)	0.015 (0.016, 0.013)

Table 6.10-52: Residues of metconazole in sugar beet after two applications of BAS 555 01 F in North America

Trial details		Crop	Country	Formulation, Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1)]	Residues found[3] (mg/kg)			
							Matrix	*Cis*-isomer	*Trans*-isomer	Total metconazole
Study code:	137729	Sugar beet	USA (WI)	BAS 555 01 F 2 x 0.11	Crop cover complete	1	Tops	2.078 (1.852, 2.303)	0.362 (0.326, 0.397)	2.440 (2.179, 2.700)
Doc ID:	2006/7006726						Roots	<0.005	<0.005	<0.01
Trial No.:	RCN R05087					7	Tops	1.363 (1.298, 1.428)	0.248 (0.246, 0.249)	1.611 (1.674, 1.547)
GLP:	yes						Roots	<0.005	<0.005	<0.01
Year:	2005					14	Tops	0.894 (0.724, 1.064)	0.130 0.112, 0.148)	1.024 (0.835, 1.212)
							Roots	<0.005	<0.005	<0.01
						21	Tops	0.765 (0.794, 0.736)	0.110 (0.111, 0.109)	0.885 (0.905, 0.845)
							Roots	<0.005	<0.005	<0.01
						28	Tops	0.661 (0.816, 0.506)	0.082 (0.104, 0.059)	0.742 (0.919, 0.565)
							Roots	<0.005	<0.005	<0.01
				BAS 555 01 F 2 x 0.17		1	Tops	2.671 (2.892, 2.450)	0.492 (0.516, 0.467)	3.163 (3.408, 2.917)
							Roots	<0.005	<0.005	<0.01
						7	Tops	2.567 (2.346, 2.788)	0.449 (0.413, 0.485)	3.016 (2.759, 3.273)
							Roots	<0.005	<0.005	<0.01
						14	Tops	1.693 (1.593, 1.792)	0.270 (0.215, 0.324)	1.598 (1.808, 2.116)
							Roots	<0.005	<0.005	<0.01
						21	Tops	1.363 (1.463, 1.263)	0.170 (0.188, 0.152)	1.533 (1.650, 1.415)
							Roots	<0.005	<0.005	<0.01
						28	Tops	0.938 (0.716, 1.160)	0.130 (0.105, 0.154)	1.067 (0.821, 1.313)
							Roots	<0.005	<0.005	<0.01
Study code:	137729	Sugar beet	USA (WI)	BAS 555 01 F 2 x 0.11	Crop cover complete	13	Tops	0.015 (0.014, 0.015)	0.127 (0.116, 0.138)	0.142 (0.130, 0.153)
Doc ID:	2006/7006726						Roots	0.025 (0.031, 0.018)	0.006 (0.008,<0.005)	0.031 (0.039, 0.023)
Trial No.:	RCN R05088									
GLP:	yes									
Year:	2005			BAS 555 01 F 2 x 0.17		13	Tops	0.028 (0.030, 0.026)	0.104 (0.005, 0.203)	0.132 (0.035, 0.229)
							Roots	0.025 (0.026, 0.024)	0.006 (0.005, 0.006)	0.030 (0.031, 0.029)
Study code:	137729	Sugar beet	USA (WI)	BAS 555 01 F 2 x 0.11	Crop cover complete	14	Tops	0.033 (0.034, 0.031)	0.006 (0.006, 0.006)	0.039 (0.040, 0.037)
Doc ID:	2006/7006726						Roots	0.036 (0.032, 0.039)	0.008 (0.007, 0.009)	0.044 (0.040, 0.048)
Trial No.:	RCN R05089									
GLP:	yes									
Year:	2005			BAS 555 01 F 2 x 0.17		14	Tops	0.055 (0.050, 0.060)	0.011 (0.010, 0.011)	0.066 (0.060, 0.071
							Roots	0.069 (0.067, 0.070)	0.015 (0.015, 0.015)	0.083 (0.082, 0.084)
Study code:	137729	Sugar beet	USA (ND)	BAS 555 01 F 2 x 0.11	Root harvestable size	14	Tops	0.038 (0.021, 0.055)	0.088 (0.164, 0.011)	0.126 (0.185, 0.066)
Doc ID:	2006/7006726						Roots	0.015 (0.019, 0.010)	<0.005 (<0.005,<0.005)	0.020 (0.024, 0.015)
Trial No.:	RCN R05090									
GLP:	yes									
Year:	2005			BAS 555 01 F 2 x 0.17		14	Tops	0.041 (0.057, 0.024)	0.103 (0.011, 0.194)	0.143 (0.068, 0.218)
							Roots	0.014 (0.012, 0.016)	<0.005 (<0.005,<0.005)	0.019 (0.017, 0.021)

Table 6.10-52: Residues of metconazole in sugar beet after two applications of BAS 555 01 F in North America

Trial details		Crop	Country	Formulation, Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1)]	Residues found[3] (mg/kg)			
							Matrix	*Cis*-isomer	*Trans*-isomer	Total metconazole
Study code:	137729	Sugar beet	USA (TX)	BAS 555 01 F 2 x 0.11	Canopy 80% closed	14	Tops	0.010 (0.010, 0.010)	0.081 (0.079, 0.082)	0.091 (0.089, 0.092)
Doc ID:	2006/7006726						Roots	<0.005 (<0.005,<0.005)	0.005 (<0.005,0.006)	0.010 (<0.01,0.011)
Trial No.:	RCN R05091									
GLP:	yes									
Year:	2005			BAS 555 01 F 2 x 0.17		14	Tops	0.019 (0.019, 0.018)	0.148 (0.144, 0.151)	0.166 (0.162, 0.170)
							Roots	0.007 (0.009, 0.005)	0.006 (<0.005,0.007)	0.014 (0.014, 0.013)
Study code:	137729	Sugar beet	USA (MT)	BAS 555 01 F 2 x 0.11	Full maturity	14	Tops	0.016 (0.014, 0.017)	0.133 (0.132, 0.133)	0.148 (0.146, 0.150)
Doc ID:	2006/7006726						Roots	0.008 (0.009, 0.006)	0.011 (0.014, 0.008)	0.019 (0.023, 0.014)
Trial No.:	RCN R05092									
GLP:	yes									
Year:	2005			BAS 555 01 F 2 x 0.17		14	Tops	0.043 (0.043, 0.042)	0.008 (0.008, 0.008)	0.051 (0.052, 0.050)
							Roots	0.027 (0.018, 0.035)	0.006 (0.005, 0.007)	0.034 (0.024, 0.043)
Study code:	137729	Sugar beet	USA (CA)	BAS 555 01 F 2 x 0.11	Crop cover complete	14	Tops	0.013 (0.012, 0.014)	0.097 (0.098, 0.096)	0.110 (0.110, 0.109)
Doc ID:	2006/7006726						Roots	0.007 (0.010,<0.005)	0.012 (0.016, 0.007)	0.019 (0.026, 0.012)
Trial No.:	RCN R05093									
GLP:	yes									
Year:	2005			BAS 555 01 F 2 x 0.17		14	Tops	0.020 (0.019, 0.021)	0.168 (0.164, 0.171)	0.188 (0.183, 0.193)
							Roots	0.005 (0.006,<0.005)	0.008 (0.008, 0.007)	0.013 (0.013, 0.012)
Study code:	137729	Sugar beet	USA (CA)	BAS 555 01 F 2 x 0.11	Crop cover complete	14	Tops	0.491 (0.459, 0.522)	0.072 (0.068, 0.075)	0.581 (0.564, 0.597)
Doc ID:	2006/7006726						Roots	0.013 (0.013, 0.013)	0.013 (0.021,<0.005)	0.026 (0.034, 0.018)
Trial No.:	RCN R05094									
GLP:	yes									
Year:	2005			BAS 555 01 F 2 x 0.17		14	Tops	0.961 (0.985, 0.956)	0.144 (0.145, 0.142)	1.015 (1.130, 1.099)
							Roots	0.013 (0.010, 0.015)	0.010 (0.016,<0.005)	0.023 (0.025, 0.020)

Table 6.10-52: Residues of metconazole in sugar beet after two applications of BAS 555 01 F in North America

Trial details		Crop	Country	Formulation, Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1)]	Residues found[3] (mg/kg)			
							Matrix	*Cis*-isomer	*Trans*-isomer	Total metconazole
Study code:	137729	Sugar beet	USA (ID)	BAS 555 01 F 2 x 0.11	Root harvestable size	1	Tops	0.027 (0.026, 0.028)	<0.005 (<0.005,<0.005)	0.032 (0.031, 0.033)
							Roots	0.008 (0.008, 0.007)	<0.005 (<0.005,<0.005)	0.013 (0.013, 0.012)
Doc ID:	2006/7006726					7	Tops	0.021 (0.021, 0.020)	0.176 (0.176, 0.176)	0.197 (0.197, 0.196)
							Roots	0.019 (0.025, 0.013)	<0.005 (<0.005,<0.005)	0.024 (0.030, 0.018)
Trial No.:	RCN R05095					15	Tops	0.014 (0.015, 0.012)	0.102 (0.109, 0.095)	0.116 (0.124, 0.107)
							Roots	0.012 (0.014, 0.009)	<0.005 (<0.005,<0.005)	0.017 (0.019, 0.014)
GLP:	yes					21	Tops	0.010 (0.008, 0.011)	0.056 (<0.005, 0.107)	0.066 (0.013, 0.118)
							Roots	0.005 (0.006,<0.005)	<0.005 (<0.005,<0.005)	0.010 (0.011,<0.01)
Year:	2005					27	Tops	0.009 (0.007, 0.011)	0.078 (0.073, 0.082)	0.087 (0.081, 0.093)
							Roots	0.007 (0.006, 0.007)	<0.005 (<0.005,<0.005)	0.012 (0.011, 0.012)
				BAS 555 01 F 2 x 0.17		1	Tops	0.035 (0.035, 0.034)	0.006 (0.006, 0.006)	0.041 (0.041, 0.041)
							Roots	0.009 (0.011, 0.006)	<0.005 (<0.005,<0.005)	0.014 (0.016, 0.011)
						7	Tops	0.028 (0.026, 0.029)	0.103 (<0.05,0.201)	0.131 (0.031, 0.230)
							Roots	0.020 (0.023, 0.017)	<0.005 (<0.005,<0.005)	0.025 (0.028, 0.022)
						15	Tops	0.016 (0.014, 0.017)	0.136 (0.135, 0.137)	0.152 (0.149, 0.154)
							Roots	0.019 (0.015, 0.023)	<0.005 (<0.005,<0.005)	0.024 (0.020, 0.028)
						21	Tops	0.019 (0.017, 0.020)	0.153 (0.132, 0.174)	0.172 (0.149, 0.194)
							Roots	0.011 (0.013, 0.009)	<0.005 (<0.005,<0.005)	0.016 (0.018, 0.014)
						27	Tops	0.015 (0.016, 0.013)	0.114 (0.130, 0.098)	0.129 (0.146, 0.111)
							Roots	0.009 (0.009, 0.008)	<0.005 (<0.005,<0.005)	0.014 (0.014, 0.013)
Study code:	137729	Sugar beet	USA (WI)	BAS 555 01 F 2 x 0.11	Root harvestable size	14	Tops	0.826 (0.783, 0.868)	0.140 (0.116, 0.164)	0.966 (0.899, 1.032)
							Roots	0.017 (0.018, 0.015)	<0.005 (<0.005,<0.005)	0.022 (0.023, 0.020)
Doc ID:	2006/7006726									
Trial No.:	RCN R05096									
GLP:	yes									
Year:	2005			BAS 555 01 F 2 x 0.17		14	Tops	1.642 (1.812, 1.471)	0.240 (0.262, 0.218)	1.882 (2.074, 1.689)
							Roots	0.033 (0.032, 0.034)	0.007 (0.007, 0.007)	0.040 (0.039, 0.041)

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
3 Mean of two samples; individual values are given in parentheses

Table 6.10-53: Residues of metconazole in sugar beet after two applications of BAS 555 01 F in North America

Trial details		Crop	Country	Formulation, Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1)]	Residues found[3,4] (mg/kg)			
							Matrix	M11	M21	M30
Study code:	137729	Sugar beet	USA (MN)	BAS 555 01 F 2 x 0.11	Crop cover complete	14	Tops	<0.01	<0.01	<0.01
Doc ID:	2006/7006726						Roots	<0.01	<0.01	<0.01
Trial No.:	RCN R05085									
GLP:	yes									
Year:	2005			BAS 555 01 F 2 x 0.17		14	Tops	<0.01	0.02 (0.01, 0.02)	<0.01
							Roots	<0.01	<0.01	<0.01
Study code:	137729	Sugar beet	USA (MN)	BAS 555 01 F 2 x 0.11	Crop cover complete	14	Tops	<0.01	<0.01	<0.01
Doc ID:	2006/7006726						Roots	<0.01	<0.01	<0.01
Trial No.:	RCN R05086									
GLP:	yes									
Year:	2005			BAS 555 01 F 2 x 0.17		14	Tops	<0.01	<0.01	<0.01
							Roots	<0.01	<0.01	<0.01
Study code:	137729	Sugar beet	USA (WI)	BAS 555 01 F 2 x 0.11	Crop cover complete	1	Tops	<0.01	<0.01	<0.01
Doc ID:	2006/7006726						Roots	<0.01	<0.01	<0.01
Trial No.:	RCN R05087					7	Tops	<0.01	<0.01	<0.01
GLP:	yes						Roots	<0.01	<0.01	<0.01
Year:	2005					14	Tops	<0.01	<0.01	<0.01
							Roots	<0.01	<0.01	<0.01
						21	Tops	<0.01	<0.01	<0.01
							Roots	<0.01	<0.01	<0.01
						28	Tops	<0.01	<0.01	<0.01
							Roots	<0.01	<0.01	<0.01
				BAS 555 01 F 2 x 0.17		1	Tops	<0.01	0.01	<0.01
							Roots	<0.01	<0.01	<0.01
						7	Tops	<0.01	0.02 (0.01, 0.02)	<0.01
							Roots	<0.01	<0.01	<0.01
						14	Tops	<0.01	0.02	<0.01
							Roots	<0.01	<0.01	<0.01
						21	Tops	<0.01	0.01	0.01
							Roots	<0.01	<0.01	<0.01
						28	Tops	<0.01	<0.01	<0.01
							Roots	<0.01	<0.01	<0.01
Study code:	137729	Sugar beet	USA (WI)	BAS 555 01 F 2 x 0.11	Crop cover complete	13	Tops	<0.01	<0.01	<0.01
Doc ID:	2006/7006726						Roots	<0.01	<0.01	<0.01
Trial No.:	RCN R05088									
GLP:	yes									
Year:	2005			BAS 555 01 F 2 x 0.17		13	Tops	<0.01	0.01	<0.01
							Roots	<0.01	<0.01	<0.01

Table 6.10-53: Residues of metconazole in sugar beet after two applications of BAS 555 01 F in North America

Trial details	Crop	Country	Formulation, Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1)]	Residues found[3,4] (mg/kg)			
						Matrix	M11	M21	M30
Study code: 137729 **Doc ID:** 2006/7006726 **Trial No.:** RCN R05089 **GLP:** yes **Year:** 2005	Sugar beet	USA (WI)	BAS 555 01 F 2 x 0.11	Crop cover complete	14	Tops	<0.01	0.01	<0.01
						Roots	<0.01	<0.01	<0.01
			BAS 555 01 F 2 x 0.17		14	Tops	<0.01	0.02 (0.01, 0.02)	<0.01
						Roots	<0.01	<0.01	<0.01
Study code: 137729 **Doc ID:** 2006/7006726 **Trial No.:** RCN R05090 **GLP:** yes **Year:** 2005	Sugar beet	USA (ND)	BAS 555 01 F 2 x 0.11	Root harvestable size	14	Tops	<0.01 (<0.01, 0.01)	0.01 (<0.01, 0.02)	<0.01
						Roots	<0.01	<0.01	<0.01
			BAS 555 01 F 2 x 0.17		14	Tops	0.01 (<0.01, 0.02)	0.01 (<0.01, 0.02)	<0.01
						Roots	<0.01	<0.01	<0.01
Study code: 137729 **Doc ID:** 2006/7006726 **Trial No.:** RCN R05091 **GLP:** yes **Year:** 2005	Sugar beet	USA (TX)	BAS 555 01 F 2 x 0.11	Canopy 80% closed	14	Tops	<0.01 (<0.01, 0.01)	<0.01	<0.01
						Roots	<0.01	<0.01	<0.01
			BAS 555 01 F 2 x 0.17		14	Tops	0.02	0.02 (0.01, 0.02)	<0.01
						Roots	<0.01	<0.01	<0.01
Study code: 137729 **Doc ID:** 2006/7006726 **Trial No.:** RCN R05092 **GLP:** yes **Year:** 2005	Sugar beet	USA (MT)	BAS 555 01 F 2 x 0.11	Full maturity	14	Tops	<0.01	<0.01	<0.01
						Roots	<0.01	<0.01	<0.01
			BAS 555 01 F 2 x 0.17		14	Tops	<0.01	0.02	<0.01
						Roots	<0.01	<0.01	<0.01
Study code: 137729 **Doc ID:** 2006/7006726 **Trial No.:** RCN R05093 **GLP:** yes **Year:** 2005	Sugar beet	USA (CA)	BAS 555 01 F 2 x 0.11	Crop cover complete	14	Tops	0.02 (0.01, 0.02)	0.01	<0.01
						Roots	<0.01	<0.01	<0.01
			BAS 555 01 F 2 x 0.17		14	Tops	0.03 (0.02, 0.03)	0.02	<0.01
						Roots	<0.01	<0.01	<0.01
Study code: 137729 **Doc ID:** 2006/7006726 **Trial No.:** RCN R05094 **GLP:** yes **Year:** 2005	Sugar beet	USA (CA)	BAS 555 01 F 2 x 0.11	Crop cover complete	14	Tops	0.02	<0.01 (<0.01, 0.01)	<0.01
						Roots	<0.01	<0.01	<0.01
			BAS 555 01 F 2 x 0.17		14	Tops	0.03 (0.02, 0.03)	0.02	<0.01
						Roots	<0.01	<0.01	<0.01

Table 6.10-53: Residues of metconazole in sugar beet after two applications of BAS 555 01 F in North America

Trial details		Crop	Country	Formulation, Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1)]	Residues found[3,4] (mg/kg)			
							Matrix	M11	M21	M30
Study code:	137729	Sugar beet	USA (ID)	BAS 555 01 F 2 x 0.11	Root harvestable size	1	Tops	<0.01	<0.01	<0.01
Doc ID:	2006/7006726						Roots	<0.01	<0.01	<0.01
Trial No.:	RCN R05095					7	Tops	<0.01	<0.01	<0.01
GLP:	yes						Roots	<0.01	<0.01	<0.01
Year:	2005					15	Tops	<0.01	<0.01	<0.01
							Roots	<0.01	<0.01	<0.01
						21	Tops	<0.01	<0.01	<0.01
							Roots	<0.01	<0.01	<0.01
						27	Tops	<0.01	<0.01	<0.01
							Roots	<0.01	<0.01	<0.01
				BAS 555 01 F 2 x 0.17		1	Tops	<0.01	<0.01	<0.01
							Roots	<0.01	<0.01	<0.01
						7	Tops	<0.01	<0.01 (<0.01, 0.01)	<0.01
							Roots	<0.01	<0.01	<0.01
						15	Tops	<0.01	<0.01	<0.01
							Roots	<0.01	<0.01	<0.01
						21	Tops	<0.01	<0.01 (<0.01, 0.01)	<0.01
							Roots	<0.01	<0.01	<0.01
						27	Tops	<0.01	<0.01 (<0.01, 0.01)	<0.01
							Roots	<0.01	<0.01	<0.01
Study code:	137729	Sugar beet	USA (WI)	BAS 555 01 F 2 x 0.11	Root harvestable size	14	Tops	<0.01 (<0.01, 0.01)	<0.01 (<0.01, 0.01)	<0.01
Doc ID:	2006/7006726						Roots	<0.01	<0.01	<0.01
Trial No.:	RCN R05096									
GLP:	yes									
Year:	2005			BAS 555 01 F 2 x 0.17		14	Tops	0.02 (0.01, 0.02)	0.02	<0.01
							Roots	<0.01	<0.01	<0.01

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
3 Mean of two samples; individual values are given in parentheses
4 Residues are reported as < LOQ of 0.01; calculated residues in analytical detail tables are reported as 0.0 mg/kg, equivalent to < LOD or < 0.002 mg/kg.

CA 6.10.1 Effect on the residue level in pollen and bee products

Report: CA 6.10.1/1
Plier S., 2014a
Determination of residues of BAS 555 F (Metconazole) in rapeseed honey after one application of BAS 555 00 F in oilseed rape during full flowering (BBCH 65) in Germany, 2013
2014/1018041

Guidelines: OECD 509 Crop Field Trial (2009), EC 1107/2009 of the European Parliament and of the Council of 21 Oct 2009, EEC 7029/VI/95 rev. 5 Appendix B (July 22 1997)

GLP: yes
(certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description: BAS 555 00 F (EC): metconazole: 60 g/L nominal
Lot/Batch #: FRE-000895
Purity:
CAS#: 125116-23-6
Development code:
Spiking levels: 0.01-16 mg/kg

2. Test Commodity:

Crop: Oilseed rape / Honey bee (bee colonies with at least 1 brood body and 1 honey body)
Type: Oilseeds
Variety: Avatar, Visby, NK Linus, Sherpa
Botanical name: *Brassica napus* / *Apis mellifera carnica*
Crop parts(s) or processed commodity: Whole plant without root, honey, pollen
Sample size: Whole plant without root: >1 kg / honey: >0.5 kg/ pollen: ≥0.03 kg

B. STUDY DESIGN

1. Test procedure

During the 2013 growing season, four field trials were conducted in different representative oilseed rape growing areas in Germany to determine the magnitude of the residues in oilseed rape, honey and pollen.

The fungicidal product BAS 555 00 F, containing metconazole, was foliar applied once at a target rate corresponding to 0.090 kg/ha of metconazole in a spray volume of 300 L/ha. The application was conducted at BBCH 65. The actual application rates were within 10% of the target rates.

Three hives with one brood body were placed at the border of each field to be treated 3 days before planned application for adaptation. Immediately before application one honey body (incl. approx. 11 empty honey combs) was set up on each brood body.

Whole plant without roots specimens for analysis were sampled on the day of the application and the honey specimens were sampled 15-18 days after application. Pollen specimens for analysis were mixed from subsamples taken at one day after application and further at an interval of approximately 5 days until sampling of the honey.

All specimens were frozen immediately after specimen preparation and remained frozen (≤-18°C) until analysis including transportation. The maximum storage interval from harvest until extraction for analysis of metconazole was 469 days.

In order to check the bee flight to the applied field, the pollen spectrum was determined. Therefore a pollen trap at one hive per location was installed and the pollen was collected. The traps were emptied before application as well as 1 day after application and further with an interval of approximately 5 days until sampling of honey.

Table 6.10.1-1: Target application rates and timings for oilseed rape (honey production)

Year	No of trials	No of appl.	F P or G	Test item	Active substance	Appl. rate (kg a.s./ha)	Water volume (L/ha)	Target date/ Timing[1]
2013	4	1	F	BAS 555 00 F (EC)	BAS 555 F	0.090	300	BBCH 65

2. Description of analytical procedures

Samples were analyzed for *cis*- and *trans*-metconazole residues using BASF method No 550/0 which was validated in the matrices of honey, pollen and nectar (M-CA 4.1.2/10). The homogenized specimens were extracted with methanol/water/hydrochloric acid. After clean-up by liquid/liquid partition with dichloromethane, the analytes in the final extract were determined by LC-MS/MS. The limit of quantitation (LOQ) in plant material was at 0.005 mg/kg for each analyte , the limit of quantitation 0.01 mg/kg for total metconazole.

Table 6.10.1-2: Summary of procedural recovery data for metconazole

Matrix	Fortification level (mg/kg)	Summary recoveries		
		n	Mean (%)	RSD (%)
BASF method No 550/0; LOQ = 0.01 mg/kg		**Metconazole**		
Whole plant w/o root	0.01-16	3	109	1.4
Honey	0.01-1.0	5	92	11
Pollen	0.01-0.1	2	85	N/A

N/A Not applicable

Additionally the honey was analyzed to determine the amount of oilseed rape pollen. The analysis of the pollen spectrum was realized by a specialized bee institute under non-GLP conditions. The results are summarized in the following table.

Table 6.10.1-3: Content of oilseed rape pollen spectrum of the honey

Trial No	L130473	L130474	L130475	L130476
Content of oilseed rape pollen in the honey (%)	62.5	54.5	91.8	87.0

II. RESULTS AND DISCUSSION

The residue ranges of metconazole treated with formulation BAS 555 00 F are shown in Table 6.10.1-4. Details are presented in Table 6.10.1-5.
Metconazole residues in whole plant without root specimens at 0 DALA (BBCH 65) ranged between 0.78 and 11 mg/kg. At 15-18 DALA residues in honey were <0.01 mg/kg. At 1-15 or 1-17 DALA, residues in pollen were also <0.01 mg/kg.
No residues of metconazole were found in the control samples (commercial products in case of honey and pollen) above the LOQ.

The analysis of the pollen spectrum in the harvested honey showed a content of the oilseed rape type in the range of 54.5-91.8%.

Table 6.10.1-4: Summary of residues in oilseed rape treated with BAS 555 00 F

Crop	Year	DALA[1]	Growth stage[2] (BBCH)	Metconazole residues (sum of *cis*- and *trans*-isomer; mg/kg)	
				Matrix	BAS 555 00 F
Oilseed rape	2013	0	65	Whole plant without roots	0.78-11
		15-18	N/A	Honey	<0.01
		1-15 / 1-17	N/A	Pollen	<0.01

1 Days after last application
2 At sampling
N/A Not applicable

III. CONCLUSION

Bee hives were set at the border of oilseed rape fields in four trial sites in Germany. The honey specimens were sampled 15-18 days after application. Analysis of pollen in the honey indicated 55-92% of pollen originated from oilseed rape. The residues of metconazole in oilseed rape honey were below the LOQ of 0.01 mg/kg.

Table 6.10.1-5: Residues of metconazole in oilseed rape honey after application of BAS 555 00 F in Northern Europe

Trial details		Crop	Country	Formulation, Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1)]	Residues found (mg/kg)	
							Matrix	Metconazole (sum of *cis*- and *trans*-isomer)
Study code:	429879	Oilseed rape	Germany	BAS 555 00 F 1 x 0.090	65	0	Whole plant without root	3.1
Doc ID:	2014/1018041					18	Honey	<u><0.01</u>
Trial No:	L130473					1-15	Pollen	<0.01
GLP:	Yes							
Year:	2013							
Study code:	429879	Oilseed rape	Germany	BAS 555 00 F 1 x 0.090	65	0	Whole plant without root	2.5
Doc ID:	2014/1018041					16	Honey	<u><0.01</u>
Trial No:	L130474					1-15	Pollen	<0.01
GLP:	Yes							
Year:	2013							
Study code:	429879	Oilseed rape	Germany	BAS 555 00 F 1 x 0.090	65	0	Whole plant without root	0.78
Doc ID:	2014/1018041					15	Honey	<u><0.01</u>
Trial No:	L130475					1-15	Pollen	<0.01
GLP:	Yes							
Year:	2013							
Study code:	429879	Oilseed rape	Germany	BAS 555 00 F 1 x 0.090	65	0	Whole plant without root	11
Doc ID:	2014/1018041					18	Honey	<u><0.01</u>
Trial No:	L130476					1-17	Pollen	<0.01
GLP:	Yes							
Year:	2013							

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
_ Underlined values were used for MRL derivation

Appendix 1 Additional information provided by the applicant

Appendix 1A Dietary risk calculations using EFSA PRIMO Model (rev 2.0)

Table 1A-1: TMDI calculations based on established MRLs for metconazole representative uses

metconazole			
Status of the active substance:		Code no.	BAS 555 F
LOQ (mg/kg bw):	0.01	proposed LOQ:	
Toxicological end points			
ADI (mg/kg bw/day):	**0.01**	ARfD (mg/kg bw):	**0.01**
Source of ADI:	**Annex II**	Source of ARfD:	
Year of evaluation:	**2006**	Year of evaluation:	

Prepare workbook for refined calculations

Undo refined calculations

Explain choice of toxicological reference values.

The risk assessment has been performed on the basis of the MRLs plus metabolite levels based on residues measured in U. S. studies'

Chronic risk assessment

	TMDI (range) in % of ADI minimum - maximum	
	0	16
No of diets exceeding ADI	---	0

Highest calculated TMDI values in % of ADI	MS Diet	Highest contributor to MS diet (in % of ADI)	Commodity / group of commodities	2nd contributor to MS diet (in % of ADI)	Commodity / group of commodities	3rd contributor to MS diet (in % of ADI)	Commodity / group of commodities	pTMRLs at LOQ (in % of ADI)
15.8	DK child	8 3	Wheat	2.7	Rye	2.5	Milk and cream	0.0
15.5	WHO Cluster diet B	12.8	Wheat	1.1	Barley	0.6	Milk and cream,	0.0
14.6	NL child	7.1	Wheat	5.9	Milk and cream,	0.4	Oats	0.0
13.2	UK Infant	7.7	Milk and cream	3.9	Wheat	1.0	Oats	0.0
12.7	FR toddler	7 9	Milk and cream,	3.9	Wheat	0.3	Bovine: Meat	0.0
12.6	WHO cluster diet D	9 8	Wheat	1.0	Milk and cream,	0.9	Barley	0.0
12.4	WHO cluster diet E	5 9	Wheat	3.2	Barley	1.2	Rape seed	0.0
11.0	DE child	6 2	Wheat	2.9	Milk and cream,	0.8	Oats	0.0
11.0	WHO Cluster diet F	5.4	Wheat	2.4	Barley	0.8	Milk and cream,	0.0
10.8	E adult	5 0	Barley	3.4	Wheat	0.7	Oats	0.0
10.6	UK Toddler	5 9	Wheat	4.1	Milk and cream,	0.2	Oats	0.0
10.2	ES child	6.7	Wheat	2.5	Milk and cream	0.3	Bovine: Meat	0.0
10.0	IT kids/toddler	10.0	Wheat	0.0	Barley	0.0	Peas (without pods)	0.0
8.2	WHO regional European diet	4 5	Wheat	1.3	Barley	1.0	Milk and cream,	0.0
7.7	SE general population 90th percentile	4 8	Wheat	2.5	Milk and cream,	0.2	Rye	0.0
7.1	ES adult	3 5	Wheat	2.0	Barley	1.0	Milk and cream,	0.0
6.8	FR infant	5.1	Milk and cream,	1.3	Wheat	0.1	Bovine: Meat	0.0
6.6	NL general	3.1	Wheat	1.5	Barley	1.3	Milk and cream,	0.0
6.3	IT adult	6 2	Wheat	0.0	Barley	0.0	Peas (without pods)	0.0
6.2	PT General population	5 9	Wheat	0.1	Barley	0.1	Barley	0.0
5.9	FR all population	4 9	Wheat	0.5	Milk and cream,	0.1	Poultry -chicken, geese, duck,	0.0
5.4	DK adult	3 0	Wheat	1.1	Milk and cream,	0.5	Oats	0.0
4.1	UK vegetarian	3.1	Wheat	0.7	Milk and cream	0.2	Oats	0.0
4.0	LT adult	1 6	Wheat	0.8	Milk and cream,	0.6	Rye	0.0
3.6	FI adult	1 5	Wheat	1.1	Milk and cream,	0.4	Rye	0.0
3.5	UK Adult	2 5	Wheat	0.6	Milk and cream,	0.1	Barley	0.0
0.0	PL general population	0 0	Peas (without pods)	0.0	Poppy seed	0.0	FRUIT (FRESH OR FROZEN)	0.0

Table 1A-2: TMDI calculations for metconazole (including all registered uses, import tolerances & default MRLs)

metconazole			
Status of the active substance:		Code no.	BAS 555 F
LOQ (mg/kg bw):	0.01	proposed LOQ:	
Toxicological end points			
ADI (mg/kg bw/day):	**0.01**	ARfD (mg/kg bw):	**0.01**
Source of ADI:	**Annex II**	Source of ARfD:	**Annex II**
Year of evaluation:	**2006**	Year of evaluation:	**2006**

Prepare workbook for refined calculations

Undo refined calculations

Explain choice of toxicological reference values.

The risk assessment has been performed on the basis of the MRLs plus metabolite levels based on residues measured in U. S. studies'

Chronic risk assessment

TMDI (range) in % of ADI minimum - maximum: 2 - 29

No of diets exceeding ADI: --- 0

Highest calculated TMDI values in % of ADI	MS Diet	Highest contributor to MS diet (in % of ADI)	Commodity / group of commodities	2nd contributor to MS diet (in % of ADI)	Commodity / group of commodities	3rd contributor to MS diet (in % of ADI)	Commodity / group of commodities	pTMRLs at LOQ (in % of ADI)
28.6	UK Toddler	13.7	Sugar beet (root)	5.9	Wheat	4.1	Milk and cream,	0.0
26.2	WHO Cluster diet B	12.8	Wheat	2.5	Maize	1.1	Barley	0.0
24.7	UK Infant	7.7	Milk and cream,	6.0	Sugar beet (root)	3.9	Wheat	0.0
22.2	NL child	7.1	Wheat	5.9	Milk and cream,	1.7	Bananas	0.0
20.0	DE child	6.2	Wheat	2.9	Milk and cream,	2.5	Pome fruit	0.0
19.6	DK child	8.3	Wheat	2.7	Rye	2.5	Milk and cream,	0.0
19.3	IE adult	5.0	Barley	3.4	Wheat	2.3	Maize	0.0
18.1	FR toddler	7.9	Milk and cream,	3.9	Wheat	1.6	Root and tuber vegetables	0.0
17.5	WHO cluster diet E	5.9	Wheat	3.2	Barley	1.2	Rape seed	0.0
16.8	WHO cluster diet D	9.8	Wheat	1.0	Milk and cream,	0.9	Root and tuber vegetables	0.0
15.0	ES child	6.7	Wheat	2.5	Milk and cream,	1.0	Bananas	0.0
14.8	WHO Cluster diet F	5.4	Wheat	2.4	Barley	0.9	Root and tuber vegetables	0.0
13.5	SE general population 90th percentile	4.8	Wheat	2.5	Milk and cream,	1.8	Bananas	0.0
13.0	IT kids/toddler	10.0	Wheat	0.5	Bananas	0.3	Peaches	0.0
12.1	WHO regional European diet	4.5	Wheat	1.3	Barley	1.0	Milk and cream,	0.0
10.6	FR infant	5.1	Milk and cream,	1.4	Root and tuber vegetables	1.3	Wheat	0.0
10.4	PT General population	5.9	Wheat	0.6	Berries & small fruit	0.5	Brassica vegetables	0.0
10.0	ES adult	3.5	Wheat	2.0	Barley	1.0	Milk and cream,	0.0
9.3	NL general	3.1	Wheat	1.5	Barley	1.3	Milk and cream,	0.0
8.7	FR all population	4.9	Wheat	0.8	Berries & small fruit	0.5	Milk and cream,	0.0
8.6	IT adult	6.2	Wheat	0.4	Peaches	0.3	Solanacea	0.0
8.6	UK vegetarian	3.1	Wheat	2.3	Sugar beet (root)	0.7	Milk and cream,	0.0
7.8	UK Adult	2.5	Wheat	2.4	Sugar beet (root)	0.6	Milk and cream,	0.0
7.2	DK adult	3.0	Wheat	1.1	Milk and cream,	0.5	Oats	0.0
5.7	LT adult	1.6	Wheat	0.8	Milk and cream,	0.7	Root and tuber vegetables	0.0
5.3	FI adult	1.5	Wheat	1.1	Milk and cream,	0.4	Rye	0.0
2.3	PL general population	0.8	Root and tuber vegetables	0.5	Pome fruit	0.2	Solanacea	0.0

Conclusion

The estimated Theoretical Maximum Daily Intakes (TMDI), based on pTMRLs were below the ADI.
A long-term intake of residues of metconazole is unlikely to present a public health concern.

Table 1A-3: Acute dietary exposure assessment (IESTI) for Metconazole based on established MRLs

Unprocessed commodities	No of commodities for which ARfD/ADI is exceeded (IESTI 1)		---	No of commodities for which ARfD/ADI is exceeded (IESTI 2)		---	No of commodities for which ARfD/ADI is exceeded (IESTI 1)		---	No of commodities for which ARfD/ADI is exceeded (IESTI 2)		---
	IESTI 1	*)	**)	IESTI 2	*)	**)	IESTI 1	*)	**)	IESTI 2	*)	**)
	Highest % of ARfD/ADI	Commodities	pTMRL/ threshold MRL (mg/kg)	Highest % of ARfD/ADI	Commodities	pTMRL/ threshold MRL (mg/kg)	Highest % of ARfD/ADI	Commodities	pTMRL/ threshold MRL (mg/kg)	Highest % of ARfD/ADI	Commodities	pTMRL/ threshold MRL (mg/kg)
	83.6	Bananas	0.1 / -	75.8	Melons	0.05 / -	29.0	Barley	0.4 / -	29.0	Barley	0.4 / -
	75.8	Melons	0.05 / -	60.6	Bananas	0.1 / -	19.7	Melons	0.05 / -	19.7	Melons	0 05 / -
	59.3	Peaches	0.1 / -	43.5	Peaches	0.1 / -	17.5	Peaches	0.1 / -	15 6	Sugar beet (root)	0 06 / -
	38.3	Sugar beet (root)	0.06 / -	38.3	Sugar beet (root)	0.06 / -	15.6	Sugar beet (root)	0 06 / -	13.6	Peaches	0.1 / -
	31.0	Apricots	0.1 / -	24.8	Milk and milk	0.02 / -	13.7	Bananas	0.1 / -	11.7	Wheat	0.15 / -
	0.0		0	0.0		0.00	0.0		0.00	0.0		0.00
	0.0		0	0.0		0.00	0.0		0.00	0.0		0.00
	0.0		0	0.0		0.00	0.0		0.00	0.0		0.00
	0.0		0	0.0		0.00	0.0		0.00	0.0		0.00
	0.0		0	0.0		0.00	0.0		0.00	0.0		0.00
	0.0		0	0.0		0.00	0.0		0.00	0.0		0.00
	0.0		0	0.0		0.00	0.0		0.00	0.0		0.00
	0.0		0	0.0		0.00	0.0		0.00	0.0		0.00
	0.0		0	0.0		0.00	0.0		0.00	0.0		0.00
	0.0		0	0.0		0.00	0.0		0.00	0.0		0.00
	0.0		0	0.0		0.00	0.0		0.00	0.0		0.00
	No of critical MRLs (IESTI 1)		0 ---				No of critical MRLs (IESTI 2)		0 ---			

Processed commodities	No of commodities for which ARfD/ADI is exceeded		---				No of commodities for which ARfD/ADI is exceeded		---			
			***)						***)			
	Highest % of ARfD/ADI	Processed commodities	pTMRL/ threshold MRL (mg/kg)				Highest % of ARfD/ADI	Processed commodities	pTMRL/ threshold MRL (mg/kg)			
	17.9	Peach juice	0.1 / -				6.6	Bread/pizza	0.15 / -			
	17.7	Wheat flour	0.15 / -				2.0	Orange juice	0 02 / -			
	10.2	Apple juice	0.02 / -				2.0	Peach preserved with	0.1 / -			
	9.9	Orange juice	0.02 / -				1.3	Apple juice	0.02 / -			
	8.6	Carrot, juice	0.02 / -				0.8	Wine	0.02 / -			
	0.0	0	0.00				0.0	0	0.00			
	0.0	0	0.00						0.00			
	0.0	0	0.00						0.00			

Table 1A-4: Evaluation of chronic exposure to metconazole metabolite M11 using the TTC threshold for genotoxic testing

				metconazole M11						**Prepare workbook for refined calculations**		
				Status of the active substance:			Code no.	**BAS 555 F**				
				LOQ (mg/kg bw):		0.01	proposed LOQ:					
				Toxicological end points						**Undo refined calculations**		
				ADI (mg/kg bw/day):		**0.0000025**	ARfD (mg/kg bw):	**0.0003**				
				Source of ADI:		**Annex II**	Source of ARfD:					
				Year of evaluation:		**2004**	Year of evaluation:					
Explain choice of toxicological reference values.												
The risk assessment has been performed on the basis of the MRLs collected from Member States in April 2006. For each pesticide/commodity the highest national MRL was identified (proposed temporary MRL = pTMRL).												
The pTMRLs have been submitted to EFSA in September 2006.												
Chronic risk assessment												
						TMDI (range) in % of ADI minimum - maximum						
						41	4517					
				No of diets exceeding ADI			26					
	Highest calculated TMDI values in % of ADI	MS Diet		Highest contributor to MS diet (in % of ADI)	Commodity / group of commodities		2nd contributor to MS diet (in % of ADI)	Commodity / group of commodities		3rd contributor to MS diet (in % of ADI)	Commodity / group of commodities	pTMRLs at LOQ (in % of ADI)
	4516.5	DK child		2201.8	Wheat		1767.3	Rye		474.5	Oats	4042.0
	4326.6	WHO Cluster diet B		3414.0	Wheat		336 0	Barley		197.9	Maize	3980.6
	3845.1	UK Toddler		1829.6	Sugar beet (root)		1567.1	Wheat		227.3	Oilseeds	3771.1
	3300.1	WHO cluster diet D		2601.3	Wheat		264 0	Barley		162.0	Rye	2952.1
	3130.1	E adult		1488.8	Barley		918.1	Wheat		218.6	Oats	1422.6
	3106.6	WHO cluster diet E		1577.3	Wheat		972 0	Barley		172.0	Rye	2020.6
	2799.7	WHO Cluster diet F		1440.0	Wheat		722 0	Barley		305.3	Rye	1899.7
	2772.7	IT kids/toddler		2658.6	Wheat		27.9	Peaches		21.4	Bananas	2759.5
	2513.4	UK Infant		1048.3	Wheat		806.4	Sugar beet (root)		303.4	Oats	2209.9
	2439.6	DE child		1644.6	Wheat		317 0	Rye		245.2	Oats	2179.6
	2324.3	NL child		1896.6	Wheat		128.4	Oats		71 6	Rye	2165.0
	1922.3	ES child		1774.2	Wheat		40.4	Bananas		23 0	Maize	1919.7
	1892.9	PT General population		1567.3	Wheat		54.7	Rye		48 5	Oilseeds	1820.9
	1773.3	WHO regional European diet		1186.7	Wheat		396 0	Barley		40 0	Oats	1337.3
	1751.0	IT adult		1654.2	Wheat		30.1	Peaches		11 2	Barley	1739.7
	1617.8	ES adult		939.2	Wheat		590 5	Barley		16 9	Peaches	1027.2
	1510.0	SE general population 90th percentile		1280.7	Wheat		118 0	Rye		72 3	Bananas	1510.0
	1413.9	NL general		829.2	Wheat		449 5	Barley		37 9	Oats	926.4
	1406.6	FR all population		1315.3	Wheat		40.3	Oilseeds		11 3	Peaches	1398.6
	1280.3	UK vegetarian		819.2	Wheat		302.1	Sugar beet (root)		57 6	Oats	1199.3
	1253.8	DK adult		805.4	Wheat		272.4	Rye		137.8	Oats	1116.0
	1193.2	UK Adult		670.5	Wheat		319 8	Sugar beet (root)		96 6	Oilseeds	1141.1
	1153.1	FR toddler		1049.1	Wheat		51.7	Bananas		27 9	Peas (without pods)	1153.1
	1048.2	LT adult		430.7	Rye		420.7	Wheat		108.9	Oats	867 5
	815.1	FI adult		393.6	Wheat		273.1	Rye		102.2	Oats	684 6
	396.2	FR infant		336.4	Wheat		28.6	Bananas		20 9	Peas (without pods)	396 2
	41.0	PL general population		10.7	Cherries		7 5	Bananas		4.8	Peaches	41.0

Table 1A-5 Evaluation of chronic exposure to metconazole metabolite M11 using the TTC threshold of 0.0003 mg/kg bw/day

				metconazole M11							**Prepare workbook for refined calculations**			
				Status of the active substance:				Code no.		**BAS 555 F**				
				LOQ (mg/kg bw):		0.01		proposed LOQ:						
				Toxicological end points							**Undo refined calculations**			
				ADI (mg/kg bw/day):		**0.0003**		ARfD (mg/kg bw):		**0.0003**				
				Source of ADI:		**Annex II**		Source of ARfD:						
				Year of evaluation:		**2004**		Year of evaluation:						
Explain choice of toxicological reference values.														
The risk assessment has been performed on the basis of the MRLs collected from Member States in April 2006. For each pesticide/commodity the highest national MRL was identified (proposed temporary MRL = pTMRL).														
The pTMRLs have been submitted to EFSA in September 2006.														
Chronic risk assessment														
						TMDI (range) in % of ADI minimum - maximum								
						0		38						
				No of diets exceeding ADI		---		0						
	Highest calculated TMDI values in % of ADI	MS Diet		Highest contributor to MS diet (in % of ADI)	Commodity / group of commodities		2nd contributor to MS diet (in % of ADI)	Commodity / group of commodities		3rd contributor to MS diet (in % of ADI)	Commodity / group of commodities		pTMRLs at LOQ (in % of ADI)	
	37.6	DK child		18 3	Wheat		14.7	Rye		4.0	Oats		33.7	
	36.1	WHO Cluster diet B		28 5	Wheat		2.8	Barley		1.6	Maize		33.2	
	32.0	UK Toddler		15 2	Sugar beet (root)		13.1	Wheat		1.9	Oilseeds		31.4	
	27.5	WHO cluster diet D		21.7	Wheat		2.2	Barley		1.4	Rye		24.6	
	26.1	IE adult		12.4	Barley		7.7	Wheat		1.8	Oats		11.9	
	25.9	WHO cluster diet E		13.1	Wheat		8.1	Barley		1.4	Rye		16.8	
	23.3	WHO Cluster diet F		12 0	Wheat		6.0	Barley		2.5	Rye		15.8	
	23.1	IT kids/toddler		22 2	Wheat		0.2	Peaches		0.2	Bananas		23.0	
	20.9	UK Infant		8.7	Wheat		6.7	Sugar beet (root)		2.5	Oats		18.4	
	20.3	DE child		13.7	Wheat		2.6	Rye		2.0	Oats		18.2	
	19.4	NL child		15 8	Wheat		1.1	Oats		0.6	Rye		18.0	
	16.0	ES child		14 8	Wheat		0.3	Bananas		0.2	Maize		16.0	
	15.8	PT General population		13.1	Wheat		0.5	Rye		0.4	Oilseeds		15.2	
	14.8	WHO regional European diet		9.9	Wheat		3.3	Barley		0.3	Oats		11.1	
	14.6	IT adult		13 8	Wheat		0.3	Peaches		0.1	Barley		14.5	
	13.5	ES adult		7.8	Wheat		4.9	Barley		0.1	Peaches		8.6	
	12.6	SE general population 90th percentile		10.7	Wheat		1.0	Rye		0.6	Bananas		12.6	
	11.8	NL general		6.9	Wheat		3.7	Barley		0.3	Oats		7.7	
	11.7	FR all population		11 0	Wheat		0.3	Oilseeds		0.1	Peaches		11.7	
	10.7	UK vegetarian		6.8	Wheat		2.5	Sugar beet (root)		0.5	Oats		10.0	
	10.4	DK adult		6.7	Wheat		2.3	Rye		1.1	Oats		9.3	
	9.9	UK Adult		5.6	Wheat		2.7	Sugar beet (root)		0.8	Oilseeds		9.5	
	9.6	FR toddler		8.7	Wheat		0.4	Bananas		0.2	Peas (without pods)		9.6	
	8.7	LT adult		3.6	Rye		3.5	Wheat		0.9	Oats		7.2	
	6.8	FI adult		3.3	Wheat		2.3	Rye		0.9	Oats		5.7	
	3.3	FR infant		2.8	Wheat		0.2	Bananas		0.2	Peas (without pods)		3.3	
	0.3	PL general population		0.1	Cherries		0.1	Bananas		0.0	Peaches		0.3	

Table 1A-6: Evaluation of acute exposure to metconazole metabolite M11 using the TTC threshold of 0.0003 mg/kg bw/day

Threshold MRL is the calculated residue level which would leads to an exposure equivalent to 100 % of the ARfD.

Unprocessed commodities

No of commodities for which ARfD/ADI is exceeded (IESTI 1)		---	**No of commodities for which ARfD/ADI is exceeded (IESTI 2)**		---	**No of commodities for which ARfD/ADI is exceeded (IESTI 1)**		---	**No of commodities for which ARfD/ADI is exceeded (IESTI 2)**		---
IESTI 1	***)**	****)**	**IESTI 2**	***)**	****)**	**IESTI 1**	***)**	****)**	**IESTI 2**	***)**	**)
Highest % of ARfD/ADI	Commodities	pTMRL/ threshold MRL (mg/kg)	Highest % of ARfD/ADI	Commodities	pTMRL/ threshold MRL (mg/kg)	Highest % of ARfD/ADI	Commodities	pTMRL/ threshold MRL (mg/kg)	Highest % of ARfD/ADI	Commodities	pTMRL/ threshold MRL (mg/kg)
50.6	Melons	0.001 / -	50.6	Melons	0.001 / -	72.4	Barley	0.03 / -	72.4	Barley	0.03 / -
48.2	Wheat	0 01 / -	48.2	Wheat	0.01 / -	26.1	Wheat	0.01 / -	26.1	Wheat	0.01 / -
42.6	Sugar beet (root)	0.002 / -	42.6	Sugar beet (root)	0.002 / -	17.3	Sugar beet (root)	0 002 / -	17 3	Sugar beet (root)	0.002 / -
40.8	Watermelons	0.001 / -	40.8	Watermelons	0.001 / -	16.2	Rye	0.01 / -	16 2	Rye	0.01 / -
39.8	Oats	0 03 / -	39.8	Oats	0.03 / -	14.3	Oats	0.03 / -	14 3	Oats	0.03 / -
0.0		0	0.0		0.00	0 0		0 00	0.0		0.00
0.0		0	0.0		0.00	0 0		0 00	0.0		0.00
0.0		0	0.0		0.00	0 0		0 00	0.0		0.00
0.0		0	0.0		0.00	0 0		0 00	0.0		0.00
0.0		0	0.0		0.00	0 0		0 00	0.0		0.00
0.0		0	0.0		0.00	0 0		0 00	0.0		0.00
0.0		0	0.0		0.00	0 0		0 00	0.0		0.00
0.0		0	0.0		0.00	0 0		0 00	0.0		0.00
0.0		0	0.0		0.00	0 0		0 00	0.0		0.00
0.0		0	0.0		0.00	0 0		0 00	0.0		0.00
0.0		0	0.0		0.00	0 0		0 00	0.0		0.00
0.0		0	0.0		0.00	0 0		0 00	0.0		0.00
No of critical MRLs (IESTI 1)		0	---			**No of critical MRLs (IESTI 2)**		0	---		

Processed commodities

No of commodities for which ARfD/ADI is exceeded		---				**No of commodities for which ARfD/ADI is exceeded**		---			
		*****)**						*****)**			
Highest % of ARfD/ADI	Processed commodities	pTMRL/ threshold MRL (mg/kg)				Highest % of ARfD/ADI	Processed commodities	pTMRL/ threshold MRL (mg/kg)			
39.4	Wheat flour	0 01 / -				14.6	Bread/pizza	0.01 / -			
11.9	Peach juice	0.002 / -				1 3	Peach preserved with syrup	0.002 / -			
2 9	Maize flour	0.002 / -				0 2	Maize flour	0.002 / -			
2 8	Plums juice	0.0006 / -				0 0		0 00			
0 0		0.00				0 0		0 00			
0 0	0	0.00				0 0	0	0 00			
0 0	0	0.00						0 00			

Table 1A-7: Evaluation of chronic exposure to metconazole metabolite M1 using the TTC threshold for genotoxic testing

					metconazole M1							**Prepare workbook for refined calculations**		
					Status of the active substance:			Code no.	**BAS 555 F**					
					LOQ (mg/kg bw):		0.01	proposed LOQ:						
					Toxicological end points							**Undo refined calculations**		
					ADI (mg/kg bw/day):		**0.0000025**	ARfD (mg/kg bw):	**0.0003**					
					Source of ADI:		**Annex II**	Source of ARfD:						
					Year of evaluation:		**2004**	Year of evaluation:						
Explain choice of toxicological reference values.														
The risk assessment has been performed on the basis of the MRLs collected from Member States in April 2006. For each pesticide/commodity the highest national MRL was identified (proposed temporary MRL = pTMRL).														
The pTMRLs have been submitted to EFSA in September 2006.														
Chronic risk assessment														
							TMDI (range) in % of ADI minimum - maximum							
							7	208						
					No of diets exceeding ADI			10						
	Highest calculated TMDI values in % of ADI	MS Diet			Highest contributor to MS diet (in % of ADI)	Commodity / group of commodities		2nd contributor to MS diet (in % of ADI)	Commodity / group of commodities		3rd contributor to MS diet (in % of ADI)	Commodity / group of commodities	pTMRLs at LOQ (in % of ADI)	
	207.6	WHO Cluster diet B			58.7	Bovine: Edible offal		40.5	Peaches		29.3	Bovine: Kidney	207.6	
	201.2	DE child			80 5	Bananas		57.5	Cherries		35.1	Apricots	201.2	
	197.9	IE adult			54 6	Peaches		42.6	Sheep: Edible offal		42.3	Melons	197.9	
	140.0	NL child			88 6	Bananas		15.6	Peaches		14.9	Cherries	140.0	
	123.2	FR toddler			67 2	Bananas		49.1	Bovine: Edible offal		4.5	Apricots	123.2	
	119.2	WHO regional European diet			40 0	Bovine: Edible offal		20.0	Peaches		19.8	Bananas	119.2	
	116.6	SE general population 90th percentile			93 9	Bananas		13.9	Peaches		3.2	Apricots	116.6	
	113.9	UK Infant			75 9	Bananas		12.1	Apricots		11.0	Cherries	113.9	
	103.7	ES child			52 6	Bananas		18.8	Peaches		14.7	Cherries	103.7	
	102.4	WHO cluster diet D			42 0	Bovine: Edible offal		16.5	Cherries		10.0	Melons	102.4	
	90.2	IT kids/toddler			33 5	Peaches		27.9	Bananas		14.3	Cherries	90.2	
	86.9	DK child			59 3	Bananas		13.7	Melons		10.5	Peaches	86.9	
	85.0	WHO cluster diet E			30.7	Bovine: Edible offal		18.9	Bananas		13.1	Peaches	85.0	
	73.7	IT adult			36.1	Peaches		10.6	Bananas		10.4	Apricots	73.7	
	72.7	WHO Cluster diet F			29 3	Bananas		26.7	Bovine: Edible offal		5.3	Peaches	72.7	
	71.7	FR all population			23 3	Bovine: Edible offal		13.6	Peaches		12.9	Bananas	71.7	
	70.1	UK Toddler			55 9	Bananas		6.6	Peaches		2.7	Bovine: Kidney	70.1	
	69.1	ES adult			20 3	Peaches		18.7	Bananas		10.7	Melons	69.1	
	63.8	PT General population			34 2	Peaches		17.6	Bananas		5.9	Cherries	63.8	
	56.3	FR infant			37 2	Bananas		13.6	Bovine: Edible offal		5.5	Peaches	56.3	
	35.0	DK adult			19.7	Bananas		9.6	Peaches		4.8	Melons	35.0	
	33.3	NL general			16 3	Bananas		5.3	Peaches		5.3	Cherries	33.3	
	29.9	PL general population			14 3	Cherries		9.8	Bananas		5.8	Peaches	29.9	
	29.4	UK vegetarian			19 6	Bananas		3.7	Melons		2.9	Peaches	29.4	
	26.4	UK Adult			18.4	Bananas		2.7	Peaches		2.3	Melons	26.4	
	13.7	FI adult			13 0	Bananas		0.6	Peaches		0.1	Apricots	13.7	
	7.2	LT adult			4.2	Cherries		3.0	Bananas		0.0	FRUIT (FRESH OR FROZEN)	7.2	

Table 1A-8: Evaluation of chronic exposure to metconazole metabolite M1 using the threshold of 0.0003 mg/kg bw/day

metconazole M1

Status of the active substance:		Code no.	**BAS 555 F**
LOQ (mg/kg bw):	0.01	proposed LOQ:	
Toxicological end points			
ADI (mg/kg bw/day):	**0.0003**	ARfD (mg/kg bw):	**0.0003**
Source of ADI:	**Annex II**	Source of ARfD:	
Year of evaluation:	**2004**	Year of evaluation:	

Prepare workbook for refined calculations

Undo refined calculations

Explain choice of toxicological reference values.

The risk assessment has been performed on the basis of the MRLs collected from Member States in April 2006. For each pesticide/commodity the highest national MRL was identified (proposed temporary MRL = pTMRL).
The pTMRLs have been submitted to EFSA in September 2006.

Chronic risk assessment

	TMDI (range) in % of ADI minimum - maximum	
	0	2
No of diets exceeding ADI	---	0

Highest calculated TMDI values in % of ADI	MS Diet	Highest contributor to MS diet (in % of ADI)	Commodity / group of commodities	2nd contributor to MS diet (in % of ADI)	Commodity / group of commodities	3rd contributor to MS diet (in % of ADI)	Commodity / group of commodities	pTMRLs at LOQ (in % of ADI)
1.7	WHO Cluster diet B	0.5	Bovine: Edible offal	0 3	Peaches	0.2	Bovine: Kidney	1.7
1.7	DE child	0.7	Bananas	0 5	Cherries	0.3	Apricots	1.7
1.6	E adult	0.5	Peaches	0.4	Sheep: Edible offal	0.4	Melons	1 6
1.2	NL child	0.7	Bananas	0.1	Peaches	0.1	Cherries	1 2
1.0	FR toddler	0.6	Bananas	0.4	Bovine: Edible offal	0.0	Apricots	1 0
1.0	WHO regional European diet	0.3	Bovine: Edible offal	0 2	Peaches	0.2	Bananas	1 0
1.0	SE general population 90th percentile	0.8	Bananas	0.1	Peaches	0.0	Apricots	1 0
0.9	UK Infant	0.6	Bananas	0.1	Apricots	0.1	Cherries	0 9
0.9	ES child	0.4	Bananas	0 2	Peaches	0.1	Cherries	0 9
0.9	WHO cluster diet D	0.4	Bovine: Edible offal	0.1	Cherries	0.1	Melons	0 9
0.8	IT kids/toddler	0.3	Peaches	0 2	Bananas	0.1	Cherries	0 8
0.7	DK child	0.5	Bananas	0.1	Melons	0.1	Peaches	0.7
0.7	WHO cluster diet E	0.3	Bovine: Edible offal	0 2	Bananas	0.1	Peaches	0.7
0.6	IT adult	0.3	Peaches	0.1	Bananas	0.1	Apricots	0 6
0.6	WHO Cluster diet F	0.2	Bananas	0 2	Bovine: Edible offal	0.0	Peaches	0 6
0.6	FR all population	0.2	Bovine: Edible offal	0.1	Peaches	0.1	Bananas	0 6
0.6	UK Toddler	0.5	Bananas	0.1	Peaches	0.0	Bovine: Kidney	0 6
0.6	ES adult	0.2	Peaches	0 2	Bananas	0.1	Melons	0 6
0.5	PT General population	0.3	Peaches	0.1	Bananas	0.0	Cherries	0 5
0.5	FR infant	0.3	Bananas	0.1	Bovine: Edible offal	0.0	Peaches	0 5
0.3	DK adult	0.2	Bananas	0.1	Peaches	0.0	Melons	0 3
0.3	NL general	0.1	Bananas	0 0	Peaches	0.0	Cherries	0 3
0.2	PL general population	0.1	Cherries	0.1	Bananas	0.0	Peaches	0 2
0.2	UK vegetarian	0.2	Bananas	0 0	Melons	0.0	Peaches	0 2
0.2	UK Adult	0.2	Bananas	0 0	Peaches	0.0	Melons	0 2
0.1	FI adult	0.1	Bananas	0 0	Peaches	0.0	Apricots	0.1
0.1	LT adult	0.0	Cherries	0 0	Bananas	0.0	FRUIT (FRESH OR FROZEN)	0.1

Table 1A-9: Evaluation of chronic exposure to metconazole metabolite M12 using the TTC threshold for genotoxic testing

metconazole M12			
Status of the active substance:		Code no.	BAS 555 F
LOQ (mg/kg bw):	0.01	proposed LOQ:	
Toxicological end points			
ADI (mg/kg bw/day):	0.0000025	ARfD (mg/kg bw):	0.005
Source of ADI:	Annex II	Source of ARfD:	
Year of evaluation:	2004	Year of evaluation:	

Prepare workbook for refined calculations

Undo refined calculations

Explain choice of toxicological reference values.

The risk assessment has been performed on the basis of the MRLs collected from Member States in April 2006. For each pesticide/commodity the highest national MRL was identified (proposed temporary MRL = pTMRL).
The pTMRLs have been submitted to EFSA in September 2006.

Chronic risk assessment

	TMDI (range) in % of ADI minimum - maximum
	0 99
No of diets exceeding ADI:	--- 0

Highest calculated TMDI values in % of ADI	MS Diet	Highest contributor to MS diet (in % of ADI)	Commodity / group of commodities	2nd contributor to MS diet (in % of ADI)	Commodity / group of commodities	3rd contributor to MS diet (in % of ADI)	Commodity / group of commodities	pTMRLs at LOQ (in % of ADI)
99.3	WHO Cluster diet B	58.7	Bovine: Edible offal	29.3	Bovine: Kidney	8.7	Sheep: Edible offal	99.3
59.3	WHO cluster diet D	42.0	Bovine: Edible offal	6.7	Sheep: Edible offal	6.0	Bovine: Kidney	59.3
50.0	WHO regional European diet	40.0	Bovine: Edible offal	8.7	Sheep: Edible offal	1.3	Bovine: Kidney	50.0
49.1	FR toddler	49.1	Bovine: Edible offal	0.0	FRUIT (FRESH OR FROZEN)	0.0	FRUIT (FRESH OR FROZEN)	49.1
42.6	IE adult	42.6	Sheep: Edible offal	0.0	FRUIT (FRESH OR FROZEN)	0.0	FRUIT (FRESH OR FROZEN)	42.6
35.3	WHO cluster diet E	30.7	Bovine: Edible offal	4.7	Sheep: Edible offal	0.0	FRUIT (FRESH OR FROZEN)	35.3
34.0	WHO Cluster diet F	26.7	Bovine: Edible offal	4.0	Bovine: Kidney	2.7	Sheep: Edible offal	34.0
23.3	FR all population	23.3	Bovine: Edible offal	0.0	FRUIT (FRESH OR FROZEN)	0.0	FRUIT (FRESH OR FROZEN)	23.3
13.6	FR infant	13.6	Bovine: Edible offal	0.0	FRUIT (FRESH OR FROZEN)	0.0	FRUIT (FRESH OR FROZEN)	13.6
9.2	UK Infant	9.2	Bovine: Kidney	0.0	FRUIT (FRESH OR FROZEN)	0.0	FRUIT (FRESH OR FROZEN)	9.2
7.6	ES child	7.5	Bovine: Edible offal	0.1	Sheep: Edible offal	0.0	FRUIT (FRESH OR FROZEN)	7.6
5.6	ES adult	5.0	Bovine: Edible offal	0.6	Sheep: Edible offal	0.0	FRUIT (FRESH OR FROZEN)	5.6
2.7	UK Toddler	2.7	Bovine: Kidney	0.0	FRUIT (FRESH OR FROZEN)	0.0	FRUIT (FRESH OR FROZEN)	2.7
2.3	NL child	2.3	Bovine: Kidney	0.0	FRUIT (FRESH OR FROZEN)	0.0	FRUIT (FRESH OR FROZEN)	2.3
1.1	UK Adult	1.1	Bovine: Kidney	0.0	FRUIT (FRESH OR FROZEN)	0.0	FRUIT (FRESH OR FROZEN)	1.1
0.0	DK adult	0.0	FRUIT (FRESH OR FROZEN)	0.0	FRUIT (FRESH OR FROZEN)	0.0	FRUIT (FRESH OR FROZEN)	0.0
0.0	DK adult	0.0	FRUIT (FRESH OR FROZEN)	0.0	FRUIT (FRESH OR FROZEN)	0.0	FRUIT (FRESH OR FROZEN)	0.0
0.0	DK adult	0.0	FRUIT (FRESH OR FROZEN)	0.0	FRUIT (FRESH OR FROZEN)	0.0	FRUIT (FRESH OR FROZEN)	0.0
0.0	DK adult	0.0	FRUIT (FRESH OR FROZEN)	0.0	FRUIT (FRESH OR FROZEN)	0.0	FRUIT (FRESH OR FROZEN)	0.0
0.0	DK adult	0.0	FRUIT (FRESH OR FROZEN)	0.0	FRUIT (FRESH OR FROZEN)	0.0	FRUIT (FRESH OR FROZEN)	0.0
0.0	DK adult	0.0	FRUIT (FRESH OR FROZEN)	0.0	FRUIT (FRESH OR FROZEN)	0.0	FRUIT (FRESH OR FROZEN)	0.0
0.0	DK adult	0.0	FRUIT (FRESH OR FROZEN)	0.0	FRUIT (FRESH OR FROZEN)	0.0	FRUIT (FRESH OR FROZEN)	0.0
0.0	DK adult	0.0	FRUIT (FRESH OR FROZEN)	0.0	FRUIT (FRESH OR FROZEN)	0.0	FRUIT (FRESH OR FROZEN)	0.0
0.0	DK adult	0.0	FRUIT (FRESH OR FROZEN)	0.0	FRUIT (FRESH OR FROZEN)	0.0	FRUIT (FRESH OR FROZEN)	0.0
0.0	DK adult	0.0	FRUIT (FRESH OR FROZEN)	0.0	FRUIT (FRESH OR FROZEN)	0.0	FRUIT (FRESH OR FROZEN)	0.0
0.0	DK adult	0.0	FRUIT (FRESH OR FROZEN)	0.0	FRUIT (FRESH OR FROZEN)	0.0	FRUIT (FRESH OR FROZEN)	0.0

Conclusion

The estimated Theoretical Maximum Daily Intakes (TMDI), based on pTMRLs were below the ADI.
A long-term intake of residues of metconazole M12 is unlikely to present a public health concern.

Table 1A-10: Evaluation of chronic exposure to metconazole metabolite M31 using the TTC threshold for genotoxic testing

metconazole M31			
Status of the active substance:		Code no.	BAS 555 F
LOQ (mg/kg bw):	0.01	proposed LOQ:	
Toxicological end points			
ADI (mg/kg bw/day):	0.0000025	ARfD (mg/kg bw):	0.0003
Source of ADI:	Annex II	Source of ARfD:	
Year of evaluation:	2004	Year of evaluation:	

Prepare workbook for refined calculations

Undo refined calculations

Explain choice of toxicological reference values.

The risk assessment has been performed on the basis of the MRLs collected from Member States in April 2006. For each pesticide/commodity the highest national MRL was identified (proposed temporary MRL = pTMRL).

The pTMRLs have been submitted to EFSA in September 2006.

Chronic risk assessment

TMDI (range) in % of ADI minimum - maximum	
0	129
No of diets exceeding ADI	1

Highest calculated TMDI values in % of ADI	MS Diet	Highest contributor to MS diet (in % of ADI)	Commodity / group of commodities	2nd contributor to MS diet (in % of ADI)	Commodity / group of commodities	3rd contributor to MS diet (in % of ADI)	Commodity / group of commodities	pTMRLs at LOQ (in % of ADI)
128.7	WHO Cluster diet B	58.7	Bovine: Edible offal	29.3	Bovine: Liver	29.3	Bovine: Liver	128.7
65.3	WHO cluster diet D	42 0	Bovine: Edible offal	6.7	Sheep: Edible offal	6.0	Bovine: Liver	65.3
52.7	WHO regional European diet	40 0	Bovine: Edible offal	8.7	Sheep: Edible offal	2.7	Bovine: Liver	52.7
50.9	DK child	50 9	Bovine: Liver	0.0	FRUIT (FRESH OR FROZEN)	0.0	FRUIT (FRESH OR FROZEN)	50.9
49.1	FR toddler	49.1	Bovine: Edible offal	0.0	FRUIT (FRESH OR FROZEN)	0.0	FRUIT (FRESH OR FROZEN)	49.1
46.0	UK Infant	36 8	Bovine: Liver	9.2	Bovine: Kidney	0.0	FRUIT (FRESH OR FROZEN)	46.0
42.6	IE adult	42 6	Sheep: Edible offal	0.0	FRUIT (FRESH OR FROZEN)	0.0	FRUIT (FRESH OR FROZEN)	42.6
42.0	WHO cluster diet E	30.7	Bovine: Edible offal	6.7	Bovine: Liver	4.7	Sheep: Edible offal	42.0
38.0	WHO Cluster diet F	26.7	Bovine: Edible offal	4.0	Bovine: Liver	4.0	Bovine: Liver	38.0
32.7	NL child	30.4	Bovine: Liver	2.3	Bovine: Kidney	0.0	FRUIT (FRESH OR FROZEN)	32.7
23.3	FR all population	23 3	Bovine: Edible offal	0.0	FRUIT (FRESH OR FROZEN)	0.0	FRUIT (FRESH OR FROZEN)	23.3
21.6	DK adult	21 6	Bovine: Liver	0.0	FRUIT (FRESH OR FROZEN)	0.0	FRUIT (FRESH OR FROZEN)	21.6
13.9	ES child	7.5	Bovine: Edible offal	6.3	Bovine: Liver	0.1	Sheep: Edible offal	13.9
13.6	FR infant	13 6	Bovine: Edible offal	0.0	FRUIT (FRESH OR FROZEN)	0.0	FRUIT (FRESH OR FROZEN)	13.6
11.0	UK Toddler	8.2	Bovine: Liver	2.7	Bovine: Kidney	0.0	FRUIT (FRESH OR FROZEN)	11.0
10.0	ES adult	5.0	Bovine: Edible offal	4.3	Bovine: Liver	0.6	Sheep: Edible offal	10.0
7.3	NL general	7.3	Bovine: Liver	0.0	FRUIT (FRESH OR FROZEN)	0.0	FRUIT (FRESH OR FROZEN)	7 3
6.6	LT adult	6.6	Bovine: Liver	0.0	FRUIT (FRESH OR FROZEN)	0.0	FRUIT (FRESH OR FROZEN)	6 6
6.3	UK Adult	5.3	Bovine: Liver	1.1	Bovine: Kidney	0.0	FRUIT (FRESH OR FROZEN)	6 3
0.0	FI adult	0.0	FRUIT (FRESH OR FROZEN)	0.0	FRUIT (FRESH OR FROZEN)	0.0	FRUIT (FRESH OR FROZEN)	0 0
0.0	FI adult	0.0	FRUIT (FRESH OR FROZEN)	0.0	FRUIT (FRESH OR FROZEN)	0.0	FRUIT (FRESH OR FROZEN)	0 0
0.0	FI adult	0.0	FRUIT (FRESH OR FROZEN)	0.0	FRUIT (FRESH OR FROZEN)	0.0	FRUIT (FRESH OR FROZEN)	0 0
0.0	FI adult	0.0	FRUIT (FRESH OR FROZEN)	0.0	FRUIT (FRESH OR FROZEN)	0.0	FRUIT (FRESH OR FROZEN)	0 0
0.0	FI adult	0.0	FRUIT (FRESH OR FROZEN)	0.0	FRUIT (FRESH OR FROZEN)	0.0	FRUIT (FRESH OR FROZEN)	0 0
0.0	FI adult	0.0	FRUIT (FRESH OR FROZEN)	0.0	FRUIT (FRESH OR FROZEN)	0.0	FRUIT (FRESH OR FROZEN)	0 0
0.0	FI adult	0.0	FRUIT (FRESH OR FROZEN)	0.0	FRUIT (FRESH OR FROZEN)	0.0	FRUIT (FRESH OR FROZEN)	0 0
0.0	FI adult	0.0	FRUIT (FRESH OR FROZEN)	0.0	FRUIT (FRESH OR FROZEN)	0.0	FRUIT (FRESH OR FROZEN)	0 0

Table 1A-11: Evaluation of chronic exposure to the total residues of metconazole and metabolites M1, M11, M12, M30, M31 and M32

				metconazole +M11,M21,M30, M1,M12, M31,M32						Prepare workbook for refined calculations			
				Status of the active substance:			Code no.	**BAS 555 F**					
				LOQ (mg/kg bw):		0.01	proposed LOQ:						
				Toxicological end points						Undo refined calculations			
				ADI (mg/kg bw/day):		**0.01**	ARfD (mg/kg bw):	**0.01**					
				Source of ADI:		**Annex II**	Source of ARfD:						
				Year of evaluation:		**2004**	Year of evaluation:						
Explain choice of toxicological reference values.													
The risk assessment has been performed on the basis of the MRLs plus metabolite levels based on residues measured in U. S. studies'													
Chronic risk assessment													
						TMDI (range) in % of ADI minimum - maximum							
						1		29					
				No of diets exceeding ADI		---		0					
	Highest calculated TMDI values in % of ADI	MS Diet		Highest contributor to MS diet (in % of ADI)	Commodity / group of commodities		2nd contributor to MS diet (in % of ADI)	Commodity / group of commodities		3rd contributor to MS diet (in % of ADI)	Commodity / group of commodities		pTMRLs at LOQ (in % of ADI)
	28.6	UK Toddler		15.1	Sugar beet (root)		7.1	Wheat		4.1	Milk and cream,		0.0
	23.2	UK Infant		7.7	Milk and cream,		6.7	Sugar beet (root)		4.7	Wheat		0.0
	21.8	WHO Cluster diet B		15.4	Wheat		1.3	Barley		0.6	Maize		0.0
	20.4	DK child		9.9	Wheat		4.0	Rye		2.5	Milk and cream,		0.0
	18.8	NL child		8.5	Wheat		5.9	Milk and cream,		1.7	Bananas		0.0
	16.2	WHO cluster diet D		11.7	Wheat		1.0	Milk and cream,		1.0	Barley		0.0
	15.8	WHO cluster diet E		7.1	Wheat		3.6	Barley		1.2	Rape seed		0.0
	15.8	DE child		7.4	Wheat		2.9	Milk and cream		1.6	Bananas		0.0
	15.5	IE adult		5.6	Barley		4.1	Wheat		0.8	Oats		0.0
	15.0	FR toddler		7.9	Milk and cream		4.7	Wheat		1.3	Bananas		0.0
	14.0	WHO Cluster diet F		6.5	Wheat		2.7	Barley		0.8	Milk and cream,		0.0
	13.4	IT kids/toddler		12 0	Wheat		0.5	Bananas		0.4	Peaches		0.0
	13.4	ES child		8.0	Wheat		2.5	Milk and cream,		1.0	Bananas		0.0
	10.9	SE general population 90th percentile		5.8	Wheat		2.5	Milk and cream,		1.8	Bananas		0.0
	10.6	WHO regional European diet		5.3	Wheat		1.5	Barley		1.0	Milk and cream,		0.0
	9.1	ES adult		4.2	Wheat		2.2	Barley		1.0	Milk and cream,		0.0
	9.0	PT General population		7.1	Wheat		0.4	Peaches		0.3	Bananas		0.0
	8.5	IT adult		7.4	Wheat		0.4	Peaches		0.2	Bananas		0.0
	8.1	NL general		3.7	Wheat		1.7	Barley		1.3	Milk and cream		0.0
	8.0	UK vegetarian		3.7	Wheat		2.5	Sugar beet (root)		0.7	Milk and cream,		0.0
	7.9	FR infant		5.1	Milk and cream		1.5	Wheat		0.7	Bananas		0.0
	7.8	FR all population		5.9	Wheat		0.5	Milk and cream,		0.3	Bananas		0.0
	7.2	UK Adult		3.0	Wheat		2.6	Sugar beet (root)		0.6	Milk and cream		0.0
	6.9	DK adult		3.6	Wheat		1.1	Milk and cream,		0.6	Rye		0.0
	4.9	LT adult		1.9	Wheat		1.0	Rye		0.8	Milk and cream,		0.0
	4.5	FI adult		1.8	Wheat		1.1	Milk and cream,		0.6	Rye		0.0
	0.5	PL general population		0.2	Bananas		0.2	Cherries		0.1	Peaches		0.0

Table 1A-12: Evaluation of acute exposure to the total residues of metconazole and metabolites M1, M11, M12, M30, M31 and M32

Unprocessed commodities

No of commodities for which ARfD/ADI is exceeded (IESTI 1):		---	No of commodities for which ARfD/ADI is exceeded (IESTI 2):		---	No of commodities for which ARfD/ADI is exceeded (IESTI 1):		---	No of commodities for which ARfD/ADI is exceeded (IESTI 2):		---
IESTI 1	***)**	****)**	**IESTI 2**	***)**	****)**	**IESTI 1**	***)**	****)**	**IESTI 2**	***)**	**)
Highest % of ARfD/ADI	Commodities	pTMRL/ threshold MRL (mg/kg)	Highest % of ARfD/ADI	Commodities	pTMRL/ threshold MRL (mg/kg)	Highest % of ARfD/ADI	Commodities	pTMRL/ threshold MRL (mg/kg)	Highest % of ARfD/ADI	Commodities	pTMRL/ threshold MRL (mg/kg)
85.3	Bananas	0.102 / -	78.9	Melons	0.052 / -	32.6	Barley	0.45 / -	32.6	Barley	0.45 / -
78.9	Melons	0.052 / -	61.8	Bananas	0.102 / -	20.5	Melons	0.052 / -	20.5	Melons	0.052 / -
61.7	Peaches	0.104 / -	45.3	Peaches	0.104 / -	18.2	Peaches	0.104 / -	17.1	Sugar beet (root)	0.066 / -
42.1	Sugar beet (root)	0.066 / -	42.1	Sugar beet (root)	0.066 / -	17.1	Sugar beet (root)	0.066 / -	14.1	Peaches	0.104 / -
32.2	Apricots	0.104 / -	26.0	Wheat	0.18 / -	14.1	Wheat	0.18 / -	14.1	Wheat	0.18 / -
0.0		0	0.0		0.00	0.0		0.00	0.0		0.00
0.0		0	0.0		0.00	0.0		0.00	0.0		0.00
0.0		0	0.0		0.00	0.0		0.00	0.0		0.00
0.0		0	0.0		0.00	0.0		0.00	0.0		0.00
0.0		0	0.0		0.00	0.0		0.00	0.0		0.00
0.0		0	0.0		0.00	0.0		0.00	0.0		0.00
0.0		0	0.0		0.00	0.0		0.00	0.0		0.00
0.0		0	0.0		0.00	0.0		0.00	0.0		0.00
0.0		0	0.0		0.00	0.0		0.00	0.0		0.00
0.0		0	0.0		0.00	0.0		0.00	0.0		0.00
0.0		0	0.0		0.00	0.0		0.00	0.0		0.00
0.0		0	0.0		0.00	0.0		0.00	0.0		0.00
No of critical MRLs (IESTI 1)		0 ---				**No of critical MRLs (IESTI 2)**		0 ---			

Processed commodities

No of commodities for which ARfD/ADI is exceeded:		---				No of commodities for which ARfD/ADI is exceeded:		---			
		***)						***)			
Highest % of ARfD/ADI	Processed commodities	pTMRL/ threshold MRL (mg/kg)				Highest % of ARfD/ADI	Processed commodities	pTMRL/ threshold MRL (mg/kg)			
21.3	Wheat flour	0.18 / -				7.9	Bread/pizza	0.18 / -			
18.6	Peach juice	0.104 / -				2.1	Peach preserved with syrup	0.104 / -			
1.1	Maize flour	0.026 / -				0.1	Maize flour	0.026 / -			
0.0		0.00				0.0		0.00			
0.0		0.00				0.0		0.00			
0.0	0	0.00				0.0	0	0.00			
0.0	0	0.00						0.00			
0.0	0	0.00						0.00			
0.0	0	0.00						0.00			
0.0	0	0.00						0.00			
		0						0			

*) The results of the IESTI calculations are reported for at least 5 commodities. If the ARfD is exceeded for more than 5 commodities, all IESTI values > 90% of ARfD are reported.
**) pTMRL: provisional temporary MRL
***) pTMRL: provisional temporary MRL for unprocessed commodity

Conclusion:

For metconazole +M11+M21+M30 IESTI 1 and IESTI 2 were calculated for food commodities for which pTMRLs were submitted and for which consumption data are available.
No exceedance of the ARfD/ADI was identified for any unprocessed commodity.

Appendix 1B Tier 1 Summaries of the Supervised Field Residue Trials

Tier 1 Summaries of the Supervised Field Residue Trials for the Representative Crops

Wheat

Treatment with BAS 555 00 F on wheat in Northern Europe

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name): Metconazole (BAS 555 F)
Crop /crop group: Wheat/cereals
Responsible body for reporting (name, address) BASF SE, 67117 Limburgerhof
Country (trial) Germany
Content of active substance (g/kg or g/L): 60 g/L
Formulation (e.g. WP) EC (BAS 555 00 F)

Commercial product:
Producer of commercial product: BASF SE, Ludwigshafen, Germany
Indoor/Glasshouse/Outdoor Outdoor
Other active substance in the formulation (common name and content)
Residue calculated as: Metconazole (BAS 555 F)

1	2	3	4	5			6	7	8	9			10	11
Report No Location (Trial No)	**Commodity/ Variety**	**Date of 1. Sowing / Planting 2. Flowering 3. Harvest**	**Method of treatment**	**Application rate[0] per treatment**			**No of treatment(s) and last date**	**Growth stage (BBCH)[2]**	**Portion analyzed**	**Residues (mg/kg)**			**DALA[1]**	**Remarks**
				kg a.s./hL	**Water L/ha**	**kg a.s./ha**				***Cis***	***Trans***	**Sum**		
358573 2010/1075867 47652, Kleve Germany (L090073)	Wheat GC 0654 (Hattrick)	1. 13.10.2008 2. 01.06.-14.06.2009 3. 17.07.2009	Foliar application with boom sprayer	0.045	200	0.090	2 04.06.2009	69	Plant w/o roots Ears Rest of plant Grain Straw Grain Straw	n.r. n.r. n.r. n.r. n.r. n.r. n.r.	n.r. n.r. n.r. n.r. n.r. n.r. n.r.	2.9 0.11 0.9 0.016 1.4 <0.01 1.1	0 27 27 35 35 43 43	BASF method No 535/1 (L0076/01) LOQ 0.01 mg/kg
358573 2010/1075867 OX15 6HT, Oxon United Kingdom (L090074)	Wheat GC 0654 (Consort)	1. 11.10.2008 2. 12.06.-23.06.2009 3. 03.08.2009	Foliar application with boom sprayer	0.045	200	0.090	2 23.06.2009	69	Plant w/o roots Grain Straw Grain Straw Grain Straw	n.r. n.r. n.r. n.r. n.r. n.r. n.r.	n.r. n.r. n.r. n.r. n.r. n.r. n.r.	1.8 0.023 1.3 <0.01 0.86 <0.01 0.84	0 28 28 35 35 41 41	
358573 2010/1075867 4930, Lolland Denmark (L090075)	Wheat GC 0654 (Frument)	1. 26.09.2008 2. 08.06.-01.07.2009 3. 08.08.2009	Foliar application with boom sprayer	0.045	200	0.090	2 01.07.2009	69	Plant w/o roots Ears Rest of plant Grain Straw Grain Straw	n.r. n.r. n.r. n.r. n.r. n.r. n.r.	n.r. n.r. n.r. n.r. n.r. n.r. n.r.	2.2 0.34 1.4 <0.01 2.8 <0.01 2.4	0 27 27 35 35 41 41	

1	2	3	4	5			6	7	8	9			10	11
Report No Location (Trial No)	**Commodity/ Variety**	**Date of 1. Sowing / Planting 2. Flowering 3. Harvest**	**Method of treatment**	**Application rate[0] per treatment**			**No of treatment(s) and last date**	**Growth stage (BBCH)[2]**	**Portion analyzed**	**Residues (mg/kg)**			**DALA[1]**	**Remarks**
				kg a.s./hL	**Water L/ha**	**kg a.s./ha**				***Cis***	***Trans***	**Sum**		
358573 2010/1075867 51220, Marne France (L090076)	Wheat GC 0654 (Maxwell)	1. 15.10.2008 2. 27.05.-05.06.2009 3. 15.07.-20.07.2009	Foliar application with boom sprayer	0.045	200	0.090	2 03.06.2009	69	Plant w/o roots Ears Rest of plant Ears Rest of plant Grain Straw	n.r. n.r. n.r. n.r. n.r. n.r. n.r.	n.r. n.r. n.r. n.r. n.r. n.r. n.r.	3.3 0.44 0.41 0.52 0.56 <0.01 1.4	0 29 29 35 35 42 42	BASF method No 535/1 (L0076/01) LOQ 0.01 mg/kg
358573 2010/1075867 47652, Kleve Germany (L090080)	Wheat GC 0654 (Rosario)	1. 22.10.2008 2. 06.06.-20.06.2009 3. 06.08.2009	Foliar application with boom sprayer	0.045	200	0.090	2 25.06.2009	71	Plant w/o roots Grain Straw Grain Straw Grain Straw	n.r. n.r. n.r. n.r. n.r. n.r. n.r.	n.r. n.r. n.r. n.r. n.r. n.r. n.r.	1.9 <0.01 0.26 <0.01 0.35 <0.01 0.44	0 29 29 34 34 42 42	
358573 2010/1075867 51490, Marne France (L090081)	Wheat GC 0654 (Oryantis)	1. 17.10.2008 2. 25.05.-04.06.2009 3. 28.07.-29.07.2009	Foliar application with boom sprayer	0.045	200	0.090	2 16.06.2009	71	Plant w/o roots Ears Rest of plant Grain Straw Grain Straw	n.r. n.r. n.r. n.r. n.r. n.r. n.r.	n.r. n.r. n.r. n.r. n.r. n.r. n.r.	2.7 0.85 1.1 0.013 3.5 0.013 2.0	0 28 28 35 35 42 42	
255298 2007/1050102 74193, Stetten a. H. Germany (L070184) (AC/07/040)	Wheat GC 0654 (Tommi)	1. 16.10.2006 2. 26.05.-02.06.2007 3. 23.07.2007	Foliar application with boom sprayer	0.045	200	0.090	2 04.06.2007	73	Plant w/o roots Ears Rest of plant Grain Straw Grain Straw	1.12 <0.005 0.675 <0.005 0.975 <0.005 0.905	0.219 <0.005 0.207 <0.005 0.315 <0.005 0.278	1.33 <0.01 0.88 <0.01 1.29 <0.01 1.18	0 35 35 42 42 49 49	BASF method No 550/0 (L0019/01) LOQ 0.005 mg/kg per isomer
255298 2007/1050102 16833, Brunne Germany (L070185) (AC/07/041)	Wheat GC 0654 (Brilliant)	1. 25.09.2006 2. 18.05.-31.05.2007 3. 19.07.2007	Foliar application with boom sprayer	0.045	200	0.090	2 31.05.2007	69	Plant w/o roots Grain Straw Grain Straw Grain Straw	1.61 <0.005 0.490 <0.005 0.795 <0.005 0.590	0.245 <0.005 0.132 <0.005 0.173 <0.005 0.128	1.85 <0.01 0.62 <0.01 0.97 <0.01 0.72	0 36 36 41 41 49 49	

1	2	3	4	5			6	7	8	9			10	11
Report No Location (Trial No)	**Commodity/ Variety**	**Date of 1. Sowing / Planting 2. Flowering 3. Harvest**	**Method of treatment**	**Application rate[0] per treatment**			**No of treatment(s) and last date**	**Growth stage (BBCH)[2]**	**Portion analyzed**	**Residues (mg/kg)**			**DALA[1]**	**Remarks**
				kg a.s./hL	**Water L/ha**	**kg a.s./ha**				***Cis***	***Trans***	**Sum**		
255298 2007/1050102 67440, Kleingoeft France (north) (L070188) (AC/07/044)	Triticale GC 0653 (Talentro)	1. 04.10.2006 2. 20.05.-26.05.2007 3. 17.07.-18.07.2007	Foliar application with boom sprayer	0.045	200	0.090	2 24.05.2007	69	Plant w/o roots Ears Rest of plant Ears Rest of plant Ears Rest of plant Grain Straw	1.16 0.103 0.264 0.038 0.201 0.062 0.162 <0.005 0.099	0.223 0.020 0.063 0.011 0.047 0.018 0.041 <0.005 0.024	1.38 0.12 0.33 0.05 0.25 0.08 0.20 <0.01 0.12	0 36 36 41 41 48 48 54 54	BASF method No 550/0 (L0019/01) LOQ 0.005 mg/kg per isomer
255298 2007/1050102 29830, Ploudalmezeau France (north) (L070189) (AC/07/044)	Wheat GC 0654 (Caphorn)	1. 30.11.2006 2. 30.05.-05.06.2007 3. 03.09.2007	Foliar application with boom sprayer	0.045	200	0.090	2 05.06.2007	69	Plant w/o roots Ears Rest of plant Ears Rest of plant Ears Rest of plant Grain Straw	1.46 0.072 0.134 0.076 0.097 0.074 0.084 <0.005 0.438	0.239 0.022 0.043 0.023 0.030 0.020 0.024 <0.005 0.121	1.69 0.09 0.18 0.10 0.13 0.09 0.11 <0.01 0.56	0 35 35 42 42 48 48 65 65	
401831 2012/1194991 21614, Buxtehude Germany (L110188)	Wheat GC 0654 (Julius)	1. 24.09.2010 2. 27.05.-07.06.2011 3. 03.08.2011	Foliar application with boom sprayer	0.045	200	0.090	2 07.06.2011	69	Plant w/o roots Ears Rest of plant w/o root Ears Rest of plant w/o root Ears Rest of plant w/o root Grain Straw	2.3 0.18 0.68 0.22 0.63 0.22 0.57 <0.005 0.66	0.46 0.045 0.17 0.055 0.16 0.056 0.15 <0.005 0.18	2.8 0.23 0.85 0.27 0.79 0.27 0.72 <0.01 0.84	0 29 29 36 36 43 43 57 57	BASF method No 535/1 (L0076/01) LOQ 0.005 mg/kg per isomer
401831 2012/1194991 HU17 9SL, Beverley United Kingdom (L110189)	Wheat GC 0654 (Oakley)	1. 05.09.2010 2. n.r. 3. 16.08.2011	Foliar application with boom sprayer	0.045	200	0.090	2 20.06.2011	69	Plant w/o roots Ears Rest of plant w/o root Grain Straw Grain Straw Grain Straw	1.1 0.090 0.54 <0.005 0.68 <0.005 0.86 <0.005 0.44	0.24 0.025 0.15 <0.005 0.20 <0.005 0.26 <0.005 0.14	1.3 0.12 0.69 <0.01 0.88 <0.01 1.1 <0.01 0.57	0 29 29 36 36 42 42 57 57	

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
n.r. Not reported

Treatment with BAS 627 00 F on wheat in Northern Europe

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name): Metconazole (BAS 555 F)
Crop /crop group: Wheat/cereals
Responsible body for reporting (name, address) BASF SE, 67117 Limburgerhof
Country (trial) Germany
Content of active substance (g/kg or g/L): 27.5 g/L
Formulation (e.g. WP) SL (BAS 627 00 F)

Commercial product:
Producer of commercial product: BASF SE, Ludwigshafen, Germany
Indoor/Glasshouse/Outdoor Outdoor
Other active substance in the formulation (common name and content) Epoxiconazole (BAS 480 F, 37.5 g/L)
Residue calculated as: Metconazole (BAS 555 F)

1	2	3	4	5			6	7	8	9			10	11
Report No Location (Trial No)	**Commodity/ Variety**	**Date of 1. Sowing / Planting 2. Flowering 3. Harvest**	**Method of treatment**	**Application rate[0] per treatment**			**No of treatment(s) and last date**	**Growth stage (BBCH)[2]**	**Portion analyzed**	**Residues (mg/kg)**			**DALA[1]**	**Remarks**
				kg a.s./hL	**Water L/ha**	**kg a.s./ha**				***Cis***	***Trans***	**Sum**		
358573 2010/1075867 47652, Kleve Germany (L090073)	Wheat GC 0654 (Hattrick)	1. 13.10.2008 2. 01.06.-14.06.2009 3. 17.07.2009	Foliar application with boom sprayer	0.04125	200	0.0825	2 04.06.2009	69	Plant w/o roots Ears Rest of plant Grain Straw Grain Straw	n.r. n.r. n.r. n.r. n.r. n.r. n.r.	n.r. n.r. n.r. n.r. n.r. n.r. n.r.	2.3 0.12 1.3 0.025 2.7 <0.01 1.2	0 27 27 35 35 43 43	BASF Method No 535/1 (L0076/01) LOQ 0.01 mg/kg
358573 2010/1075867 OX15 6HT, Oxon United Kingdom (L090074)	Wheat GC 0654 (Consort)	1. 11.10.2008 2. 12.06.-23.06.2009 3. 03.08.2009	Foliar application with boom sprayer	0.04125	200	0.0825	2 23.06.2009	69	Plant w/o roots Grain Straw Grain Straw Grain Straw	n.r. n.r. n.r. n.r. n.r. n.r. n.r.	n.r. n.r. n.r. n.r. n.r. n.r. n.r.	1.3 0.047 1.6 0.01 1.4 <0.01 1.3	0 28 28 35 35 41 41	
358573 2010/1075867 4930, Lolland Denmark (L090075)	Wheat GC 0654 (Frument)	1. 26.09.2008 2. 08.06.-01.07.2009 3. 08.08.2009	Foliar application with boom sprayer	0.04125	200	0.0825	2 01.07.2009	69	Plant w/o roots Ears Rest of plant Grain Straw Grain Straw	 n.r. n.r. n.r. n.r. n.r. n.r.	 n.r. n.r. n.r. n.r. n.r. n.r.	1.9 0.29 1.6 0.011 2 <0.01 2.2	0 27 27 35 35 41 41	
358573 2010/1075867 51220, Marne France (L090076)	Wheat GC 0654 (Maxwell)	1. 15.10.2008 2. 27.05.-05.06.2009 3. 15.07.-20.07.2009	Foliar application with boom sprayer	0.04125	200	0.0825	2 03.06.2009	69	Plant w/o roots Ears Rest of plant Ears Rest of plant Grain Straw	n.r. n.r. n.r. n.r. n.r. n.r. n.r.	n.r. n.r. n.r. n.r. n.r. n.r. n.r.	2.0 0.31 0.33 0.23 0.29 0.012 2.1	0 29 29 35 35 42 42	

1	2	3	4	5			6	7	8	9			10	11
Report No Location (Trial No)	**Commodity/ Variety**	**Date of 1. Sowing / Planting 2. Flowering 3. Harvest**	**Method of treatment**	**Application rate[0] per treatment**			**No of treatment(s) and last date**	**Growth stage (BBCH)[2]**	**Portion analyzed**	**Residues (mg/kg)**			**DALA[1]**	**Remarks**
				kg a.s./hL	**Water L/ha**	**kg a.s./ha**				***Cis***	***Trans***	**Sum**		
358573 2010/1075867 47652, Kleve Germany (L090080)	Wheat GC 0654 (Rosario)	1. 22.10.2008 2. 06.06.-20.06.2009 3. 06.08.2009	Foliar application with boom sprayer	0.04125	200	0.0825	2 25.06.2009	71	Plant w/o roots Grain Straw Grain Straw Grain Straw	n.r. n.r. n.r. n.r. n.r. n.r. n.r.	n.r. n.r. n.r. n.r. n.r. n.r. n.r.	1.8 <0.01 0.44 0.011 0.71 0.012 1.0	0 29 29 34 34 42 42	
358573 2010/1075867 51490, Marne France (L090081)	Wheat GC 0654 (Oryantis)	1. 17.10.2008 2. 25.05.-04.06.2009 3. 28.07.-29.07.2009	Foliar application with boom sprayer	0.04125	200	0.0825	2 16.06.2009	71	Plant w/o roots Ears Rest of plant Grain Straw Grain Straw	n.r. n.r. n.r. n.r. n.r. n.r. n.r.	n.r. n.r. n.r. n.r. n.r. n.r. n.r.	2.6 0.19 2 0.023 2.3 0.018 2.5	0 28 28 35 35 42 42	
255298 2007/1050102 74193, Stetten a. H. Germany (L070184) (AC/07/040)	Wheat GC 0654 (Tommi)	1. 16.10.2006 2. 26.05.-02.06.2007 3. 23.07.2007	Foliar application with boom sprayer	0.04125	200	0.0825	2 04.06.2007	73	Plant w/o roots Ears Rest of plant Grain Straw Grain Straw	1.12 0.005 0.900 0.005 1.31 <0.005 2.12	0.249 <0.005 0.412 <0.005 0.412 <0.005 0.715	1.37 0.01 1.31 0.01 1.72 <0.01 2.83	0 35 35 42 42 49 49	BASF method No 550/0 (L0019/01) LOQ 0.005 mg/kg per isomer
255298 2007/1050102 16833, Brunne Germany (L070185) (AC/07/041)	Wheat GC 0654 (Brilliant)	1. 25.09.2006 2. 18.05.-31.05.2007 3. 19.07.2007	Foliar application with boom sprayer	0.04125	200	0.0825	2 31.05.2007	69	Plant w/o roots Grain Straw Grain Straw Grain Straw	1.42 <0.005 0.825 <0.005 0.585 <0.005 0.890	0.258 <0.005 0.322 <0.005 0.161 <0.005 0.264	1.68 <0.01 1.15 <0.01 0.75 <0.01 1.15	0 36 36 41 41 49 49	
255298 2007/1050102 67440, Kleingoeft France (north) (L070188) (AC/07/044)	Wheat GC 0654 (Talerno)	1. 04.10.2006 2. 20.05.-26.05.2007 3. 17.07.-18.07.2007	Foliar application with boom sprayer	0.04125	200	0.0825	2 24.05.2007	69	Plant w/o roots Ears Rest of plant Ears Rest of plant Ears Rest of plant Grain Straw	1.56 0.124 0.209 0.088 0.133 0.066 0.290 <0.005 0.143	0.458 0.036 0.050 0.029 0.032 0.023 0.067 <0.005 0.056	2.02 0.16 0.26 0.12 0.17 0.09 0.36 <0.01 0.20	0 36 36 41 41 48 48 54 54	

1	2	3	4	5			6	7	8	9			10	11
Report No Location (Trial No)	**Commodity/ Variety**	**Date of 1. Sowing / Planting 2. Flowering 3. Harvest**	**Method of treatment**	**Application rate[0] per treatment**			**No of treatment(s) and last date**	**Growth stage (BBCH)[2]**	**Portion analyzed**	**Residues (mg/kg)**			**DALA[1]**	**Remarks**
				kg a.s./hL	**Water L/ha**	**kg a.s./ha**				***Cis***	***Trans***	**Sum**		
255298 2007/1050102 29830, Ploudalmezeau France (north) (L070189) (AC/07/044)	Wheat GC 0654 (Caphorn)	1. 30.11.2006 2. 30.05.-05.06.2007 3. 03.09.2007	Foliar application with boom sprayer	0.04125	200	0.0825	2 05.06.2007	69	Plant w/o roots Ears Rest of plant Ears Rest of plant Ears Rest of plant Grain Straw	1.24 0.077 0.193 0.065 0.125 0.075 0.093 <0.005 0.345	0.256 0.028 0.068 0.026 0.045 0.026 0.031 <0.005 0.146	1.49 0.10 0.26 0.09 0.17 0.10 0.12 <0.01 0.49	0 35 35 42 42 48 48 65 65	
339326 2010/1144333 47574 Goch- Nierswalde Kleve Germany (L100105)	GC 0654/ Wheat Winnetou	1 27.10.2009 2 10.06.- 18.06.2010 3 06.08.2010	Foliar application with boom	0.0563	200	0.1125	2 18.06.2010	69	Plant w/o roots Grain Straw Grain Straw Grain Straw	2.0 0.006 1.3 0.005 1.3 0.005 0.76	0.47 <0.005 0.36 <0.005 0.37 <0.005 0.24	2.4 0.011 1.7 0.010 1.7 0.010 1.0	0 34 34 42 42 49 49	BASF method No 550/0 (L0019/01) LOQ 0.005 mg/kg per isomer
339326 2010/1144333 CV35 9ES Wellesbourne Warwick UK (L100106)	GC 0654/ Wheat Hereward	1 05.10.2009 2 07.06.- 17.06.2010 3 07.08.2010	Foliar application with boom	0.0563	200	0.1125	2 17.06.2010	69	Plant w/o roots Grain Straw Grain Straw Grain Straw	2.4 <0.005 3.6 <0.005 4.1 0.011 3.2	0.55 <0.005 0.90 <0.005 1.0 <0.005 0.77	2.9 <0.01 4.5 <0.01 5.1 0.016 4.0	0 35 35 41 41 49 49	

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
n.r. Not reported

Treatment with BAS 627 02 F on wheat in Northern Europe

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name): Metconazole (BAS 555 F)
Crop /crop group: Wheat/cereals
Responsible body for reporting (name, address) BASF SE, 67117 Limburgerhof
Country (trial) Germany

Content of active substance (g/kg or g/L): 41.25 g/L
Formulation (e.g. WP) EC (BAS 627 02 F)

Commercial product:
Producer of commercial product: BASF SE, Ludwigshafen, Germany
Indoor/Glasshouse/Outdoor Outdoor
Other active substance in the formulation (common name and content) Epoxiconazole (BAS 480 F, 56.25 g/L)

Residue calculated as: Metconazole (BAS 555 F)

1	2	3	4	5			6	7	8	9			10	11
Report No Location (Trial No)	**Commodity/ Variety**	**Date of 1. Sowing / Planting 2. Flowering 3. Harvest**	**Method of treatment**	**Application rate[0] per treatment**			**No of treatment(s) and last date**	**Growth stage (BBCH)[2]**	**Portion analyzed**	**Residues (mg/kg)**			**DALA[1]**	**Remarks**
				kg a.s./hL	**Water L/ha**	**kg a.s./ha**				***Cis***	***Trans***	**Sum**		
339326 2010/1144333 47574 Goch-Nierswalde Kleve Germany (L100105)	GC 0654/ Wheat Winnetou	1 27.10 2009 2 10.06.- 18.06 2010 3 06.08 2010	Foliar application with boom	0.0563	200	0.1125	2 18.06.2010	69	Plant w/o roots Grain Straw Grain Straw Grain Straw	1.9 0.006 1.4 <0.005 0.69 0.005 0.87	0.42 <0.005 0.41 <0.005 0.21 <0.005 0.27	2.4 0.011 1.8 <0.010 0.90 0.010 1.1	0 34 34 42 42 49 49	BASF method No 550/0 (L0019/01) LOQ 0.005 mg/kg per isomer
339326 2010/1144333 CV35 9ES Wellesbourne Warwick UK (L100106)	GC 0654/ Wheat Hereward	1 05.10 2009 2 07.06.- 17.06 2010 3 07.08 2010	Foliar application with boom	0.0563	200	0.1125	2 17.06.2010	69	Plant w/o roots Grain Straw Grain Straw Grain Straw	2.3 <0.005 5.1 0.005 4.3 0.009 5.8	0.53 <0.005 1.3 <0.005 1.1 <0.005 1.5	2.9 <0.010 6.4 0.010 5.4 0.014 7.3	0 35 35 41 41 49 49	

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
n.r. Not reported

Treatment with BAS 556 03 F on wheat in Northern Europe

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name): Metconazole (BAS 555 F)
Crop /crop group: Wheat/cereals
Responsible body for reporting (name, address) BASF SE, 67117 Limburgerhof
Country (trial) Germany
Content of active substance (g/kg or g/L): 80 g/L
Formulation (e.g. WP) EC (BAS 556 03 F)

Commercial product:
Producer of commercial product: BASF SE, Ludwigshafen, Germany
Indoor/Glasshous/Outdoor Outdoor
Other active substance in the formulation (common name and content) Pyraclostrobin (BAS 500 F, 130 g/L)
Residue calculated as: Metconazole (BAS 555 F)

1	2	3	4	5			6	7	8	9			10	11
Report No Location (Trial No)	**Commodity/ Variety**	**Date of 1. Sowing / Planting 2. Flowering 3. Harvest**	**Method of treatment**	**Application rate[0] per treatment**			**No of treatment(s) and last date**	**Growth stage (BBCH)[2]**	**Portion analyzed**	**Residues (mg/kg)**			**DALA[1]**	**Remarks**
				kg a.s./hL	**Water L/ha**	**kg a.s./ha**				**Cis**	**Trans**	**Sum**		
401831 2012/1194991 21614, Buxtehude Germany (L110188)	Wheat GC 0654 (Julius)	1. 24.09.2010 2. 27.05.-07.06.2011 3. 03.08.2011	Foliar application with boom sprayer	0.044	200	0.088	2 07.06.2011	69	Plant w/o roots Ears Rest of plant w/o root Ears Rest of plant w/o root Ears Rest of plant w/o root Grain Straw	1 9 0.095 0.42 0.13 0.53 0.13 0.44 <0.005 0.56	0.38 0.024 0.11 0.032 0.14 0.035 0.12 <0.005 0.16	2.3 0.19 0.53 0.16 0.67 0.17 0.56 <0.01 1.9	0 29 29 36 36 43 43 57 57	BASF method No 535/1 (L0076/01) LOQ 0.005 mg/kg per isomer
401831 2012/1194991 HU17 9SL, Beverley United Kingdom (L110189)	Wheat GC 0654 (Oakley)	1. 05.09.2010 2. n.r. 3. 16.08.2011	Foliar application with boom sprayer	0.044	200	0.088	2 20.06.2011	69	Plant w/o roots Ears Rest of plant w/o root Grain Straw Grain Straw Grain Straw	0.91 0.11 0.41 0.006 <0.005 <0.005 0.91 <0.005 0.48	0.20 0.031 0.13 <0.005 <0.005 <0.005 0.28 <0.005 0.15	1.1 0.14 0.54 0.011 <0.01 <0.01 1.2 <0.01 0.63	0 29 29 36 36 42 42 57 57	

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
n.r. Not reported

Treatment with BAS 555 00 F on wheat in Southern Europe

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name): Metconazole (BAS 555 F)
Crop /crop group: Wheat/cereals
Responsible body for reporting (name, address) BASF SE, 67117 Limburgerhof
Country (trial) Germany
Content of active substance (g/kg or g/L): 60 g/L
Formulation (e.g. WP) EC (BAS 555 00 F)

Commercial product:
Producer of commercial product: BASF SE, Ludwigshafen, Germany
Indoor/Glasshouse/Outdoor Outdoor
Other active substance in the formulation (common name and content)
Residue calculated as: Metconazole (BAS 555 F)

1	2	3	4	5			6	7	8	9			10	11
Report No Location (Trial No)	**Commodity/ Variety**	**Date of 1. Sowing / Planting 2. Flowering 3. Harvest**	**Method of treatment**	**Application rate[0] per treatment**			**No of treatment(s) and last date**	**Growth stage (BBCH)[2]**	**Portion analyzed**	**Residues (mg/kg)**			**DALA[1]**	**Remarks**
				kg a.s./hL	**Water L/ha**	**kg a.s./ha**				***Cis***	***Trans***	**Sum**		
358573 2010/1075867 48010, Ravenna Italy (L090077)	Wheat GC 0654 (Bologna)	1. 15.11.2008 2. 01.05.-11.05.2009 3. 22.06.2009	Foliar application with boom sprayer	0.045	200	0.090	2 11.05.2009	69	Plant w/o roots Ears Rest of plant Ears Rest of plant Grain Straw	n.r. n.r. n.r. n.r. n.r. n.r. n.r.	n.r. n.r. n.r. n.r. n.r. n.r. n.r.	2.4 0.14 1.1 0.16 1.2 <0.01 1.2	0 28 28 35 35 42 42	BASF method No 535/1 (L0076/01) LOQ 0.01 mg/kg
358573 2010/1075867 58300, Pella Greece (L090078)	Wheat GC 0654 (Panifor)	1. 25.11.2008 2. 10.04.-28.04.2009 3. 10.06.-20.06.2009	Foliar application with boom sprayer	0.045	200	0.090	2 29.04.2009	69	Plant w/o roots Ears Rest of plant Ears Rest of plant Ears Rest of plant Grain Straw	n.r. n.r. n.r. n.r. n.r. n.r. n.r. n.r. n.r.	n.r. n.r. n.r. n.r. n.r. n.r. n.r. n.r. n.r.	2.0 0.2 0.61 0.22 0.58 0.37 0.73 <0.01 1.4	0 29 29 35 35 42 42 50 50	
358573 2010/1075867 41727, Sevilla Spain (L090079)	Wheat GC 0654 (Califa Sur)	1. 24.12.2008 2. 08.04.-24.04.2009 3. 05.06.2009	Foliar application with boom sprayer	0.045	200	0.090	2 22.04.2009	69	Plant w/o roots Ears Rest of plant Grain Straw Grain Straw	n.r. n.r. n.r. n.r. n.r. n.r. n.r.	n.r. n.r. n.r. n.r. n.r. n.r. n.r.	4.9 0.76 3.1 <0.01 3.5 <0.01 4.4	0 28 28 35 35 41 41	
358573 2010/1075867 71240, Charente-Maritime France (L090082)	Wheat GC 0654 (Premio)	1. 28.11.2008 2. 25.05.-30.05.2009 3. 15.07.-11.07.2009	Foliar application with boom sprayer	0.045	200	0.090	2 02.06.2009	71	Plant w/o roots Ears Rest of plant Ears Rest of plant Grain Straw	n.r. n.r. n.r. n.r. n.r. n.r. n.r.	n.r. n.r. n.r. n.r. n.r. n.r. n.r.	2.8 0.51 1.8 0.43 2.6 <0.01 2.1	0 28 28 34 34 41 41	

1	2	3	4	5			6	7	8	9			10	11
Report No Location (Trial No)	**Commodity/ Variety**	**Date of 1. Sowing / Planting 2. Flowering 3. Harvest**	**Method of treatment**	**Application rate[0] per treatment**			**No of treatment(s) and last date**	**Growth stage (BBCH)[2]**	**Portion analyzed**	**Residues (mg/kg)**			**DALA[1]**	**Remarks**
				kg a.s./hL	**Water L/ha**	**kg a.s./ha**				***Cis***	***Trans***	**Sum**		
255298 2007/1050102 31330, Grenade France (L070186) (AC/07/042)	Wheat GC 0654 (Kalango)	1. 20.11.2006 2. 05.05.-10.05.2007 3. 22.06.-23.06.2007	Foliar application with boom sprayer	0.045	200	0.090	2 24.05.2007	85	Plant w/o roots Grain Straw Grain Straw Grain Straw	1.40 <0.005 0.204 <0.005 0.275 <0.005 0.265	0.208 <0.005 0.052 <0.005 0.065 <0.005 0.064	1.60 <0.01 0.26 <0.01 0.34 <0.01 0.33	0 34 34 42 42 49 49	BASF method No 550/0 (L0019/01) LOQ 0.005 mg/kg per isomer
255298 2007/1050102 47120, Duras Aquitaine France (L070187) (AC/07/043)	Wheat GC 0654 (Andalou)	1. 20.10.2006 2. 30.04.-07.05.2007 3. 28.06.-15.07.2007	Foliar application with boom sprayer	0.045	200	0.090	2 25.05.2007	75	Plant w/o roots Grain Straw Grain Straw Grain Straw	1.21 0.006 0.270 <0.005 0.375 0.005 0.300	0.188 <0.005 0.067 <0.005 0.089 <0.005 0.065	1.39 0.01 0.34 <0.01 0.46 0.01 0.37	0 35 35 43 43 49 49	
MK-FR-00-731 MK-730-049 32370, Espas France (south) (00-731-283)	Wheat GC 0654 (Aztec)	1. 05.11.1999 2. 05.05.-15.05.2000 3. Early July 2000	Foliar application with boom	0.030	300	0.090	2 12.05.2000	64-65	Whole plant Whole plant Plant without ears Ears Grain Straw Grain Straw	1.23 0.48 0.42 0.08 0.02 0.24 <0.01 0.30	0.27 0.09 0.10 0.02 <0.01 0.06 <0.01 0.08	1.50 0.57 0.52 0.10 0.03 0.30 <0.02 0.38	0 14 26 26 34 34 45 45	Method FAMS 050-01 LOQ 0.01 mg/kg per isomer
MK-FR-00-731 MK-730-049 84260, Sarrians France (south) (00-731-639)	Wheat GC 0654 (Manital)	1. 07.12.1999 2. 05.05.-12.05.2000 3. Early July 2000	Foliar application	0.023	400	0.090	2 22.05.2000	75-77	Whole plant Whole plant Plant without ears Ears Grain Straw Grain Straw	1.48 0.32 0.15 0.05 <0.01 0.13 <0.01 0.31	0.16 0.06 0.04 0.01 <0.01 0.03 <0.01 0.08	1.64 0.38 0.19 0.06 <0.02 0.16 <0.02 0.39	0 14 28 28 35 35 42 42	
401831 2012/1194991 82290, Lacourt- Saint-Pierre France (L110190)	Wheat GC 0654 (PR22 R58)	1. 02.11.2010 2. n.r. 3. 15.06.2011	Foliar application with boom sprayer	0.045	200	0.090	2 04.05.2011	69	Plant w/o roots Ears Rest of plant w/o root Ears Rest of plant w/o root Grain Straw	2.0 0.22 3.0 0.2 2.1 <0.005 2.4	0.36 0.054 0.63 0.051 0.47 <0.005 0.55	2.4 0.28 3.6 0.25 2.6 <0.01 2.9	0 28 28 35 35 42 42	BASF method No 535/1 (L0076/01) LOQ 0.005 mg/kg per isomer

1	2	3	4	5			6	7	8	9			10	11
Report No Location (Trial No)	**Commodity/ Variety**	**Date of 1. Sowing / Planting 2. Flowering 3. Harvest**	**Method of treatment**	**Application rate[0] per treatment**			**No of treatment(s) and last date**	**Growth stage (BBCH)[2]**	**Portion analyzed**	**Residues (mg/kg)**			**DALA[1]**	**Remarks**
				kg a.s./hL	**Water L/ha**	**kg a.s./ha**				***Cis***	***Trans***	**Sum**		
401831 2012/1194991 40057, Quarto Inferiore Italy (L110191)	Wheat GC 0654 (Bologna)	1. 20.10.2010 2. n.r. 3. 21.06.2011	Foliar application with boom sprayer	0.045	200	0.090	2 13.05.2011	69	Plant w/o roots Ears Rest of plant w/o root Ears Rest of plant w/o root Grain Straw	2.9 0.14 1.5 0.27 1.4 <0.005 2.0	0.52 0.034 0.32 0.070 0.32 <0.005 0.52	3.4 0.17 1.8 0.34 1.7 <0.01 2.56	0 27 27 34 34 39 39	

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
n.r. Not reported

Treatment with BAS 555 01 F on wheat in Southern Europe

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name): Metconazole (BAS 555 F)
Crop /crop group: Wheat/cereals
Responsible body for reporting (name, address) BASF SE, 67117 Limburgerhof
Country (trial) Germany
Content of active substance (g/kg or g/L): 90 g/L
Formulation (e.g. WP) SL (BAS 555 01 F)

Commercial product:
Producer of commercial product: BASF SE, Ludwigshafen, Germany
Indoor/Glasshouse/Outdoor Outdoor
Other active substance in the formulation (common name and content)
Residue calculated as: Metconazole (BAS 555 F)

1	2	3	4	5			6	7	8	9			10	11
Report No Location (Trial No)	**Commodity/ Variety**	**Date of 1. Sowing / Planting 2. Flowering 3. Harvest**	**Method of treatment**	**Application rate[0] per treatment**			**No of treatment(s) and last date**	**Growth stage (BBCH)[2]**	**Portion analyzed**	**Residues (mg/kg)**			**DALA[1]**	**Remarks**
				kg a.s./hL	**Water L/ha**	**kg a.s./ha**				**Cis**	**Trans**	**Sum**		
MK-FR-00-731 MK-730-049 32370, Espas France (south) (00-731-283)	Wheat GC 0654 (Aztec)	1. 05.11.1999 2. 05.05.-15.05.2000 3. Early July 2000	Foliar application with boom	0.030	300	0.090	2 12.05.2000	64-65	Whole plant Whole plant Plant without ears Ears Grain Straw Grain Straw	1.12 0.50 0.36 0.09 0.02 0.17 <0.01 0.22	0.25 0.11 0.09 0.02 <0.01 0.05 <0.01 0.07	1.37 0.61 0.45 0.11 0.03 0.22 <0.02 0.29	0 14 26 26 34 34 45 45	Method FAMS 050-01 LOQ 0.01 mg/kg per isomer
MK-FR-00-731 MK-730-049 84260, Sarrians France (south) (00-731-639)	Wheat GC 0654 (Manital)	1. 07.12.1999 2. 05.05.-12.05.2000 3. Early July 2000	Foliar application	0.023	400	0.090	2 22.05.2000	75-77	Whole plant Whole plant Plant without ears Ears Grain Straw Grain Straw	1.44 0.24 0.19 0.05 <0.01 0.23 <0.01 0.30	0.24 0.06 0.05 0.01 <0.01 0.06 <0.01 0.07	1.68 0.30 0.24 0.06 <0.02 0.29 <0.02 0.37	0 14 28 28 35 35 42 42	

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
n.r. Not reported

Treatment with BAS 627 00 F on wheat in Southern Europe

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name): Metconazole (BAS 555 F)
Crop /crop group: Wheat/cereals
Responsible body for reporting (name, address) BASF SE, 67117 Limburgerhof
Country (trial) Germany
Content of active substance (g/kg or g/L): 27.5 g/L
Formulation (e.g. WP) SL (BAS 627 00 F)

Commercial product:
Producer of commercial product: BASF SE, Ludwigshafen, Germany
Indoor/Glasshouse/Outdoor Outdoor
Other active substance in the formulation (common name and content) Epoxiconazole (BAS 480 F, 37.5 g/L)
Residue calculated as: Metconazole (BAS 555 F)

1	2	3	4	5			6	7	8	9			10	11
Report No Location (Trial No)	**Commodity/ Variety**	**Date of 1. Sowing / Planting 2. Flowering 3. Harvest**	**Method of treatment**	**Application rate[0] per treatment**			**No of treatment(s) and last date**	**Growth stage (BBCH)[2]**	**Portion analyzed**	**Residues (mg/kg)**			**DALA[1]**	**Remarks**
				kg a.s./hL	**Water L/ha**	**kg a.s./ha**				***Cis***	***Trans***	**Sum**		
358573 2010/1075867 48010, Ravenna Italy (L090077)	Wheat GC 0654 (Bologna)	1. 15.11.2008 2. 01.05.-11.05.2009 3. 22.06.2009	Foliar application with boom sprayer	0.04125	200	0.0825	2 11.05.2009	69	Plant w/o roots Ears Rest of plant Ears Rest of plant Grain Straw	n.r. n.r. n.r. n.r. n.r. n.r. n.r.	n.r. n.r. n.r. n.r. n.r. n.r. n.r.	1.9 0.19 1.2 0.23 1.2 <0.01 1.2	0 28 28 35 35 42 42	BASF Method No 535/1 (L0076/01) LOQ 0.01 mg/kg
358573 2010/1075867 58300, Pella Greece (L090078)	Wheat GC 0654 (Panifor)	1. 25.11.2008 2. 10.04.-28.04.2009 3. 10.06.-20.06.2009	Foliar application with boom sprayer	0.04125	200	0.0825	2 29.04.2009	69	Plant w/o roots Ears Rest of plant Ears Rest of plant Ears Rest of plant Grain Straw	n.r. n.r. n.r. n.r. n.r. n.r. n.r. n.r. n.r.	n.r. n.r. n.r. n.r. n.r. n.r. n.r. n.r. n.r.	1.7 0.23 0.98 0.32 0.89 0.45 0.93 <0.01 1.4	0 29 29 35 35 42 42 50 50	
358573 2010/1075867 41727, Sevilla Spain (L090079)	Wheat GC 0654 (Califa Sur)	1. 24.12.2008 2. 08.04.-24.04.2009 3. 05.06.2009	Foliar application with boom sprayer	0.04125	200	0.0825	2 22.04.2009	69	Plant w/o roots Ears Rest of plant Grain Straw Grain Straw	n.r. n.r. n.r. n.r. n.r. n.r. n.r.	n.r. n.r. n.r. n.r. n.r. n.r. n.r.	4.5 1.8 4 <0.01 6.6 <0.01 8.4	0 28 28 35 35 41 41	
358573 2010/1075867 71240, Charente-Maritime France (L090082)	Wheat GC 0654 (Premio)	1. 28.11.2008 2. 25.05.-30.05.2009 3. 15.07.-11.07.2009	Foliar application with boom sprayer	0.04125	200	0.0825	2 02.06.2009	71	Plant w/o roots Ears Rest of plant Ears Rest of plant Grain Straw	n.r. n.r. n.r. n.r. n.r. n.r. n.r.	n.r. n.r. n.r. n.r. n.r. n.r. n.r.	2.8 0.68 2.5 0.74 4.2 <0.01 3.0	0 28 28 34 34 41 41	

1	2	3	4	5			6	7	8	9			10	11
Report No Location (Trial No)	**Commodity/ Variety**	**Date of 1. Sowing / Planting 2. Flowering 3. Harvest**	**Method of treatment**	**Application rate[0] per treatment**			**No of treatment(s) and last date**	**Growth stage (BBCH)[2]**	**Portion analyzed**	**Residues (mg/kg)**			**DALA[1]**	**Remarks**
				kg a.s./hL	**Water L/ha**	**kg a.s./ha**				***Cis***	***Trans***	**Sum**		
255298 2007/1050102 31330, Grenade France (L070186) (AC/07/042)	Wheat GC 0654 (Kalango)	1. 20.11.2006 2. 05.05.-10.05.2007 3. 22.06.-23.06.2007	Foliar application with boom sprayer	0.04125	200	0.0825	2 24.05.2007	85	Plant w/o roots Grain Straw Grain Straw Grain Straw	1.46 <0.005 0.285 <0.005 0.225 <0.005 0.198	0.267 <0.005 0.079 <0.005 0.065 <0.005 0.054	1.73 <0.01 0.36 <0.01 0.29 <0.01 0.25	0 34 34 42 42 49 49	BASF method No 550/0 (L0019/01) LOQ 0.005 mg/kg per isomer
255298 2007/1050102 47120, Duras Aquitaine France (L070187) (AC/07/043)	Wheat GC 0654 (Andalou)	1. 20.10.2006 2. 30.04.-07.05.2007 3. 28.06.-15.07.2007	Foliar application with boom sprayer	0.04125	200	0.0825	2 25.05.2007	75	Plant w/o roots Grain Straw Grain Straw Grain Straw	1.33 0.006 0.455 0.005 0.430 <0.005 0.278	0.263 <0.005 0.100 <0.005 0.093 <0.005 0.065	1.59 0.01 0.55 0.01 0.52 <0.01 0.34	0 35 35 43 43 49 49	
339326 2010/1144333 47140 Trentels Lot-Et-Garonne France (L100107)	GC 0654/ Wheat Apache	1 20.10.2009 2 20.04.- 28.04.2010 3 16.07.2010	Foliar application with boom	0.0563	200	0.1125	2 28.05.2010	69	Plant w/o roots Ears Rest of plant Grain Straw Grain Straw	2.0 0.25 0.49 0.005 0.75 0.005 1.1	0.44 0.067 0.15 <0.005 0.23 <0.005 0.32	2.5 0.31 0.64 0.010 0.98 0.010 1.4	0 35 35 42 42 49 49	BASF method No 550/1 (L0019/01) LOQ 0.005 mg/kg per isomer
339326 2010/1144333 41710 Utrera Sevilla Spain (L100108)	GC 0654/ Wheat Prospero	1 13.12.2009 2 15.04.- 25.04.2010 3 08.06.2010	Foliar application with boom	0.0563	200	0.1125	2 21.04.2010	69	Plant w/o roots Grain Straw Grain Straw Grain Straw	3.7 <0.005 2.4 <0.005 2.1 <0.005 1.8	0.79 <0.005 0.51 <0.005 0.46 <0.005 0.39	4.5 <0.01 2.9 <0.01 2.6 <0.01 2.2	0 35 35 42 42 48 48	

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
n.r. Not reported

Treatment with BAS 627 02 F on wheat in Southern Europe

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name): Metconazole (BAS 555 F)
Crop /crop group: Wheat/cereals
Responsible body for reporting (name, address) BASF SE, 67117 Limburgerhof
Country (trial) Germany

Content of active substance (g/kg or g/L): 41.25 g/L
Formulation (e.g. WP) EC (BAS 627 02 F)

Commercial product:
Producer of commercial product: BASF SE, Ludwigshafen, Germany
Indoor/Glasshouse/Outdoor Outdoor
Other active substance in the formulation (common name and content) Epoxiconazole (BAS 480 F, 56.25 g/L)

Residue calculated as: Metconazole (BAS 555 F)

1	2	3	4	5			6	7	8	9			10	11
Report No Location (Trial No)	**Commodity/ Variety**	**Date of 1. Sowing / Planting 2. Flowering 3. Harvest**	**Method of treatment**	**Application rate[0] per treatment**			**No of treatment(s) and last date**	**Growth stage (BBCH)[2]**	**Portion analyzed**	**Residues (mg/kg)**			**DALA[1]**	**Remarks**
				kg a.s./hL	**Water L/ha**	**kg a.s./ha**				***Cis***	***Trans***	**Sum**		
339326 2010/1144333 47140 Trentels Lot-Et-Garonne France (L100107)	GC 0654/ Wheat Apache	1 20.10 2009 2 20.04.- 28.04 2010 3 16.07 2010	Foliar application with boom	0.0563	200	0.1125	2 28.05.2010	69	Plant w/o roots Ears Rest of plant Grain Straw Grain Straw	2.3 0.37 0.52 <0.005 0.47 0.007 0.77	0.52 0.10 0.15 <0.005 0.15 <0.005 0.24	2.8 0.47 0.67 <0.010 0.62 0.012 1.0	0 35 35 42 42 49 49	BASF method No 550/1 (L0019/01) LOQ 0.005 mg/kg per isomer
339326 2010/1144333 41710 Utrera Sevilla Spain (L100108)	GC 0654/ Wheat Prospero	1 13.12 2009 2 15.04.- 25.04 2010 3 08.06 2010	Foliar application with boom	0.0563	200	0.1125	2 21.04.2010	69	Plant w/o roots Grain Straw Grain Straw Grain Straw	4.2 0.005 3.1 <0.005 3.3 <0.005 2.5	0.89 <0.005 0.68 <0.005 0.71 <0.005 0.54	5.1 0.010 3.8 <0.010 4.0 <0.010 3.0	0 35 35 42 42 48 48	

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
n.r. Not reported

Treatment with BAS 556 03 F on wheat in Southern Europe

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name): Metconazole (BAS 555 F)
Crop /crop group: Wheat/cereals
Responsible body for reporting (name, address) BASF SE, 67117 Limburgerhof
Country (trial) Germany
Content of active substance (g/kg or g/L): 80 g/L
Formulation (e.g. WP) EC (BAS 556 03 F)

Commercial product:
Producer of commercial product: BASF SE, Ludwigshafen, Germany
Indoor/Glasshous/Outdoor Outdoor
Other active substance in the formulation (common name and content) Pyraclostrobin (BAS 500 F, 130 g/L)
Residue calculated as: Metconazole (BAS 555 F)

1	2	3	4	5			6	7	8	9			10	11
Report No Location (Trial No)	**Commodity/ Variety**	**Date of 1. Sowing / Planting 2. Flowering 3. Harvest**	**Method of treatment**	**Application rate[0] per treatment**			**No of treatment(s) and last date**	**Growth stage (BBCH)[2]**	**Portion analyzed**	**Residues (mg/kg)**			**DALA[1]**	**Remarks**
				kg a.s./hL	**Water L/ha**	**kg a.s./ha**				**Cis**	**Trans**	**Sum**		
401831 2012/1194991 82290, Lacourt-Saint-Pierre France (L110190)	Wheat GC 0654 (PR22 R58)	1. 02.11.2010 2. n.r. 3. 15.06.2011	Foliar application with boom sprayer	0.044	200	0.088	2 04.05.2011	69	Plant w/o roots Ears Rest of plant w/o root Ears Rest of plant w/o root Grain Straw	1 9 0.14 2.7 0.19 1.4 <0.005 0.72	0.37 0.035 0.57 0.048 0.33 <0.005 0.19	2.2 0.18 3.2 0.24 1.8 <0.01 0.91	0 28 28 35 35 42 42	BASF method No 535/1 (L0076/01) LOQ 0.005 mg/kg per isomer
401831 2012/1194991 40057, Quarto Inferiore Italy (L110191)	Wheat GC 0654 (Bologna)	1. 20.10.2010 2. n.r. 3. 21.06.2011	Foliar application with boom sprayer	0.044	200	0.088	2 13.05.2011	69	Plant w/o roots Ears Rest of plant w/o root Ears Rest of plant w/o root Grain Straw	2 5 0.077 0.81 0.048 0.79 <0.005 0.62	0.47 0.019 0.20 0.012 0.21 <0.005 0.19	2.9 0.10 1.0 0.06 1.0 <0.01 0.81	0 27 27 34 34 39 39	

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
n.r. Not reported

Barley

Treatment with BAS 555 01 F on barley in Southern Europe

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Metconazole (BAS 555 F)	**Commercial Product (name)**	--
Crop/crop group:	Barley/Cereals	**Producer of commercial product**	BASF SE, Ludwigshafen, Germany
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	**Indoor/Glasshouse/Outdoor**	outdoor
Country	Germany	**Other active substance in the formulation**	NA
Content of active substance (g/kg or g/L)	90 g/L	**(common name and content)**	
Formulation (e.g. WP)	SL (code BAS 555 01 F)	**Residues calculated as:**	Metconazole (BAS 555 F)

1	2	3	4	5			6	7	8	9			10	11
Report-No. Location (trial no.)	**Commodity/ Variety**	**1. 2. 3. Date of Sowing / planting Flowering Harvest**	**Method of treatment**	**Application rate per treatment**			**No of treat-ment(s) and last date**	**Growth stage at last treat. BBCH**	**Portion analyzed**	**Residues (mg/kg)**			**DALA (days)**	**Remarks**
				kg a.s./hL	**Water L/ha**	**kg a.s./ha**				***Cis***	***Trans***	**Sum**		
2008/1009268 E-41730 Las Cabezas Sevilla, Spain (L070190)	GC 0640 Belen	1. 12.01.07 2. 24.-30.04.07 3. 12.06.07	Foliar appl. with boom	0.06	150	0.09	2 24.04.07	69	whole plant [3)] ears rest of plant [3)] grain straw grain straw	1.740 0.034 0.258 0.012 0.329 0.013 0.279	0.329 0.005 0.050 0.003 0.060 0.004 0.048	2.07 0.04 0.31 <u>0.02</u> <u>0.39</u> 0.02 0.33		BASF method 550/0
2008/1009268 E-11500 Puerto de Sta Maria,Cadiz, Spain (L070191)	GC 0640 Cecilia	1. 18.01.07 2. 19.-27.04.07 3. 11.06.07	Foliar appl. with boom	0.06	150	0.09	2 23.04.07	69	whole plant [3)] ears rest of plant [3)] grain straw grain straw	1.670 0.029 0.160 0.009 0.217 0.011 0.290	0.295 0.007 0.034 0.002 0.037 0.003 0.053	1.97 0.04 0.19 0.01 0.25 <u>0.01</u> <u>0.34</u>		BASF method 550/0
2008/1009268 I-21010 Cardano di Campo, Varese, Italy (L070192)	GC 0640 Aliseo	1. 20.10.06 2. 03.-10.05.07 3. 27.06.07	Foliar appl. with boom	0.06	150	0.09	2 09.05.07	69	whole plant [3)] grain straw grain straw grain straw	0.747 0.04 0.145 0.030 0.118 0.034 0.103	0.135 0.012 0.039 0.010 0.032 0.011 0.028	0.88 <u>0.05</u> <u>0.18</u> 0.04 0.15 0.05 0.13		BASF method 550/0
2008/1009268 I-20020 Lazzaate Milano, Italy (L070193)	GC 0640 Trasimeno	1. 30.10.06 2. 02.-07.05.07 3. 22.06.07	Foliar appl. with boom	0.06	150	0.09	2 07.05.07	69	whole plant [3)] grain straw grain straw grain straw	1.440 0.015 0.037 0.016 0.064 0.015 0.050	0.283 0.004 0.010 0.004 0.018 0.004 0.013	1.72 0.02 0.05 <u>0.02</u> <u>0.08</u> 0.02 0.06		BASF method 550/0

Treatment with BAS 555 00 F on barley in Northern Europe

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name): Metconazole (BAS 555 F)
Crop /crop group: Barley/cereals
Responsible body for reporting (name, address) BASF SE, 67117 Limburgerhof
Country (trial) Germany
Content of active substance (g/kg or g/L): 60 g/L
Formulation (e.g. WP) EC (BAS 555 00 F)

Commercial product:
Producer of commercial product: BASF SE, Ludwigshafen, Germany
Indoor/Glasshouse/Outdoor Outdoor
Other active substance in the formulation (common name and content)
Residue calculated as: Metconazole (BAS 555 F)

1	2	3	4	5			6	7	8	9			10	11
Report No Location (Trial No)	**Commodity/ Variety**	**Date of 1. Sowing / Planting 2. Flowering 3. Harvest**	**Method of treatment**	**Application rate[0] per treatment**			**No of treatment(s) and last date**	**Growth stage (BBCH)[2]**	**Portion analyzed**	**Residues (mg/kg)**			**DALA[1]**	**Remarks**
				kg a.s./hL	**Water L/ha**	**kg a.s./ha**				***Cis***	***Trans***	**Sum**		
366605 2010/1110643 47625, Kleve Germany (L090063)	Barley GC 0640 (Campanele)	1. 28.09 2008 2. 14.05.-28.05.2009 3. 09.07 2009	Foliar application with boom sprayer	0.045	200	0.090	2 14.05 2009	69	Plant w/o roots Grain Straw Grain Straw Grain Straw	n.r. n.r. n.r. n.r. n.r. n.r. n.r.	n.r. n.r. n.r. n.r. n.r. n.r. n.r.	2.9 0.077 0.56 0.075 0.43 0.056 0.6	0 43 43 49 49 56 56	BASF method No 535/1 (L0076/01) LOQ 0.01 mg/kg
366605 2010/1110643 6595, Gennep The Netherlands (L090064)	Barley GC 0640 (Sequel)	1. 06.10 2008 2. 02.05.-16.05.2009 3. 10.07 2009	Foliar application with boom sprayer	0.045	200	0.090	2 15.05 2009	69	Plant w/o roots Grain Straw Grain Straw Grain Straw	n.r. n.r. n.r. n.r. n.r. n.r. n.r.	n.r. n.r. n.r. n.r. n.r. n.r. n.r.	2.3 0.021 0.65 0.017 0.59 0.013 0.25	0 42 42 48 48 56 56	
366605 2010/1110643 CV35 9EJ, Warwick United Kingdom (L090065)	Barley GC 0640 (Suzuka)	1. 23.10 2008 2. 22.05.-03.06.2009 3. 13.07 2009	Foliar application with boom sprayer	0.045	200	0.090	2 03.06 2009	69	Plant w/o roots Grain Straw Grain Straw Grain Straw	n.r. n.r. n.r. n.r. n.r. n.r. n.r.	n.r. n.r. n.r. n.r. n.r. n.r. n.r.	2.0 0.019 0.18 0.014 0.20 <0.01 0.15	0 29 29 34 34 41 41	
366605 2010/1110643 4930, Lolland Denmark (L090066)	Barley GC 0640 (Suzuka)	1. 05.04 2009 2. 26.06.-09.07.2009 3. 10.07.-11.08.2009	Foliar application with boom sprayer	0.045	200	0.090	2 08.07 2009	69	Plant w/o roots Ears Rest of plant Grain Straw Grain Straw	n.r. n.r. n.r. n.r. n.r. n.r. n.r.	n.r. n.r. n.r. n.r. n.r. n.r. n.r.	2.1 0.24 0.86 0.031 0.76 0.032 0.74	0 28 28 34 34 41 41	

1	2	3	4	5			6	7	8	9			10	11
Report No Location (Trial No)	**Commodity/ Variety**	**Date of 1. Sowing / Planting 2. Flowering 3. Harvest**	**Method of treatment**	**Application rate[0] per treatment**			**No of treatment(s) and last date**	**Growth stage (BBCH)[2]**	**Portion analyzed**	**Residues (mg/kg)**			**DALA[1]**	**Remarks**
				kg a.s./hL	**Water L/ha**	**kg a.s./ha**				***Cis***	***Trans***	**Sum**		
366605 2010/1110643 08190, Marne France (L090070)	Barley GC 0640 (Prestige)	1. 03.10 2008 2. 10.04.-24.04.2009 3. 03.06 2009	Foliar application with boom sprayer	0.045	200	0.090	2 03.06 2009	71	Plant w/o roots Grain Straw	n.r. n.r. n.r.	n.r. n.r. n.r.	1.1 0.055 0.45	0 27 27	
366605 2010/1110643 47574, Kleve Germany (L090071)	Barley GC 0640 (Alinghi)	1. 23.09 2008 2. 14.05.-28.05.2009 3. 15.07 2009	Foliar application with boom sprayer	0.045	200	0.090	2 03.06 2009	77	Plant w/o roots Grain Straw Grain Straw Grain Straw	n.r. n.r. n.r. n.r. n.r. n.r. n.r.	n.r. n.r. n.r. n.r. n.r. n.r. n.r.	3.3 0.12 0.95 0.079 0.59 0.074 0.55	0 28 28 36 36 42 42	
255301 2007/1050101 67294 Mauchenheim Germany (L070178) (AC/07/046)	Barley GC 0640 (Braemar)	1. 12.03.2007 2. 06.06.-13.06.2007 3. 15.07.2007	Foliar application with boom sprayer	0.045	200	0.090	2 06.06 2007	61	Plant w/o roots Ears Rest of plant Grain Straw Grain Straw	1.72 0.021 0.148 0.009 0.535 0.008 0.373	0.256 0.006 0.048 <0.005 0.097 <0.005 0.074	1.98 0.03 0.20 0.01 0.63 0.01 0.45	0 35 35 42 42 48 48	BASF method No 550/0 (L0019/01) LOQ 0.005 mg/kg per isomer
255301 2007/1050101 16833 Brunne Germany (L070179) (AC/07/047)	Barley GC 0640 (Campanile)	1. 15.09.2006 2. 06.05.-13.05.2007 3. 11.07.2007	Foliar application with boom sprayer	0.045	200	0.090	2 24.05 2007	77-83	Plant w/o roots Grain Straw Grain Straw Grain Straw	1.30 0.041 0.061 0.045 0.067 0.043 0.061	0.170 0.010 0.013 0.010 0.014 0.010 0.013	1.47 0.05 0.07 0.06 0.08 0.05 0.07	0 34 34 41 41 48 48	
255301 2007/1050101 67160 Seebach France (L070182) (AC/07/050)	Barley GC 0640 (Platine)	1. 20.10.2006 2. 10.05.-17.05.2007 3. 06.07.-07.07.2007	Foliar application with boom sprayer	0.045	200	0.090	2 16.05 2007	69	Plant w/o roots Grain Straw Grain Straw Grain Straw	1.38 0.008 0.121 0.007 0.075 0.005 0.031	0.251 <0.005 0.031 <0.005 0.020 <0.005 0.008	1.63 0.01 0.15 0.01 0.09 0.01 0.04	0 34 34 41 41 50 50	

1	2	3	4	5			6	7	8	9			10	11
Report No Location (Trial No)	**Commodity/ Variety**	**Date of 1. Sowing / Planting 2. Flowering 3. Harvest**	**Method of treatment**	**Application rate[0] per treatment**			**No of treatment(s) and last date**	**Growth stage (BBCH)[2]**	**Portion analyzed**	**Residues (mg/kg)**			**DALA[1]**	**Remarks**
				kg a.s./hL	**Water L/ha**	**kg a.s./ha**				***Cis***	***Trans***	**Sum**		
255301 2007/1050101 36110 Levroux France (L070183) (AC/07/051)	Barley GC 0640 (Regalia)	1. 18.10.2006 2. 25.04.-30.04.2007 3. 19.06.2007	Foliar application with boom sprayer	0.045	200	0.090	2 30.04 2007	69	Plant w/o roots Ears Rest of plant Ears Rest of plant Grain Straw	1.72 0.011 0.245 0.027 0.251 0.011 0.167	0.278 < 0.005 0.073 0.006 0.079 < 0.005 0.035	1.99 0.02 0.32 0.03 0.33 0.02 0.20	0 35 35 42 42 50 50	
401831_1 2012/1194990 21717, Schwinge Germany (L110192)	Barley GC 0640 (Souleyka)	1. 22.09 2010 2. 19.05.-29.05.2011 3. 11.07 2011	Foliar application with boom sprayer	0.045	200	0.090	2 30.05.2011	69-73	Plant w/o roots Ears Rest of plant Ears Rest of plant Grain Straw	3.0 0.047 0.73 0.037 0.45 0.0095 0.88	0.57 0.013 0.18 0.010 0.12 <0.005 0.24	3.5 0.060 0.90 0.047 0.57 0.014 1.1	0 28 28 36 36 42 42	BASF method No 535/1 (L0076/01) LOQ 0.005 mg/kg per isomer
401831_1 2012/1194990 HU17 8TB, Bishop Burton United Kingdom (L110193)	Barley GC 0640 (Cavia)	1. 16.09 2010 2. n.r. 3. 03.08 2011	Foliar application with boom sprayer	0.045	200	0.090	2 10.06.2011	69	Plant w/o roots Ears Rest of plant Ears Rest of plant Grain Straw Grain Straw	1 3 0.023 0 16 0.048 0 17 0.0093 0 20 0.011 0 12	0.25 0.0059 0.041 0.012 0.043 <0.005 0.055 <0.005 0.033	1.5 0.029 0.20 0.061 0.21 0.014 0.26 0.016 0.16	0 28 28 34 34 41 41 54 54	

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
n.r. Not reported

Treatment with BAS 627 00 F on barley in Northern Europe

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name): Metconazole (BAS 555 F)
Crop /crop group: Barley/cereals
Responsible body for reporting (name, address) BASF SE, 67117 Limburgerhof
Country (trial) Germany

Commercial product:
Producer of commercial product: BASF SE, Ludwigshafen, Germany
Indoor/Glasshouse/Outdoor Outdoor
Other active substance in the formulation (common name and content) Epoxiconazole (BAS 480 F, 37.5 g/L)

Content of active substance (g/kg or g/L): 27.5 g/L
Formulation (e.g. WP) EC (BAS 627 00 F)

Residue calculated as: Metconazole (BAS 555 F)

1	2	3	4	5			6	7	8	9			10	11
Report No Location (Trial No)	**Commodity/ Variety**	**Date of 1. Sowing / Planting 2. Flowering 3. Harvest**	**Method of treatment**	**Application rate[0] per treatment**			**No of treatment(s) and last date**	**Growth stage (BBCH)[2]**	**Portion analyzed**	**Residues (mg/kg)**			**DALA[1]**	**Remarks**
				kg a.s./hL	**Water L/ha**	**kg a.s./ha**				***Cis***	***Trans***	**Sum**		
366605 2010/1110643 47625, Kleve Germany (L090063)	Barley GC 0640 (Campanele)	1. 28.09.2008 2. 14.05.-28.05.2009 3. 09.07.2009	Foliar application with boom sprayer	0.04125	200	0.0825	2 14.05.2009	69	Plant w/o roots Grain Straw Grain Straw Grain Straw	n.r. n.r. n.r. n.r. n.r. n.r. n.r.	n.r. n.r. n.r. n.r. n.r. n.r. n.r.	3.6 0.20 1.6 0.11 1.1 0.16 1.4	0 43 43 49 49 56 56	BASF Method No 535/1 (L0076/01) LOQ 0.01 mg/kg
366605 2010/1110643 6595, Gennep The Netherlands (L090064)	Barley GC 0640 (Sequel)	1. 06.10.2008 2. 02.05.-16.05.2009 3. 10.07.2009	Foliar application with boom sprayer	0.04125	200	0.0825	2 15.05.2009	69	Plant w/o roots Grain Straw Grain Straw Grain Straw	n.r. n.r. n.r. n.r. n.r. n.r. n.r.	n.r. n.r. n.r. n.r. n.r. n.r. n.r.	2.2 0.012 0.35 0.013 0.41 <0.01 0.16	0 42 42 48 48 56 56	
366605 2010/1110643 CV35 9EJ, Warwick United Kingdom (L090065)	Barley GC 0640 (Suzuka)	1. 23.10.2008 2. 22.05.-03.06.2009 3. 13.07.2009	Foliar application with boom sprayer	0.04125	200	0.0825	2 03.06.2009	69	Plant w/o roots Grain Straw Grain Straw Grain Straw	n.r. n.r. n.r. n.r. n.r. n.r. n.r.	n.r. n.r. n.r. n.r. n.r. n.r. n.r.	1.8 0.023 0.25 0.017 0.28 0.015 0.22	0 29 29 34 34 41 41	
366605 2010/1110643 4930, Lolland Denmark (L090066)	Barley GC 0640 (Suzuka)	1. 05.04.2009 2. 26.06.-09.07.2009 3. 10.07.-11.08.2009	Foliar application with boom sprayer	0.04125	200	0.0825	2 08.07.2009	69	Plant w/o roots Ears Rest of plant Grain Straw Grain Straw	n.r. n.r. n.r. n.r. n.r. n.r. n.r.	n.r. n.r. n.r. n.r. n.r. n.r. n.r.	2.4 0.55 1.2 0.058 1.3 0.056 1.2	0 28 28 34 34 41 41	

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name): Metconazole (BAS 555 F)
Crop /crop group: Barley/cereals
Responsible body for reporting (name, address) BASF SE, 67117 Limburgerhof
Country (trial) Germany

Content of active substance (g/kg or g/L): 27.5 g/L
Formulation (e.g. WP) EC (BAS 627 00 F)

Commercial product:
Producer of commercial product: BASF SE, Ludwigshafen, Germany
Indoor/Glasshouse/Outdoor Outdoor
Other active substance in the formulation (common name and content) Epoxiconazole (BAS 480 F, 37.5 g/L)

Residue calculated as: Metconazole (BAS 555 F)

1	2	3	4	5			6	7	8	9			10	11
Report No Location (Trial No)	**Commodity/ Variety**	**Date of 1. Sowing / Planting 2. Flowering 3. Harvest**	**Method of treatment**	**Application rate[0] per treatment**			**No of treatment(s) and last date**	**Growth stage (BBCH)[2]**	**Portion analyzed**	**Residues (mg/kg)**			**DALA[1]**	**Remarks**
				kg a.s./hL	**Water L/ha**	**kg a.s./ha**				***Cis***	***Trans***	**Sum**		
366605 2010/1110643 08190, Marne France (L090070)	Barley GC 0640 (Prestige)	1. 03.10.2008 2. 10.04.-24.04.2009 3. 03.06.2009	Foliar application with boom sprayer	0.04125	200	0.0825	2 03.06.2009	71	Plant w/o roots Grain Straw	n.r. n.r. n.r.	n.r. n.r. n.r.	1.4 0.065 0.24	0 27 27	
366605 2010/1110643 47574, Kleve Germany (L090071)	Barley GC 0640 (Alinghi)	1. 23.09.2008 2. 14.05.-28.05.2009 3. 15.07.2009	Foliar application with boom sprayer	0.04125	200	0.0825	2 03.06.2009	77	Plant w/o roots Grain Straw Grain Straw Grain Straw	n.r. n.r. n.r. n.r. n.r. n.r. n.r.	n.r. n.r. n.r. n.r. n.r. n.r. n.r.	2.3 0.091 0.59 0.087 0.34 0.064 0.26	0 28 28 36 36 42 42	
255301 2007/1050101 67294 Mauchenheim Germany (L070178) (AC/07/046)	Barley GC 0640 (Braemar)	1. 12.03.2007 2. 06.06.-13.06.2007 3. 15.07.2007	Foliar application with boom sprayer	0.04125	200	0.0825	2 06.06.2007	61	Plant w/o roots Ears Rest of plant Grain Straw Grain Straw	1.40 0.018 0.187 0.008 0.830 0.008 0.411	0.309 0.006 0.057 < 0.005 0.178 < 0.005 0.091	1.71 0.02 0.24 0.01 1.01 0.01 0.50	0 35 35 42 42 48 48	BASF method No 550/0 (L0019/01) LOQ 0.005 mg/kg per isomer
255301 2007/1050101 16833 Brunne Germany (L070179) (AC/07/047)	Barley GC 0640 (Campanile)	1. 15.09.2006 2. 06.05.-13.05.2007 3. 11.07.2007	Foliar application with boom sprayer	0.04125	200	0.0825	2 24.05.2007	77-83	Plant w/o roots Grain Straw Grain Straw Grain Straw	1.03 0.037 0.070 0.032 0.056 0.028 0.062	0.184 0.011 0.018 0.010 0.015 0.008 0.016	1.21 0.05 0.09 0.04 0.07 0.04 0.08	0 34 34 41 41 48 48	

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name): Metconazole (BAS 555 F)
Crop /crop group: Barley/cereals
Responsible body for reporting (name, address) BASF SE, 67117 Limburgerhof
Country (trial) Germany

Content of active substance (g/kg or g/L): 27.5 g/L
Formulation (e.g. WP) EC (BAS 627 00 F)

Commercial product:
Producer of commercial product: BASF SE, Ludwigshafen, Germany
Indoor/Glasshouse/Outdoor Outdoor
Other active substance in the formulation (common name and content) Epoxiconazole (BAS 480 F, 37.5 g/L)

Residue calculated as: Metconazole (BAS 555 F)

1	2	3	4	5			6	7	8	9			10	11
Report No Location (Trial No)	**Commodity/ Variety**	**Date of 1. Sowing / Planting 2. Flowering 3. Harvest**	**Method of treatment**	**Application rate[0] per treatment**			**No of treatment(s) and last date**	**Growth stage (BBCH)[2]**	**Portion analyzed**	**Residues (mg/kg)**			**DALA[1]**	**Remarks**
				kg a.s./hL	**Water L/ha**	**kg a.s./ha**				***Cis***	***Trans***	**Sum**		
255301 2007/1050101 67160 Seebach France (L070182) (AC/07/050)	Barley GC 0640 (Platine)	1. 20.10.2006 2. 10.05.-17.05.2007 3. 06.07.-07.07.2007	Foliar application with boom sprayer	0.04125	200	0.0825	2 16.05.2007	69	Plant w/o roots Grain Straw Grain Straw Grain Straw	1.14 0.009 0.263 0.007 0.159 0.007 0.054	0.237 <0.005 0.072 <0.005 0.048 <0.005 0.017	1.37 0.01 0.33 0.01 0.21 0.01 0.07	0 34 34 41 41 50 50	
255301 2007/1050101 36110 Levroux France (L070183) (AC/07/051)	Barley GC 0640 (Regalia)	1. 18.10.2006 2. 25.04.-30.04.2007 3. 19.06.2007	Foliar application with boom sprayer	0.04125	200	0.0825	2 30.04.2007	69	Plant w/o roots Ears Rest of plant Ears Rest of plant Grain Straw	1.55 0.021 0.129 0.010 0.264 0.016 0.216	0.303 0.005 0.032 <0.005 0.078 0.005 0.056	1.85 0.03 0.16 0.02 0.34 0.02 0.27	0 35 35 42 42 50 50	
339327 2010/1144334 67294 Mauchenheim Rheinhessen Germany (L100101)	GC 0640/ Barley Braemar	1 13.03.2010 2 07.06.- 15.06.2010 3 30.07.2010	Foliar application with boom	0.0413	200	0.0825	2 15.06.2010	69	Plant w/o roots Grain Straw Grain Straw Grain Straw	2.1 0.018 1.5 0.018 1.5 0.015 1.8	0.33 0.006 0.34 0.005 0.32 0.005 0.37	2.4 0.023 1.9 0.023 1.8 0.020 2.2	0 28 28 34 34 42 42	BASF method No 550/0 (L0019/01) LOQ 0.01 mg/kg
339327 2010/1144334 CV35 9EJ Charlecote Warwick UK (L100102)	GC 0640/ Barley Suzaka	1 19.10.2009 2 24.05.- 02.06.2010 3 14.07.2010	Foliar application with boom	0.0413	200	0.0825	2 02.06.2010	69	Plant w/o roots Grain Straw Grain Straw Grain Straw	1.7 0.056 1.0 0.033 0.70 0.068 1.3	0.28 0.017 0.22 0.010 0.16 0.019 0.29	1.9 0.073 1.2 0.044 0.86 0.087 1.6	0 27 27 35 35 41 41	

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
n.r. Not reported

Treatment with BAS 627 02 F on barley in Northern Europe

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name): Metconazole (BAS 555 F)
Crop /crop group: Barley/cereals
Responsible body for reporting (name, address) BASF SE, 67117 Limburgerhof
Country (trial) Germany

Content of active substance (g/kg or g/L): 41.25 g/L
Formulation (e.g. WP) EC (BAS 627 02 F)

Commercial product:
Producer of commercial product: BASF SE, Ludwigshafen, Germany
Indoor/Glasshouse/Outdoor Outdoor
Other active substance in the formulation (common name and content) Epoxiconazole (BAS 480 F, 56.25 g/L)

Residue calculated as: Metconazole (BAS 555 F)

1	2	3	4	5			6	7	8	9			10	11
Report No Location (Trial No)	**Commodity/ Variety**	**Date of 1. Sowing / Planting 2. Flowering 3. Harvest**	**Method of treatment**	**Application rate[0] per treatment**			**No of treatment(s) and last date**	**Growth stage (BBCH)[2]**	**Portion analyzed**	**Residues (mg/kg)**			**DALA[1]**	**Remarks**
				kg a.s./hL	**Water L/ha**	**kg a.s./ha**				***Cis***	***Trans***	**Sum**		
339327 2010/1144334 67294 Mauchenheim Rheinhessen Germany (L100101)	GC 0640/ Barley Braemar	1 13.03.2010 2 07.06.- 15.06.2010 3 30.07.2010	Foliar application with boom	0.0413	200	0.0825	2 15.06.2010	69	Plant w/o roots Grain Straw Grain Straw Grain Straw	2.8 0.011 2.0 0.014 1.9 0.016 2.6	0.47 <0.005 0.46 <0.005 0.41 0.005 0.52	3.3 0.016 2.5 0.019 2.3 0.021 3.1	0 28 28 34 34 42 42	BASF method No 550/0 (L0019/01) LOQ 0.01 mg/kg
339327 2010/1144334 CV35 9EJ Charlecote Warwick UK (L100102)	GC 0640/ Barley Suzaka	1 19.10.2009 2 24.05.- 02.06.2010 3 14.07.2010	Foliar application with boom	0.0413	200	0.0825	2 02.06.2010	69	Plant w/o roots Grain Straw Grain Straw Grain Straw	2.0 0.045 1.3 0.039 0.94 0.043 2.0	0.34 0.014 0.32 0.011 0.23 0.013 0.46	2.3 0.058 1.7 0.050 1.2 0.056 2.5	0 27 27 35 35 41 41	

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
n.r. Not reported

Treatment with BAS 556 03 F on barley in Northern Europe

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name): Metconazole (BAS 555 F)
Crop /crop group: Barley/cereals
Responsible body for reporting (name, address) BASF SE, 67117 Limburgerhof
Country (trial) Germany
Content of active substance (g/kg or g/L): 80 g/L
Formulation (e.g. WP) EC (BAS 556 03 F)

Commercial product:
Producer of commercial product: BASF SE, Ludwigshafen, Germany
Indoor/Glasshous/Outdoor Outdoor
Other active substance in the formulation (common name and content) Pyraclostrobin (BAS 500 F, 130 g/L)
Residue calculated as: Metconazole (BAS 555 F)

1	2	3	4	5			6	7	8	9			10	11
Report No Location (Trial No)	**Commodity/ Variety**	**Date of 1. Sowing / Planting 2. Flowering 3. Harvest**	**Method of treatment**	**Application rate[0] per treatment**			**No of treatment(s) and last date**	**Growth stage (BBCH)[2]**	**Portion analyzed**	**Residues (mg/kg)**			**DALA[1]**	**Remarks**
				kg a.s./hL	**Water L/ha**	**kg a.s./ha**				**Cis**	**Trans**	**Sum**		
401831_1 2012/1194990 21717, Schwinge Germany (L110192)	Barley GC 0640 (Souleyka)	1. 22.09.2010 2. 19.05.-29.05.2011 3. 11.07.2011	Foliar application with boom sprayer	0.044	200	0.088	2 30.05.2011	69-73	Plant w/o roots Ears Rest of plant Ears Rest of plant Grain Straw	2 9 0.069 0.71 0.051 0.39 0.012 0.83	0.57 0.018 0.18 0.013 0.11 <0.005 0.23	3.4 0.087 0.89 0.064 0.50 0.017 1.1	0 28 28 36 36 42 42	BASF method No 535/1 (L0076/01) LOQ 0.005 mg/kg per isomer
401831_1 2012/1194990 HU17 8TB, Bishop Burton United Kingdom (L110193)	Barley GC 0640 (Cavia)	1. 16.09.2010 2. n.r. 3. 03.08.2011	Foliar application with boom sprayer	0.044	200	0.088	2 10.06.2011	69	Plant w/o roots Ears Rest of plant Ears Rest of plant Grain Straw Grain Straw	1.0 0.016 0.091 0.015 0.093 0.0054 0.13 0.0059 0.092	0.22 <0.005 0.025 <0.005 0.026 <0.005 0.038 <0.005 0.027	1.3 0.021 0.12 0.020 0.12 0.010 0.17 0.011 0.12	0 28 28 34 34 41 41 54 54	

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
n.r. Not reported

Treatment with BAS 555 00 F on barley in Southern Europe

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name): Metconazole (BAS 555 F)
Crop /crop group: Barley/cereals
Responsible body for reporting (name, address) BASF SE, 67117 Limburgerhof
Country (trial) Germany, UK, France, Italy
Content of active substance (g/kg or g/L): 60 g/L
Formulation (e.g. WP) EC (BAS 555 00 F)
Commercial product: ---
Producer of commercial product: BASF SE, Ludwigshafen, Germany
Indoor/Glasshouse/Outdoor Outdoor
Other active substance in the formulation (common name and content) ---
Residue calculated as: Metconazole (BAS 555 F)

1	2	3	4	5			6	7	8	9			10	11
Report No Location (Trial No)	**Commodity/ Variety**	**Date of 1. Sowing / Planting 2. Flowering 3. Harvest**	**Method of treatment**	**Application rate[0] per treatment**			**No of treatment(s) and last date**	**Growth stage (BBCH)[2]**	**Portion analyzed**	**Residues (mg/kg)**			**DALA[1]**	**Remarks**
				kg a.s./hL	**Water L/ha**	**kg a.s./ha**				***Cis***	***Trans***	**Sum**		
366605 2010/1110643 59032, Imathia Greece (L090067)	Barley GC 0640 (Moutso)	1. 10.11.2008 2. 05.04.-20.04.2009 3. 05.06.-15.06.2009	Foliar application with boom sprayer	0.045	200	0.090	2 16.04.2009	69	Plant w/o roots Ears Rest of plant Ears Rest of plant Ears Rest of plant Grain Straw	n.r. n.r. n.r. n.r. n.r. n.r. n.r. n.r. n.r.	n.r. n.r. n.r. n.r. n.r. n.r. n.r. n.r. n.r.	2.7 0.085 0.19 0.065 0.14 0.075 0.23 0.011 0.12	0 29 29 36 36 41 41 50 50	BASF method No 535/1 (L0076/01) LOQ 0.01 mg/kg
366605 2010/1110643 40062, Bologna Italy (L090068)	Barley GC 0640 (Otis)	1. 19.02.2009 2. 09.05.-13.05.2009 3. 24.06.2009	Foliar application with boom sprayer	0.045	200	0.090	2 13.05.2009	69	Plant w/o roots Ears Rest of plant Grain Straw Grain Straw	n.r. n.r. n.r. n.r. n.r. n.r. n.r.	n.r. n.r. n.r. n.r. n.r. n.r. n.r.	2.3 0.29 2.6 0.041 1.2 0.024 1.2	0 28 28 35 35 42 42	

1	2	3	4	5			6	7	8	9			10	11
Report No Location (Trial No)	**Commodity/ Variety**	**Date of 1. Sowing / Planting 2. Flowering 3. Harvest**	**Method of treatment**	**Application rate[0] per treatment**			**No of treatment(s) and last date**	**Growth stage (BBCH)[2]**	**Portion analyzed**	**Residues (mg/kg)**			**DALA[1]**	**Remarks**
				kg a.s./hL	**Water L/ha**	**kg a.s./ha**				***Cis***	***Trans***	**Sum**		
366605 2010/1110643 41710, Sevilla Spain (L090069)	Barley GC 0640 (Cecilia)	1. 16.12.2008 2. 10.04.-24.04.2009 3. 03.06.2009	Foliar application with boom sprayer	0.045	200	0.090	2 22.04.2009	69	Plant w/o roots Ears Rest of plant Grain Straw Grain Straw	n.r. n.r. n.r. n.r. n.r. n.r. n.r.	n.r. n.r. n.r. n.r. n.r. n.r. n.r.	2.6 0.3 3.5 0.037 2.5 0.033 2.3	0 29 29 36 36 42 42	
366605 2010/1110643 32600, Gers France (south) (L090072)	Barley GC 0640 (Franzi)	1. 14.12.2008 2. 19.05.-23.05.2009 3. 01.07.-08.07.2009	Foliar application with boom sprayer	0.045	200	0.090	2 25.05.2009	71	Plant w/o roots Ears Rest of plant Grain Straw Grain Straw	n.r. n.r. n.r. n.r. n.r. n.r. n.r.	n.r. n.r. n.r. n.r. n.r. n.r. n.r.	2.6 0.43 1.7 0.035 2.0 0.027 1.6	0 28 28 34 34 43 43	
255301 2007/1050101 31620 Castelnau D'estretefonds France (L070180) (AC/07/048)	Barley GC 0640 (Platine)	1. 18.10.2006 2. 05.05.-10.05.2007 3. 15.06.-22.06.2007	Foliar application with boom sprayer	0.045	200	0.090	2 15.05.2007	83	Plant w/o roots Grain Straw Grain Straw Grain Straw	1.97 0.021 0.241 0.019 0.107 0.014 0.238	0.255 0.005 0.053 <0.005 0.023 <0.005 0.047	2.22 0.03 0.29 0.02 0.13 0.02 0.28	0 35 35 42 42 49 49	BASF method No 550/0 (L0019/01) LOQ 0.005 mg/kg per isomer
255301 2007/1050101 47120 Duras France (L070181) (AC/07/049)	Barley GC 0640 (Nikel)	1. 18.10.2006 2. 02.05.-10.05.2007 3. 25.06.-02.07.2007	Foliar application with boom sprayer	0.045	200	0.090	2 22.05.2007	83	Plant w/o roots Grain Straw Grain Straw Grain Straw	1.81 0.132 0.480 0.117 0.865 0.112 0.910	0.305 0.025 0.089 0.023 0.172 0.022 0.175	2.11 0.16 0.57 0.14 1.04 0.13 1.08	0 36 36 43 43 49 49	
401831_1 2012/1194990 82130, Lafrançaise France (L110194)	Barley GC 0640 (Azurel)	1. 05.10.2010 2. End of April 3. 10.06.2011	Foliar application with boom sprayer	0.045	200	0.090	2 29.04.2011	69	Plant w/o roots Ears Rest of plant Ears Rest of plant Grain Straw	1.6 0.027 0.54 0.021 0.62 <0.005 0.59	0.30 0.0059 0.11 <0.005 0.13 <0.005 0.12	1.9 0.033 0.65 0.026 0.74 <0.01 0.71	0 28 28 35 35 42 42	BASF method No 535/1 (L0076/01) LOQ 0.005 mg/kg per isomer

1	2	3	4	5			6	7	8	9			10	11
Report No Location (Trial No)	**Commodity/ Variety**	**Date of 1. Sowing / Planting 2. Flowering 3. Harvest**	**Method of treatment**	**Application rate[0] per treatment**			**No of treatment(s) and last date**	**Growth stage (BBCH)[2]**	**Portion analyzed**	**Residues (mg/kg)**			**DALA[1]**	**Remarks**
				kg a.s./hL	**Water L/ha**	**kg a.s./ha**				***Cis***	***Trans***	**Sum**		
401831_1 2012/1194990 S11-00713-04 40055, Castenasco Italy (L110195)	Barley GC 0640 (Amorosa)	1. 15.12.2010 2. May 3. 20.06.2011	Foliar application with boom sprayer	0.045	200	0.090	2 16.05.2011	69-71	Plant w/o roots Ears Rest of plant Ears Rest of plant Grain Straw	2.2 0.035 0.29 0.034 0.26 0.0090 0.32	0.42 0.0095 0.060 0.0086 0.055 <0.005 0.070	2.7 0.044 0.35 0.042 0.31 0.014 0.39	0 29 29 35 35 35 35	

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
n.r. Not reported

Treatment with BAS 627 00 F on barley in Southern Europe

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name): Metconazole (BAS 555 F)
Crop /crop group: Barley/cereals
Responsible body for reporting (name, address) BASF SE, 67117 Limburgerhof
Country (trial) Germany

Content of active substance (g/kg or g/L): 27.5 g/L
Formulation (e.g. WP) EC (BAS 627 00 F)

Commercial product:
Producer of commercial product: BASF SE, Ludwigshafen, Germany
Indoor/Glasshouse/Outdoor Outdoor
Other active substance in the formulation (common name and content) Epoxiconazole (BAS 480 F, 37.5 g/L)

Residue calculated as: Metconazole (BAS 555 F)

1	2	3	4	5			6	7	8	9			10	11
Report No Location (Trial No)	Commodity / Variety	1. Date of Sowing / Planting 2. Flowering 3. Harvest	Method of treatment	Application rate[0] per treatment			No of treatment(s) and last date	Growth stage (BBCH)[2]	Portion analyzed	Residues (mg/kg)			DALA[1]	Remarks
				kg a.s /hL	Water L/ha	kg a.s./ha				*Cis*	*Trans*	Sum		
366605 2010/1110643 59032, Imathia Greece (L090067)	Barley GC 0640 (Moutso)	1. 10.11.2008 2. 05.04.-20.04.2009 3. 05.06.-15.06.2009	Foliar application with boom sprayer	0.04125	200	0.0825	2 16.04.2009	69	Plant w/o roots Ears Rest of plant Ears Rest of plant Ears Rest of plant Grain Straw	n.r. n.r. n.r. n.r. n.r. n.r. n.r. n.r. n.r.	n.r. n.r. n.r. n.r. n.r. n.r. n.r. n.r. n.r.	2.4 0.19 0.64 0.17 0.69 0.23 0.96 0.02 0.76	0 29 29 36 36 41 41 50 50	BASF Method No 535/1 (L0076/01) LOQ 0.01 mg/kg
366605 2010/1110643 40062, Bologna Italy (L090068)	Barley GC 0640 (Otis)	1. 19.02.2009 2. 09.05.-13.05.2009 3. 24.06.2009	Foliar application with boom sprayer	0.04125	200	0.0825	2 13.05.2009	69	Plant w/o roots Ears Rest of plant Grain Straw Grain Straw	n.r. n.r. n.r. n.r. n.r. n.r. n.r.	n.r. n.r. n.r. n.r. n.r. n.r. n.r.	2.1 0.22 2.1 0.04 2.5 0.025 1.4	0 28 28 35 35 42 42	
366605 2010/1110643 41710, Sevilla Spain (L090069)	Barley GC 0640 (Cecilia)	1. 16.12.2008 2. 10.04.-24.04.2009 3. 03.06.2009	Foliar application with boom sprayer	0.04125	200	0.0825	2 22.04.2009	69	Plant w/o roots Ears Rest of plant Grain Straw Grain Straw	n.r. n.r. n.r. n.r. n.r. n.r. n.r.	n.r. n.r. n.r. n.r. n.r. n.r. n.r.	2.9 0.23 2.9 0.02 1.9 0.025 2.2	0 29 29 36 36 42 42	

1	2	3	4	5			6	7	8	9			10	11
Report No Location (Trial No)	**Commodity / Variety**	**1. Date of Sowing / Planting 2. Flowering 3. Harvest**	**Method of treatment**	**Application rate[0] per treatment**			**No of treatment(s) and last date**	**Growth stage (BBCH)[2]**	**Portion analyzed**	**Residues (mg/kg)**			**DALA[1]**	**Remarks**
				kg a.s /hL	**Water L/ha**	**kg a.s./ha**				***Cis***	***Trans***	**Sum**		
366605 2010/1110643 32600, Gers France (south) (L090072)	Barley GC 0640 (Franzi)	1. 14.12.2008 2. 19.05.-23.05.2009 3. 01.07.-08.07.2009	Foliar application with boom sprayer	0.04125	200	0.0825	2 25.05.2009	71	Plant w/o roots Ears Rest of plant Grain Straw Grain Straw	n.r. n.r. n.r. n.r. n.r. n.r. n.r.	n.r. n.r. n.r. n.r. n.r. n.r. n.r.	1.8 0.4 2.3 0.067 1.2 0.06 0.97	0 28 28 34 34 43 43	
255301 2007/1050101 31620 Castelnau D'estretefonds France (L070180) (AC/07/048)	Barley GC 0640 (Platine)	1. 18.10.2006 2. 05.05.-10.05.2007 3. 15.06.-22.06.2007	Foliar application with boom sprayer	0.04125	200	0.0825	2 15.05.2007	83	Plant w/o roots Grain Straw Grain Straw Grain Straw	1.43 0.018 0.109 0.022 0.043 0.016 0.074	0 220 0.006 0.028 0.007 0.011 0.005 0.018	1.65 0.02 0.14 0.03 0.05 0.02 0.09	0 35 35 42 42 49 49	BASF method No 550/0 (L0019/01) LOQ 0.005 mg/kg per isomer
255301 2007/1050101 47120 Duras France (L070181) (AC/07/049)	Barley GC 0640 (Nikel)	1. 18.10.2006 2. 02.05.-10.05.2007 3. 25.06.-02.07.2007	Foliar application with boom sprayer	0.04125	200	0.0825	2 22.05.2007	83	Plant w/o roots Grain Straw Grain Straw Grain Straw	2.28 0.157 0.635 0.126 0.644 0.131 0.520	0.455 0.035 0 150 0.029 0 158 0.031 0 130	2.73 0.19 0.79 0.15 0.80 0.16 0.65	0 36 36 43 43 49 49	
339327 2010/1144334 46250 Goujounac Lot France (L100103)	GC 0640/ Barley Diadéme	1 15.11.2009 2 24.05.-02.06.2010 3 14.07.-17.07.2010	Foliar application with boom	0.0413	200	0.0825	2 02.06.2010	69	Plant w/o roots Ears Rest of plant[3] Grain Straw Grain Straw	1.2 0.061 0.19 0.030 0.30 0.028 0.15	0 19 0.015 0.041 0.009 0.076 0.009 0.043	1.4 0.075 0.23 0.039 0.38 0.036 0.20	0 28 28 35 35 42 42	BASF method No 550/0 (L0019/01) LOQ 0.01 mg/kg
339327 2010/1144334 11500 Puerto Sta Maria Cádiz Spain (L100104)	GC 0640/ Barley Henley	1 04.12.2009 2 15.04.-24.04.2010 3 04.06.2010	Foliar application with boom	0.0413	200	0.0825	2 23.04.2010	69	Plant w/o roots Grain Straw Grain Straw Grain Straw	3.5 0.11 2.4 0.21 4.7 0.16 3.4	0 57 0.020 0.40 0.045 0 90 0.035 0.65	4.1 0.13 2.8 0.26 5.6 0.19 4.1	0 27 27 34 34 41 41	

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
3 Without roots
n.r. Not reported

Treatment with BAS 627 02 F on barley in Southern Europe

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name): Metconazole (BAS 555 F)
Crop /crop group: Barley/cereals
Responsible body for reporting (name, address) BASF SE, 67117 Limburgerhof
Country (trial) Germany

Content of active substance (g/kg or g/L): 41.25 g/L
Formulation (e.g. WP) EC (BAS 627 02 F)

Commercial product:
Producer of commercial product: BASF SE, Ludwigshafen, Germany
Indoor/Glasshouse/Outdoor Outdoor
Other active substance in the formulation (common name and content) Epoxiconazole (BAS 480 F, 56.25 g/L)

Residue calculated as: Metconazole (BAS 555 F)

1	2	3	4	5			6	7	8	9			10	11
Report No Location (Trial No)	**Commodity/ Variety**	**Date of 1. Sowing / Planting 2. Flowering 3. Harvest**	**Method of treatment**	**Application rate[0] per treatment**			**No of treatment(s) and last date**	**Growth stage (BBCH)[2]**	**Portion analyzed**	**Residues (mg/kg)**			**DALA[1]**	**Remarks**
				kg a.s./hL	**Water L/ha**	**kg a.s./ha**				***Cis***	***Trans***	**Sum**		
339327 2010/1144334 46250 Goujounac Lot France (L100103)	GC 0640/ Barley Diadéme	1 15.11.2009 2 24.05.- 02.06.2010 3 14.07.- 17.07.2010	Foliar application with boom	0.0413	200	0.0825	2 02.06 2010	69	Plant w/o roots Ears Rest of plant[3] Grain Straw Grain Straw	1.2 0.054 0.28 0.025 0.47 0.027 0.33	0.23 0.014 0.056 0.008 0.11 0.008 0.084	1 5 0.068 0.33 0.033 0.58 0.035 0.41	0 28 28 35 35 42 42	BASF method No 550/0 (L0019/01) LOQ 0.01 mg/kg
339327 2010/1144334 11500 Puerto Sta Maria Cádiz Spain (L100104)	GC 0640/ Barley Henley	1 04.12.2009 2 15.04.- 24.04.2010 3 04.06.2010	Foliar application with boom	0.0413	200	0.0825	2 23.04 2010	69	Plant w/o roots Grain Straw Grain Straw Grain Straw	3.5 0.061 1.7 0.15 5.7 0.083 3.7	0.61 0.014 0.33 0.033 1.1 0.019 0.74	4 2 0.075 2.0 0.18 6.7 0.10 4 5	0 27 27 34 34 41 41	

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
3 Without roots
n.r. Not reported

Treatment with BAS 556 03 F on barley in Southern Europe

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name):	Metconazole (BAS 555 F)	**Commercial product:**	---
Crop /crop group:	Barley/cereals	**Producer of commercial product:**	BASF SE, Ludwigshafen, Germany
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	**Indoor/Glasshous/Outdoor**	Outdoor
Country (trial)	Germany, UK, France, Italy	**Other active substance in the formulation**	---
Content of active substance (g/kg or g/L):	60 g/L	**(common name and content)**	
Formulation (e.g. WP)	EC (BAS 555 00 F)	**Residue calculated as:**	Metconazole (BAS 555 F)

1	2	3	4	5			6	7	8	9			10	11
Report No Location (Trial No)	**Commodity/ Variety**	**Date of 1. Sowing / Planting 2. Flowering 3. Harvest**	**Method of treatment**	**Application rate[0] per treatment**			**No of treatment(s) and last date**	**Growth stage (BBCH)[2]**	**Portion analyzed**	**Residues (mg/kg)**			**DALA[1]**	**Remarks**
				kg a.s./hL	**Water L/ha**	**kg a.s./ha**				**Cis**	**Trans**	**Sum**		
401831_1 2012/1194990 82130, Lafrançaise France (L110194)	Barley GC 0640 (Azurel)	1. 05.10.2010 2. End of April 3. 10.06.2011	Foliar application with boom sprayer	0.044	200	0.088	2 29.04.2011	69	Plant w/o roots Ears Rest of plant Ears Rest of plant Grain Straw	2.0 0.033 0.52 0.030 0.51 0.0068 0.41	0.39 0.0071 0.11 0.0059 0.11 <0.005 0.084	2.4 0.040 0.63 0.036 0.62 0.012 0.49	0 28 28 35 35 42 42	BASF method No 535/1 (L0076/01) LOQ 0.005 mg/kg per isomer
401831_1 2012/1194990 S11-00713-04 40055, Castenasco Italy (L110195)	Barley GC 0640 (Amorosa)	1. 15.12.2010 2. May 3. 20.06.2011	Foliar application with boom sprayer	0.044	200	0.088	2 16.05.2011	69-71	Plant w/o roots Ears Rest of plant Ears Rest of plant Grain Straw	2 2 0.032 0.75 0.030 0.61 0.0077 0.54	0.43 0.0078 0.15 0.0075 0.12 <0.005 0.11	2.6 0.040 0.90 0.038 0.73 0.013 0.65	0 29 29 35 35 35 35	

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
n.r. Not reported

Oilseed rape

Treatment with BAS 556 03 F on oilseed rape in Northern Europe

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Metconazole (BAS 555 F)	**Commercial Product (name)**	--
Crop/crop group:	Oilseed rape/Oilseeds	**Producer of commercial product**	BASF SE, Ludwigshafen, Germany
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	**Indoor/Glasshouse/Outdoor**	Outdoor
Country	Germany	**Other active substance in the formulation**	Pyraclostrobin (BAS 500 F)
Content of active substance (g/kg or g/L)	80 g/L	**(common name and content)**	130 g/L
Formulation (e.g. WP)	EC (code BAS 556 03 F)	**Residues calculated as:**	Metconazole (BAS 555 F)

1	2	3	4	5			6	7	8	9			10	11
Report No Location (Trial No)	**Commodity/ Variety**	**Date of 1. Sowing / Planting 2. Flowering 3. Harvest**	**Method of treatment**	**Application rate[0] per treatment**			**No of treat-ment(s) and last date**	**Growth stage (BBCH)[2]**	**Portion analyzed**	**Residues (mg/kg)**			**DALA[1]**	**Remarks**
				kg a.s./hL	**Water L/ha**	**kg a.s./ha**				***Cis*-isomer**	***Trans*-isomer**	**Total[6]**		
364929 2012/1255033 Brandenburg Germany L110180	AV 0495 Petrol	1. 29.08.10 2. 20.04.-15.05.11 3. 26.07.11	Foliar spray	0.04	200	0.08	2 20.05.11	71	Plant[3] Seed Rest[4] Pods[5] Seed Rest[4]	1.4 0.019 0.056 0.042 0.0093 0.042	0.26 <0.005 0.012 0.0078 <0.005 0.0087	1.6 0.024 0.068 0.050 0.014 0.051	0 56 56 56 67 67	BASF method No 535/1 LOQ 0.005 mg/kg per isomer
364929 2012/1255033 Cambridge United Kingdom L110181	AV 0495 DK Cabernet	1. 28.08.10 2. 08.04.-20.05.11 3. 28.07.11	Foliar spray	0.04	200	0.08	2 20.05.11	71	Plant[3] Seed Rest[4] Pods[5] Seed Rest[4]	2.1 0.014 0.15 0.26 0.040 0.12	0.41 <0.005 0.028 0.046 0.0060 0.024	2.6 0.019 0.18 0.31 0.046 0.15	0 56 56 56 69 69	BASF method No 535/1 LOQ 0.005 mg/kg per isomer
364929 2012/1255033 Loiret France L110182	AV 0495 Ovation	1. 07.09.10 2. April 3. 28.06.11	Foliar spray	0.04	200	0.08	2 02.05.11	71	Plant[3] Seed Rest[4]	0.99 0.065 0.15	0.19 0.0073 0.028	1.2 0.072 0.18	0 57 57	BASF method No 535/1 LOQ 0.005 mg/kg per isomer
364929 2012/1255033 Middelfart Denmark L110183	AV 0495 Cabernet	1. 20.08.10 2. 02.05.-26.05.11 3. 21.07.11	Foliar spray	0.04	200	0.08	2 26.05.11	74	Plant[3] Seed Rest[4]	0.78 0.020 0.12	0.15 <0.005 0.022	0.93 0.025 0.15	0 56 56	BASF method No 535/1 LOQ 0.005 mg/kg per isomer

0 Actual application rates varied by 10% at most except where noted otherwise
1 Days after last application
2 At last treatment
3 Whole plant without roots
4 Rest of plant without roots
5 With seeds
6 Differences in calculations of total metconazole are due to rounding

Treatment with BAS 556 03 F on oilseed rape in Southern Europe

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Metconazole (BAS 555 F)	**Commercial Product (name)**	--
Crop/crop group:	Oilseed rape/Oilseeds	**Producer of commercial product**	BASF SE, Ludwigshafen, Germany
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	**Indoor/Glasshouse/Outdoor**	Outdoor
Country	Germany	**Other active substance in the formulation**	Pyraclostrobin (BAS 500 F)
Content of active substance (g/kg or g/L)	80 g/L	**(common name and content)**	130 g/L
Formulation (e.g. WP)	EC (code BAS 556 03 F)	**Residues calculated as:**	Metconazole (BAS 555 F)

1	2	3	4	5			6	7	8	9			10	11
Report No Location (Trial No)	**Commodity/ Variety**	**Date of 1. Sowing / Planting 2. Flowering 3. Harvest**	**Method of treatment**	**Application rate[0] per treatment**			**No of treat-ment(s) and last date**	**Growth stage (BBCH)[2]**	**Portion analyzed**	**Residues (mg/kg)**			**DALA[1]**	**Remarks**
				kg a.s./hL	**Water L/ha**	**kg a.s./ha**				***Cis*-isomer**	***Trans*-isomer**	**Total[6]**		
364929 2012/1255033 Tarn Et Garonne France L110184	AV 0495 Coklicot	1. 06.09.10 2. April/May 3. 23.06.11	Foliar spray	0.04	200	0.08	2 27.04.11	71	Plant[3] Seed Rest[4]	1.1 0.032 0.45	0.23 <0.005 0.095	1.34 0.037 0.56	0 57 57	BASF method No 535/1 LOQ 0.005 mg/kg per isomer
364929 2012/1255033 Serres Greece L110185	AV 0495 Nelson	1. 20.10.10 2. 08.04.-18.04.11 3. 22.06.11	Foliar spray	0.04	200	0.08	2 28.04.11	71	Plant[3] Seed Rest[4]	1.1 0.05 0.03	0.22 <0.005 0.0067	1.30 0.051 0.04	0 55 55	BASF method No 535/1 LOQ 0.005 mg/kg per isomer
364929 2012/1255033 Emilia-Romagna Italy L110186	AV 0495 Vectra	1. 20.09.10 2. April 3. 15.06.11	Foliar spray	0.04	200	0.08	2 03.05.11	71	Plant[3] Rest[4] Pods[5] Seed Rest[4]	0.67 0.085 0.11 0.011 0.095	0.14 0.018 0.021 <0.005 0.019	0.81 0.10 0.13 0.016 0.11	0 35 35 43 43	BASF method No 535/1 LOQ 0.005 mg/kg per isomer
364929 2012/1255033 Aragon Spain L110187	AV 0495 Artist	1. 20.09.10 2. n.r. 3. 27.06.11	Foliar spray	0.04	200	0.08	2 17.05.11	75	Plant[3] Seed Rest[4]	0.47 0.010 0.20	0.094 <0.005 0.041	0.56 0.015 0.24	0 41 41	BASF method No 535/1 LOQ 0.005 mg/kg per isomer

0 Actual application rates varied by 10% at most except where noted otherwise n.r. Not reported
1 Days after last application
2 At last treatment
3 Whole plant without roots
4 Rest of plant without roots
5 With seeds
6 Differences in calculations of total metconazole are due to rounding

Studies used for discussion of *cis*/*trans* isomers only

Oilseed rape

Treatment with BAS 134 00 W on oilseed rape in Northern Europe

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name): Metconazole (BAS 555 F)
Crop /crop group: Oilseed rape/oilseeds
Responsible body for reporting (name, address) BASF SE, 67117 Limburgerhof
Country (trial) Germany
Content of active substance (g/kg or g/L): 30 g/L
Formulation (e.g. WP) SL (BAS 134 00 W)

Commercial product:
Producer of commercial product: BASF SE, Ludwigshafen, Germany
Indoor/Glasshouse/Outdoor Outdoor
Other active substance in the formulation (common name and content) Mepiquat-chloride 210 g/L
Residue calculated as: Metconazole (BAS 555 F)

1	2	3	4	5			6	7	8	9			10	11
Report No Location (Trial No)	**Commodity/ Variety**	**Date of 1. Sowing / Planting 2. Flowering 3. Harvest**	**Method of treatment**	**Application rate[0] per treatment**			**No of treatment(s) and last date**	**Growth stage (BBCH)[2]**	**Portion analyzed**	**Residues (mg/kg)**			**DALA[1]**	**Remarks**
				kg a.s./hL	**Water L/ha**	**kg a.s./ha**				***Cis***	***Trans***	**Sum**		
133933 2004/1015943 74193 Stetten a.H. Germany (DU2/14/02)	Oilseed rape SO 0495 (Smart)	1. 29.08.02 2. 10.04.-20.05.03 3. 04.07.03	Foliar application	0.014	300	0.042	2 04.04.03	59	Whole plant w/o roots Whole plant w/o roots Seeds	1.08 0.010 <0.005	0.19 <0.005 <0.005	1.27 0.015 <0.01	0 46 87	BASF method No 550/0 (L0019/01) LOQ
133933 2004/1015943 Böhl-Iggelheim 67459 Germany (DU4/15/02)	Oilseed rape SO 0495 (Express)	1. 31.08.02 2. 06.04.-16.05.03 3. 30.06.03	Foliar application	0.014	300	0.042	2 02.04.03	59	Whole plant w/o roots Whole plant w/o roots Seeds	1.00 0.010 <0.005	0.18 <0.005 <0.005	1.18 <0.015 <0.01	0 44 89	0.005 mg/kg per isomer
133933 2004/1015943 67160 Seebach France (N) (FAN/14/02)	Oilseed rape SO 0495 (Pollen)	1. 06.09.02 2. 01.-20.05.03 3. 07.07.03	Foliar application	0.014	300	0.042	2 15.04.03	55	Whole plant w/o roots Whole plant w/o roots Whole plant w/o roots Seeds	0.74 0.030 <0.005 <0.005	0.13 <0.005 <0.005 <0.005	0.87 0.035 <0.01 <0.01	0 30 56 79	
133933 2004/1015943 OX27 0BL Bicester United Kingdom (OAT/20/02)	Oilseed rape SO 0495 (Winner)	1. 28.08.02 2. 03.-12.05.03 3. 20.-21.07.03	Foliar application	0.014	300	0.042	2 27.03.03	53	Whole plant w/o roots Whole plant w/o roots Seeds	0.92 0.030 <0.005	0.16 <0.005 <0.005	1.08 0.035 <0.01	0 48 113	
144055 2004/1015942 Böhl-Iggelheim 67459 Germany (DU4/08/03)	Oilseed rape SO 0495 (Express)	1. 31.08.03 2. 17.04.-12.05.04 3. 16.07.04	Foliar application	0.014	300	0.042	2 16.04.04	55	Whole plant w/o roots Whole plant w/o roots Whole plant w/o roots Dried seeds	0.47 0.03 0.01 <0.0085	0.08 0.005 0.002 <0.0016	0.56 0.04 0.01 <0.01	0 28 54 89	BASF method No 550/0 (L0019/01) LOQ

1	2	3	4	5			6	7	8	9			10	11
Report No Location (Trial No)	Commodity/ Variety	Date of 1. Sowing / Planting 2. Flowering 3. Harvest	Method of treatment	Application rate[0] per treatment			No of treatment(s) and last date	Growth stage (BBCH)[2]	Portion analyzed	Residues (mg/kg)			DALA[1]	Remarks
				kg a.s./hL	Water L/ha	kg a.s./ha				*Cis*	*Trans*	Sum		
144055 2004/1015942 67160 Seebach France (N) (FAN/06/03)	Oilseed rape SO 0495 (Hearthy)	1. 28.08.03 2. 20.04.-12.05.04 3. 07.07.04	Foliar application	0.014	300	0.042	2 16.04.04	59	Whole plant w/o roots Whole plant w/o roots Shoots w/o roots Pods Dried seeds	0.57 0.09 0.01 <0.0085 <0.0085	0.10 0.01 0.002 <0.0016 <0.0016	0.67 0.10 0.02 <0.01 <0.01	0 26 56 56 82	<0.01 mg/kg for total metconazole
144055 2004/1015942 72800 Luché-pringé France (FBM/03/03)	Oilseed rape SO 0495 (Standing)	1. 26.08.03 2. 20.04.-03.05.04 3. 07.07.-08.07.04	Foliar application	0.014	300	0.042	2 26.03.04	59	Whole plant w/o roots Whole plant w/o roots Whole plant w/o roots Dried seeds	0.74 0.06 0.009 <0.0085	0.13 0.008 <0.0016 <0.0016	0.87 0.07 0.011 <0.01	0 38 54 102	
144055 2004/1015942 OX27 9AS Bicester United Kingdom (OAT/03/03)	Oilseed rape SO 0495 (Winner)	1. 01.09.03 2. 20.04.-06.05.04 3. 01.08.-02.08.04	Foliar application	0.014	300	0.042	2 08.04.04	55	Whole plant w/o roots Whole plant w/o roots Whole plant w/o roots Dried seeds	0.91 0.01 <0.0085 <0.0085	0.16 0.002 <0.0016 <0.0016	1.07 0.02 <0.01 <0.01	0 48 56 109	
144055 2004/1015942 MK18 4AB Buckinghamshire United Kingdom (OAT/04/03)	Oilseed rape SO 0495 (Winner)	1. 01.09.03 2. 15.04.-01.05.04 3. 05.08.-06.08.04	Foliar application	0.014	300	0.042	2 13.04.04	55	Whole plant w/o roots Whole plant w/o roots Whole plant w/o roots Dried seeds	1.01 0.02 <0.0085 <0.0085	0.17 0.003 <0.0016 <0.0016	1.18 0.02 <0.01 <0.01	0 43 55 108	
182053 2006/1026865 82130, La Francaise France AF/10166/BA/1	Oilseed rape SO 0495 (Libri)	1. 02.09.05 2. N/A 3. 29.03.-15.06.06	foliar spray	0.014	300	0.042	2 29.03.2006	57	Whole plant w/o roots Whole plant w/o roots Whole plant w/o roots Seeds	0.086 0.008 <0.005 <0.005	0.52 0.074 <0.005 <0.005	0.61 0.082 <0.01 <0.01	0 28 55 78	BASF method No 550/0 (L0019/01) LOQ 0.005 mg/kg per isomer
182053 2006/1026865 82000, Montauban France AF/10166/BA/2	Oilseed rape SO 0495 (Libri)	1. 01.09.05 2. N/A 3. 24.03.-15.06.06	foliar spray	0.014	300	0.042	2 24.03.2006		Whole plant w/o roots Whole plant w/o roots Whole plant w/o roots Seeds	0.071 <0.005 <0.005 <0.005	0.44 0.016 <0.005 <0.005	0.51 0.021 <0.01 <0.01	0 33 56 83	
240079 2007/1057852 Dange St. Romain 86220, France L06901	Oilseed rape SO 0495 (Mendel)	1. 05.09.06 2. 02.04.-05.05.06 3. 28.06.06	foliar spray	0.014	300	0.042	2 03.04.2007	59	Whole plant w/o roots Whole plant w/o roots Pods Shoots w/o pods Seeds	0.088 0.0058 <0.005 <0.005 <0.005	0.42 0.033 0.008 <0.005 <0.005	0.51 0.04 0.013 <0.01 <0.01	0 31 55 55 84	

1	2	3	4	5			6	7	8	9			10	11
Report No Location (Trial No)	**Commodity/ Variety**	**Date of 1. Sowing / Planting 2. Flowering 3. Harvest**	**Method of treatment**	**Application rate[0] per treatment**			**No of treatment(s) and last date**	**Growth stage (BBCH)[2]**	**Portion analyzed**	**Residues (mg/kg)**			**DALA[1]**	**Remarks**
				kg a.s./hL	**Water L/ha**	**kg a.s./ha**				***Cis***	***Trans***	**Sum**		
240079 2007/1057852 86250 Charroux France L06902	Oilseed rape SO 0495 (Aviso)	1. 22.08.06 2. 07.04.-10.05.07 3. 24.06.07	foliar spray	0.014	300	0.042	2 26.03.2007	59	Whole plant w/o roots Whole plant w/o roots Pods Shoots w/o pods Seeds	0.10 <0.005 <0.005 <0.005 <0.005	0.45 0.017 <0.005 0.01 <0.005	0.55 0.022 <0.01 0.017 <0.01	0 39 56 56 92	

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application

Treatment with BAS 134 00 W on oilseed rape in Southern Europe

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name): Metconazole (BAS 555 F)
Crop /crop group: Oilseed rape/oilseeds
Responsible body for reporting (name, address) BASF SE, 67117 Limburgerhof
Country (trial) Germany
Content of active substance (g/kg or g/L): 30 g/L
Formulation (e.g. WP) SL (BAS 134 00 W)

Commercial product:
Producer of commercial product: BASF SE, Ludwigshafen, Germany
Indoor/Glasshouse/Outdoor Outdoor
Other active substance in the formulation (common name and content) Mepiquat-chloride 210 g/L
Residue calculated as: Metconazole (BAS 555 F)

1	2	3	4	5			6	7	8	9			10	11
Report No Location (Trial No)	**Commodity/ Variety**	**Date of 1. Sowing / Planting 2. Flowering 3. Harvest**	**Method of treatment**	**Application rate[0] per treatment**			**No of treatment(s) and last date**	**Growth stage (BBCH)[2]**	**Portion analyzed**	**Residues (mg/kg)**			**DALA[1]**	**Remarks**
				kg a.s./hL	**Water L/ha**	**kg a.s./ha**				***Cis***	***Trans***	**Sum**		
182053 2006/1026865 82130, La Francaise France AF/10166/BA/1	Oilseed rape SO 0495 (Libri)	1. 02.09.05 2. N/A 3. 29.03.-15.06.06	foliar spray	0.014	300	0.042	2 29.03.2006	57	Whole plant w/o roots Whole plant w/o roots Whole plant w/o roots Seeds	0.086 0.008 <0.005 <0.005	0.52 0.074 <0.005 <0.005	0.61 0.082 <0.01 <0.01	0 28 55 78	BASF method No 550/0 (L0019/01) LOQ 0.005 mg/kg per isomer
182053 2006/1026865 82000, Montauban France AF/10166/BA/2	Oilseed rape SO 0495 (Libri)	1. 01.09.05 2. N/A 3. 24.03.-15.06.06	foliar spray	0.014	300	0.042	2 24.03.2006		Whole plant w/o roots Whole plant w/o roots Whole plant w/o roots Seeds	0.071 <0.005 <0.005 <0.005	0.44 0.016 <0.005 <0.005	0.51 0.021 <0.01 <0.01	0 33 56 83	
240079 2007/1057852 Dange St. Romain 86220, France L06901	Oilseed rape SO 0495 (Mendel)	1. 05.09.06 2. 02.04.-05.05.06 3. 28.06.06	foliar spray	0.014	300	0.042	2 03.04.2007	59	Whole plant w/o roots Whole plant w/o roots Pods Shoots w/o pods Seeds	0.088 0.0058 <0.005 <0.005 <0.005	0.42 0.033 0.008 <0.005 <0.005	0.51 0.04 0.013 <0.01 <0.01	0 31 55 55 84	
240079 2007/1057852 86250 Charroux France L06902	Oilseed rape SO 0495 (Aviso)	1. 22.08.06 2. 07.04.-10.05.07 3. 24.06.07	foliar spray	0.014	300	0.042	2 26.03.2007	59	Whole plant w/o roots Whole plant w/o roots Pods Shoots w/o pods Seeds	0.10 <0.005 <0.005 <0.005 <0.005	0.45 0.017 <0.005 0.01 <0.005	0.55 0.022 <0.01 0.017 <0.01	0 39 56 56 92	

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application

Studies used for metabolite discussion (toxicological relevance)

Soya bean

Treatment with BAS 555 01 F on soya bean in North America

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Metconazole (BAS 555 F)	**Commercial Product (name)**	--
Crop/crop group:	Oilseed rape/Oilseeds	**Producer of commercial product**	BASF SE, Ludwigshafen, Germany
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	**Indoor/Glasshouse/Outdoor**	Outdoor
Country	Germany	**Other active substance in the formulation**	None
Content of active substance (g/kg or g/L)	90 g/L	**(common name and content)**	
Formulation (e.g. WP)	SL (code BAS 555 01 F)	**Residues calculated as:**	Metconazole (BAS 555 F)

1	2	3	4	5			6	7	8	9			10	11
Report No Location (Trial No)	**Commodity/ Variety**	**Date of 1. Sowing / Planting 2. Flowering 3. Harvest**	**Method of treatment**	**Application rate[0] per treatment**			**No of treat-ment(s) and last date**	**Growth stage (BBCH)[2]**	**Portion analyzed**	**Residues[3] (mg/kg)**			**DALA[1]**	**Remarks**
				kg a.s./hL	**Water L/ha**	**kg a.s./ha**				***Cis*-isomer**	***Trans*-isomer**	**Total**		
137726 2006/7006995 Tift, GA USA R05111	VD 0541 Dekalb H7242	1. 23.05.2005 2. n.r. 3. 03.10.-26.10.05	Foliar spray	0.04	180	0.08	2 26.09.05	n.r.	Seed	<0.005	<0.005	<0.01	30	BASF method No D0508 LOQ 0.005 mg/kg per isomer
137726 2006/7006995 Wabasha, MN USA R05112	VD 0541 Asgrow AG1603	1. 25.05.2005 2. n.r. 3. 08.09.-01.10.05	Foliar spray	0.04	190	0.08	2 01.09.05	n.r.	Seed	<0.005	<0.005	<0.01	30	BASF method No D0508 LOQ 0.005 mg/kg per isomer
137726 2006/7006995 Wabasha, MN USA R05113	VD 0541 Asgrow AG1603	1. 25.05.2005 2. n.r. 3. 08.09.-01.10.05	Foliar spray	0.04	190	0.08	2 01.09.05	n.r.	Seed	<0.005	<0.005	<0.01	30	BASF method No D0508 LOQ 0.005 mg/kg per isomer
137726 2006/7006995 Madison, IL USA R05114	VD 0541 DKB38-52	1. 17.05.2005 2. n.r. 3. 19.09.-12.10.05	Foliar spray	0.05	150	0.08	2 12.09.05	n.r.	Seed	0.006 (0.007, 0.005)	<0.005	0.011 (0.012, 0.010)	30	BASF method No D0508 LOQ 0.005 mg/kg per isomer

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Metconazole (BAS 555 F)	**Commercial Product (name)**	--
Crop/crop group:	Oilseed rape/Oilseeds	**Producer of commercial product**	BASF SE, Ludwigshafen, Germany
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	**Indoor/Glasshouse/Outdoor**	Outdoor
Country	Germany	**Other active substance in the formulation**	None
Content of active substance (g/kg or g/L)	90 g/L	**(common name and content)**	
Formulation (e.g. WP)	SL (code BAS 555 01 F)	**Residues calculated as:**	Metconazole (BAS 555 F)

1	2	3	4	5			6	7	8	9			10	11
Report No Location (Trial No)	**Commodity/ Variety**	**Date of 1. Sowing / Planting 2. Flowering 3. Harvest**	**Method of treatment**	**Application rate[0] per treatment**			**No of treat-ment(s) and last date**	**Growth stage (BBCH)[2]**	**Portion analyzed**	**Residues[3] (mg/kg)**			**DALA[1]**	**Remarks**
				kg a.s./hL	**Water L/ha**	**kg a.s./ha**				***Cis*-isomer**	***Trans*-isomer**	**Total**		
137726 2006/7006995 Clinton, IL USA R05115	VD 0541 NK 43-B1	1. 17.06.2005 2. n.r. 3. 27.09.-19.10.05	Foliar spray	0.04	180	0.08	2 20.09.05	n.r.	Seed	<0.005	<0.005	<0.01	29	BASF method No D0508 LOQ 0.005 mg/kg per isomer
137726 2006/7006995 Stark, IL USA R05116	VD 0541 Asgrow 3202	1. 06.05.2005 2. n.r. 3. 27.09.-19.10.05	Foliar spray	0.04	180	0.08	2 20.09.05	n.r.	Seed	<0.005	<0.005	<0.01	29	BASF method No D0508 LOQ 0.005 mg/kg per isomer
137726 2006/7006995 Polk, NE USA R05117	VD 0541 Pioneer 92M80	1. 18.05.2005 2. n.r. 3. 29.08.-20.09.05	Foliar spray	0.04	190	0.08	2 22.08.05	n.r.	Seed	<0.005	<0.005	<0.01	29	BASF method No D0508 LOQ 0.005 mg/kg per isomer
137726 2006/7006995 York, NE USA R05118	VD 0541 Pioneer 92M80	1. 21.05.2005 2. n.r. 3. 24.08.-06.10.05	Foliar spray	0.04	190	0.08	2 24.08.05	n.r.	Seed Seed Seed Seed	0.005 (<0.005, 0.006) <0.005 <0.005 0.005 (0.005, <0.005)	<0.005 <0.005 <0.005 <0.005	0.01 (<0.01, 0.011) <0.01 <0.01 0.01 (0.010, <0.01)	23 30 36 43	BASF method No D0508 LOQ 0.005 mg/kg per isomer
137726 2006/7006995 Hall, NE USA R05119	VD 0541 Dyna-Gro-37B28RR	1. 19.05.2005 2. n.r. 3. 30.08.-21.09.05	Foliar spray	0.04	190	0.08	2 22.08.05	n.r.	Seed	<0.005	<0.005	<0.01	30	BASF method No D0508 LOQ 0.005 mg/kg per isomer
137726 2006/7006995 Branchton, ON Canada R05120	VD 0541 Mycogen 5140 RR	1. 21.05.2005 2. n.r. 3. 26.08.-20.09.05	Foliar spray	0.03	280	0.08	2 19.08.05	n.r.	Seed	0.024 (0.023, 0.025)	0.006	0.030 (0.029, 0.031)	32	BASF method No D0508 LOQ 0.005 mg/kg per isomer

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Metconazole (BAS 555 F)	**Commercial Product (name)**	--
Crop/crop group:	Oilseed rape/Oilseeds	**Producer of commercial product**	BASF SE, Ludwigshafen, Germany
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	**Indoor/Glasshouse/Outdoor**	Outdoor
Country	Germany	**Other active substance in the formulation**	None
Content of active substance (g/kg or g/L)	90 g/L	**(common name and content)**	
Formulation (e.g. WP)	SL (code BAS 555 01 F)	**Residues calculated as:**	Metconazole (BAS 555 F)

1	2	3	4	5			6	7	8	9			10	11
Report No Location (Trial No)	**Commodity/ Variety**	**Date of 1. Sowing / Planting 2. Flowering 3. Harvest**	**Method of treatment**	**Application rate[0] per treatment**			**No of treat-ment(s) and last date**	**Growth stage (BBCH)[2]**	**Portion analyzed**	**Residues[3] (mg/kg)**			**DALA[1]**	**Remarks**
				kg a.s./hL	**Water L/ha**	**kg a.s./ha**				***Cis*-isomer**	***Trans*-isomer**	**Total**		
137726 2006/7006995 Wapello, IA USA R05121	VD 0541 Pioneer 93M11	1. 08.05.2005 2. n.r. 3. 15.09.-10.10.05	Foliar spray	0.06	140	0.08	2 09.09.05	n.r.	Seed	0.007 (0.006, 0.007)	<0.005	0.012 (0.011, 0.012)	31	BASF method No D0508 LOQ 0.005 mg/kg per isomer
137726 2006/7006995 Jefferson, IA USA R05122	VD 0541 Pioneer 93B87	1. 07.06.2005 2. n.r. 3. 07.09.-21.10.05	Foliar spray	0.06	130	0.08	2 07.09.05	n.r.	Seed Seed Seed Seed	<0.005 <0.005 <0.005 <0.005	<0.005 <0.005 <0.005 <0.005	<0.01 <0.01 <0.01 <0.01	23 30 37 44	BASF method No D0508 LOQ 0.005 mg/kg per isomer
137726 2006/7006995 Keokuk, IA USA R05123	VD 0541 DeKalb 3451	1. 30.05.2005 2. n.r. 3. 16.09.-08.10.05	Foliar spray	0.06	140	0.08	2 08.09.05	n.r.	Seed	<0.005	<0.005	<0.01	30	BASF method No D0508 LOQ 0.005 mg/kg per isomer
137726 2006/7006995 Ottawa, MI USA R05124	VD 0541 Asgrow: AG1903	1. 18.05.2005 2. n.r. 3. 19.09.-10.10.05	Foliar spray	0.04	190	0.08	2 12.09.05	n.r.	Seed	<0.005	<0.005	<0.01	28	BASF method No D0508 LOQ 0.005 mg/kg per isomer
137726 2006/7006995 St-Pie-de Bagot, QB Canada R05125	VD 0541 26-02R RR	1. 27.05.2005 2. n.r. 3. 12.09.-05.10.05	Foliar spray	0.084	220	0.08	2 05.09..05	n.r.	Seed	<0.005	<0.005	<0.01	30	BASF method No D0508 LOQ 0.005 mg/kg per isomer

0 Actual application rates varied by 10% at most except where noted otherwise
1 Days after last application
2 At last treatment
3 Mean of two samples; individual values are given in parentheses
n.r. Not reported

Cotton

Treatment with BAS 555 01 F on cotton in North America

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Metconazole (BAS 555 F)	**Commercial Product (name)**	--
Crop/crop group:	Cotton/Oilseeds	**Producer of commercial product**	BASF SE, Ludwigshafen, Germany
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	**Indoor/Glasshouse/Outdoor**	Outdoor
Country	Germany	**Other active substance in the formulation**	None
Content of active substance (g/kg or g/L)	90 g/L	**(common name and content)**	
Formulation (e.g. WP)	SL (code BAS 555 01 F)	**Residues calculated as:**	Metconazole (BAS 555 F)

1	2	3	4	5			6	7	8	9			10	11
Report No Location (Trial No)	**Commodity/ Variety**	**Date of 1. Sowing / Planting 2. Flowering 3. Harvest**	**Method of treatment**	**Application rate[0] per treatment**			**No of treat-ment(s) and last date**	**Growth stage (BBCH)[2]**	**Portion analyzed**	**Residues[3] (mg/kg)**			**DALA[1]**	**Remarks**
				kg a.s./hL	**Water L/ha**	**kg a.s./ha**				***Cis*-isomer**	***Trans*-isomer**	**Total[6]**		
254317 2007/7001663 Chula, GA USA RCN R06450	SO 0691 PHY 480 WR	1. 07.06.06 2. n.r. 3. 09.11.06	Foliar spray	0.06	180	0.11	3 10.10.06	50% open bolls	Seed[4]	0.055 (0.062, 0.048)	0.013 (0.015, 0.011)	0.068 (0.077, 0.059)	30	BASF method No D0604 LOQ 0.005 mg/kg per isomer
254317 2007/7001663 Newport, AR USA RCN R06451	SO 0691 DP444 BG/RR	1. 22.05.06 2. n.r. 3. 04.10.06	Foliar spray	0.12	90	0.11	3 04.09.06	81	Seed[4] Gin bpdts[5]	0.019 (0.017, 0.020) 2.012 (1.945, 2.078)	0.006 (0.005, 0.006) 0.499 (0.492, 0.505)	0.024 (0.022, 0.026) 2.52 (2.44, 2.58)	30 30	BASF method No D0604 LOQ 0.005 mg/kg per isomer
254317 2007/7001663 Cheneyville, LA USA RCN R06452	SO 0691 PHY 485 WRF	1. 18.05.06 2. n.r. 3. 12.10.06	Foliar spray	0.05-0.07	160-220	0.11	3 13.09.06	81	Seed[4]	0.021 (0.020, 0.021)	0.006 (0.006, 0.006)	0.027 (0.026, 0.027)	29	BASF method No D0604 LOQ 0.005 mg/kg per isomer
254317 2007/7001663 Proctor, AR USA RCN R06453	SO 0691 DP444BR	1. 18.05.06 2. n.r. 3. 27.09.06	Foliar spray	0.09	120	0.11	3 28.08.06	15% open bolls	Seed[4] Gin bpdts[5]	0.015 (0.017, 0.012) 0.142 (0.136, 0.148)	0.005 (0.006, <0.005) 0.035 (0.034, 0.036)	0.020 (0.023, 0.017) 0.18 (0.17, 0.18)	30 30	BASF method No D0604 LOQ 0.005 mg/kg per isomer

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Metconazole (BAS 555 F)	**Commercial Product (name)**	--
Crop/crop group:	Cotton/Oilseeds	**Producer of commercial product**	BASF SE, Ludwigshafen, Germany
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	**Indoor/Glasshouse/Outdoor**	Outdoor
Country	Germany	**Other active substance in the formulation**	None
Content of active substance (g/kg or g/L)	90 g/L	**(common name and content)**	
Formulation (e.g. WP)	SL (code BAS 555 01 F)	**Residues calculated as:**	Metconazole (BAS 555 F)

1	2	3	4	5			6	7	8	9			10	11
Report No Location (Trial No)	**Commodity/ Variety**	**Date of 1. Sowing / Planting 2. Flowering 3. Harvest**	**Method of treatment**	**Application rate[0] per treatment**			**No of treat-ment(s) and last date**	**Growth stage (BBCH)[2]**	**Portion analyzed**	**Residues[3] (mg/kg)**			**DALA[1]**	**Remarks**
				kg a.s./hL	**Water L/ha**	**kg a.s./ha**				***Cis*-isomer**	***Trans*-isomer**	**Total[6]**		
254317 2007/7001663 Uvalde, TX USA RCN R06454	SO 0691 DPL 444	1. 20.04.06 2. n.r. 3. 08.09.06	Foliar spray	0.08	130	0.11	3 08.08.06	81	Seed[4]	0.017 (0.011, 0.023)	<0.005 (<0.005, 0.005)	0.022 (0.016, 0.028)	31	BASF method No D0604 LOQ 0.005 mg/kg per isomer
									Gin bpdts[5]	0.139 (0.116, 0.161)	0.032 (0.027, 0.037)	0.17 (0.14, 0.20)	31	
				0.39	140	0.55			Seed	0.106 (0.116, 0.097)	0.026 (0.029, 0.023)	0.132 (0.144, 0.120)	31	
									Meal	<0.005	<0.005	<0.01		
									Hulls	0.011 (0.007, 0.014)	<0.005 (<0.005,<0.005)	0.016 (0.012, 0.019)		
									Refined oil	0.011 (0.011, 0.011)	<0.005 (<0.005,<0.005)	0.016 (0.016, 0.016)		
254317 2007/7001663 Levelland, TX USA RCN R06455	SO 0691 FM9063 B2F	1. 01.06.06 2. n.r. 3. 16.11.06	Foliar spray	0.08	140	0.11	3 17.10.06	79-80	Seed[4]	0.033 (0.023, 0.042)	0.009 (0.006, 0.011)	0.042 (0.030, 0.053)	30	BASF method No D0604 LOQ 0.005 mg/kg per isomer
									Gin bpdts[5]	3.368 (2.952, 3.783)	0.780 (0.703, 0.856)	4.15 (3.65, 4.64)	30	
254317 2007/7001663 Wolfforth, TX USA RCN R06456	SO 0691 Fiber Max 960 BG II	1. 29.05.06 2. n.r. 3. 12.11.06	Foliar spray	0.08	140	0.11	3 13.10.06	79	Seed[4]	0.058 (0.065, 0.050)	0.014 (0.015, 0.012)	0.071 (0.080, 0.062)	30	BASF method No D0604 LOQ 0.005 mg/kg per isomer
									Gin bpdts[5]	3.155 (3.203, 3.106)	0.516 (0.529, 0.502)	3.67 (3.73, 3.61)	30	
254317 2007/7001663 Hinton, OK USA RCN R06457	SO 0691 DG 2242 B2RF	1. 25.05.06 2. n.r. 3. 03.11.06	Foliar spray	0.06-0.10	110-200	0.11	3 02.10.06	87	Seed[4]	0.061 (0.063, 0.058)	0.015 (0.016, 0.014)	0.075 (0.078, 0.071)	32	BASF method No D0604 LOQ 0.005 mg/kg per isomer
									Gin bpdts[5]	2.288 (2.098, 2.478)	0.530 (0.512, 0.547)	2.82 (2.61, 3.02)	32	
254317 2007/7001663 Dill City, OK USA RCN R06458	SO 0691 FM 960 B2R	1. 21.06.06 2. n.r. 3. 06.11.06	Foliar spray	0.06	200	0.11	3 06.10.06	5% open bolls	Seed[4]	0.015 (0.016, 0.014)	0.005 (0.006, <0.005)	0.021 (0.022, 0.019)	31	BASF method No D0604 LOQ 0.005 mg/kg per isomer

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Metconazole (BAS 555 F)	**Commercial Product (name)**	--
Crop/crop group:	Cotton/Oilseeds	**Producer of commercial product**	BASF SE, Ludwigshafen, Germany
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	**Indoor/Glasshouse/Outdoor**	Outdoor
Country	Germany	**Other active substance in the formulation**	None
Content of active substance (g/kg or g/L)	90 g/L	**(common name and content)**	
Formulation (e.g. WP)	SL (code BAS 555 01 F)	**Residues calculated as:**	Metconazole (BAS 555 F)

1	2	3	4	5			6	7	8	9			10	11
Report No Location (Trial No)	**Commodity/ Variety**	**Date of 1. Sowing / Planting 2. Flowering 3. Harvest**	**Method of treatment**	**Application rate[0] per treatment**			**No of treat-ment(s) and last date**	**Growth stage (BBCH)[2]**	**Portion analyzed**	**Residues[3] (mg/kg)**			**DALA[1]**	**Remarks**
				kg a.s./hL	**Water L/ha**	**kg a.s./ha**				***Cis*-isomer**	***Trans*-isomer**	**Total[6]**		
254317 2007/7001663 Tulare, CA USA RCN R06459	SO 0691 Sierra RR	1. 05.05.06 2. n.r. 3. 20.10.06	Foliar spray	0.06	200	0.11	3 20.09.06	76	Seed[4]	0.041 (0.028, 0.053)	0.010 (0.007, 0.012)	0.051 (0.036, 0.065)	30	BASF method No D0604 LOQ 0.005 mg/kg per isomer
254317 2007/7001663 Terra Bella, CA USA RCN R06460	SO 0691 Roundup Ready	1. 25.05.06 2. n.r. 3. 26.10.06	Foliar spray	0.06	200	0.11	3 26.09.06	76	Seed[4]	0.018 (0.017, 0.019)	0.005 (0.005, 0.005)	0.024 (0.023, 0.025)	30	BASF method No D0604 LOQ 0.005 mg/kg per isomer
254317 2007/7001663 Yuma, AZ USA RCN R06461	SO 0691 45001 G	1. 19.04.06 2. n.r. 3. 13.10.06	Foliar spray	0.05	230	0.11	3 14.09.06	50% open bolls	Seed[4]	0.188 (0.177, 0.199)	0.044 (0.041, 0.047)	0.232 (0.218, 0.246)	29	BASF method No D0604 LOQ 0.005 mg/kg per isomer

0 Actual application rates varied by 10% at most except where noted otherwise
1 Days after last application
2 At last treatment
3 Mean of two samples; individual values are given in parentheses
4 Undelinted
5 Gin byproducts
6 Differences in calculations of total metconazole (mean values) are due to rounding
n.r. Not reported

Maize / Sweet corn

Treatment with BAS 555 01 F on maize and sweet corn in North America

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Metconazole (BAS 555 F)	**Commercial Product (name)**	--
Crop/crop group:	Maize/Cereals Sweet corn/Fruiting vegetables	**Producer of commercial product** **Indoor/Glasshouse/Outdoor**	BASF SE, Ludwigshafen, Germany Outdoor
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	**Other active substance in the formulation**	None
Country	Germany	**(common name and content)**	
Content of active substance (g/kg or g/L)	90 g/L	**Residues calculated as:**	Metconazole (BAS 555 F)
Formulation (e.g. WP)	SL (code BAS 555 01 F)		

1	2	3	4	5			6	7	8	9			10	11
Report No Location (Trial No)	**Commodity/ Variety**	**Date of 1. Sowing / Planting 2. Flowering 3. Harvest**	**Method of treatment**	**Application rate[0] per treatment**			**No of treat-ment(s) and last date**	**Growth stage (BBCH)[2]**	**Portion analyzed**	**Residues[3] (mg/kg)**			**DALA[1]**	**Remarks**
				kg a.s./hL	**Water L/ha**	**kg a.s./ha**				***Cis*-isomer**	***Trans*-isomer**	**Total[6]**		
254314 2006/7012839 South Sodus, NY USA RCN R06425	VO 0447 Speedy Sweet	1. 08.07.06 2. n.r. 3. 08.09.06	Foliar spray	0.04	280	0.11	4 01.09.06	59-71	Forage K+CWHR	0.03 (0.02, 0.03) 0.005 (<0.005, 0.006)	0.01 (0.01, 0.01) <0.005 (<0.005,<0.005)	0.04 (0.03, 0.04) 0.010 (<0.01,0.011)	7 7	BASF method No D0604 LOQ 0.005 mg/kg per isomer
254314 2006/7012839 Germansville, PA USA RCN R06426	GC 0645 TA5750	1. 27.05.06 2. n.r. 3. 23.08.-04.10.06	Foliar spray	0.04	290	0.11	4 16.08.06	Milk stage	Forage K+CWHR	0.69 (0.71, 0.66) <0.005	0.14 (0.13, 0.14) <0.005	0.82 (0.83, 0.80) <0.01	7 7	BASF method No D0604 LOQ 0.005 mg/kg per isomer
				0.04	290	0.11	4 13.09.06	87	Grain Stover	<0.005 0.88 (0.74, 1.01)	<0.005 0.27 (0.18, 0.36)	<0.01 1.15 (0.93, 1.37)	21 21	
254314 2006/7012839 Seven Springs, NC USA RCN R06427	GC 0645 Garst 8377	1. 11.04.06 2. n.r. 3. 18.07.-11.09.06	Foliar spray	0.04- 0.07	150- 280	0.11	4 11.07.06	67	Forage K+CWHR	1.42 (1.48, 1.36) <0.005	0.45 (0.44, 0.45) <0.005	1.87 (1.92, 1.81) <0.01	7 7	BASF method No D0604 LOQ 0.005 mg/kg per isomer
				0.04	270	0.11	4 21.08.06	87	Grain Stover	<0.005 0.11	<0.005 0.03	<0.01 0.14	21 21	
254314 2006/7012839 O'Brien, FL USA RCN R06428	VO 0447 8102 R Bicolor	1. 09.08.06 2. n.r. 3. 18.10.06	Foliar spray	0.05	220	0.11	4 11.10.06	75	Forage K+CWHR	0.95 (0.89, 1.00) <0.005	0.20 (0.19, 0.21) <0.005	1.15 (1.08, 1.21) <0.01	7 7	BASF method No D0604 LOQ 0.005 mg/kg per isomer
254314 2006/7012839 New Holland, OH USA RCN R06429	GC 0645 Crows 7R154	1. 04.05.06 2. n.r. 3. 16.08.-14.10.06	Foliar spray	0.06	200	0.11	4 09.08.06	73	Forage K+CWHR	0.01 <0.005	0.09 <0.005	0.10 <0.01	7 7	BASF method No D0604
				0.06	200	0.11	4 24.09.06	87	Grain Stover	<0.005 1.66 (1.66, 1.65)	<0.005 0.37 (0.34, 0.39)	<0.01 2.02 (2.00, 2.04)	20 20	LOQ 0.005 mg/kg per isomer

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Metconazole (BAS 555 F)	**Commercial Product (name)**	--
Crop/crop group:	Maize/Cereals Sweet corn/Fruiting vegetables	**Producer of commercial product** **Indoor/Glasshouse/Outdoor**	BASF SE, Ludwigshafen, Germany Outdoor
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	**Other active substance in the formulation**	None
Country	Germany	**(common name and content)**	
Content of active substance (g/kg or g/L)	90 g/L	**Residues calculated as:**	Metconazole (BAS 555 F)
Formulation (e.g. WP)	SL (code BAS 555 01 F)		

1	2	3	4	5			6	7	8	9			10	11
Report No Location (Trial No)	**Commodity/ Variety**	**Date of 1. Sowing / Planting 2. Flowering 3. Harvest**	**Method of treatment**	**Application rate[0] per treatment**			**No of treat-ment(s) and last date**	**Growth stage (BBCH)[2]**	**Portion analyzed**	**Residues[3] (mg/kg)**			**DALA[1]**	**Remarks**
				kg a.s./hL	**Water L/ha**	**kg a.s./ha**				***Cis*-isomer**	***Trans*-isomer**	**Total[6]**		
254314 2006/7012839 Atlanta, OH USA RCN R06430	GC 0645 Crows 5151	1. 09.05.06 2. n.r. 3. 31.08.-14.10.06	Foliar spray	0.07	150	0.11	4 24.08.06	85	Forage	1.21 (1.21, 1.21)	0.29 (0.30, 0.27)	1.49 (1.51, 1.47)	7	BASF method No D0604 LOQ 0.005 mg/kg per isomer
				0.07	150	0.11	4 24.09.06	87	Grain Stover	<0.005 1.33 (1.21, 1.45)	<0.005 0.28 (0.21, 0.35)	<0.01 1.61 (1.41, 1.80)	20 20	
254314 2006/7012839 Carlyle, IL USA RCN R06431	GC 0645 BT6516 RR2YG	1. 20.04.06 2. n.r. 3. 16.08.-05.10.06	Foliar spray	0.06	170	0.11	4 09.08.06	R3-R4	Forage	0.36	0.17	0.53	7	BASF method No D0604 LOQ 0.005 mg/kg per isomer
				0.06	170	0.11	4 14.09.06	R6	Grain Stover Grain AGF	<0.005 2.23 (2.59, 1.87) <0.005 0.032 (0.027, 0.037)	<0.005 0.46 (0.54, 0.37) <0.005 0.007 (0.006, 0.008)	<0.01 2.69 (3.13, 2.24) <0.01 0.04 (0.03, 0.04)	21 21 21	
				0.05-0.08	130-220	0.55	4 14.09.06	R6	Grain Grits Meal Flour Ref. oil[4] Starch Ref. oil[5]	<0.005 <0.005 <0.005 <0.005 <0.005 <0.005 0.006 (0.007, 0.006)	<0.005 <0.005 <0.005 <0.005 <0.005 <0.005 <0.005 (<0.005,<0.005)	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01 0.011 (0.012, 0.011)	21	
254314 2006/7012839 Mason, IL USA RCN R06432	GC 0645 Burrus 664 RWR-PX4	1. 24.05.06 2. n.r. 3. 25.08.-29.09.06	Foliar spray	0.07	150	0.11	4 18.08.06	85	Forage	0.25 (0.20, 0.29)	1.01 (0.96, 1.05)	1.25 (1.16, 1.34)	7	BASF method No D0604 LOQ 0.005 mg/kg per isomer
				0.07	150	0.11	4 08.09.06	87-89	Grain Stover	<0.005 1.16 (1.17, 1.14)	<0.005 0.22 (0.22, 0.21)	<0.01 1.37 (1.39, 1.35)	21 21	

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Metconazole (BAS 555 F)	**Commercial Product (name)**	--
Crop/crop group:	Maize/Cereals Sweet corn/Fruiting vegetables	**Producer of commercial product** **Indoor/Glasshouse/Outdoor**	BASF SE, Ludwigshafen, Germany Outdoor
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	**Other active substance in the formulation**	None
Country	Germany	**(common name and content)**	
Content of active substance (g/kg or g/L)	90 g/L	**Residues calculated as:**	Metconazole (BAS 555 F)
Formulation (e.g. WP)	SL (code BAS 555 01 F)		

1	2	3	4	5			6	7	8	9			10	11
Report No Location (Trial No)	**Commodity/ Variety**	**Date of 1. Sowing / Planting 2. Flowering 3. Harvest**	**Method of treatment**	**Application rate[0] per treatment**			**No of treat-ment(s) and last date**	**Growth stage (BBCH)[2]**	**Portion analyzed**	**Residues[3] (mg/kg)**			**DALA[1]**	**Remarks**
				kg a.s./hL	**Water L/ha**	**kg a.s./ha**				***Cis*-isomer**	***Trans*-isomer**	**Total[6]**		
254314 2006/7012839 Wyoming, IL USA RCN R06433	GC 0645 Burrus 644 RWR	1. 20.05.06 2. n.r. 3. 22.08.-12.10.06	Foliar spray	0.08	140	0.11	4 16.08.06	R3	Forage K+CWHR	0.02 (0.02, 0.01) <0.005	0.15 (0.17, 0.13) <0.005	0.17 (0.19, 0.14) <0.01	6 6	BASF method No D0604 LOQ
				0.07	160	0.11	4 20.09.06	R5	Grain Stover	<0.005 1.69 (1.58, 1.80)	<0.005 0.40 (0.37, 0.43)	<0.01 2.10 (1.96, 2.23)	22 22	0.005 mg/kg per isomer
254314 2006/7012839 Danville, IN USA RCN R06434	GC 0645 Wyffels W5531	1. 09.05.06 2. n.r. 3. 23.08.-27.09.06	Foliar spray	0.06- 0.08	140- 180	0.11	4 16.08.06	83	Forage	1.02 (1.16, 0.88)	0.15 (0.13, 0.17)	0.17 (1.29, 1.05)	7	BASF method No D0604
				0.07	150	0.11	4 06.09.06	87-89	Grain Stover	<0.005 1.50 (1.09, 1.91)	<0.005 0.30 (0.21, 0.39)	<0.01 1.81 (1.31, 2.30)	21 21	LOQ 0.005 mg/kg per isomer
254314 2006/7012839 Bellmore, IN USA RCN R06435	GC 0645 Wyffels W5531	1. 31.05.06 2. n.r. 3. 23.08.-18.10.06	Foliar spray	0.06- 0.07	150- 180	0.11	4 16.08.06	71-73	Forage K+CWHR	0.83 (0.90, 0.76) <0.005	0.08 (0.06, 0.09) <0.005	0.91 (0.96, 0.85) <0.01	7 7	BASF method No D0604 LOQ
				0.07	160	0.11	4 27.09.06	87	Grain Stover	<0.005 2.61 (2.84, 2.38)	<0.005 0.62 (0.69, 0.54)	<0.01 3.23 (3.53, 2.93)	21 21	0.005 mg/kg per isomer
254314 2006/7012839 Richland, IA USA RCN R06436	GC 0645 Golden Harvest HX 9323	1. 27.04.06 2. n.r. 3. 17.08.-05.10.06	Foliar spray	0.06- 0.09	120- 180	0.11	4 10.08.06	R4	Forage	0.38 (0.31, 0.45)	0.09 (0.07, 0.11)	0.48 (0.38, 0.57)	7	BASF method No D0604
				0.06- 0.07	150- 180	0.11	4 14.09.06	R5	Grain Stover	<0.005 1.14 (1.13, 1.15)	<0.005 0.32 (0.32, 0.32)	<0.01 1.46 (1.45, 1.47)	21 21	LOQ 0.005 mg/kg per isomer
254314 2006/7012839 Hedrick, IA USA RCN R06437	GC 0645 Pioneer 34A16	1. 19.04.06 2. n.r. 3. 21.08.-26.09.06	Foliar spray	0.07	150	0.11	4 14.08.06	R4	Forage	0.39 (0.45, 0.32)	0.14 (0.11, 0.16)	0.52 (0.56, 0.48)	7	BASF method No D0604
				0.07	150	0.11	4 05.09.06	R5	Grain Stover	<0.005 1.50 (1.33, 1.66)	<0.005 0.27 (0.19, 0.35)	<0.01 1.77 (1.52, 2.01)	21 21	LOQ 0.005 mg/kg per isomer

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Metconazole (BAS 555 F)	**Commercial Product (name)**	--
Crop/crop group:	Maize/Cereals Sweet corn/Fruiting vegetables	**Producer of commercial product** **Indoor/Glasshouse/Outdoor**	BASF SE, Ludwigshafen, Germany Outdoor
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	**Other active substance in the formulation**	None
Country	Germany	**(common name and content)**	
Content of active substance (g/kg or g/L)	90 g/L	**Residues calculated as:**	Metconazole (BAS 555 F)
Formulation (e.g. WP)	SL (code BAS 555 01 F)		

1	2	3	4	5			6	7	8	9			10	11
Report No Location (Trial No)	**Commodity/ Variety**	**Date of 1. Sowing / Planting 2. Flowering 3. Harvest**	**Method of treatment**	**Application rate[0] per treatment**			**No of treat-ment(s) and last date**	**Growth stage (BBCH)[2]**	**Portion analyzed**	**Residues[3] (mg/kg)**			**DALA[1]**	**Remarks**
				kg a.s./hL	**Water L/ha**	**kg a.s./ha**				***Cis*-isomer**	***Trans*-isomer**	**Total[6]**		
254314 2006/7012839 Ollie, IA USA RCN R06438	GC 0645 Middlekoop 2212	1. 38.04.06 2. n.r. 3. 16.08.-05.10.06	Foliar spray	0.06-0.09	120-170	0.11	4 10.08.06	R2.5	Forage K+CWHR	0.81 (0.95, 0.66) <0.005	0.17 (0.14, 0.19) <0.005	0.97 (1.09, 0.85) <0.01	7 7	BASF method No D0604 LOQ 0.005 mg/kg per isomer
				0.06-0.07	150-180	0.11	4 14.09.06	R5	Grain Stover	<0.005 1.91 (2.16, 1.66)	<0.005 0.39 (0.44, 0.34)	<0.01 2.30 (2.60, 2.00)	21 21	
254314 2006/7012839 Bagley, IA USA RCN R06439	GC 0645 33P65	1. 11.05.06 2. n.r. 3. 15.08.-18.10.06	Foliar spray	0.06	200	0.11	4 08.08.06	75	Forage	0.69 (0.73, 0.65)	0.11 (0.10, 0.11)	0.80 (0.83, 0.76)	7	BASF method No D0604 LOQ 0.005 mg/kg per isomer
				0.06	200	0.11	4 27.09.06	85	Grain Stover	0.010 (0.013, 0.006) 1.61 (1.87, 1.35)	<0.005 (<0.005,<0.005) 0.30 (0.37, 0.22)	0.015 (0.018, 0.011) 1.91 (2.25, 1.57)	21 21	
254314 2006/7012839 Delavan, WI USA RCN R06440	GC 0645 DKC52-40 (RR2/YGPL)	1. 20.05.06 2. n.r. 3. 29.08.-26.10.06	Foliar spray	0.06	170	0.11	4 22.08.06	R3	Forage K+CWHR	0.97 (1.02, 0.92) <0.005	0.18 (0.19, 0.17) <0.005	1.15 (1.21, 1.08) <0.01	7 7	BASF method No D0604 LOQ 0.005 mg/kg per isomer
				0.06	170	0.11	4 05.10.06	R5	Grain Stover	<0.005 1.49 (1.19, 1.78)	<0.005 0.28 (0.22, 0.33)	<0.01 1.76 (1.41, 2.11)	21 21	
254314 2006/7012839 Ellendale, MN USA RCN R06441	GC 0645 Pioneer 38H66	1. 05.05.06 2. n.r. 3. 27.08.-11.10.06	Foliar spray	0.07	160	0.11	4 20.08.06	R4	Forage	1.02 (0.96, 1.07)	0.24 (0.18, 0.30)	1.26 (1.14, 1.38)	7	BASF method No D0604 LOQ 0.005 mg/kg per isomer
				0.07	160	0.11	4 20.09.06	R5/R6	Grain Stover	<0.005 2.25 (2.14, 2.36)	<0.005 0.48 (0.47, 0.48)	<0.01 2.72 (2.61, 2.83)	21 21	
254314 2006/7012839 Geneva, MN USA RCN R06442	GC 0645 Pioneer 38H66	1. 07.05.06 2. n.r. 3. 27.08.-11.10.06	Foliar spray	0.07	160	0.11	4 20.08.06	R4	Forage	0.88 (0.92, 0.84)	0.17 (0.18, 0.16)	1.05 (1.10, 1.00)	7	BASF method No D0604 LOQ 0.005 mg/kg per isomer
				0.07	160	0.11	4 20.09.06	R5/R6	Grain Stover	<0.005 1.59 (1.84, 1.34)	<0.005 0.35 (0.41, 0.28)	<0.01 1.94 (2.25, 1.63)	21 21	

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Metconazole (BAS 555 F)	**Commercial Product (name)**	--
Crop/crop group:	Maize/Cereals Sweet corn/Fruiting vegetables	**Producer of commercial product** **Indoor/Glasshouse/Outdoor**	BASF SE, Ludwigshafen, Germany Outdoor
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	**Other active substance in the formulation**	None
Country	Germany	**(common name and content)**	
Content of active substance (g/kg or g/L)	90 g/L	**Residues calculated as:**	Metconazole (BAS 555 F)
Formulation (e.g. WP)	SL (code BAS 555 01 F)		

1	2	3	4	5			6	7	8	9			10	11
Report No Location (Trial No)	**Commodity/ Variety**	**Date of 1. Sowing / Planting 2. Flowering 3. Harvest**	**Method of treatment**	**Application rate[0] per treatment**			**No of treat-ment(s) and last date**	**Growth stage (BBCH)[2]**	**Portion analyzed**	**Residues[3] (mg/kg)**			**DALA[1]**	**Remarks**
				kg a.s./hL	**Water L/ha**	**kg a.s./ha**				***Cis*-isomer**	***Trans*-isomer**	**Total[6]**		
254314 2006/7012839 York, NE USA RCN R06443	GC 0645 Pioneer 34N45 RR/YG	1. 20.04.06 2. n.r. 3. 21.08.-18.09.06	Foliar spray	0.06	180	0.11	4 14.08.06	85	Forage	0.01 (0.01, 0.01)	0.10 (0.09, 0.10)	0.11 (0.10, 0.11)	7	BASF method No D0604 LOQ 0.005 mg/kg per isomer
				0.06	180	0.11	4 28.08.06	87	Grain Stover	<0.005 1.30 (1.38, 1.22)	<0.005 0.21 (0.22, 0.20)	<0.01 1.51 (1.60, 1.42)	21 21	
254314 2006/7012839 Grand Island, NE USA RCN R06444	GC 0645 NK N73-F7 RR/LL/YG	1. 09.05.06 2. n.r. 3. 23.08.-20.09.06	Foliar spray	0.06	180	0.11	4 16.08.06	85	Forage	0.01 (0.01, 0.01)	0.09 (0.09, 0.08)	0.10 (0.10, 0.09)	7	BASF method No D0604 LOQ 0.005 mg/kg per isomer
				0.06	180	0.11	4 30.08.06	87	Grain Stover	<0.005 0.79 (0.84, 0.73)	<0.005 0.15 (0.16, 0.14)	<0.01 0.94 (1.00, 0.88)	21 21	
254314 2006/7012839 Osceola, NE USA RCN R06445	GC 0645 NK N73-F7	1. 26.04.06 2. n.r. 3. 21.08.-19.09.06	Foliar spray	0.06	190	0.11	4 14.08.06	85	Forage	0.01 (0.01, 0.01)	0.08 (0.08, 0.07)	0.09 (0.09, 0.08)	7	BASF method No D0604 LOQ 0.005 mg/kg per isomer
				0.06	190	0.11	4 29.08.06	87	Grain Stover	<0.005 1.70 (1.60, 1.79)	<0.005 0.33 (0.32, 0.34)	<0.01 2.03 (1.92, 2.13)	21 21	
254314 2006/7012839 Dill City, OK USA RCN R06446	GC 0645 DK C48-53 AF2	1. 08.06.06 2. n.r. 3. 01.09.-20.10.06	Foliar spray	0.06	200	0.11	4 25.08.06	83	Forage	1.62 (1.79, 1.45)	0.15 (0.13, 0.16)	1.77 (1.92, 1.61)	7	BASF method No D0604 LOQ 0.005 mg/kg per isomer
				0.06	200	0.11	4 29.09.06	87	Grain Stover	0.005 2.05 (1.72, 2.37)	<0.005 0.40 (0.35, 0.45)	0.01 2.45 (2.07, 2.82)	21 21	
254314 2006/7012839 Porterville, CA USA RCN R06447	VO 0447 Bodacious	1. 08.05.06 2. n.r. 3. 28.07.06	Foliar spray	0.04	290	0.11	4 21.07.06	78	Forage K+CWHR	2.16 (2.15, 2.17) <0.005	0.50 (0.46, 0.54) <0.005	2.66 (2.61, 2.71) <0.01	7 7	BASF method No D0604 LOQ 0.005 mg/kg per isomer
254314 2006/7012839 Ephrata, WA USA RCN R06448	VO 0447 Golden Jubilee	1. 10.05.06 2. n.r. 3. 15.08.06	Foliar spray	0.08	140	0.11	4 08.08.06	73	Forage K+CWHR	0.02 (0.02, 0.02) <0.005	0.17 (0.14, 0.20) <0.005	0.19 (0.16, 0.22) <0.01	7 7	BASF method No D0604 LOQ 0.005 mg/kg per isomer

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Metconazole (BAS 555 F)	**Commercial Product (name)**	--
Crop/crop group:	Maize/Cereals Sweet corn/Fruiting vegetables	**Producer of commercial product** **Indoor/Glasshouse/Outdoor**	BASF SE, Ludwigshafen, Germany Outdoor
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	**Other active substance in the formulation**	None
Country	Germany	**(common name and content)**	
Content of active substance (g/kg or g/L)	90 g/L	**Residues calculated as:**	Metconazole (BAS 555 F)
Formulation (e.g. WP)	SL (code BAS 555 01 F)		

1	2	3	4	5			6	7	8	9			10	11
Report No Location (Trial No)	**Commodity/ Variety**	**Date of 1. Sowing / Planting 2. Flowering 3. Harvest**	**Method of treatment**	**Application rate[0] per treatment**			**No of treat-ment(s) and last date**	**Growth stage (BBCH)[2]**	**Portion analyzed**	**Residues[3] (mg/kg)**			**DALA[1]**	**Remarks**
				kg a.s./hL	**Water L/ha**	**kg a.s./ha**				***Cis*-isomer**	***Trans*-isomer**	**Total[6]**		
254314 2006/7012839 Corvallis, OR USA RCN R06449	VO 0447 Super Sweet Jubilee Plus	1. 11.05.06 2. n.r. 3. 29.08.06	Foliar spray	0.09	120	0.11	4 22.08.06	73	Forage K+CWHR	0.96 (1.01, 0.91) <0.005	0.17 (0.17, 0.16) <0.005	1.13 (1.19, 1.06) <0.01	7 7	BASF method No D0604 LOQ 0.005 mg/kg per isomer

0 Actual application rates varied by 10% at most except where noted otherwise
1 Days after last application
2 At last treatment
3 Mean of two samples; individual values are given in parentheses
4 Dry milling
5 Wet milling
6 Differences in calculations of total metconazole (mean values) are due to rounding
K+CWHR Kernel plus cob with husk removed
n.r. Not reported

Wheat

Treatment with BAS 555 01 F on wheat in North America

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Metconazole (BAS 555 F)	**Commercial Product (name)**	--
Crop/crop group:	Wheat/Cereals	**Producer of commercial product**	BASF SE, Ludwigshafen, Germany
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	**Indoor/Glasshouse/Outdoor**	Outdoor
Country	Germany	**Other active substance in the formulation**	None
Content of active substance (g/kg or g/L)	90 g/L	**(common name and content)**	
Formulation (e.g. WP)	SL (code BAS 555 01 F)	**Residues calculated as:**	Metconazole (BAS 555 F)

1	2	3	4	5			6	7	8	9			10	11
Report No Location (Trial No)	**Commodity/ Variety**	**Date of 1. Sowing / Planting 2. Flowering 3. Harvest**	**Method of treatment**	**Application rate[0] per treatment**			**No of treat-ment(s) and last date**	**Growth stage (BBCH)[2]**	**Portion analyzed**	**Residues[3] (mg/kg)**			**DALA[1]**	**Remarks**
				kg a.s /hL	**Water L/ha**	**kg a.s./ha**				***Cis*-isomer**	***Trans*-isomer**	**Total[6]**		
137711 2006/7006723 Sunsweet, GA USA RCN R05044	GC 0654 Pioneer 26R24 (winter)	1. 07.12.04 2. n.r. 3. 14.04.-27.05.05	Foliar spray	0.06	170	0.11	2 30.03.05	Flag leaf stage	Hay	3.480 (3.160, 3.800)	0.682 (0.628, 0.736)	4.162 (3.788, 4 536)	7	BASF method No D0508 LOQ 0.005 mg/kg per isomer
				0.08	140	0.11	2 06.05.05	Soft dough	Grain	0.029 (0.030, 0.027)	0.007 (0.007, 0.007)	0.036 (0.037, 0.035)	21	
									Straw	1.415 (1.200, 1.630)	0.309 (0.269, 0 349)	1.724 (1.469, 1 979)	21	
137711 2006/7006723 Newport, AR USA RCN R05045	GC 0654 Genesis R033 (winter)	1. 05.11.04 2. n.r. 3. 14.05.-06.06.05	Foliar spray	0.06	190	0.11	2 02.05.05	Full flowering	Hay	5.280 (5.140, 5.420)	0.904 (0.868, 0 940)	6.184 (6.008, 6 360)	7	BASF method No D0508 LOQ 0.005 mg/kg per isomer
				0.06	190	0.11	2 16.05.05	Medium milk	Grain	0.080 (0.076, 0.083)	0.014 (0.011, 0.017)	0.094 (0.087, 0 100)	21	
									Straw	6.650 (6.500, 6.800)	1.205 (1.130, 1 280)	7.855 (7.630, 8.080)	21	
137711 2006/7006723 York, NE USA RCN R05046	GC 0654 Millennium (winter)	1. 27.09.04 2. n.r. 3. 10.06.05-06.07.05	Foliar spray	0.06	190	0.11	2 02.06.05	End of flowering	Hay	1.184 (1.164, 1.204)	0.173 (0.168, 0 178)	1.357 (1.332, 1 382)	8	BASF method No D0508 LOQ 0.005 mg/kg per isomer
				0.06	190	0.11	2 16.06.05	Early dough	Grain	0.014 (0.012, 0.016)	0.005 (0.005,<0.005)	0.019 (0.017, 0.021	20	
									Straw	4.525 (3.300, 5.750)	0.776 (0.553, 0 999)	5.301 (3.853, 6.749)	20	

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Metconazole (BAS 555 F)	**Commercial Product (name)**	--
Crop/crop group:	Wheat/Cereals	**Producer of commercial product**	BASF SE, Ludwigshafen, Germany
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	**Indoor/Glasshouse/Outdoor**	Outdoor
Country	Germany	**Other active substance in the formulation**	None
Content of active substance (g/kg or g/L)	90 g/L	**(common name and content)**	
Formulation (e.g. WP)	SL (code BAS 555 01 F)	**Residues calculated as:**	Metconazole (BAS 555 F)

1	2	3	4	5			6	7	8	9			10	11
Report No Location (Trial No)	**Commodity/ Variety**	**Date of 1. Sowing / Planting 2. Flowering 3. Harvest**	**Method of treatment**	**Application rate[0] per treatment**			**No of treat-ment(s) and last date**	**Growth stage (BBCH)[2]**	**Portion analyzed**	**Residues[3] (mg/kg)**			**DALA[1]**	**Remarks**
				kg a.s /hL	**Water L/ha**	**kg a.s./ha**				***Cis*-isomer**	***Trans*-isomer**	**Total[6]**		
137711 2006/7006723 Carlyle, IL USA RCN R05047	GC 0654 Excel 201 (winter)	1. 16.10.04 2. n.r. 3. 17.05.-07.07.05	Foliar spray	0.08	140	0.11	2 12.05.05	Full flowering: 50% of anthers mature	Hay Hay Hay	9.430 (9.000, 9.860) 6.190 (6.740, 5.640) 4.380 (4.940, 3.820)	1.504 (1.544, 1.464) 1.094 (1.176, 1.012) 0.822 (0.820, 0.824)	10.934 (10 544, 11.324) 7.284 (7.916, 6.652) 5.202 (5.760, 4.644)	0 7 14	BASF method No D0508 LOQ 0.005 mg/kg per isomer
				0.07-0.10	110-160	0.11	2 02.06.05	Early dough	Grain Straw Grain Straw Grain Straw Grain Straw	0.034 (0.028, 0.040) 2.793 (2.510, 3.075) 0.058 (0.065, 0.051) 3.833 (3.860, 3.805) 0.044 (0.042, 0.046) 3.955 (3.660, 4.250) 0.035 (0.031, 0.039) 3.315 (3.435, 3.195)	0.006 (0.007,<0.005) 0.547 (0.515, 0 578) 0.011 (0.011, 0.010) 0.779 (0.816, 0.741) 0.007 (0.008,<0.005) 0.796 (0.743, 0.849) 0.007 (<0.005,0.009) 0.645 (0.661, 0.628)	0.040 (0.034, 0.045 3.339 (3.025, 3.653) 0.070 (0.077, 0.062) 4.611 (4.676, 4 546) 0.051 (0.051, 0.051) 4.751 (4.403, 5.099) 0.042 (0.036, 0.048) 3.960 (4.096, 3.823)	14 21 28 35	
137711 2006/7006723 Grand Island, NE USA RCN R05048	GC 0654 Jagalene HRW (winter)	1. 29.09.04 2. n.r. 3. 08.06.-07.07.05	Foliar spray	0.06	190	0.11	2 01.06.05	End of flowering	Hay	1.672 (1.824, 1.520)	0.334 (0.362, 0 306)	2.006 (2.186, 1.826)	7	BASF method No D0508 LOQ 0.005 mg/kg per isomer
				0.06	190	0.11	2 15.06.05	Late milk	Grain Straw	0.010 (0.012, 0.008) 1.875 (1.410, 2.340)	<0.005 (<0.005,<0.005) 0.422 (0.305, 0 538)	0.015 (0.017, 0.013) 2.297 (1.715, 2.878)	22	

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Metconazole (BAS 555 F)	**Commercial Product (name)**	--
Crop/crop group:	Wheat/Cereals	**Producer of commercial product**	BASF SE, Ludwigshafen, Germany
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	**Indoor/Glasshouse/Outdoor**	Outdoor
Country	Germany	**Other active substance in the formulation**	None
Content of active substance (g/kg or g/L)	90 g/L	**(common name and content)**	
Formulation (e.g. WP)	SL (code BAS 555 01 F)	**Residues calculated as:**	Metconazole (BAS 555 F)

1	2	3	4	5			6	7	8	9			10	11
Report No Location (Trial No)	**Commodity/ Variety**	**Date of 1. Sowing / Planting 2. Flowering 3. Harvest**	**Method of treatment**	**Application rate[0] per treatment**			**No of treat-ment(s) and last date**	**Growth stage (BBCH)[2]**	**Portion analyzed**	**Residues[3] (mg/kg)**			**DALA[1]**	**Remarks**
				kg a.s /hL	**Water L/ha**	**kg a.s./ha**				***Cis*-isomer**	***Trans*-isomer**	**Total[6]**		
137711 2006/7006723 Velva, ND USA RCN R05049	GC 0654 Dapps (spring)	1. 15.04.05 2. n.r. 3. 06.07.-01.08.05	Foliar spray	0.10	110	0.11	2 27.06.05	Full flowering	Hay	5.000 (4.080, 5.920)	0.916 (0.740, 1.092)	5.916 (4.820, 7.012)	8	BASF method No D0508 LOQ 0.005 mg/kg per isomer
				0.10	110	0.11	2 12.07.05	Medium milk	Grain	0.032 (0.031, 0.032)	0.009 (0.009, 0.009)	0.040 (0.039, 0.040)	20	
									Straw	2.108 (2.004, 2.212)	0.380 (0.347, 0.412)	2.488 (2.351, 2.624)		
137711 2006/7006723 Gardner, ND USA RCN R05050	GC 0654 Knudson (spring)	1. 06.05.05 2. n.r. 3. 20.07.-29.08.05	Foliar spray	0.07	150	0.11	2 16.07.05	Early milk	Hay	10.160 (10.000, 10.320)	1.682 (1.764, 1.600)	11.842 (11.764, 11.920)	0	BASF method No D0508 LOQ 0.005 mg/kg per isomer
									Hay	5.130 (5.080, 5.180)	0.904 (0.868, 0 940)	6.034 (5.948, 6 120)	8	
									Hay	3.324 (3.180, 3.468)	0.616 (0.592, 0.640)	3.940 (3.772, 4 108)	14	
				0.07	150	0.11	2 24.07.05	Late milk	Grain	0.015 (0.013, 0.017)	<0.005 (<0.005,<0.005)	0.020 (0.018, 0.022)	14	
									Straw	1.108 (1.108, 1.108)	0.174 (0.177, 0 170)	1.282 (1.285, 1 278)		
									Grain	0.010 (0.009, 0.011)	<0.005 (<0.005,<0.005)	0.015 (0.014, 0.016)	22	
									Straw	1.098 (1.260, 0.936)	0.144 (0.161, 0 126)	1.242 (1.421, 1.062)		
									Grain	0.008	<0.005	0.013	28	
									Straw	1.038 (1.044, 1.032)	0.151 (0.154, 0 147)	1.189 (1.198, 1 179)		
									Grain	0.008 (0.007, 0.008)	<0.005 (<0.005,<0.005)	0.013 (0.012, 0.013)	36	
									Straw	0.760 (0.660, 0.860)	0.108 (0.097, 0 118)	0.868 (0.757, 0 978)		

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Metconazole (BAS 555 F)	**Commercial Product (name)**	--
Crop/crop group:	Wheat/Cereals	**Producer of commercial product**	BASF SE, Ludwigshafen, Germany
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	**Indoor/Glasshouse/Outdoor**	Outdoor
Country	Germany	**Other active substance in the formulation**	None
Content of active substance (g/kg or g/L)	90 g/L	**(common name and content)**	
Formulation (e.g. WP)	SL (code BAS 555 01 F)	**Residues calculated as:**	Metconazole (BAS 555 F)

1	2	3	4	5			6	7	8	9			10	11
Report No Location (Trial No)	**Commodity/ Variety**	**Date of 1. Sowing / Planting 2. Flowering 3. Harvest**	**Method of treatment**	**Application rate[0] per treatment**			**No of treat-ment(s) and last date**	**Growth stage (BBCH)[2]**	**Portion analyzed**	**Residues[3] (mg/kg)**			**DALA[1]**	**Remarks**
				kg a.s /hL	**Water L/ha**	**kg a.s./ha**				***Cis*-isomer**	***Trans*-isomer**	**Total[6]**		
137711 2006/7006723 Hinton, OK USA RCN R05051	GC 0654 Jagalene (winter)	1. 15.10.04 2. n.r. 3. 18.05.-10.06.05	Foliar spray	0.08	130	0.11	2 05.05.05	First grains half final size	Hay	6.220 (6.720, 5.720)	1.050 (1.136, 0 964)	7.270 (7.856, 6.684)	7	BASF method No D0508 LOQ 0.005 mg/kg per isomer
				0.08	130	0.11	2 19.05.05	Soft dough	Grain Straw	0.006 0.361 (0.218, 0.504)	<0.005 0.059 (0.048, 0.070)	0.011 0.420 (0.266, 0 574)	22 22	
137711 2006/7006723 Colony, OK USA RCN R05052	GC 0654 Jagger (winter)	1. 10.10.04 2. n.r. 3. 19.05.-09.06.05	Foliar spray	0.08	130	0.11	2 06.05.05	Late milk	Hay	8.330 (8.540, 8.120)	1.396 (1.400, 1 392)	9.726 (9.940, 9 512)	6	BASF method No D0508 LOQ 0.005 mg/kg per isomer
				0.08-0.11	100-130	0.11	2 19.05.05	Soft dough	Grain Straw	0.080 (0.097, 0.063) 8.285 (8.180, 8.390)	0.016 (0.019, 0.013) 1.444 (1.436, 1.452)	0.096 (0.116, 0.075) 9.729 (9.616, 9.842)	21 21	
137711 2006/7006723 Lubbock, TX USA RCN R05053	GC 0654 TAM 200 (winter)	1. 06.09.04 2. n.r. 3. 12.05.-17.06.05	Foliar spray	0.08	140	0.11	2 26.04.05	End of heading	Hay	8.970 (8.440, 9.500)	1.682 (1.644, 1.720)	10.652 (10.084, 11.220)	7	BASF method No D0508 LOQ 0.005 mg/kg per isomer
				0.08	140	0.11	2 27.05.05	Hard dough	Grain Straw	0.029 (0.026, 0.031) 3.466 (3.080, 3.852)	0.006 (0.005, 0.006) 0.662 (0.564, 0.760)	0.035 (0.032, 0.037) 4.138 (3.664, 4.612)	21 21	
137711 2006/7006723 Payette, ID USA RCN R05054	GC 0654 Penawawa (spring)	1. 22.04.05 2. n.r. 3. 05.07.-17.08.05	Foliar spray	0.04	280	0.11	2 23.06.05	Full flowering	Hay	5.080 (4.860, 5.300)	0.828 (0.916, 0.740)	5.908 (5.776, 6.040)	7	BASF method No D0508 LOQ 0.005 mg/kg per isomer
				0.04	280	0.11	2 27.07.05	Hard dough	Grain Straw	0.041 (0.043, 0.038) 0.772 (0.632, 0.912)	0.008 (0.008, 0.007) 4.132 (3.544, 4.720)	0.048 (0.051, 0.045) 4.904 (4.176, 5.632)	21 21	
137711 2006/7006723 Laird, SK Canada RCN R05055	GC 0654 Bounty (spring)	1. 23.05.05 2. n.r. 3. 11.08.-06.09.05	Foliar spray	0.08	140	0.11	2 27.07.05	Full flowering	Hay	10.940 (10.980, 10.900)	1.640 (1.684, 1 596)	12.580 (12.664, 12.496)	7	BASF method No D0508 LOQ 0.005 mg/kg per isomer
				0.08	140	0.11	2 16.08.05	Soft dough	Grain Straw	0.018 (0.019, 0.017) 0.157 (0.153, 0.160)	<0.005 (<0.005,<0.005) 1.032 (1.044, 1.020)	0.023 (0.024, 0.022) 1.189 (1.197, 1 180)	21 21	

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Metconazole (BAS 555 F)	**Commercial Product (name)**	--
Crop/crop group:	Wheat/Cereals	**Producer of commercial product**	BASF SE, Ludwigshafen, Germany
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	**Indoor/Glasshouse/Outdoor**	Outdoor
Country	Germany	**Other active substance in the formulation**	None
Content of active substance (g/kg or g/L)	90 g/L	**(common name and content)**	
Formulation (e.g. WP)	SL (code BAS 555 01 F)	**Residues calculated as:**	Metconazole (BAS 555 F)

1	2	3	4	5			6	7	8	9			10	11
Report No Location (Trial No)	**Commodity/ Variety**	**Date of 1. Sowing / Planting 2. Flowering 3. Harvest**	**Method of treatment**	**Application rate[0] per treatment**			**No of treat-ment(s) and last date**	**Growth stage (BBCH)[2]**	**Portion analyzed**	**Residues[3] (mg/kg)**			**DALA[1]**	**Remarks**
				kg a.s /hL	**Water L/ha**	**kg a.s./ha**				***Cis*-isomer**	***Trans*-isomer**	**Total[6]**		
137711 2006/7006723 Minto, MB Canada RCN R05056	GC 0654 AC Barrie (spring)	1. 05.05.05 2. n.r. 3. 23.07.-29.08.05	Foliar spray	0.05	210	0.11	2 12.07.05	First grains half final size	Hay	5.380 (5.520, 5.240)	0.886 (0.956, 0.816)	6.266 (6.476, 6.056)	7	BASF method No D0508 LOQ 0.005 mg/kg per isomer
				0.05	210	0.11	2 08.08.05	Soft dough	Grain Straw	0.022 (0.020, 0.023) 0.387 (0.416, 0.358)	<0.005 (<0.005,<0.005) 2.118 (2.040, 2 196)	0.027 (0.025, 0.028) 2.505 (2.456, 2 554)	21 21	
137711 2006/7006723 Innisfail, AB Canada RCN R05057	GC 0654 AC Intrepid (spring)	1. 13.05.05 2. n.r. 3. 04.08.-23.09.05	Foliar spray	0.05	240	0.11	2 15.07.05	Begin of flowering	Hay	4.720 (4.780, 4.660)	0.790 (0.744, 0.836)	5.510 (5.524, 5.496)	7	BASF method No D0508 LOQ 0.005 mg/kg per isomer
				0.05	230	0.11	2 02.09.05	Soft dough	Grain Straw	0.022 (0.022, 0.022) 0.379 (0.353, 0.404)	0.005 (0.005, 0.005) 2.090 (2.068, 2 112)	0.028 (0.028, 0.027) 2.469 (2.421, 2 516)	21 21	
137711 2006/7006723 Innisfail, AB Canada RCN R05058	GC 0654 5700PR (spring)	1. 06.05.05 2. n.r. 3. 04.08.-19.09.05	Foliar spray	0.05	230	0.11	2 14.07.05	Full flowering	Hay	5.740 (5.500, 5.980)	0.918 (1.012, 0.824)	6.658 (6.512, 6.804)	7	BASF method No D0508 LOQ 0.005 mg/kg per isomer
				0.05	210	0.11	2 29.08.05	Soft dough	Grain Straw	0.044 0.554 (0.554, 0.554)	0.010 2.944 (2.820, 3.068)	0.054 3.488 (3.364, 3.612)	21 21	

0 Actual application rates varied by 10% at most except where noted otherwise
1 Days after last application
2 At last treatment
3 Mean of two samples; individual values are given in parentheses
6 Differences in calculations of total metconazole (mean values) are due to rounding
n.r. Not reported

Sugar beet

Treatment with BAS 555 01 F on sugar beet in North America

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Metconazole (BAS 555 F)	**Commercial Product (name)**	--
Crop/crop group:	Sugar beet/Sugar plants	**Producer of commercial product**	BASF SE, Ludwigshafen, Germany
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	**Indoor/Glasshouse/Outdoor**	Outdoor
Country	Germany	**Other active substance in the formulation**	None
Content of active substance (g/kg or g/L)	90 g/L	**(common name and content)**	
Formulation (e.g. WP)	SL (code BAS 555 01 F)	**Residues calculated as:**	Metconazole (BAS 555 F)

1	2	3	4	5			6	7	8	9			10	11
Report No Location (Trial No)	**Commodity/ Variety**	**Date of 1. Sowing / Planting 2. Flowering 3. Harvest**	**Method of treatment**	**Application rate[0] per treatment**			**No of treat-ment(s) and last date**	**Growth stage (BBCH)[2]**	**Portion analyzed**	**Residues[3] (mg/kg)**			**DALA[1]**	**Remarks**
				kg a.s./hL	**Water L/ha**	**kg a.s./ha**				***Cis*-isomer**	***Trans*-isomer**	**Total[6]**		
137729 2006/7006726 Dumfries, MN USA RCN R05085	VR 0596 VDH66556 8232 Medium	1. 25.05.05 2. n.r. 3. 29.09.05	Foliar spray	0.06	190	0.11	2 15.09.05	Crop cover complete	Tops	1.047 (0.997, 1.077)	0.173 (0.167, 0.178)	1.210 (1.163, 1.256)	14	BASF method No D0508 LOQ 0.005 mg/kg per isomer
									Roots	0.005 (<0.005,0.006)	<0.005 (<0.005,<0.005)	0.010 (0.011,<0.01)		
				0.09		0.17			Tops	1.865 (1.662, 2.067)	0.384 (0.342, 0.426)	2.249 (2.005, 2.493)	14	
									Roots	0.010 (0.007, 0.012)	<0.005 (<0.005,<0.005)	0.015 (0.012, 0.017)		
137729 2006/7006726 Theilman, MN USA RCN R05086	VR 0596 VDH66556 8232 Medium	1. 25.05.05 2. n.r. 3. 29.09.05	Foliar spray	0.06	190	0.11	2 15.09.05	Crop cover complete	Tops	0.898 (0.870, 0.926)	0.145 (0.145, 0.145)	1.043 (1.015, 1.070)	14	BASF method No D0508 LOQ 0.005 mg/kg per isomer
									Roots	0.005 (<0.005,0.005)	<0.005 (<0.005,<0.005)	0.010 (<0.01,0.010)		
				0.09		0.17			Tops	0.871 (0.811, 0.931)	0.140 (0.131, 0.148)	1.011 (0.942, 1.079)	14	
									Roots	0.010 (0.011, 0.008)	<0.005 (<0.005,<0.005)	0.015 (0.016, 0.013)		

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Metconazole (BAS 555 F)	**Commercial Product (name)**	--
Crop/crop group:	Sugar beet/Sugar plants	**Producer of commercial product**	BASF SE, Ludwigshafen, Germany
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	**Indoor/Glasshouse/Outdoor**	Outdoor
Country	Germany	**Other active substance in the formulation**	None
Content of active substance (g/kg or g/L)	90 g/L	**(common name and content)**	
Formulation (e.g. WP)	SL (code BAS 555 01 F)	**Residues calculated as:**	Metconazole (BAS 555 F)

1	2	3	4	5			6	7	8	9			10	11
Report No Location (Trial No)	**Commodity/ Variety**	**Date of 1. Sowing / Planting 2. Flowering 3. Harvest**	**Method of treatment**	**Application rate[0] per treatment**			**No of treat-ment(s) and last date**	**Growth stage (BBCH)[2]**	**Portion analyzed**	**Residues[3] (mg/kg)**			**DALA[1]**	**Remarks**
				kg a.s./hL	**Water L/ha**	**kg a.s./ha**				***Cis*-isomer**	***Trans*-isomer**	**Total[6]**		
137729 2006/7006726 Arkansaw, WI USA RCN R05087	VR 0596 VDH66556 8232 Medium	1. 08.04.05 2. n.r. 3. 21.09.-18.10.05	Foliar spray	0.06	190	0.11	2 20.09.05	Crop cover complete	Tops	2.078 (1.852, 2.303)	0.362 (0.326, 0.397)	2.440 (2.179, 2.700)	1	BASF method No D0508 LOQ 0.005 mg/kg per isomer
									Roots	<0.005	<0.005	<0.01		
									Tops	1.363 (1.298, 1.428)	0.248 (0.246, 0.249)	1.611 (1.674, 1.547)	7	
									Roots	<0.005	<0.005	<0.01		
									Tops	0.894 (0.724, 1.064)	0.130 0.112, 0.148)	1.024 (0.835, 1.212)	14	
									Roots	<0.005	<0.005	<0.01		
									Tops	0.765 (0.794, 0.736)	0.110 (0.111, 0.109)	0.885 (0.905, 0.845)	21	
									Roots	<0.005	<0.005	<0.01		
									Tops	0.661 (0.816, 0.506)	0.082 (0.104, 0.059)	0.742 (0.919, 0.565)	28	
									Roots	<0.005	<0.005	<0.01		
				0.09		0.17			Tops	2.671 (2.892, 2.450)	0.492 (0.516, 0.467)	3.163 (3.408, 2.917)	1	
									Roots	<0.005	<0.005	<0.01		
									Tops	2.567 (2.346, 2.788)	0.449 (0.413, 0.485)	3.016 (2.759, 3.273)	7	
									Roots	<0.005	<0.005	<0.01		
									Tops	1.693 (1.593, 1.792)	0.270 (0.215, 0.324)	1.598 (1.808, 2.116)	14	
									Roots	<0.005	<0.005	<0.01		
									Tops	1.363 (1.463, 1.263)	0.170 (0.188, 0.152)	1.533 (1.650, 1.415)	21	
									Roots	<0.005	<0.005	<0.01		
									Tops	0.938 (0.716, 1.160)	0.130 (0.105, 0.154)	1.067 (0.821, 1.313)	28	
									Roots	<0.005	<0.005	<0.01		

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Metconazole (BAS 555 F)	**Commercial Product (name)**	--
Crop/crop group:	Sugar beet/Sugar plants	**Producer of commercial product**	BASF SE, Ludwigshafen, Germany
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	**Indoor/Glasshouse/Outdoor**	Outdoor
Country	Germany	**Other active substance in the formulation**	None
Content of active substance (g/kg or g/L)	90 g/L	**(common name and content)**	
Formulation (e.g. WP)	SL (code BAS 555 01 F)	**Residues calculated as:**	Metconazole (BAS 555 F)

1	2	3	4	5			6	7	8	9			10	11
Report No Location (Trial No)	**Commodity/ Variety**	**Date of 1. Sowing / Planting 2. Flowering 3. Harvest**	**Method of treatment**	**Application rate[0] per treatment**			**No of treat-ment(s) and last date**	**Growth stage (BBCH)[2]**	**Portion analyzed**	**Residues[3] (mg/kg)**			**DALA[1]**	**Remarks**
				kg a.s./hL	**Water L/ha**	**kg a.s./ha**				***Cis*-isomer**	***Trans*-isomer**	**Total[6]**		
137729 2006/7006726 Fitchburg, WI USA	VR 0596 Vanderhave VDH 66556	1. 01.07.05 2. n.r. 3. 12.10.05	Foliar spray	0.05	220	0.11	2 29.09.05	Crop cover complete	Tops	0.015 (0.014, 0.015)	0.127 (0.116, 0.138)	0.142 (0.130, 0.153)	13	BASF method No D0508 LOQ 0.005 mg/kg per isomer
									Roots	0.025 (0.031, 0.018)	0.006 (0.008,<0.005)	0.031 (0.039, 0.023)		
RCN R05088				0.08		0.17			Tops	0.028 (0.030, 0.026)	0.104 (0.005, 0.203)	0.132 (0.035, 0.229)	13	
									Roots	0.025 (0.026, 0.024)	0.006 (0.005, 0.006)	0.030 (0.031, 0.029)		
137729 2006/7006726 Fitchburg, WI USA	VR 0596 Vanderhave VDH 66556	1. 01.07.05 2. n.r. 3. 12.10.05	Foliar spray	0.05	220	0.11	2 28.09.05	Crop cover complete	Tops	0.033 (0.034, 0.031)	0.006 (0.006, 0.006)	0.039 (0.040, 0.037)	14	BASF method No D0508 LOQ 0.005 mg/kg per isomer
									Roots	0.036 (0.032, 0.039)	0.008 (0.007, 0.009)	0.044 (0.040, 0.048)		
RCN R05089				0.08		0.17			Tops	0.055 (0.050, 0.060)	0.011 (0.010, 0.011)	0.066 (0.060, 0.071	14	
									Roots	0.069 (0.067, 0.070)	0.015 (0.015, 0.015)	0.083 (0.082, 0.084)		
137729 2006/7006726 Eldridge, ND USA	VR 0596 66453 (Vanderhave)	1. 18.05.05 2. n.r. 3. 28.09.05	Foliar spray	0.06	190	0.11	2 14.09.05	Root harvestable size	Tops	0.038 (0.021, 0.055)	0.088 (0.164, 0.011)	0.126 (0.185, 0.066)	14	BASF method No D0508 LOQ 0.005 mg/kg per isomer
									Roots	0.015 (0.019, 0.010)	<0.005 (<0.005,<0.005)	0.020 (0.024, 0.015)		
RCN R05090				0.09		0.17			Tops	0.041 (0.057, 0.024)	0.103 (0.011, 0.194)	0.143 (0.068, 0.218)	14	
									Roots	0.014 (0.012, 0.016)	<0.005 (<0.005,<0.005)	0.019 (0.017, 0.021)		
137729 2006/7006726 Levelland, TX USA	VR 0596 Eagle R	1. 12.05.05 2. n.r. 3. 13.10.05	Foliar spray	0.06	190	0.11	2 29.09.05	Canopy 80% closed	Tops	0.010 (0.010, 0.010)	0.081 (0.079, 0.082)	0.091 (0.089, 0.092)	14	BASF method No D0508 LOQ 0.005 mg/kg per isomer
									Roots	<0.005 (<0.005,<0.005)	0.005 (<0.005,0.006)	0.010 (<0.01,0.011)		
RCN R05091				0.09		0.17			Tops	0.019 (0.019, 0.018)	0.148 (0.144, 0.151)	0.166 (0.162, 0.170)	14	
									Roots	0.007 (0.009, 0.005)	0.006 (<0.005,0.007)	0.014 (0.014, 0.013)		

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Metconazole (BAS 555 F)	**Commercial Product (name)**	--
Crop/crop group:	Sugar beet/Sugar plants	**Producer of commercial product**	BASF SE, Ludwigshafen, Germany
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	**Indoor/Glasshouse/Outdoor**	Outdoor
Country	Germany	**Other active substance in the formulation**	None
Content of active substance (g/kg or g/L)	90 g/L	**(common name and content)**	
Formulation (e.g. WP)	SL (code BAS 555 01 F)	**Residues calculated as:**	Metconazole (BAS 555 F)

1	2	3	4	5			6	7	8	9			10	11
Report No Location (Trial No)	**Commodity/ Variety**	**Date of 1. Sowing / Planting 2. Flowering 3. Harvest**	**Method of treatment**	**Application rate[0] per treatment**			**No of treat-ment(s) and last date**	**Growth stage (BBCH)[2]**	**Portion analyzed**	**Residues[3] (mg/kg)**			**DALA[1]**	**Remarks**
				kg a.s./hL	**Water L/ha**	**kg a.s./ha**				***Cis*-isomer**	***Trans*-isomer**	**Total[6]**		
137729 2006/7006726 Pavillion, MT USA RCN R05092	VR 0596 Treasure	1. 13.04.05 2. n.r. 3. 27.09.05	Foliar spray	0.06	170	0.11	2 13.09.05	Full maturity	Tops	0.016 (0.014, 0.017)	0.133 (0.132, 0.133)	0.148 (0.146, 0.150)	14	BASF method No D0508 LOQ 0.005 mg/kg per isomer
									Roots	0.008 (0.009, 0.006)	0.011 (0.014, 0.008)	0.019 (0.023, 0.014)		
				0.10		0.17			Tops	0.043 (0.043, 0.042)	0.008 (0.008, 0.008)	0.051 (0.052, 0.050)	14	
									Roots	0.027 (0.018, 0.035)	0.006 (0.005, 0.007)	0.034 (0.024, 0.043)		
137729 2006/7006726 Hughson, CA USA RCN R05093	VR 0596 Unknown	1. 22.04.05 2. n.r. 3. 28.09.05	Foliar spray	0.05	240	0.11	2 14.09.05	Crop cover complete	Tops	0.013 (0.012, 0.014)	0.097 (0.098, 0.096)	0.110 (0.110, 0.109)	14	BASF method No D0508 LOQ 0.005 mg/kg per isomer
									Roots	0.007 (0.010,<0.005)	0.012 (0.016, 0.007)	0.019 (0.026, 0.012)		
				0.07		0.17			Tops	0.020 (0.019, 0.021)	0.168 (0.164, 0.171)	0.188 (0.183, 0.193)	14	
									Roots	0.005 (0.006,<0.005)	0.008 (0.008, 0.007)	0.013 (0.013, 0.012)		
137729 2006/7006726 Hickman, CA USA RCN R05094	VR 0596 Unknown	1. 16.04.05 2. n.r. 3. 28.09.05	Foliar spray	0.05	240	0.11	2 14.09.05	Crop cover complete	Tops	0.491 (0.459, 0.522)	0.072 (0.068, 0.075)	0.581 (0.564, 0.597)	14	BASF method No D0508 LOQ 0.005 mg/kg per isomer
									Roots	0.013 (0.013, 0.013)	0.013 (0.021,<0.005)	0.026 (0.034, 0.018)		
				0.07		0.17			Tops	0.961 (0.985, 0.956)	0.144 (0.145, 0.142)	1.015 (1.130, 1.099)	14	
									Roots	0.013 (0.010, 0.015)	0.010 (0.016,<0.005)	0.023 (0.025, 0.020)		

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Metconazole (BAS 555 F)	**Commercial Product (name)**	--
Crop/crop group:	Sugar beet/Sugar plants	**Producer of commercial product**	BASF SE, Ludwigshafen, Germany
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	**Indoor/Glasshouse/Outdoor**	Outdoor
Country	Germany	**Other active substance in the formulation**	None
Content of active substance (g/kg or g/L)	90 g/L	**(common name and content)**	
Formulation (e.g. WP)	SL (code BAS 555 01 F)	**Residues calculated as:**	Metconazole (BAS 555 F)

1	2	3	4	5			6	7	8	9			10	11
Report No Location (Trial No)	**Commodity/ Variety**	**Date of 1. Sowing / Planting 2. Flowering 3. Harvest**	**Method of treatment**	**Application rate[0] per treatment**			**No of treat-ment(s) and last date**	**Growth stage (BBCH)[2]**	**Portion analyzed**	**Residues[3] (mg/kg)**			**DALA[1]**	**Remarks**
				kg a.s./hL	**Water L/ha**	**kg a.s./ha**				***Cis*-isomer**	***Trans*-isomer**	**Total[6]**		
137729 2006/7006726 Payette, ID USA RCN R05095	VR 0596 HM WS91	1. 03.05.05 2. n.r. 3. 28.09.-24.10.05	Foliar spray	0.04	280	0.11	2 27.09.05	Root harvestable size	Tops	0.027 (0.026, 0.028)	<0.005 (<0.005,<0.005)	0.032 (0.031, 0.033)	1	BASF method No D0508 LOQ 0.005 mg/kg per isomer
									Roots	0.008 (0.008, 0.007)	<0.005 (<0.005,<0.005)	0.013 (0.013, 0.012)		
									Tops	0.021 (0.021, 0.020)	0.176 (0.176, 0.176)	0.197 (0.197, 0.196)	7	
									Roots	0.019 (0.025, 0.013)	<0.005 (<0.005,<0.005)	0.024 (0.030, 0.018)		
									Tops	0.014 (0.015, 0.012)	0.102 (0.109, 0.095)	0.116 (0.124, 0.107)	15	
									Roots	0.012 (0.014, 0.009)	<0.005 (<0.005,<0.005)	0.017 (0.019, 0.014)		
									Tops	0.010 (0.008, 0.011)	0.056 (<0.005, 0.107)	0.066 (0.013, 0.118)	21	
									Roots	0.005 (0.006,<0.005)	<0.005 (<0.005,<0.005)	0.010 (0.011,<0.01)		
									Tops	0.009 (0.007, 0.011)	0.078 (0.073, 0.082)	0.087 (0.081, 0.093)	27	
									Roots	0.007 (0.006, 0.007)	<0.005 (<0.005,<0.005)	0.012 (0.011, 0.012)		
				0.06		0.17			Tops	0.035 (0.035, 0.034)	0.006 (0.006, 0.006)	0.041 (0.041, 0.041)	1	
									Roots	0.009 (0.011, 0.006)	<0.005 (<0.005,<0.005)	0.014 (0.016, 0.011)		
									Tops	0.028 (0.026, 0.029)	0.103 (<0.05,0.201)	0.131 (0.031, 0.230)	7	
									Roots	0.020 (0.023, 0.017)	<0.005 (<0.005,<0.005)	0.025 (0.028, 0.022)		
									Tops	0.016 (0.014, 0.017)	0.136 (0.135, 0.137)	0.152 (0.149, 0.154)	15	
									Roots	0.019 (0.015, 0.023)	<0.005 (<0.005,<0.005)	0.024 (0.020, 0.028)		

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Metconazole (BAS 555 F)	**Commercial Product (name)**	--
Crop/crop group:	Sugar beet/Sugar plants	**Producer of commercial product**	BASF SE, Ludwigshafen, Germany
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	**Indoor/Glasshouse/Outdoor**	Outdoor
Country	Germany	**Other active substance in the formulation**	None
Content of active substance (g/kg or g/L)	90 g/L	**(common name and content)**	
Formulation (e.g. WP)	SL (code BAS 555 01 F)	**Residues calculated as:**	Metconazole (BAS 555 F)

1	2	3	4	5			6	7	8	9			10	11
Report No Location (Trial No)	**Commodity/ Variety**	**Date of 1. Sowing / Planting 2. Flowering 3. Harvest**	**Method of treatment**	**Application rate[0] per treatment**			**No of treat-ment(s) and last date**	**Growth stage (BBCH)[2]**	**Portion analyzed**	**Residues[3] (mg/kg)**			**DALA[1]**	**Remarks**
				kg a.s./hL	**Water L/ha**	**kg a.s./ha**				***Cis*-isomer**	***Trans*-isomer**	**Total[6]**		
									Tops	0.019 (0.017, 0.020)	0.153 (0.132, 0.174)	0.172 (0.149, 0.194)	21	
									Roots	0.011 (0.013, 0.009)	<0.005 (<0.005,<0.005)	0.016 (0.018, 0.014)		
									Tops	0.015 (0.016, 0.013)	0.114 (0.130, 0.098)	0.129 (0.146, 0.111)	27	
									Roots	0.009 (0.009, 0.008)	<0.005 (<0.005,<0.005)	0.014 (0.014, 0.013)		
137729 2006/7006726 Jerome, WI USA RCN R05096	VR 0596 Beta 8422	1. 15.04.05 2. n.r. 3. 30.09.05	Foliar spray	0.06	180	0.11	2 16.09.05	Root harvestable size	Tops	0.826 (0.783, 0.868)	0.140 (0.116, 0.164)	0.966 (0.899, 1.032)	14	BASF method No D0508 LOQ 0.005 mg/kg per isomer
									Roots	0.017 (0.018, 0.015)	<0.005 (<0.005,<0.005)	0.022 (0.023, 0.020)		
				0.09		0.17			Tops	1.642 (1.812, 1.471)	0.240 (0.262, 0.218)	1.882 (2.074, 1.689)	14	
									Roots	0.033 (0.032, 0.034)	0.007 (0.007, 0.007)	0.040 (0.039, 0.041)		

0 Actual application rates varied by 10% at most except where noted otherwise
1 Days after last application
2 At last treatment
3 Mean of two samples; individual values are given in parentheses
6 Differences in calculations of total metconazole (mean values) are due to rounding
n.r. Not reported

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Metconazole

Document M-CA, Section 7

FATE AND BEHAVIOUR IN THE ENVIRONMENT

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Version history[1]

Date	Data points containing amendments or additions and brief description	Document identifier and version number
08/Jan/2015	KCA 7.1.2.2.1/4 Interim report 2014/1001261 was replaced by final report 2015/1204922 KCA 7.1.2.2.1/1 Reference to interim report 2014/1001261 was replaced by final report 2015/1204922	Document MCA Section 7 Version 2 (BASF DocID 2016/1030846)

[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 How to revise an Assessment Report

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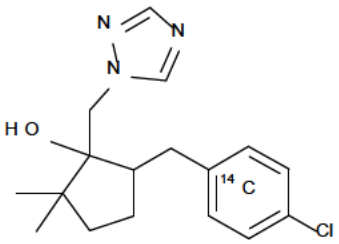
CA 7 FATE AND BEHAVIOUR IN THE ENVIRONMENT

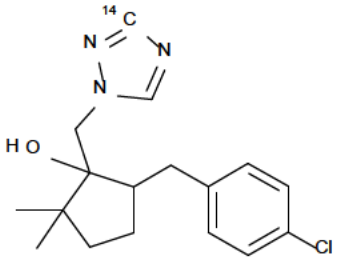
All relevant information on the first Annex I review and the endpoints used in environmental risk assessments can be found in the EFSA Scientific Report (2006) 64, 1-71 and in the SANCO/10027/2006 final document (EU Review Report). In this Supplemental Dossier for renewal of approval, only those environmental fate studies are summarized which are submitted for the first time. However, relevant results of the studies already evaluated are also summarized briefly in order to provide an overall picture of the fate and behaviour of metconazole in the environment.

For the current registration renewal under Regulation 1107/2009, a data gap analysis according to new guidelines, new guidance documents and new procedures in kinetic evaluations and exposure assessments was performed and new studies or kinetic evaluations were initiated where considered necessary. All new data are provided in this section.

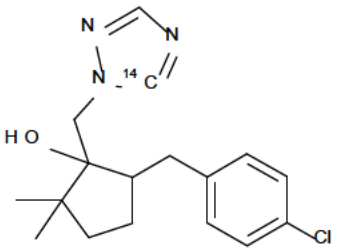
Studies on the route of degradation were usually performed using the chlorophenyl-^{14}C or triazole-^{14}C labeled metconazole. In one study metconazole labeled in the cyclopentanol ring (third possible label) was used to determine the rate of degradation in five soils. No cleavage of the molecule between the cyclopentyl ring and chlorophenyl ring was observed in any of the (peer-reviewed) studies; thus, labelling to the above-mentioned rings is deemed sufficient. Where reasonable, new studies were conducted under consideration of the respective second label or both labels.



U-^{14}C-phenyl-label



3,(5)-triazole-^{14}C-label



Most information on the overall route of degradation in soil was already derived from the peer-reviewed studies. New soil studies were initiated in order to further elucidate the degradation pathway and to provide updated information, i.e. if in a study an unknown metabolite occurred >5% but <10% total applied radioactivity (TAR) and thus was not identified due to the relevant trigger value at that time. An overview of metabolites discussed in this section is provided in Document N3.

Special emphasis was given to the investigation of the fate of the enantiomers of metconazole in various study types. No shift was observed in any of the studies, proving that metconazole isomers do not have to be addressed separately in further data processing (kinetic evaluation, modelling).

Furthermore, a literature search was performed and scientific publications were evaluated for their endpoint relevance and quality. No publications were found that provided a reliable endpoint for usage in the risk assessment. Consequently, for environmental fate no summaries of public literature data on metconazole are provided in this section. Further information on the literature assessment and respective justifications can be found in M-CA 9.

CA 7.1 Fate and behaviour in soil

CA 7.1.1 Route of degradation in soil

CA 7.1.1.1 Aerobic degradation

For the previous Annex I listing, information on the route of degradation of metconazole in aerobic soil were available from two studies [*BASF DocID 2002/7004457 (MK-620-020); BASF DocID 1990/7000168 (MK-620-002)*]. In the first study [*BASF DocID 2002/7004457 (MK-620-020)*], [triazole-3(5)-^{14}C]-labeled metconazole degraded in the sandy loam soil at 20 C to minor metabolites (<5% TAR), an unknown component corresponding to polar material (>5% TAR) and CO_2. The minor metabolites consisted of M555F030 (CL 382389, Reg. No. 4110625) and an unidentified metabolite, the polar material was found to be composed of several polar components, the major component represented an average of 9.1% TAR. In the second study [*BASF DocID 1990/7000168 (MK-620-002)*] metconazole labeled in the cyclopentanol ring was used to determine the rate of degradation in five soils. Results showed that metconazole slowly degraded in all soils incubated at 22 C. The majority of the radioactivity after 112 days was metconazole. Three minor metabolites were detected, each accounting for less than 3.3% of the total applied radioactivity (TAR).

For further elucidation, an additional aerobic soil metabolism study was conducted. The study was performed according to guideline OECD 307 considering [phenyl-U-^{14}C]- and [triazole-3(5)-^{14}C]- metconazole. In addition, enantiomeric separation was considered. The study is submitted under CA 7.1.1.1/1.

In the new study metconazole degraded slowly in sandy loam soil LUFA 5M during incubation under aerobic conditions with a $DegT_{50}$ of 128 days (SFO). Metabolite M555F020 (1,2,4-triazole) was only detected in soil samples treated with triazole-^{14}C-BAS 555 F with a maximum of 1.2% TAR at the end of the incubation period. In addition, four (triazole label) to five (phenyl label) peaks appeared in chromatograms. None of them exceeded 2% TAR at any sampling time. Nevertheless, under one of the peaks metabolite M555F040 was detected by LC-MS/MS. However, it could not be further quantified due to a co-eluting unknown metabolite.

In the already peer-reviewed study the unidentified component >5% was measured at a retention time of approximately 5.6 min. In the new study metabolite M555F020 (1,2,4-triazole) was measured at a retention time of 6.3 min. This metabolite was not analysed in the already peer-reviewed study. Although there is no further evidence, it is highly likely that the unknown polar peak in the already peer-reviewed study contained for a large sum the polar metabolite 1,2,4-triazole. Therefore, 1,2,4-triazole will be considered in the risk assessment using, albeit very conservative due to other potential polar constituents, with the maximum occurrence of 9.1%TAR that was attributed to the main component of the unidentified polar peak.
The amount of non-extractable residues (NER) as well as the distribution between the different humic substance fractions was evaluated in the old study [*BASF DocID 2002/7004457 (MK-620-020)*] as well as in the new study. In the already peer-reviewed study the amount of bound residues increased steadily from 1.9% at day 0 to 39.2% at 120 days. Further analysis of the bound residues at 120 days showed 26, 10, and 1% of the applied radioactivity was associated with the humin, fulvic acid and humic acid fractions, respectively.

In the new study, the non-extractable residues increased from 1.0% TAR at day 0 to 23.1% TAR at 119 DAT for the phenyl-labeled test item and from 1.2% to 41.7% TAR for the triazole-labeled test item. The distribution between the different humic substance fractions was related to the position of the radiolabel. With phenyl-labeled metconazole, where the amount of NER was lower, the major part of radioactivity was associated to the humin fraction. For the triazole-labeled metconazole, the total NER was higher and found predominantly in the fulvic acid fraction. HPLC-MS/MS analysis of the processed NaOH solubilizate of a solvent extracted soil residue (triazole label) revealed that the major portion of the radioactive residues related to the fulvic acid fraction can be attributed to metabolite M555F020 corresponding to 22.8% TAR. This is considered an artificial detection which is most likely caused by the demolishment of the soil structure by NaOH after the parent was bound to the NER. The major part of radioactivity though was found in the humin fraction. In conclusion, the already peer-reviewed study and the new study show comparable results on non-extractable residues.

The major routes of metconazole degradation in soil were either mineralization (phenyl label) or formation of non-extractable residues (triazole label). Throughout the incubation period, enantiomers of cis- and trans-metconazole were almost equally distributed in the pooled soil extracts revealed by chiral radio-HPLC analyses.

These results confirm the already peer-reviewed aerobic degradation pathway; moreover, it enlarges the view on the degradation behaviour in soil, since additional compounds were identified and an unidentified component larger than 5% in the already peer-reviewed study can with acceptable certainty be attributed to one of the identified metabolites in the new study (1,2,4-triazole).

In addition, the results show that investigation of two labels (triazole and chlorophenyl) is sufficient to analyse the degradation behaviour in soil. Based on the results of the degradation study with cyclopenthanol-ring-labeled metconazole [*BASF DocID 1990/7000168 (MK-620-002)*] no major unknown degradation product were observed in five soils. An additional new study with the cyclopentyl label as third label is not required as no further information is expected. Besides the fact that no cleavage of the molecule between the cyclopentyl ring and chlorophenyl ring was observed in any of the (peer-reviewed) studies, the maximum total amount of non-extractable bound residues that would potentially be available for further degradation (fulvic acid fraction) is low. In the total humic substance fraction the unidentified fulvic acid fraction accounted for 5.8% and 8.3% TAR for the phenyl and triazole label, respectively (triazole label: 31.1% TAR minus 22.8% TAR attributed to metabolite M555F020). Under the assumption that these amounts contain multiple peaks it is most likely that individual peaks are <5%. In conclusion, a further study under consideration of the cyclopentyl label was not deemed necessary, as no new information on major unknown degradation products will be generated.

Report:	CA 7.1.1.1/1 Dalkmann P.,Kibat H., 2015a Soil metabolism of Metconazole (BAS 555 F) under aerobic conditions 2014/1000901
Guidelines:	OECD 307, EPA 835.4100
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

The objective of this study was to investigate route and rate of degradation of [phenyl-U-^{14}C]- and [3,(5)-triazole-^{14}C]-metconazole (BAS 555 F; Reg. No. 4056343) in soil.

Each test item was incubated aerobically in a loamy sand (sandy loam, according to USDA) soil at 20ºC and 45% maximum water holding capacity in the dark for 120 days. The nominal application rate was 0.27 mg kg^{-1} dry soil corresponding to a field application rate of 100 g active substance per hectare, calculated on the basis of an equal distribution in the top 2.5 cm soil layer and a soil density of 1.5 g cm^{-3}. Soil aliquots were weighed into test vessels and placed into an incubation cabinet. A closed incubation system with continuous aeration was used with an attached trapping system for the determination of volatile compounds.

At 58 DAT and 119 days after treatment (DAT) the microbial biomass was determined by the substrate induced respiration method.

Soil samples were taken at 0, 3, 7, 14, 28, 43, 63, 91 and 119 DAT for both labels. At all sampling times, soil samples were worked up in duplicate. Soil samples were extracted three times with acetonitrile and twice with acetonitrile:water (50/50, v/v). The individual extracts were analyzed by liquid scintillation counting (LSC). Combined extracts were analyzed by LSC and by radio-HPLC. The remaining soil after extraction was combusted to determine the amount of non-extractable residues (NER). The volatile trapping solutions were checked for radioactivity by LSC so that a full material balance was provided for each sampling interval. Since the fraction of NER was > 10% of the total applied radioactivity (TAR) in some cases, NER were further characterized by treatment with NaOH.

For both radiolabels, the mean material balances of the incubation experiments throughout the incubation period were in the range of 94.0 to 101.5% TAR.

The amount of extractable radioactive residues decreased continuously in the aerated soil from 97.7 (phenyl label) and 99.3% TAR (triazole label) at day 0 to 56.9% TAR (both labels) at 119 DAT.

The non-extractable residues increased from 1.0% TAR at day 0 to 23.1% TAR at 119 DAT for the phenyl-labeled test item and from 1.2% to 41.7% TAR for the triazole-labeled test item. The formation of volatiles was exclusively related to the mineralization to $^{14}CO_2$ and reached a total amount of 14.1% TAR for the phenyl-labeled and only 1.1% TAR for the triazole-labeled metconazole after 119 days of incubation.

Also the distribution between the different humic substance fractions was related to the position of the radiolabel. With phenyl-labeled metconazole, where the amount of NER was lower, the major part of radioactivity was associated to the humin fraction. With the triazole-labeled metconazole, the NER were higher and found predominantly in the fulvic acid fraction.

In the soil extracts, the unchanged test item ^{14}C-metconazole (both labels) represented the major radioactive component. Although metabolites were detected only in amounts < 5% TAR, identification of some peaks could be achieved by co-chromatography with reference items and also LC-MS/MS analyses.

Metabolite M555F030 was found in soil extracts of both radiolabels, metabolite M555F020 (1,2,4-(1H)-triazole) was only detected in extracts of the soil samples treated with triazole-^{14}C-BAS 555 F.

In addition, four (triazole label) to five (phenyl label) peaks appeared in chromatograms. None of them exceeded 2% TAR at any sampling time. Nevertheless, under one of the peaks metabolite M555F040 was detected by LC-MS/MS. However, it could not be further quantified due to a co-eluting unknown metabolite.

Throughout the incubation period, enantiomers of cis- and trans-metconazole were almost equally distributed in the pooled soil extracts revealed by chiral radio-HPLC analyses.

Kinetic analysis and calculation of $DegT_{50}$ and $DegT_{90}$ values for BAS 555 F in soil was performed following the recommendations of the FOCUS Kinetics workgroup. The analysis was conducted by non-linear regression methods employing the software tool KinGUI 2. A $DegT_{50}$ value of 128.4 days and a $DegT_{90}$ value of 426.6 days were calculated according to the best fit kinetics for metconazole in soil LUFA 5M.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

BAS-Code:	BAS 555 F (metaconazole)
Chemical name:	(1RS,5RS,1RS,5SR)-5-(4-chlorobenzyl)-2,2-dimethyl-1-(1H-1,2,4-triazol-1-ylmethyl)cyclopentanol
Molecular formula:	$C_{17}H_{22}ClN_3O$
Molar mass:	319.83 g mol^{-1} (unlabeled)

Label 1 (phenyl label)

Label:	phenyl-U-^{14}C
Isomeric ratio cis/trans:	81.4 : 18.6
Batch No.:	1065-1029
Specific radioactivity of a.s.:	9.26 MBq mg^{-1}
Radiochemical purity:	99.4%
Chemical purity:	92.7%

Label 2 (triazole label)

Label:	3,(5)-triazole-^{14}C
Isomeric ratio cis/trans:	82 : 18
Batch No.:	811-1101
Specific radioactivity of a.s.:	4.84 MBq mg^{-1}
Radiochemical purity:	97.0%
Chemical purity:	92.5%

2. Soil

The German soil LUFA 5M from LUFA (Landwirtschaftliche Untersuchungs- und Forschungsanstalt, Speyer, Germany) was used in this study. It was sampled from 0-20 cm depth. The soil was passed through a 2 mm sieve and stored for no longer than three months at 4°C before use. The soil characteristics are summarized in Table 7.1.1.1-1.

Table 7.1.1.1-1: Characteristics of soil LUFA 5M

Soil designation	**LUFA 5 M** **13/1651/01** (Germany, Origin: LUFA Speyer; sampled in Mechtersheim, Rhineland-Palatinate)
DIN Particle size distribution [%] sand 0.063 – 2 mm silt 0.002 – 0.063 mm clay < 0.002 mm textural class	 53.6 33.3 13.1 loamy sand (Sl4)
USDA Particle size distribution [%] sand 0.050 – 2 mm silt 0.002 – 0.050 mm clay < 0.002 mm textural class	 57.0 29.9 13.1 sandy loam
Total organic C [%] Organic matter [%] [a]	1.98 3.41
pH [H_2O] pH [$CaCl_2$]	7.9 7.4
cation exchange capacity [cmol+ kg^{-1} dry weight]	10.2
max. water holding capacity pF0 [g/100 g dry weight]	27.0
microbial biomass (start of study, according to certificate) [mg C/100 g dry soil]	27.8
microbial biomass (after 58 days) [mg C/100 g dry soil]	24.0 [b]
microbial biomass (end of study) [mg C/100 g dry soil]	19.5 [b]

[a] Organic matter = organic carbon x 1.724

[b] Determined at BASF test facility Limburgerhof

B. STUDY DESIGN

1. Experimental conditions

The test items were applied at a nominal concentration of 0.27 mg ^{14}C-metconazole per kg dry soil (phenyl and triazole label) which corresponds to a field application rate of 100 g a.s. ha^{-1} (calculated on the basis of an equal distribution in the top 2.5 cm soil layer and a soil density of 1.5 g cm^{-3}).

Before application, the soil was readjusted to 45% of the maximum water holding capacity (MWHC). After application to bulk soil, portions of 100 g soil (dry weight basis) were filled into test vessels. The actual application rate was 0.271 mg kg^{-1} for the phenyl-labeled test item and 0.270 mg kg^{-1} for the triazole-labeled test item.

For incubation, all test vessels were connected in line with aeration tubes in the soil metabolism apparatus. For trapping of volatiles possibly evolving from soil during the incubation, test vessels were connected to two gas washing flasks containing ethylene glycol and 0.5 M NaOH. The treated soils were incubated at 20 ± 2°C in the dark.

To determine the microbial biomass at 58 and 119 days after treatment, additional soil portions were incubated under the same conditions as the ^{14}C-treated samples.

2. Sampling

Sampling dates were 0, 3, 7, 14, 28, 43, 63, 91 and 119 days after treatment (DAT) for both labels. At each sampling time, three flasks per label were removed from the incubator. Two flasks were worked up immediately; one flask was stored in a freezer as a reserve. The volatile traps were collected at each sampling day (except day 0) and replaced by flasks with fresh solutions. The trapping solutions were measured for radioactivity by LSC.

3. Description of analytical procedures

For the determination of ERR, the 100 g (dry weight basis) soil samples were consecutively extracted three times with 100 mL of acetonitrile and two times with 100 mL of acetonitrile/water (50:50, v/v) on a laboratory shaker.

After each extraction step, the samples were centrifuged and aliquots of each supernatant were radio-assayed. For HPLC analysis, all five extracts were combined. An aliquot of each pooled extract was evaporated to dryness, re-dissolved in methanol and then analyzed by radio-HPLC.

Radio-HPLC was used to investigate the metabolism of the phenyl- and the triazole-labeled test item. As metconazole is composed of cis/trans isomers as well as enantiomers, the pooled sample extracts were additionally analyzed by chiral radio-HPLC to check for potential shifts in the isomeric and enantiomeric ratio during incubation.

The soil residues remaining after extraction were dried at room temperature under a stream of nitrogen, homogenized, and aliquots were combusted in an oxidizer. The evolved $^{14}CO_2$ was trapped and measured by LSC to determine the amount of the non-extractable radioactive residues (NER).

Since amounts of NER exceeded 10% TAR in several soil samples, some selected dry soil residues (a 28 and a 119 DAT replicate of each label) were further characterized by NaOH treatment and subsequent fractionation into fulvic acids, humic acids, and humins.

Aliquots were extracted three times with 0.5 M NaOH on a laboratory shaker for 8 – 13 hours. All extracts were pooled and acidified with concentrated hydrochloric acid to pH 1.5 to precipitate the humic acid fraction. After centrifugation, the supernatant (fulvic acids) was separated from the precipitate. The precipitate (humic acid fraction) was re-dissolved in NaOH. The humic acid fraction and the fulvic acid fraction were measured for radioactivity. The remaining soil samples after NaOH and water extraction were dried at room temperature. Afterwards, aliquots were combusted. The released $^{14}CO_2$ was trapped and analyzed by LSC to determine the ^{14}C-residues in the humin fraction.

For the determination of the microbial biomass, extra test vessels with untreated soil were analyzed after 58 and 119 days of incubation. The method was based on the determination of oxygen consumption upon addition of glucose. Measurements were made in quadruplicate. The microbial biomass slightly declined over the incubation phase (see Table 7.1.1.1-1). However, the results demonstrate that the soil was still viable and microbially active at days 58 and 119 (end) of the study.

4. Calculation of the degradation rate

Kinetic evaluation was performed in order to derive degradation parameters as triggers for additional work (trigger endpoints) as well as modeling endpoints. Kinetic analysis and calculation of $DegT_{50}$ and $DegT_{90}$ values was performed following the recommendations of the FOCUS Kinetics workgroup [*FOCUS (2006) "Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration" Report of the FOCUS Work Group on Degradation Kinetics, EC Document Reference Sanco/10058/2005 version 1.0 (November 2011), 434 pp.*].

The software package KinGUII (version 2.2014.224.1704) was used for parameter fitting [*SCHÄFER, D., MIKOLASCH, M., RAINBIRD, P., HARVEY, B. (2007) KinGUI: A new kinetic software tool for evaluations according to FOCUS Degradation Kinetics. In: Del Re, A.A.M. et al. (Eds.): Proceedings of the XIII Symposium on Pesticide Chemistry, Piacenza, 2007, p. 916-923., WITT, J., GAO, Z., MEYER, H. (2014) KinGUII, Version 2.2014.224.1704 Bayer CropScience AG*]. The error tolerance and the number of iterations of the optimization tool were set to 0.00001 and 100, respectively.

No metabolites occurred at amounts above 5% TAR during the study period; thus, only the parent substance was considered in the kinetic evaluation.

During the incubation period, no shift in the isomeric ratio cis/trans was observed for any of the labels. Further, findings show that both labels (^{14}C-phenyl and ^{14}C-triazole) provide comparable residue data throughout the study duration. Thus, the respective results for the isomers were summed up for each label and replicate, and radiolabels were considered as experimental replicates in the kinetic evaluation. This results in quadruplicate measurements for metconazole per sampling time, which were considered for the parameter estimation.

II. RESULTS AND DISCUSSION

A. MASS BALANCE

The detailed results on the extractability of radioactive residues and the overall mass balances are presented in Table 7.1.1.1-2 and Table 7.1.1.1-3. The mass balance ranged from 94.0 to 101.5% TAR for the phenyl-labeled test item and from 97.1 to 100.7% TAR for the triazole-labeled test item, respectively.

B. EXTRACTABLE AND BOUND RESIDUES

The amount of extractable radioactive residues in soil decreased continuously from 97.7 (phenyl label) and 99.3% TAR (triazole label) at 0 DAT to 56.9% TAR (both labels) after 119 days of incubation (Table 7.1.1.1-2 and Table 7.1.1.1-3).

The amount of non-extractable residues (NER) increased during the study with both labels, reaching 23.1% TAR (phenyl label) and 41.7% TAR (triazole label) after 119 days.

Table 7.1.1.1-2: Mass balance and distribution of radioactivity after application of phenyl-^{14}C-metconazole to soil LUFA 5M [% TAR]

Days after treatment	Extractable residues						NER	Volatiles [a]			Material balance
	ACN 1	ACN 2	ACN 3	ACN/ H_2O 1	ACN/ H_2O 2	Total		Ethylene glycol	NaOH (CO_2)	Total	
0	77.0	13.4	2.4	3.8	1.0	97.6	0.9	n.d.	n.d.	-	98.5
0	75.3	14.8	2.6	4.0	1.0	97.7	1.0	n.d.	n.d.	-	98.7
0 mean	**76.2**	**14.1**	**2.5**	**3.9**	**1.0**	**97.7**	**1.0**	**-**	**-**	**-**	**98.6**
3	71.5	16.4	2.9	4.9	1.7	97.5	4.0	0.0	0.3	0.3	101.7
3	72.3	15.5	2.8	4.9	1.7	97.2	3.7	0.0	0.3	0.3	101.2
3 mean	**71.9**	**16.0**	**2.9**	**4.9**	**1.7**	**97.3**	**3.9**	**0.0**	**0.3**	**0.3**	**101.5**
7	65.9	17.5	3.4	5.3	1.8	93.9	4.5	0.0	0.6	0.6	99.0
7	67.5	17.4	3.3	5.5	1.8	95.5	4.6	0.0	0.7	0.7	100.8
7 mean	**66.7**	**17.4**	**3.4**	**5.4**	**1.8**	**94.7**	**4.5**	**0.0**	**0.6**	**0.6**	**99.9**
14	64.7	15.1	2.8	5.4	2.1	90.1	6.8	0.0	1.3	1.3	98.1
14	65.7	15.7	3.0	5.8	2.2	92.3	7.2	0.0	1.3	1.3	100.8
14 mean	**65.2**	**15.4**	**2.9**	**5.6**	**2.1**	**91.2**	**7.0**	**0.0**	**1.3**	**1.3**	**99.5**
28	64.2	12.8	2.4	5.7	2.2	87.3	9.2	0.0	2.9	2.9	99.5
28	62.1	13.1	2.5	5.7	2.1	85.6	9.1	0.0	3.1	3.1	97.8
28 mean	**63.2**	**13.0**	**2.5**	**5.7**	**2.1**	**86.5**	**9.2**	**0.0**	**3.0**	**3.0**	**98.6**
43	56.8	14.3	2.9	5.6	2.8	82.4	11.3	0.0	5.1	5.1	98.9
43	56.5	14.2	2.9	5.8	2.9	82.3	11.5	0.0	5.4	5.4	99.3
43 mean	**56.7**	**14.3**	**2.9**	**5.7**	**2.9**	**82.4**	**11.4**	**0.0**	**5.3**	**5.3**	**99.1**
63	51.3	14.0	2.9	5.6	2.3	76.2	15.3	0.0	8.1	8.1	99.6
63	50.3	13.8	2.8	5.7	2.4	75.0	15.2	0.0	8.8	8.8	99.0
63 mean	**50.8**	**13.9**	**2.9**	**5.7**	**2.3**	**75.6**	**15.2**	**0.0**	**8.4**	**8.4**	**99.3**
91	44.7	11.9	2.4	5.0	2.2	66.2	19.1	0.0	9.2	9.2	94.5
91	44.0	10.9	2.1	5.2	2.2	64.4	18.7	0.0	11.5	11.5	94.6
91 mean	**44.3**	**11.4**	**2.2**	**5.1**	**2.2**	**65.3**	**18.9**	**0.0**	**10.3**	**10.3**	**94.5**
119	38.3	10.4	2.2	4.9	2.3	58.1	23.0	0.0	12.7	12.7	93.8
119	36.7	9.7	2.1	4.8	2.3	55.6	23.2	0.0	15.4	15.4	94.2
119 mean	**37.5**	**10.0**	**2.1**	**4.9**	**2.3**	**56.9**	**23.1**	**0.0**	**14.1**	**14.1**	**94.0**

TAR = Total applied radioactivity (100% TAR = 0.271 mg kg^{-1})
NER = Non-extractable residues
ACN = Acetonitrile
n.d. = Not determined
[a] No other volatiles than CO_2 were found values for volatile radioactive residues were calculated cumulatively.

Table 7.1.1.1-3: Mass balance and distribution of radioactivity after application of triazole-^{14}C-metconazole to soil LUFA 5M [% TAR]

Days after treatment	Extractable residues						NER	Volatiles [a]			Material balance
	ACN 1	ACN 2	ACN 3	ACN/ H_2O 1	ACN/ H_2O 2	Total		Ethylene glycol	NaOH (CO_2)	Total	
0	76.2	15.1	2.6	4.1	1.1	99.1	1.2	n.d.	n.d.	-	100.3
0	78.4	13.6	2.4	4.0	1.1	99.5	1.2	n.d.	n.d.	-	100.7
0 mean	**77.3**	**14.4**	**2.5**	**4.1**	**1.1**	**99.3**	**1.2**	**-**	**-**	**-**	**100.5**
3	71.6	15.8	2.8	5.0	1.8	97.0	3.8	0.0	0.0	0.0	100.8
3	71.0	15.8	2.8	5.2	1.9	96.7	3.9	0.0	0.0	0.0	100.6
3 mean	**71.3**	**15.8**	**2.8**	**5.1**	**1.8**	**96.8**	**3.8**	**0.0**	**0.0**	**0.0**	**100.7**
7	65.6	16.9	3.3	5.6	1.9	93.4	5.7	0.0	0.0	0.0	99.1
7	66.0	17.2	3.3	6.1	2.0	94.7	5.7	0.0	0.0	0.0	100.4
7 mean	**65.8**	**17.1**	**3.3**	**5.8**	**2.0**	**94.0**	**5.7**	**0.0**	**0.0**	**0.0**	**99.7**
14	64.6	15.4	2.9	6.0	2.3	91.3	9.1	0.0	0.1	0.1	100.5
14	64.6	15.8	2.9	6.1	2.3	91.7	8.8	0.0	0.1	0.1	100.6
14 mean	**64.6**	**15.6**	**2.9**	**6.1**	**2.3**	**91.5**	**9.0**	**0.0**	**0.1**	**0.1**	**100.6**
28	63.7	13.3	2.6	6.0	2.4	88.1	13.0	0.0	0.2	0.2	101.2
28	60.1	13.5	2.5	5.9	2.3	84.4	12.8	0.0	0.1	0.1	97.4
28 mean	**61.9**	**13.4**	**2.6**	**5.9**	**2.3**	**86.2**	**12.9**	**0.0**	**0.2**	**0.2**	**99.3**
43	55.4	14.4	2.8	5.9	3.2	81.6	18.0	0.0	0.3	0.3	100.0
43	56.3	14.1	2.9	6.2	3.2	82.7	17.2	0.0	0.3	0.3	100.1
43 mean	**55.8**	**14.2**	**2.8**	**6.1**	**3.2**	**82.1**	**17.6**	**0.0**	**0.3**	**0.3**	**100.0**
63	50.0	13.8	2.8	5.9	2.6	75.2	23.7	0.0	0.7	0.7	99.5
63	49.7	13.7	2.7	6.0	2.7	74.8	22.3	0.0	0.4	0.4	97.5
63 mean	**49.9**	**13.8**	**2.8**	**6.0**	**2.6**	**75.0**	**23.0**	**0.0**	**0.5**	**0.5**	**98.5**
91	43.6	11.3	2.3	5.6	2.7	65.5	31.3	0.0	1.0	1.0	97.9
91	43.3	11.3	2.3	6.1	2.8	65.9	30.0	0.0	0.5	0.5	96.4
91 mean	**43.5**	**11.3**	**2.3**	**5.9**	**2.8**	**65.7**	**30.7**	**0.0**	**0.8**	**0.8**	**97.1**
119	36.3	9.8	2.0	5.2	3.0	56.2	42.4	0.0	1.3	1.3	99.9
119	37.0	10.1	2.1	5.5	2.9	57.6	41.0	0.0	0.9	0.9	99.5
119 mean	**36.6**	**9.9**	**2.1**	**5.3**	**3.0**	**56.9**	**41.7**	**0.0**	**1.1**	**1.1**	**99.7**

TAR = Total applied radioactivity (100% TAR = 0.270 mg kg^{-1})
ACN = Acetonitrile
NER = Non-extractable residues
n.d. = Not determined
[a] No other volatiles than CO_2 were found values for volatile radioactive residues were calculated cumulatively.

C. VOLATILIZATION

Carbon dioxide was the only trapped volatile degradation product found in the sodium hydroxide traps reaching 14.1% TAR for the phenyl-labeled and only 1.1% TAR for the triazole-labeled metconazole after 119 days of incubation. In ethylene glycol traps no radioactive residues were found.

D. TRANSFORMATION OF PARENT COMPOUND

All soil extracts of each sampling point were pooled and analyzed by radio-HPLC. The summarized results are presented in Table 7.1.1.1-4.

The test item metconazole (sum of cis and trans isomer) decreased in soil from about 97% TAR to about 50% TAR after 119 days of incubation.

With both radio-labels, metabolite M555F030 was detected in the soil extracts, reaching a maximum of 2.0% TAR at 91 DAT (phenyl label) and 3.0% TAR at 63 DAT (triazole label), respectively.

Metabolite M555F040 and a non-identified metabolite were assigned in the pooled soil extracts of 119 DAT with both labels by HPLC-MS analysis. Since only a selected pooled extract of 119 DAT per label was analyzed by HPLC-MS, it cannot be excluded that the co-eluting metabolite was present in the other extracts not analyzed by HPLC-MS. M555F040 and the non-identified metabolite were detected as a sum in maximum amounts of 1.8% TAR (phenyl label) and 1.3% TAR (triazole label), respectively.

M555F020 (1,2,4-(1H)-triazole; Reg. No. 87084) reached only very low amounts of maximum 1.2% TAR at the end of the incubation period (triazole label only).

Besides the known metabolites, four (triazole label) to five (phenyl label) additional peaks could be detected in the chromatograms. However, they never exceeded 2% TAR at any sampling time.

Chiral HPLC results

Throughout the incubation period, the ratio between enantiomers of cis- and trans-metconazole remained almost constant in the pooled soil extracts. No significant shift was observed.

Table 7.1.1.1-4: Radio-HPLC analysis of extracts of soil LUFA 5M treated with phenyl-labeled metconazole[% TAR]

Days after treatment	Total extractable (ERR)	unknown	unknown	unknown	M555F030	Metconazole (BAS 555 F)			M555F040 and/or Unknown [a]	unknown
						trans-isomer	cis-isomer	sum of isomer		
		t_{Ret} ~5.1	23.8	29.2	36.5	43.5	44.6		48.7	56.5
0	97.6	-	-	-	-	17.5	79.6	97.0	0.6	-
0	97.7	-	-	-	-	16.9	80.4	97.3	0.4	-
0 mean	**97.7**	**-**	**-**	**-**	**-**	**17.2**	**80.0**	**97.2**	**0.5**	**-**
3	97.5	-	-	-	-	17.9	79.1	97.0	0.5	-
3	97.2	-	-	-	-	16.7	80.4	97.2	-	-
3 mean	**97.3**	**-**	**-**	**-**	**-**	**17.3**	**79.8**	**97.1**	**0.2**	**-**
7	93.9	-	-	-	-	16.9	76.4	93.3	0.6	-
7	95.5	-	-	-	-	17.2	77.8	95.0	0.5	-
7 mean	**94.7**	**-**	**-**	**-**	**-**	**17.1**	**77.1**	**94.1**	**0.6**	**-**
14	90.1	-	-	-	0.9	15.5	73.2	88.7	0.5	-
14	92.3	-	-	0.7	0.9	16.1	74.0	90.1	0.7	-
14 mean	**91.2**	**-**	**-**	**0.3**	**0.9**	**15.8**	**73.6**	**89.4**	**0.6**	**-**
28	87.3	-	-	0.3	1.1	14.6	71.2	85.9	-	-
28	85.6	-	-	0.6	1.1	14.7	68.5	83.1	0.7	-
28 mean	**86.5**	**-**	**-**	**0.5**	**1.1**	**14.7**	**69.8**	**84.5**	**0.3**	**-**
43	82.4	-	-	0.8	1.8	14.4	64.5	78.9	0.9	-
43	82.3	-	-	0.6	1.3	14.3	65.3	79.6	0.9	-
43 mean	**82.4**	**-**	**-**	**0.7**	**1.5**	**14.4**	**64.9**	**79.2**	**0.9**	**-**
63	76.2	-	-	0.8	2.0	13.4	59.1	72.5	0.9	-
63	75.0	-	0.4	1.1	1.9	13.2	57.5	70.7	0.9	-
63 mean	**75.6**	**-**	**0.2**	**0.9**	**1.9**	**13.3**	**58.3**	**71.6**	**0.9**	**-**
91	66.2	-	0.6	1.1	1.9	12.3	49.2	61.5	1.1	-
91	64.4	-	0.6	0.9	2.1	13.3	46.0	59.3	1.5	-
91 mean	**65.3**	**-**	**0.6**	**1.0**	**2.0**	**12.8**	**47.6**	**60.4**	**1.3**	**-**
119	58.1	0.5	0.9	0.9	2.0	11.9	40.0	51.8	1.5	0.5
119	55.6	0.6	0.5	1.0	1.8	11.9	37.2	49.1	2.1	0.6
119 mean	**56.9**	**0.5**	**0.7**	**1.0**	**1.9**	**11.9**	**38.6**	**50.5**	**1.8**	**0.5**

TAR = Total applied radioactivity (100% TAR = 0.271 mg kg^{-1})
t_{Ret} = retention time [min]
[a] Metabolite M555F040 and a co-eluting unknown metabolite could not be quantified separately.

Table 7.1.1.1-5: Radio-HPLC analysis of extracts of soil LUFA 5M treated with triazole-labeled metconazole[% TAR]

Days after treatment	Total extractable (ERR)	M555F020	unknown	unknown	M555F030	Metconazole (BAS 555 F)			M555F040/ and/or unknown [a]	unknown
						trans-isomer	cis-isomer	sum of isomer		
		t_{Ret} ~5.8	23.8	29.2	36.5	43.5	44.6		48.7	56.5
0	99.1	-	-	-	1.6	16.4	81.2	97.6	-	-
0	99.5	-	-	-	1.0	17.2	81.2	98.4	-	-
0 mean	**99.3**	**-**	**-**	**-**	**1.3**	**16.8**	**81.2**	**98.0**	**-**	**-**
3	97.0	-	-	-	1.0	16.4	79.6	96.0	-	-
3	96.7	-	-	-	1.4	15.9	79.4	95.3	-	-
3 mean	**96.8**	**-**	**-**	**-**	**1.2**	**16.2**	**79.5**	**95.7**	**-**	**-**
7	93.4	-	-	-	1.3	15.7	76.5	92.1	-	-
7	94.7	-	-	-	2.0	15.7	76.9	92.6	-	-
7 mean	**94.0**	**-**	**-**	**-**	**1.7**	**15.7**	**76.7**	**92.4**	**-**	**-**
14	91.3	-	-	0.5	1.9	15.8	73.1	88.9	-	-
14	91.7	-	-	-	1.3	16.1	74.4	90.4	-	-
14 mean	**91.5**	**-**	**-**	**0.3**	**1.6**	**15.9**	**73.7**	**89.7**	**-**	**-**
28	88.1	-	-	-	1.9	14.7	71.5	86.1	-	-
28	84.4	-	-	-	2.1	13.5	68.8	82.3	-	-
28 mean	**86.2**	**-**	**-**	**-**	**2.0**	**14.1**	**70.1**	**84.2**	**-**	**-**
43	81.6	-	-	-	2.2	14.2	65.2	79.4	-	-
43	82.7	-	-	-	2.6	13.2	66.9	80.1	-	-
43 mean	**82.1**	**-**	**-**	**-**	**2.4**	**13.7**	**66.0**	**79.7**	**-**	**-**
63	75.2	-	-	-	3.4	14.1	57.6	71.7	-	-
63	74.8	-	-	-	2.5	14.2	57.4	71.6	0.6	-
63 mean	**75.0**	**-**	**-**	**-**	**3.0**	**14.1**	**57.5**	**71.7**	**0.3**	**-**
91	65.5	1.2	0.9	0.9	2.9	11.5	47.0	58.5	1.0	-
91	65.9	0.6	-	1.0	2.9	11.8	48.5	60.4	1.0	-
91 mean	**65.7**	**0.9**	**0.5**	**1.0**	**2.9**	**11.7**	**47.8**	**59.4**	**1.0**	**-**
119	56.2	1.5	-	1.4	2.8	10.3	38.4	48.7	1.3	0.6
119	57.6	1.0	0.7	0.9	2.2	10.7	40.4	51.1	1.3	0.6
119 mean	**56.9**	**1.2**	**0.3**	**1.1**	**2.5**	**10.5**	**39.4**	**49.9**	**1.3**	**0.6**

TAR = Total applied radioactivity (100% TAR = 0.270 mg kg^{-1})
t_{Ret} = retention time [min]
[a] Metabolite M555F040 and a co-eluting unknown metabolite could not be quantified separately.

E. CHARACTERIZATION OF NON-EXTRACTABLE RESIDUES (NER)

The total amount of NER as well as the distribution of radioactivity between the different humic substance fractions was related to the position of the radiolabel. Regarding the phenyl-labeled metconazole, the amount of NER was smaller and predominantly related to the humin fraction (5.1 and 11.4% TAR at 28 and 119 DAT, respectively). Residual radioactive residues of triazole-labeled metconazole comprised a larger fraction, being mainly related to the fulvic acid fraction (7.5% at day 28 and 31.1% TAR at 119 DAT).

HPLC-MS/MS analysis of the processed NaOH solubilizate of a solvent extracted soil residue (triazole label) revealed that the major portion of the radioactive residues related to the fulvic acid fraction can be attributed to metabolite M555F020 corresponding to 22.8% TAR.

Results of the non-extractable residues characterization performed by humic substance fractionation are given in Table 7.1.1.1-6.

Table 7.1.1.1-6: Characterization of bound residues [% TAR] of ^{14}C-metconazole

DAT	^{14}C-label position	NER	NaOH treatment				humic substance fraction				
			1	2	3	sum	fulvic acids	humic acids	humins	sum	% recovery
28	phenyl	9.2	2.3	1.3	0.8	4.3	2.7	1.5	5.1	9.3	100.8
119	phenyl	23.2	6.5	2.8	1.6	10.8	5.8	4.8	11.4	22.0	94.9
28	triazole	13.0	6.1	2.0	0.9	9.0	7.5	1.3	4.8	13.5	104.2
119	triazole	42.4	25.2	6.2	2.2	33.6	31.1	2.5	8.6	42.1	99.4

TAR = Total applied radioactivity
DAT = days after treatment
NER = non-extractable residues

F. KINETIC MODELING RESULTS

The degradation of metconazole could be best described by the fit with the SFO model. A summary of the $DegT_{50}$ and $DegT_{90}$ values of metconazole derived as trigger and modeling endpoints are given in Table 7.1.1.1-7.

Table 7.1.1.1-7: $DegT_{50}$ and $DegT_{90}$ values as trigger and modeling endpoints for metconazole

Compound	$DegT_{50}$ [d]	$DegT_{90}$ [d]	Kinetic model	χ^2 error
Metconazole	128.4	426.6	SFO	1.23

III. CONCLUSION

Metconazole degraded slowly in sandy loam soil LUFA 5M during incubation under aerobic conditions with a $DegT_{50}$ of 128 days (SFO). No metabolite > 3% TAR was detected. The major routes of metconazole degradation in soil were either mineralization (phenyl label) or formation of non-extractable residues (triazole label). Throughout the incubation period, enantiomers of cis- and trans-metconazole were almost equally distributed in the pooled soil extracts revealed by chiral radio-HPLC analyses.

Normalization of the degradation rate to reference conditions

Since for environmental fate modeling $DegT_{50}$ values at reference conditions (temperature of 20°C and soil moisture at field capacity, i.e. pF2) are required, the reported $DegT_{50}$ value for modeling was normalized following the recommendations of FOCUS (2014) [*Generic Guidance for Tier 1 FOCUS Ground Water Assessments. Version: 2.2 May 2014, 66 pp*].

Since the study was performed at 20°C no temperature correction was necessary. The moisture normalization was performed using the moisture dependency equations by Walker as described in Equation 7.1.1.1-1.

Equation 7.1.1.1-1 Calculation of the moisture correction factor according to Walker

$$f_{moist} = \begin{cases} \left(\frac{\theta_{act}}{\theta_{ref}}\right)^{0.7} & if \quad \theta_{act} < \theta_{ref} \\ 1 & if \quad \theta_{act} \geq \theta_{ref} \end{cases}$$

with:

f_{moist}	moisture correction factor	[-]
θ_{ref}	reference soil moisture at field capacity (pF2, 10 kPa)	[g / 100 g dry soil]
θ_{act}	actual soil moisture during incubation	[g / 100 g dry soil]

The actual soil moisture was taken from the study report and the corresponding reference moisture at pF 2 was derived from FOCUS (2014). The normalized DT_{50} values were calculated by multiplying the DT_{50} values at study conditions by the correction factor f_{moist} as described in Equation 7.1.1.1-2.

Equation 7.1.1.1-2 Calculation of the DT_{50} at reference conditions (20°C, pF2)

$$DegT_{50,ref} = DegT_{50,act} \cdot f_{moist}$$

with:	$DegT_{50,ref}$	normalized $DegT_{50}$	[d]
	$DegT_{50,act}$	$DegT_{50}$ at study conditions	[d]
	f_{moist}	moisture correction factor	[-]

The normalized half-live of metconazole is given below:

Table 7.1.1.1-8: Normalization of the $DegT_{50}$ value to reference conditions

Compound	Soil	pH ($CaCl_2$)	Kinetic model	θ_{act}	θ_{ref}	f_{moist}	$DegT_{50,act}$	$DegT_{50,ref}$
Metconazole	LUFA 5M	7.4	SFO	12.2	19	0.731	128.4	93.9

θ_{act}	actual soil moisture	[g / 100 g dry soil]
θ_{ref}	reference soil moisture at field capacity (pF 2) according to FOCUS (2012)	[g / 100 g dry soil]
f_{moist}	moisture correction factor	[-]
$DegT_{50,act}$	$DegT_{50}$ at study conditions	[d]
$DegT_{50,ref}$	$DegT_{50}$ at reference conditions	[d]

CA 7.1.1.2 Anaerobic degradation

An anaerobic soil degradation study with triazole-3(5)-^{14}C labeled metconazole was submitted for the previous Annex I listing [*BASF DocID 2001/7000226 (MK-620-018)*]. Metconazole degraded slowly in soil under anaerobic conditions. The degradation pathway was similar to the pathway noted in the aerobic soil metabolism studies. Low levels of M555F030 (CL 382389, Reg. No. 4110625) and a minor unidentified component were detected at levels <3% of total applied radioactivity (TAR). No major metabolites were detected. The DT_{50} for the Ipswich, UK anaerobic soil could not be accurately calculated and was stated as >120 days, which was the length of the study.

For further elucidation, an additional anaerobic soil degradation study was conducted. The study was performed according to guideline OECD 307 considering another radiolabel, [phenyl-U-^{14}C]-metconazole. It is submitted under CA 7.1.1.2/1.

Results of the new study confirm the degradation behavior observed previously. Metconazole degraded only slowly in soil under anaerobic conditions, with an estimated half-live of 555 days. The degradation of metconazole was initially established during the aerobic incubation phase and metconazole continued to degrade with the same pattern under anaerobic conditions although at a slower rate. Under the conditions of the study metconazole was metabolized to M555F030cis (Reg. No. 4110625) by oxidation (≤ 3.0%), and then to minor metabolites (≤ 2.0%), bound residues and finally mineralized to carbon dioxide. No additional degradates were formed during the anaerobic phase, and no degradation products >5% AR were observed.

Report:	CA 7.1.1.2/1 Crowe A., 2015a Metconazole: Route and rate of degradation in anaerobic soil 2014/1000922
Guidelines:	EC 1107/2009 of the European Parliament, SETAC Procedures for assessing the environmental fate and behaviour and ecotoxicity of pesticides (March 1995), JMAFF No 12 Nosan No 8147 24 November 2000 revised 16 March 2005 (2-5-3), EPA 835.4200, OECD 307 (2002)
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Executive Summary

The fate of metconazole was investigated in a UK sandy loam soil (Bromsgrove) under anaerobic conditions in the laboratory. The sandy loam soil had an organic carbon content of 1.4% and a pH of 5.7 ($CaCl_2$). The soil was acclimatized under aerobic conditions before being treated with [phenyl-U-^{14}C]-metconazole at a rate of 0.133 mg kg^{-1} dry soil, equivalent to a field application rate of 100 g a.s. ha^{-1}. Individual samples were incorporated into flow through systems with traps to collect volatile radioactivity. Treated soil samples were initially incubated under aerobic conditions at a moisture content equivalent to pF 2 and 20°C in darkness for 30 days before being flooded (degassed water to a depth of 3 cm). After 30 days, treated soil samples were flushed with nitrogen and incubated under anaerobic conditions for 119 days.

There was little or no oxygen in the water from day 3. After 10 days, moderately reducing conditions had been established in the waterlogged soil that were maintained and further developed throughout the incubation period. From day 21, redox potentials in the soil phase were indicative of reducing conditions.

Duplicate samples were taken for processing and analysis at time zero, after 30 days aerobic incubation and after 1, 3, 6, 10, 14, 21, 31, 60, 90 and 119 days of anaerobic incubation. Samples of soil or soil/water slurry, as appropriate, were extracted with three portions of acetonitrile at room temperature. From day 30, samples were further extracted with acetonitrile : water (1:1, v:v or 1:3 v:v). Post extraction, soil was dried prior to combustion and liquid scintillation counting (LSC). Radioactivity in the extracts and trapping solutions was quantified using LSC. Extracts were pooled and concentrated prior to chromatographic analysis.

Total recoveries of radioactivity (mass balances) were between 94.6% and 102.2% TAR. Most of the radioactivity was extracted from the soil or soil/water slurry, with acetonitrile or acetonitrile/water, throughout both the aerobic and anaerobic incubation periods. The amount of radioactivity remaining unextracted increased to a mean of 19.4% TAR after 119 days of anaerobic incubation. The amount of radioactivity recovered in the CO_2 trapping solutions increased to a mean of 2.9% TAR after 119 days of anaerobic incubation. Non-extractable radioactivity was mainly associated with the humin fraction.

The quantity of metconazole in the soil extracts declined from a mean of 94.4% TAR at time zero to 85.9% TAR after 30 days of aerobic incubation and then to 71.5% TAR after 119 days of anaerobic incubation. Degradation under anaerobic conditions was slower than under aerobic conditions.

Kinetic analysis and calculation of the $DegT_{50}$ and $DegT_{90}$ values for parent metconazole was performed following the recommendation of the FOCUS Kinetics workgroup. Only data from the anaerobic incubation phase (i. e. samples taken after flooding of the soil in each test vessel with water) were considered. The analysis was done by non-linear regression methods using the software package KinGUI version 2.1.

The following best fit $DegT_{50}$ and $DegT_{90}$ values were calculated for metconazole:

Table 7.1.1.2-1: Best-fit endpoints for metconazole

Compound	Kinetic model	χ^2 error	$DegT_{50}$ [d]	$DegT_{90}$ [d]
Metconazole	SFO	1.9	555	> 1000

Two minor metabolites were formed during the study, metabolite M555F030cis (Reg. No. 4110625) (≤ 3.0%) and one low level unidentified metabolite (≤ 2.0%).

Under the conditions of the study metconazole was metabolized to M555F030cis (Reg. No. 4110625) by oxidation, and then to minor metabolites, bound residues and finally mineralized to carbon dioxide. The degradation of metconazole was initially established during the aerobic incubation phase and metconazole continued to degrade with the same pattern under anaerobic conditions although at a slower rate. No additional degradates were formed during the anaerobic phase. The dissipation of metconazole was considered to be mainly due to the formation of bound residues. Non-extractable radioactivity was mainly associated with the humin fraction while only low levels of radioactivity were associated with the fulvic and humic acid fractions. These results demonstrate that even under harsh extraction conditions most of the radioactivity was bound to the soil matrix.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

The test item metconazole (BAS 555 F) was used in one ^{14}C-labeled form.

BAS code:	BAS 555 F
Reg. No.:	4056343
Common name:	Metconazole
CAS No.:	125116-23-6
Chemical name (IUPAC):	(1RS,5RS;1RS,5SR)-5-(4-chlorobenzyl)-2,2-dimethyl-1-(1*H*-1,2,4-triazol-1-ylmethyl)cyclopentanol
Molecular mass:	319.8 g mol^{-1}
Molecular formula:	$C_{17}H_{22}ClN_3O$

Phenyl-U-^{14}C-label

Batch No.:	1065-1029
Cis/trans isomer ratio:	81.4 : 18.6
Specific radioactivity of a.s.:	9.26 MBq mg^{-1}
Radiochemical purity:	99.4%, see certificate of analysis (measurement prior to application: 99.6%)
Purity:	92.7%

Unlabeled

Batch No.:	AC12140-17
Cis/trans isomer ratio:	85 : 15
Purity:	98.1%

2. Soil

The sandy loam soil (Bromsgrove) derived from LandLook (Warwickshire, UK) was used in this study. It was sampled from 5-20 cm depth from a grassland site with no pesticide use for at least five years. The soil was stored for no longer than three months at 4°C and was passed through a 2 mm sieve before use. The soil characteristics are summarized in Table 7.1.1.2-2.

Table 7.1.1.2-2: Soil characteristics

Soil designation	**Bromsgrove** **HLS soil No. 291013A** United Kingdom (Warwickshire)
UK Particle size distribution *[%]* sand 0.063 – 2 mm silt 0.002 – 0.063 mm clay < 0.002 mm textural class	 71 16 13 sandy loam
USDA Particle size distribution *[%]* sand 0.050 – 2 mm silt 0.002 – 0.050 mm clay < 0.002 mm textural class	 72 16 12 sandy loam
Organic C *[%]*	1.4
pH [H_2O] pH [$CaCl_2$]	6.1 5.7
cation exchange capacity *[$cmol^+ kg^{-1}$]*	9.0
Water content at pF 2 *[%]*	19.9
microbial biomass (start of study) *[mg C 100 g^{-1} dry soil]*	30.0

B. STUDY DESIGN

1. Experimental conditions

Portions of 100 g soil (dry weight equivalents) were filled in 250 mL glass bottles, adjusted with distilled water to a soil moisture of pF 2, and acclimatized connected to a flow-through incubation system (20 ± 2°C, dark) for seven days until application.

Aliquots (100 µL) of the application solution were applied to the surface of the soil samples. The actual application rate was 0.140 mg active substance (a.s.) per kg dry soil. Following application, the solvent was allowed to evaporate and the soil mixed by gently shaking. The soil moisture in each vessel was adjusted to pF 2, if necessary. Following application, each vessel (with the exception of those taken for zero-time analysis) was reincorporated to its flow-through incubation system. A trapping system consisting of flasks of ethyl digol, 1 M KOH (+ phenolphthalein indicator), and 1 M KOH (+ phenolphthalein indicator) were connected in series. For the aerobic sampling on day 0, no absorption traps were set up.

Control samples used for microbial biomass determination were not treated.

The incubation system was aerated with moist air for 30 days during the initial aerobic phase. After flooding, the samples were ventilated with nitrogen. Based on the parameter measurements, the gas flow was changed from continuous to intermittent (three periods of 20 minutes each day) 52 days after flooding.

All test systems were maintained in darkness at 20 ± 2°C in a temperature-controlled room.

2. Sampling

Duplicate samples of soil treated with [^{14}C]-metconazole were taken for analysis immediately after application and after 30 days of aerobic incubation. Following flooding, duplicate samples of soil treated with [^{14}C]-metconazole were taken for analysis after 1, 3, 6, 10, 14, 21, 31, 60, 90 and 119 days of anaerobic incubation. Trapping solutions were taken for analysis when the associated sample was taken for analysis. Additionally, all remaining traps were taken for analysis and replaced with fresh media as necessary after 14 and 30 days of aerobic incubation and after 7, 14, 21, 31, 45, 60, 73, 90, 103 and 119 days of anaerobic incubation.

3. Description of analytical procedures

Each soil or soil/water slurry sample was extracted with three portions of acetonitrile by shaking at ambient temperature by sonication. The soil and extract were separated by centrifugation. The extracts were pooled, weighed, and duplicate weighed aliquots taken for liquid scintillation counting (LSC).

The day 30 soil samples were further extracted with a portion of acetonitrile : water (1 : 1, v:v) by sonication followed by shaking at ambient temperature. The soil and extract were separated by centrifugation. The extracts were weighed and duplicate weighed aliquots taken for LSC. From day 1 following, flooding soil/water slurry samples were further extracted with a portion of acetonitrile : water (1 : 3, v:v) by sonication followed by shaking at ambient temperature. The soil and extract were separated by centrifugation. The extracts were weighed and duplicate weighed aliquots taken for LSC.

The non-extractable radioactivity (NER) was characterized in the soil debris from samples taken at the end of the aerobic phase (30 days) and after 119 days of anaerobic incubation using an acid/base fractionation procedure. Duplicate weighed aliquots of each extract were taken for radioassay. The soil debris remaining after extraction was air-dried, the total weight measured, and triplicate weighed portions were taken for combustion and radioassay.

The volumes of trapping solutions were measured and duplicate aliquots taken for radioassay.

For each sample, portions of acetonitrile and acetonitrile/water extracts were pooled and concentrated to a small volume under vacuum and a stream of nitrogen. Acetonitrile was added to each sample to give a ratio of approximately 1:1, by volume. These concentrated extracts were used for HPLC and where appropriate for TLC analysis. Recoveries of radioactivity following concentration were in the range 96.0% to 115.1% and were considered quantitative.

4. Calculation of the degradation rate

Kinetic evaluation was performed in order to derive degradation parameters as triggers for additional work (trigger endpoints). Kinetic analysis and calculation of $DegT_{50}$ and $DegT_{90}$ values was performed following the recommendations of the FOCUS Kinetics workgroup [*FOCUS (2006)*]. The software package KinGUII (version 2.2014.224.1704) was used for parameter fitting.

None of the transformation products occurred at amounts above 5% of the total applied radioactivity (TAR) in the test system treated with [phenyl-U-^{14}C]-metconazole and were therefore not considered in the kinetic evaluation.

For the kinetic evaluation of the parent substance, true replicates were used in the evaluation. Only data from the anaerobic incubation phase (i.e. samplings after flooding of the soil in each test vessel with water) were considered.

The appropriateness of the distinct kinetic model to describe soil degradation was tested by visual assessment, estimation of the error percentage at which the χ^2 test was passed, and the t-test to evaluate whether estimated degradation parameters differ from zero.

The trigger endpoints were derived from the kinetic models that provided the best-fit to the measured data, generally indicated by the lowest χ^2 – error and a visual assessment of the fit.

II. RESULTS AND DISCUSSION

A. TEST CONDITIONS

Anaerobic conditions were checked by measuring the oxygen content, the redox potential, and the pH of the test system at each sampling time from 3 days after flooding onwards until 119 days after flooding. The data show that there was little or no oxygen in the water from day 3. After 10 days moderately reducing conditions had been established in the water-logged soil that were maintained and further developed throughout the incubation period. Redox potentials in the soil were negative (-4 to -105 mV) from 21 days after flooding and indicative of an anaerobic, reducing soil environment.

B. MASS BALANCE

The detailed results on the extractability of radioactive residues and the overall mass balances are presented in Table 7.1.1.2-3. The mass balance was in the range 94.6% to 102.2% total applied radioactivity (TAR) (mean values of 95.9 to 101.8% TAR).

Table 7.1.1.2-3: Distribution of radioactivity and material balance after application of phenyl-U-^{14}C-labeled test item to soil Bromsgrove [% TAR]

DAT	Sample code	ERR			NER	Volatiles [a]			Material balance
		Acetonitrile extract	Acetonitrile /water extract	Total ERR		CO_2	Ethyl digol	Total	
0	A1	93.8	n.a.	93.8	5.1	n.a.	n.a.	n.a.	98.9
	A2	95.6	n.a.	95.6	2.8	n.a.	n.a.	n.a.	98.4
	mean	**94.7**	**n.a.**	**94.7**	**4.0**	**n.a.**	**n.a.**	**n.a.**	**98.7**
30 [b]	A3	83.1	5.0	88.1	7.8	1.6	n.d.	1.6	97.5
	A4	83.3	5.3	88.6	6.7	1.4	n.d.	1.4	96.7
	mean	**83.2**	**5.2**	**88.4**	**7.3**	**1.5**	**n.d.**	**1.5**	**97.1**
1	A5	77.9	7.2	85.1	8.3	1.8	n.d.	1.8	95.2
	A6	77.8	7.3	85.1	10.1	1.5	n.d.	1.5	96.7
	mean	**77.9**	**7.3**	**85.1**	**9.2**	**1.7**	**n.d.**	**1.7**	**96.0**
3	A7	88.1	2.3	90.4	5.2	1.4	n.d.	1.4	97.0
	A8	86.6	3.5	90.1	7.2	1.3	n.d.	1.3	98.6
	mean	**87.4**	**2.9**	**90.3**	**6.2**	**1.4**	**n.d.**	**1.4**	**97.8**
6	A9	87.4	1.9	89.3	9.4	2.6	n.d.	2.6	101.3
	A10	82.1	2.5	84.6	9.8	2.6	n.d.	2.6	97.0
	mean	**84.8**	**2.2**	**87.0**	**9.6**	**2.6**	**n.d.**	**2.6**	**99.2**
10	A11	84.2	2.0	86.2	11.8	3.4	n.d.	3.4	101.4
	A12	85.9	2.0	87.9	11.9	2.4	n.d.	2.4	102.2
	mean	**85.1**	**2.0**	**87.1**	**11.9**	**2.9**	**n.d.**	**2.9**	**101.8**
14	A13	85.0	2.1	87.1	6.3	2.2	n.d.	2.2	95.6
	A14	83.7	2.2	85.9	7.4	2.8	n.d.	2.8	96.1
	mean	**84.4**	**2.2**	**86.5**	**6.9**	**2.5**	**n.d.**	**2.5**	**95.9**
21	A15	84.1	2.0	86.1	10.5	2.3	n.d.	2.3	98.9
	A16	82.7	1.9	84.6	7.7	2.3	n.d.	2.3	94.6
	mean	**83.4**	**2.0**	**85.4**	**9.1**	**2.3**	**n.d.**	**2.3**	**96.8**
31	A17	82.5	2.3	84.8	12.0	2.3	n.d.	2.3	99.1
	A18	84.3	2.3	86.6	11.2	1.9	n.d.	1.9	99.7
	mean	**83.4**	**2.3**	**85.7**	**11.6**	**2.1**	**n.d.**	**2.1**	**99.4**
60	A19	79.5	2.3	81.8	15.2	2.6	n.d.	2.6	99.6
	A20	80.7	2.3	83.0	14.7	2.8	n.d.	2.8	100.5
	mean	**80.1**	**2.3**	**82.4**	**15.0**	**2.7**	**n.d.**	**2.7**	**100.1**
90	A21	81.8	2.5	84.3	13.5	1.8	n.d.	1.8	99.6
	A22	77.4	2.8	80.2	15.6	3.7	n.d.	3.7	99.5
	mean	**79.6**	**2.7**	**82.3**	**14.6**	**2.8**	**n.d.**	**2.8**	**99.6**
119	A23	75.1	2.3	77.4	18.5	2.7	n.d.	2.7	98.6
	A24	73.2	2.4	75.6	20.3	3.0	n.d.	3.0	98.9
	mean	**74.2**	**2.4**	**76.5**	**19.4**	**2.9**	**n.d.**	**2.9**	**98.7**

TAR = Total applied radioactivity
DAT = Days after treatment
ERR = Extractable radioactive residues
NER = Non-extractable radioactive residues
n.a. = Not analyzed
n.d. = Not detected
[a] Cumulative values
[b] Soils remaining after 30 DAT sampling flooded and anaerobic incubation started

C. EXTRACTABLE AND BOUND RESIDUES

Following application of [phenyl-U-^{14}C]-metconazole, the amount of radioactivity extracted from the soil declined from a mean of 94.7% TAR at time zero to 88.4% TAR after 30 days. There was a corresponding increase in non-extractable radioactivity to 7.3% TAR after 30 days.

Following flooding, the amount of radioactivity extracted from the soil/water slurry declined to a mean of 76.5% TAR after 119 days. There was a corresponding increase with time in non-extractable radioactivity to 19.4% TAR after 119 days.

D. VOLATILISATION

Following application of [^{14}C]-metconazole, volatile radioactivity collected in the CO_2 trapping solutions increased to 1.5% TAR after 30 days. Following flooding, it increased to 2.9% TAR after 119 days. No other volatile radioactive compounds were detected.

E. TRANSFORMATION OF PARENT COMPOUND

The summarized results of radio-HPLC analyses are presented in Table 7.1.1.2-4.

The proportion of metconazole declined from a mean of 94.4% TAR at time zero to 85.9% TAR at 30 days. Following flooding, the proportion of metconazole declined to 71.5% TAR at 119 days.

Metconazole was metabolized to M555F030cis (Reg. No. 4110625) (up to a mean of 3.0% TAR) and one low level unidentified degradate (up to a mean of 2.0% TAR). Both of these degradates were present in samples from the aerobic and anaerobic phases of the study and remained at low levels throughout the incubation period. No additional degradates were formed during the anaerobic phase.

The identities of metconazole and the metabolite M555F030cis (Reg. No. 4110625) were confirmed by co-chromatography using HPLC and TLC. The unidentified degradate did not co-chromatograph with any of the available reference standards available. Due to the low levels formed, no attempt was made to identify the unknown.

Table 7.1.1.2-4: Radio HPLC analysis of combined acetonitrile/water + acetonitrile extracts of soil Bromsgrove treated with phenyl-U-^{14}C -metconazole [% TAR]

DAT	Sample code	Cis isomer (Rt 40'30)	Trans isomer (Rt 40'0)	Total metconazole	M555F030cis (Reg. No. 4110625) (Rt 37'50)	Unknown (Rt 33'0)	Total
0	A1	77.4	15.9	93.2	0.6	n.d.	93.8
	A2	78.9	16.7	95.6	n.d.	n.d.	95.6
	mean	**78.2**	**16.3**	**94.4**	**0.3**	**n.d.**	**94.7**
30 [a]	A3	69.7	15.8	85.5	1.8	0.9	88.2
	A4	71.6	14.7	86.3	1.9	0.3	88.5
	mean	**70.7**	**15.3**	**85.9**	**1.9**	**0.6**	**88.4**
1	A5	68.4	14.3	82.7	2.4	n.d.	85.1
	A6	70.5	13.4	83.9	1.2	n.d.	85.1
	mean	**69.5**	**13.9**	**83.3**	**1.8**	**n.d.**	**85.1**
3	A7	74.9	14.5	89.4	1.0	n.d.	90.4
	A8	73.8	14.9	88.8	1.3	n.d.	90.1
	mean	**74.4**	**14.7**	**89.1**	**1.2**	**n.d.**	**90.3**
6	A9	71.9	15.7	87.6	1.6	n.d.	89.3
	A10	67.9	14.0	81.9	2.7	n.d.	84.6
	mean	**69.9**	**14.9**	**84.8**	**2.2**	**n.d.**	**87.0**
10	A11	67.4	14.4	81.8	2.8	1.6	86.2
	A12	70.5	15.0	85.5	1.9	0.4	87.9
	mean	**69.0**	**14.7**	**83.7**	**2.4**	**1.0**	**87.1**
14	A13	70.5	15.0	85.4	1.1	0.5	87.1
	A14	69.2	14.1	83.3	2.1	0.3	85.9
	mean	**69.9**	**14.6**	**84.4**	**1.6**	**0.4**	**86.5**
21	A15	69.1	14.6	83.6	1.9	0.6	86.1
	A16	67.8	14.3	82.1	2.0	0.4	84.6
	mean	**68.5**	**14.5**	**82.9**	**2.0**	**0.5**	**85.4**
31	A17	69.1	13.8	82.9	1.4	0.4	84.8
	A18	69.9	14.8	84.7	1.6	0.3	86.6
	mean	**69.5**	**14.3**	**83.8**	**1.5**	**0.4**	**85.7**
60	A19	64.9	14.8	79.8	2.0	n.d.	81.8
	A20	68.7	11.7	80.4	1.9	0.7	83.0
	mean	**66.8**	**13.3**	**80.1**	**2.0**	**0.4**	**82.4**
90	A21	67.6	15.3	82.8	1.2	0.3	84.3
	A22	62.4	14.4	76.8	2.6	0.9	80.2
	mean	**65.0**	**14.9**	**79.8**	**1.9**	**0.6**	**82.3**
119	A23	62.6	8.3	70.9	3.3	3.2	77.4
	A24	60.4	11.7	72.1	2.6	0.8	75.6
	mean	**61.5**	**10.0**	**71.5**	**3.0**	**2.0**	**76.5**

TAR = Total applied radioactivity
DAT = Days after treatment
n.d. = Not detected
[a] Soils remaining after 30 DAT sampling flooded and anaerobic incubation started

F. CHARACTERIZATION OF NON-EXTRACTABLE RESIDUES (NER)

On 30 DAT (end of aerobic phase) and on 119 DAT (end of anaerobic phase), NER were further characterized by NaOH treatment and subsequent fractionation into fulvic acids, humic acids, and humins. Results are shown in Table 7.1.1.2-5.

Non-extractable radioactivity was mainly associated with the humin fraction (mean values of 7.5 and 16.8% TAR at 30 and 119 days, respectively) while only low levels (mean values of ≤ 2.5% TAR) of radioactivity were associated with the fulvic and humic acid fractions. No further characterization was conducted on these fractions.

Table 7.1.1.2-5: Characterization of non-extractable residues in soil treated with phenyl-U-^{14}C-metconazole [% TAR]

Days after treatment	Non-extractable residues	Fulvic acids	Humic acids	Humins
30 [a]	7.8	1.6	1.2	6.5
30 [a]	6.7	1.6	1.1	8.4
30 [a] **(mean)**	**7.3**	**1.6**	**1.2**	**7.5**
119	18.5	2.4	1.5	16.2
119	20.3	2.6	1.4	17.3
119 (mean)	**19.4**	**2.5**	**1.5**	**16.8**

TAR = Total applied radioactivity
[a] Soils remaining after 30 DAT sampling flooded and anaerobic incubation started

G. KINETIC MODELING RESULTS

The resulting $DegT_{50}$ and $DegT_{90}$ values for metconazole are summarized in Table 7.1.1.2-6.

Table 7.1.1.2-6: Best-fit endpoints for metconazole

Compound	Kinetic model	χ^2 error	$DegT_{50}$ [d]	$DegT_{90}$ [d]
Metconazole	SFO	1.9	555	> 1000

III. CONCLUSION

Metconazole was degraded in anaerobic soil with a $DegT_{50}$ value of 555 days.

Metconazole was metabolized to M555F030cis (Reg. No. 4110625) (≤ 3.0% TAR), one low level unidentified degradate (≤ 2.0% TAR), bound residues (≤ 19.4% TAR), and finally carbon dioxide (≤ 2.9% TAR). Both metabolites had already been observed in trace amounts during the aerobic incubation phase. No additional metabolites were formed during the anaerobic phase.

The dissipation of metconazole was considered to be mainly due to the formation of bound residues. Non-extractable radioactivity was mainly associated with the humin fraction while only low levels of radioactivity were associated with the fulvic and humic acid fractions. These results demonstrate that even under harsh extraction conditions most of the radioactivity was bound to the soil matrix.

CA 7.1.1.3 Soil photolysis

Two soil photolysis studies with triazole-3(5)-^{14}C labeled metconazole were submitted for the previous Annex I listing [*BASF DocID 1992/7000199 (MK-620-005); BASF DocID 1996/7000411 (MK-620-013)*]. Metconazole photodegraded slowly on the soil surface. In one study two minor metabolites <5% total applied radioactivity (TAR) were observed. In the other study, four minor unidentified metabolites were formed. The maximum concentration of unidentified metabolites reached 6.7% TAR on day 4, the other metabolites increased to 5.0, 2.8, and 1.0% TAR within 15 days. Overall, it was concluded that photodegradation on the soil surface is not expected to have a significant impact on the degradation rate or pathway of metconazole in soil.

For further elucidation, an additional soil photolysis study was conducted. The study was performed according to current guidelines considering two radiolabels, [phenyl-U-^{14}C]- and [3,(5)-triazole-^{14}C]-metconazole. In addition, enantiomeric separation was considered. It is submitted under CA 7.1.1.3/1.

Findings of the new study confirm that irradiation has only a minor influence on the degradation behavior and metabolite formation in soil. Under irradiated and dark conditions similar degradation processes were observed. No major metabolite and no metabolite specific for one of the labels was detected. The enantiomer ratio was about 4:4:1:1 (R,S: S,R: S,S: R,R metconazole), demonstrating that the ratio of isomers remained stable during the course of the study.

Report:	CA 7.1.1.3/1 Knight L., 2015b Metconazole (BAS 555 F): Soil photolysis 2014/1000923
Guidelines:	SETAC Procedures for assessing the environmental fate and ecotoxicity for pesticides (March 1995), EPA 835.2410, OECD Draft Guideline Phototransformation of Chemicals on Soil Surfaces (January 2002), 2004/10/EC of 11 February 2004
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Executive Summary

The photodegradation of metconazole (BAS 555 F) on soil has been studied separately with [phenyl-U-^{14}C]- and [3,(5)-triazole-^{14}C]-metconazole.

The soil was passed through a 2 mm sieve before use and treated with radiolabeled metconazole at a nominal rate of 0.6 mg per kg dry soil, equivalent to a use rate of 90 g a.s. ha^{-1} calculated on the basis of an equal distribution in the soil layer and a soil density of 1.5 g cm^{-3}.

Soil aliquots of 5 g (dry weight basis) were weighed into test dishes and placed into a Suntest apparatus for continuous irradiation (light intensity 27.2 - 34.9 W m^{-2}) and aeration. The incubation temperature was kept at 22°C ± 1°C and the soil moisture was daily adjusted to pF 2.0. A closed incubation system with continuous aeration was used with an attached trapping system for the determination of volatile compounds. The samples for the dark control were kept in an incubation cabinet and treated analogously but without irradiation.

Samples were taken at 0, 2, 5, 8, 10, 13 and 15 days after treatment (DAT). All soil samples were worked up in duplicate. The soil samples were extracted three times with acetonitrile and up to three times with acetonitrile : water (1:1, v:v). The amount of radioactivity in extracts was determined by liquid scintillation counting (LSC) and in non-extractable residues (NER) by combustion and LSC. Volatiles were trapped in appropriate trapping solutions and analyzed by LSC. A full material balance was provided for each sample. Bound residue characterization was also performed with selected soil samples by NaOH extraction and subsequent fractionation into fulvic acids, humic acids and humins.

The overall mean values for the material balance in the photolysis and the dark control were in the range of 97.4 - 106.8% TAR. Carbon dioxide was the only volatile degradation product trapped reaching a maximum of 2.9% TAR after 13 days in the photolysis test and 0.2% TAR in the dark control.

The sample extractability between the photolysis test and the dark control did not significantly differ. At the end of the study 5.2 - 9.0% TAR were not extractable from the irradiated and dark control soil samples. The humin fraction generally accounted for 1.9 - 4.0% TAR. The fulvic acid fraction accounted for 1.4 - 5.1% TAR in NER after application of triazole-^{14}C-metconazole but was less predominant in NER after application of phenyl-^{14}C-metconazole, ≤ 2% TAR. The humic acid fraction accounted for lower amounts, up to 1.4% TAR. Values were similar for dark control samples.

HPLC analysis was used for routine quantification of metconazole and its degradates for all extract samples. The extracts for each sample were pooled prior to chromatographic analysis. Metconazole declined in the photolysis experiment from an average of 101.5 – 102.6% TAR to an average of 85.0 - 88.6% TAR within the study period of 15 days under continuous irradiation. A slightly slower degradation was observed in dark control samples with an average of 93.8 - 93.9% TAR metconazole present in the extracts at the end of the study. No isomeric shift between cis- and trans-metconazole was observed during the study. HPLC and TLC analysis showed that metconazole was metabolized to M555F030cis (Reg. No. 4110625) up to mean value of 3.7% TAR and up to two low level unidentified degradates (mean ≤ 4% and ≤ 2.5% TAR, respectively) under the influence of light and in the dark control. No degradation product exceeded 4.0% TAR at any sampling time. It can be concluded that degradation in irradiated samples was slightly faster but similar to that in dark control samples and no major degradation product was detected.

Representative extract samples were analyzed by TLC for confirmation of identity for metconazole and degradates, mass spectrometry for confirmation of metconazole, and chiral HPLC for determination of enantiomer ratios. No change in the enantiomer ratios was observed for metconazole.
Kinetic analysis and calculations of DT_{50} and DT_{90} values was performed following the recommendations of the FOCUS Kinetics workgroup in order to derive persistence endpoints. The analysis was conducted by non-linear regression methods employing the software tool KinGUI2. The DT_{50} and DT_{90} values were calculated using data from both radiolabeled forms of metconazole.

The following best fit DT_{50} and DT_{90} values were calculated:

Test system	**DegT_{50} [d]**	**DegT_{90} [d]**	**Best-fit model**
Photolysis	68.3	226.9	SFO
Dark control	163.8	544.0	SFO

Transformation products occurred at minor amounts below 5% TAR in the test systems treated with phenyl-^{14}C- and triazole-^{14}C-metconazole and were therefore not considered in the kinetic evaluation.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

The test item metconazole (BAS 555 F) was used in two ^{14}C-labeled forms.

BAS code:	BAS 555 F
Reg. No.:	4056343
CAS No.:	125116-23-6
Chemical name (IUPAC):	(1*RS*,5*RS*;1*RS*,5*SR*)-5-(4-chlorobenzyl)-2,2-dimethyl-1-(1*H*-1,2,4-triazol-1-ylmethyl)cyclopentanol
Molecular mass:	319.8 g mol^{-1}
Molecular formula:	$C_{17}H_{22}ClN_3O$

1. Phenyl-U-^{14}C-label

Batch No.:	1065-1029
Cis/trans isomer ratio:	81.4 : 18.6
Specific radioactivity of a.s.:	9.26 MBq mg^{-1}
Radiochemical purity:	99.4%, see certificate of analysis attached to the final report (measurement prior to application: 99.6%)
Purity:	92.7%

2. Triazole-3(5)-^{14}C-label

Batch No.:	811-1101
Cis/trans isomer ratio:	82 : 18
Specific radioactivity of a.s.:	4.84 MBq mg^{-1}
Radiochemical purity:	97.0%, see certificate of analysis attached to the final report (measurement prior to application: 99.7%)
Purity:	92.5%

Unlabeled

Batch No.:	AC12140-17
Cis/trans isomer ratio:	85 : 15
Purity:	98.1%

2. Soil

Two batches of German agricultural soil Speyer 5M from LUFA (Landwirtschaftliche Untersuchungs- und Forschungsanstalt, Speyer, Germany) were used in this study sampled from 0-20 cm depth. After collecting the soil from the field, the soil was kept at room temperature until sieving. The soil was passed through a 2 mm sieve, remoistened to approximately 8-12% soil moisture and stored at about 4°C in the dark no longer than 3 months before use. An overview of soil parameters is listed in Table 7.1.1.3-1.

Table 7.1.1.3-1: Soil characteristics

Soil designation	**Speyer 5M** Germany (Origin LUFA Speyer)
DIN 4220 Particle size distribution *[%]* sand 0.063 – 2 mm silt 0.002 – 0.063 mm clay < 0.002 mm textural class	 53.3 35.6 11.1 loamy sand
USDA Particle size distribution *[%]* sand 0.050 – 2 mm silt 0.002 – 0.050 mm clay < 0.002 mm textural class	 58.0 30.8 11.2 sandy loam
Organic C *[%]*	0.98
pH [$CaCl_2$]	7.3
Cation exchange capacity *[$cmol^+ kg^{-1}$]*	16.1
Max. water holding capacity *[g per 100 g dry weight]*	39.8
Microbial biomass (start of study) *[mg C per 100 g dry soil]*	80.5

B. STUDY DESIGN

1. Experimental conditions

Fourteen small glass dishes (30 mm diameter, 10 cm deep) per incubation group were filled with soil for photolysis testing and for dark control, respectively, containing 5 g dry soil per dish. The soil was adjusted to a moisture content equivalent to that at pF 2.0 (53.5% of the maximum water holding capacity) The dishes, which were later treated with the test item, were placed into a separate temperatured-controlled (22 ± 1°C) water jacket, placed in the Suntest apparatus, and then incorporated into a flow-through system. The dishes for the dark control were put into an incubator at 22 ± 1°C. The light intensity for the photolysis test group was set to 3 mW cm^{-2} (UVA range). The test vessel was closed airtight with a quartz glass covering and the whole incubation device was continuously aerated with humidified air via an air inlet and an air outlet.

In order to trap potentially evolving volatiles (including $^{14}CO_2$), the emergent air was bubbled through four different trapping solutions located between dish and pump: 1.ethyl digol; 2. 1 M KOH (+ phenolphthalein); 3. 1 M KOH (+ phenolphthalein); 4. 1 M H_2SO_4.

The study was conducted using a Suntest accelerated exposure unit (Heraeus) fitted with a xenon arc light source emitting light with a spectrum similar to the intensity of sunlight, about 3 mW cm^{-2} (UVA range). This corresponds to a clear summer day in Southern Germany (about 49° N). Wavelengths < 290 nm were filtered off to simulate natural sunlight.

To maintain the initial water content as constant as possible, dishes were weighed at each incubation day and the evaporated water was replaced.

The amount of test item to be applied on the soil surface was calculated based on a recommended field application rate of 90 g ha^{-1}. If a soil layer of 1 cm and bulk density of 1.5 kg L^{-1} is assumed the application rate corresponds to about 0.6 mg test item per kg dry soil (and about 3 µg per dish).

2. Sampling

The sampling dates were 0, 2, 5, 8, 10, 13, 15 days after treatment (DAT) for both labels and for the irradiated, and the dark control samples. Two vessels were taken at each sampling time from each photolysis test system and the dark control. At each sampling time (with exception of DAT 0, dark control), the respective volatile trapping solutions were removed.

3. Description of analytical procedures

Each soil sample was consecutively extracted three times with acetonitrile and three times with acetonitrile/water (1:1, v/v). For each extraction step, the suspension was shaken for 15 min. After each extraction step, solid and extract were separated by centrifugation, and supernatants were measured by liquid scintillation counting (LSC). All extracts were combined and measured by LSC and radio-HPLC.

After the last extraction, the soil residues were air-dried and stored at room temperature. For determination of the amount of non-extractable residues by combustion, the residues were homogenized by milling. Aliquots of each sample were combusted in a sample oxidizer. The trapped $^{14}CO_2$ was analyzed by LSC.

The samples of day 0 and day 15 for the two labels (irradiated and dark control) were characterized on a chiral HPLC column to verify the enantiomeric composition of the radiolabeled test substance materials. All samples were measured for radioactivity (LSC) and were analyzed by HPLC to determine the metabolite pattern. Herefore, prior to injection, the solvent of the extracts was completely evaporated by a rotary evaporator and afterwards, the residues were redissolved in a well-defined volume of acetonitrile. Selected irradiated and dark control samples were analyzed by TLC for co-chromatography with reference standards and by LC-MS for confirmation of parent.

Since the samples of the photolysis and the dark control ($\geq$ 10/13 DAT [phenyl label]; $\geq$ 2/8 DAT [triazole label]) showed > 5% of the total applied radioactivity (TAR) of non-extractable residues, further analyses were performed before combustion to separate humic and fulvic acids from humin.

Samples were extracted three times with 0.5 M NaOH on a rotary shaker and twice washed with water. Aliquots were analyzed by LSC. After pooling of NaOH and water extracts, the combined extract was adjusted to pH 1 with HCl to precipitate the humic acids. After centrifugation the supernatant was removed. The precipitate was washed with 1 M HCl and the supernatant combined with these washings. This solution, the fulvic acid fraction, was measured by LSC. The precipitate, the humic acid fraction, was dissolved in 0.5 M NaOH and analyzed by LSC.

The remaining non-extractable radioactivity in the sediment debris (humins) was determined by combustion after air drying of the sediments and subsequent LSC measurement.

4. Calculation of the degradation rate

Kinetic evaluation was performed in order to derive degradation parameters as triggers for additional work (trigger endpoints). Kinetic analysis and calculation of $DegT_{50}$ and $DegT_{90}$ values was performed following the recommendations of the FOCUS Kinetics workgroup [*FOCUS (2006)*]. The software package KinGUI2 (version 2.2014.224.1704) was used for parameter fitting [*Schmitt et al. (2014)*]. The error tolerance and the number of iterations of the optimization tool were set to 0.00001 and 100, respectively.

For each data set, the single first order (SFO) kinetic model as proposed by the FOCUS Kinetics guidance document was applied [*FOCUS (2006)*]. The Goodness-of-fit was evaluated by visual assessment, χ^2 minimum error, and type-I-error rate (t-test) [for details see Chapter 6.3 in *FOCUS (2006)*].

As no isomeric shift was observed, the sum of both isomers at each sampling was included in the evaluation. Further, chromatographic analysis was qualitatively similar between the two radiolabeled forms. Thus, results of both radiolabeled forms were combined to one dataset, resulting in quadruplicate measurements per sampling time point. The initial concentration of the applied test item was set to the material balance recovered at day 0, corrected by the amount of transformation products occurring at day 0, if feasible. The χ^2 value was calculated for the kinetic model as recommended by FOCUS, considering the average of replicate measurements. For the individual parameters, the t-test statistics were based on number of individual measurements.

II. RESULTS AND DISCUSSION

A. MASS BALANCE

Total recoveries of radioactivity extracted from soil are summarized in Table 7.1.1.3-2 to Table 7.1.1.3-5. The overall mean values for the material balance in the photolysis and in the dark control were in the range of 97.4 - 106.8% TAR.

Table 7.1.1.3-2: Recovery and distribution of radioactivity in soil Speyer 5M after treatment with phenyl-U-^{14}C-labeled metconazole and incubation under irradiated conditions [% TAR]

DAT	ACN [a]	ACN/water [b]	Total extractable	Non-extractable	Volatiles [c]	Material balance
2/I	89.5	8.4	97.9	2.5	0.6	101.0
2/II	91.9	8.4	100.3	2.1	0.7	103.1
2 mean	**90.7**	**8.4**	**99.1**	**2.3**	**0.7**	**102.1**
5/I	86.7	5.7	92.4	5.6	1.3	99.3
5/II	93.4	4.5	97.9	4.5	1.0	103.4
5 mean	**90.1**	**5.1**	**95.2**	**5.1**	**1.2**	**101.4**
8/I	88.8	4.9	93.7	4.7	2.1	100.5
8/II	90.6	4.5	95.1	4.6	1.4	101.1
8 mean	**89.7**	**4.7**	**94.4**	**4.7**	**1.8**	**100.8**
10/I	88.4	7.7	96.1	5.3	4.0	105.4
10/II	88.2	6.1	94.3	7.7	1.4	103.4
10 mean	**88.3**	**6.9**	**95.2**	**6.5**	**2.7**	**104.4**
13/I	87.6	8.0	95.6	6.4	2.9	104.9
13/II	87.8	5.7	93.5	6.4	2.8	102.7
13 mean	**87.7**	**6.9**	**94.6**	**6.4**	**2.9**	**103.8**
15/I	86.7	5.9	92.6	7.5	2.2	102.3
15/II	87.4	7.4	94.8	7.2	0.8	102.8
15 mean	**87.1**	**6.7**	**93.7**	**7.4**	**1.5**	**102.6**

TAR = total applied radioactivity
DAT = days after treatment
ACN = acetonitrile
[a] sum of three extraction steps; calculated from individual values given in original study report
[b] sum of two extraction steps; calculated from individual values given in original study report
[c] no other volatiles than CO_2 were found

Table 7.1.1.3-3: Recovery and distribution of radioactivity in soil Speyer 5M after treatment with phenyl-U-^{14}C-labeled metconazole and incubation under dark conditions [% TAR]

DAT	ACN [a]	ACN/water [b]	Total extractable	Non-extractable	Volatiles [c]	Material balance
0/I	96.9	3.2	100.1	0.5	n.a.	100.6
0/II	101.8	2.5	104.5	0.5	n.a.	104.8
0 mean	**99.4**	**2.9**	**102.3**	**0.5**	**n.a.**	**102.7**
2/I	91.5	5.0	96.5	1.9	0.1	98.5
2/II	91.8	3.8	95.6	2.3	0.1	98.0
2 mean	**91.7**	**4.4**	**96.1**	**2.1**	**0.1**	**98.3**
5/I	95.5	3.4	98.9	3.9	0.1	102.9
5/II	95.1	4.0	99.1	3.8	0.1	103.0
5 mean	**95.3**	**3.7**	**99.0**	**3.9**	**0.1**	**103.0**
8/I	95.6	4.4	100.0	3.5	0.1	103.6
8/II	93.3	5.0	98.3	3.6	0.1	102.0
8 mean	**94.5**	**4.7**	**99.2**	**3.6**	**0.1**	**102.8**
10/I	86.9	5.7	92.6	4.2	0.1	96.9
10/II	89.3	6.2	95.5	4.1	0.1	99.7
10 mean	**88.1**	**6.0**	**94.1**	**4.2**	**0.1**	**98.3**
13/I	92.4	6.6	99.0	5.4	0.1	104.5
13/II	94.7	6.7	101.4	4.7	0.1	106.2
13 mean	**93.6**	**6.7**	**100.2**	**5.1**	**0.1**	**105.4**
15/I	91.5	6.0	97.5	5.1	0.2	102.8
15/II	88.5	6.0	94.5	5.3	0.2	100.0
15 mean	**90.0**	**6.0**	**96.0**	**5.2**	**0.2**	**101.4**

TAR = total applied radioactivity
DAT = days after treatment
ACN = acetonitrile
n.a. = not analyzed
[a] sum of three extraction steps; calculated from individual values given in original study report
[b] sum of two extraction steps; calculated from individual values given in original study report
[c] no other volatiles than CO_2 were found

Table 7.1.1.3-4: Recovery and distribution of radioactivity in soil Speyer 5M after treatment with triazole-3(5)-^{14}C-labeled metconazole and incubation under irradiated conditions [% TAR]

DAT	ACN [a]	ACN/water [b]	Total extractable	Non-extractable	Volatiles [c]	Material balance
2/I	92.4	5.3	97.7	2.9	n.d.	100.6
2/II	90.2	8.7	98.9	5.8	n.d.	104.7
2 mean	**91.3**	**7.0**	**98.3**	**4.4**	**n.d.**	**102.7**
5/I	81.5	15.2	96.7	5.2	0.2	102.1
5/II	89.7	6.2	95.9	4.2	0.2	100.3
5 mean	**85.6**	**10.7**	**96.3**	**4.7**	**0.2**	**101.2**
8/I	83.7	13.0	96.7	8.1	0.2	105.0
8/II	84.0	12.9	96.9	6.4	0.4	103.7
8 mean	**83.9**	**13.0**	**96.8**	**7.3**	**0.3**	**104.4**
10/I	81.9	13.6	95.5	5.9	0.4	101.8
10/II	85.3	10.3	95.6	5.7	0.3	101.6
10 mean	**83.6**	**12.0**	**95.6**	**5.8**	**0.4**	**101.7**
13/I	79.3	12.0	91.3	10.0	1.0	102.3
13/II	82.4	9.4	91.8	7.6	2.5	101.9
13 mean	**80.9**	**10.7**	**91.6**	**8.8**	**1.8**	**102.1**
15/I	83.8	11.2	95.0	8.1	0.3	103.4
15/II	76.1	15.1	91.2	9.9	1.3	102.4
15 mean	**80.0**	**13.2**	**93.1**	**9.0**	**0.8**	**102.9**

TAR = total applied radioactivity
DAT = days after treatment
ACN = acetonitrile
n.d. = not detected
[a] sum of three extraction steps; calculated from individual values given in original study report
[b] sum of up to three extraction steps; calculated from individual values given in original study report
[c] no other volatiles than CO_2 were found

Table 7.1.1.3-5: Recovery and distribution of radioactivity in soil Speyer 5M after treatment with triazole-3(5)-^{14}C-labeled metconazole and incubation under dark conditions [% TAR]

DAT	ACN [a]	ACN/water [b]	Total extractable	Non-extractable	Volatiles [c]	Material balance
0/I	97.3	5.1	102.4	1.0	n.a.	103.4
0/II	98.0	4.7	102.7	0.7	n.a.	103.4
0 mean	**97.7**	**4.9**	**102.6**	**0.9**	**n.a.**	**103.4**
2/I	92.9	4.5	97.4	2.3	n.d.	99.7
2/II	95.0	4.4	99.4	2.4	n.d.	101.8
2 mean	**94.0**	**4.5**	**98.4**	**2.4**	**n.d.**	**100.8**
5/I	91.0	6.9	97.9	4.8	0.1	102.8
5/II	92.8	5.2	98.0	4.8	0.1	102.9
5 mean	**91.9**	**6.1**	**98.0**	**4.8**	**0.1**	**102.9**
8/I	81.9	14.4	96.3	7.0	0.1	103.4
8/II	80.7	16.8	97.5	12.6	0.1	110.2
8 mean	**81.3**	**15.6**	**96.9**	**9.8**	**0.1**	**106.8**
10/I	89.0	5.1	94.1	6.3	n.d.	100.4
10/II	83.2	5.0	88.2	6.2	n.d.	94.4
10 mean	**86.1**	**5.1**	**91.2**	**6.3**	**n.d.**	**97.4**
13/I	91.4	5.8	97.2	7.7	0.1	105.0
13/II	90.8	6.0	96.8	6.7	0.1	103.6
13 mean	**91.1**	**5.9**	**97.0**	**7.2**	**0.1**	**104.36**
15/I	87.4	6.7	94.1	7.7	0.1	101.9
15/II	91.1	6.0	97.1	7.4	0.1	104.6
15 mean	**89.3**	**6.4**	**95.6**	**7.6**	**0.1**	**103.3**

TAR = total applied radioactivity
DAT = days after treatment
ACN = acetonitrile
n.a. = not analyzed
n.d. = not detected
[a] sum of three extraction steps; calculated from individual values given in original study report
[b] sum of up to three extraction steps; calculated from individual values given in original study report
[c] no other volatiles than CO_2 were found

B. EXTRACTABLE AND BOUND RESIDUES

The amount of extractable radioactive residues in the study with the phenyl-label decreased from 102.3% TAR on day 0 to 93.7% TAR on day 15 in the photolysis test and to 96.0% TAR on day 15 in the dark control.

In the study with the triazole-label the amount of extractable radioactive residues decreased from 102.6% TAR on day 0 to 93.1% TAR on day 15 in the photolysis test and to 95.6% TAR in the dark control.

In the study with the phenyl-label, the amount of non-extractable residues (NER) increased from 0.5% TAR on day 0 to 7.4% TAR in photolysis compared to 5.2% TAR in dark control after 15 days. The amount of NER in the study with the triazole-label increased from 0.9% TAR on day 0 to 9.0% TAR in photolysis compared to 7.6% TAR in dark control after 15 days.

C. VOLATILIZATION

Carbon dioxide was the only trapped volatile degradation product found in the trapping solutions and accounted for up to 2.9% TAR (phenyl label) and 1.8% TAR (triazole label) in the photolysis test and up to 0.2% and 0.1% TAR in the dark control.

D. TRANSFORMATION OF PARENT COMPOUND

Results of radio-HPLC analyses are presented in Table 7.1.1.3-6 to Table 7.1.1.3-9.

After 15 days, the amount of [phenyl-U-^{14}C]-labeled metconazole decreased to 85.0% TAR in the photolysis experiment and to 93.8% TAR in the dark control samples. The amount of triazole-3(5)-labeled metconazole decreased in the same time (15 days) to 88.6% TAR in the photolysis experiment and to 93.9% TAR in the dark control samples.

Metconazole degraded to M555F030cis (Reg. No. 4110625) (up to 3.7% TAR) and up to two unidentified degradates. The unidentified degradates were present at levels of ≤ 4.0% TAR and ≤ 2.5% TAR, respectively.

Table 7.1.1.3-6: Radio-HPLC analysis of soil extracts after treatment of soil Speyer 5M with phenyl-U-^{14}C-labeled metconazole and incubation under irradiated conditions [% TAR]

DAT (days)	trans-metconazole (Rt 40 mins)	cis-metconazole (Rt 41 mins)	Total metconazole (BAS 555 F)	M555F030cis (Reg. No. 4110625) (Rt 37 mins)	unknown 1 (Rt 33 mins)	unknown 2 (Rt 38 mins)
2/I	20.3	77.6	97.9	-	-	-
2/II	17.6	82.7	100.3	-	-	-
2 mean	**19.0**	**80.2**	**99.1**	**-**	**-**	**-**
5/I	12.5	79.9	92.4	1.3	-	-
5/II	16.7	78.3	95.0	-	-	1.6
5 mean	**14.6**	**79.1**	**93.7**	**0.7**	**-**	**0.8**
8/I	19.4	71.8	91.2	1.9	-	2.5
8/II	16.4	72.8	89.2	-	2.0	2.0
8 mean	**17.9**	**72.3**	**90.2**	**1.0**	**1.0**	**2.3**
10/I	17.1	79.0	96.1	-	-	-
10/II	16.9	72.9	89.8	2.0	2.5	-
10 mean	**17.0**	**76.0**	**93.0**	**1.0**	**1.3**	**-**
13/I	15.4	74.5	89.9	4.0	-	1.7
13/II	18.5	66.1	84.6	2.9	2.7	3.3
13 mean	**17.0**	**70.3**	**87.3**	**3.5**	**1.4**	**2.5**
15/I	16.7	66.8	83.5	3.5	3.9	1.8
15/II	15.8	70.7	86.5	1.4	4.0	2.8
15 mean	**16.3**	**68.8**	**85.0**	**2.5**	**4.0**	**2.3**

TAR = total applied radioactivity
DAT = days after treatment
Rt = retention time [min]

Table 7.1.1.3-7: Radio-HPLC analysis of soil extracts after treatment of soil Speyer 5M with phenyl-U-^{14}C-labeled metconazole and incubation under dark conditions [% TAR]

DAT (days)	trans-metconazole (Rt 40 mins)	cis-metconazole (Rt 41 mins)	Total metconazole (BAS 555 F)	M555F030cis (Reg. No. 4110625) (Rt 37 mins)	unknown 1 (Rt 33 mins)	unknown 2 (Rt 38 mins)
0/I	16.8	83.3	100.1	-	-	-
0/II	19.7	83.2	102.9	-	-	1.4
0 mean	**18.3**	**83.3**	**101.5**	**-**	**-**	**0.7**
2/I	24.1	72.4	96.5	-	-	-
2/II	15.9	79.7	95.6	-	-	-
2 mean	**20.0**	**76.1**	**96.1**	**-**	**-**	**-**
5/I	18.7	80.2	98.9	-	-	-
5/II	18.5	80.6	99.1	-	-	-
5 mean	**18.6**	**80.4**	**99.0**	**-**	**-**	**-**
8/I	16.4	83.6	100.0	-	-	-
8/II	20.6	77.7	98.3	-	-	-
8 mean	**18.5**	**80.7**	**99.2**	**-**	**-**	**-**
10/I	16.7	74.7	91.4	1.2	-	-
10/II	15.1	80.4	95.5	-	-	-
10 mean	**15.9**	**77.6**	**93.5**	**0.6**	**-**	**-**
13/I	18.1	78.5	96.6	2.4	-	-
13/II	19.8	81.6	101.4	-	-	-
13 mean	**19.0**	**80.1**	**99.0**	**1.2**	**-**	**-**
15/I	18.7	76.4	95.1	2.3	-	-
15/II	17.3	75.2	92.5	2.0	-	-
15 mean	**18.0**	**75.8**	**93.8**	**2.2**	**-**	**-**

TAR = total applied radioactivity
DAT = days after treatment
Rt = retention time [min]

Table 7.1.1.3-8: Radio-HPLC analysis of soil extracts after treatment of soil Speyer 5M with triazole-3(5)-U-^{14}C-labeled metconazole and incubation under irradiated conditions [% TAR]

DAT (days)	trans-metconazole (Rt 40 mins)	cis-metconazole (Rt 41 mins)	Total metconazole (BAS 555 F)	M555F030cis (Reg. No. 4110625) (Rt 37 mins)	unknown 1 (Rt 33 mins)	unknown 2 (Rt 38 mins)
2/I	14.3	79.8	94.1	3.5	-	-
2/II	16.7	82.2	98.9	-	-	-
2 mean	**15.5**	**81.0**	**96.5**	**1.8**	**-**	**-**
5/I	16.0	78.0	94.0	2.7	-	-
5/II	17.6	74.8	92.4	1.3	1.1	1.1
5 mean	**16.8**	**76.4**	**93.2**	**2.0**	**0.6**	**0.6**
8/I	13.7	78.8	92.5	4.2	-	-
8/II	14.4	79.4	93.8	3.1	-	-
8 mean	**14.1**	**79.1**	**93.2**	**3.7**	**-**	**-**
10/I	18.0	73.9	91.9	3.6	-	-
10/II	13.9	75.7	89.6	3.8	-	2.2
10 mean	**16.0**	**74.8**	**90.8**	**3.7**	**-**	**1.1**
13/I	13.8	74.5	88.3	3.0	-	-
13/II	19.3	72.5	91.8	-	-	-
13 mean	**16.6**	**73.5**	**90.1**	**1.5**	**-**	**-**
15/I	19.2	66.7	85.9	4.6	2.9	1.7
15/II	12.3	78.9	91.2	-	-	-
15 mean	**15.8**	**72.8**	**88.6**	**2.3**	**1.5**	**0.9**

TAR = total applied radioactivity
DAT = days after treatment
Rt = retention time [min]

Table 7.1.1.3-9: Radio-HPLC analysis of soil extracts after treatment of soil Speyer 5M with triazole-3(5)-^{14}C-labeled metconazole and incubation under dark conditions [% TAR]

DAT (days)	trans-metconazole (Rt 40 mins)	cis-metconazole (Rt 41 mins)	Total metconazole (BAS 555 F)	M555F030cis (Reg. No. 4110625) (Rt 37 mins)	unknown 1 (Rt 33 mins)	unknown 2 (Rt 38 mins)
0/I	18.5	83.9	102.4	-	-	-
0/II	17.4	85.3	102.7	-	-	-
0 mean	**18.0**	**84.6**	**102.6**	**-**	**-**	**-**
2/I	18.5	78.9	97.4	-	-	-
2/II	19.0	80.4	99.4	-	-	-
2 mean	**18.8**	**79.7**	**98.4**	**-**	**-**	**-**
5/I	15.1	80.6	95.7	2.3	-	-
5/II	13.2	80.3	93.5	4.5	-	-
5 mean	**14.2**	**80.5**	**94.6**	**3.4**	**-**	**-**
8/I	16.9	79.4	96.3	-	-	-
8/II	18.9	78.6	97.5	-	-	-
8 mean	**17.9**	**79.0**	**96.9**	**-**	**-**	**-**
10/I	18.1	76.0	94.1	-	-	-
10/II	15.8	72.4	88.2	-	-	-
10 mean	**17.0**	**74.2**	**91.2**	**-**	**-**	**-**
13/I	21.3	74.0	95.3	1.9	-	-
13/II	15.7	81.1	96.8	-	-	-
13 mean	**18.5**	**77.6**	**96.1**	**1.0**	**-**	**-**
15/I	17.9	76.2	94.1	-	-	-
15/II	19.8	73.8	93.6	3.5	-	-
15 mean	**18.9**	**75.0**	**93.9**	**1.8**	**-**	**-**

TAR = total applied radioactivity
DAT = days after treatment
Rt = retention time [min]

The enantiomer ratio was about 4:4:1:1 (R,S: S,R: S,S: R,R metconazole (BAS 555 F)) for all analyzed samples (see Table 7.1.1.3-10). This result suggests that the ratio is stable over time and no isomerization occurs.

Table 7.1.1.3-10: Chiral HPLC analysis of selected soil extracts after treatment of soil Speyer 5M with phenyl-U-^{14}C- and triazole-3(5)-^{14}C-labeled metconazole [% TAR]

Metconazole (BAS 555 F)	Sample/ timepoint	Enantiomer ratio (% sample radioactivity)			
		R,S	S,R	S,S	R,R
[phenyl-U-^{14}C]	Zero	41.0	41.1	9.3	8.6
	Irradiated: day 15	43.9	40.3	8.6	7.2
	Dark: day 15	37.8	46.2	7.7	8.3
[3,(5)-triazole-^{14}C]	Zero	41.4	44.9	6.4	7.2
	Irradiated: day 15	40.0	49.4	6.1	4.6
	Dark: day 15	41.6	46.2	8.3	3.8

E. CHARACTERIZATION OF NON-EXTRACTABLE RESIDUES (NER)

Results of the non-extractable residue characterization perfomed by humic substance fractionation are given in Table 7.1.1.3-11 and Table 7.1.1.3-12.

Non-extractable radioactivity was mainly associated with the humin fraction (2.9 - 4.0% TAR) for samples treated with phenyl-labeled metconazole and the fulvic acid (1.4 - 5.1% TAR) and humin (1.9 - 7.7% TAR) fractions for samples treated with triazole-labeled metconazole. No further characterization was conducted on these fractions. The results demonstrate that even under harsh extraction conditions most of the radioactivity was bound to the soil matrix.

Table 7.1.1.3-11: Characterization of non-extractable residues (NER) in soil Speyer 5M after treatment with phenyl-U-^{14}C-metconazole [% TAR]

Incubation	Days after treatment	Total non-extractable radioactivity	Fulvic acids	Humic acids	Humins
Irradiated	10	5.3	1.2	0.9	3.3
	10	7.7	2.0	1.4	3.8
	13	6.4	1.8	1.1	3.6
	13	6.4	1.5	1.1	3.6
	15	7.5	2.0	1.4	4.0
	15	7.2	1.9	1.3	3.9
Dark control	13	5.4	1.3	1.0	2.9
	15	5.1	1.3	0.8	3.0
	15	5.3	1.4	1.0	3.0

TAR = total applied radioactivity

Table 7.1.1.3-12: Characterization of non-extractable residues (NER) in soil Speyer 5M after treatment with triazole-3(5)-^{14}C-metconazole [% TAR]

Incubation	Days after treatment	Total non-extractable radioactivity	Fulvic acids	Humic acids	Humins
Irradiated	2	5.8	1.4	0.5	3.6
	5	5.2	2.2	0.6	2.0
	8	8.1	3.3	0.9	3.5
	8	6.4	2.7	0.6	2.4
	10	5.9	2.9	0.6	2.0
	10	5.7	2.5	0.5	1.9
	13	10.0	4.9	0.9	3.0
	13	7.6	3.8	0.8	2.8
	15	8.1	3.8	0.6	2.9
	15	9.9	5.1	0.8	3.4
Dark control	8	7.0	3.4	0.6	2.4
	8	12.6	3.2	0.5	7.7
	10	6.3	3.1	0.6	2.0
	10	6.2	3.3	0.5	2.0
	13	7.7	3.9	0.6	2.6
	13	6.7	3.8	0.6	2.2
	15	7.7	4.0	0.6	2.5
	15	7.4	3.9	0.5	2.5

TAR = total applied radioactivity

F. KINETIC MODELING RESULTS

Degradation rates of metconazole were estimated using the software package KinGUI2 following the recommendations of the FOCUS Kinetics workgroup. The $DegT_{50}$ values were calculated using data from both radiolabeled forms of metconazole. The soil residues for the irradiated and the dark control experiment could both be best described by the SFO kinetic fit approach. The $DegT_{50}$/$DegT_{90}$ values obtained are presented in Table 7.1.1.3-13.

Table 7.1.1.3-13: Trigger endpoints for ^{14}C-labeled metconazole

Test system	$DegT_{50}$ [d]	$DegT_{90}$ [d]	Best-fit model	χ^2 error [%]
Photolysis	68.3	226.9	SFO	1.1
Dark control	163.8	544.0	SFO	1.8

III. CONCLUSION

Metconazole (BAS 555 F) was degraded on irradiated and non-irradiated soil surfaces, with DT_{50} values of 68.3 days and 163.8 days respectively.

Metconazole was metabolized to M555F030cis (Reg. No. 4110625) (≤ 3.7% TAR), two low level unidentified degradates (≤ 4.0% TAR and ≤ 2.5% TAR, respectively), bound residues, and finally carbon dioxide.

Irradiation in the soil photolysis experiment with [phenyl-U-^{14}C]- and [3,(5)-triazole-^{14}C]-metconazole showed a minor but significant influence on the degradation behavior and metabolite formation in soil. Under irradiated and dark conditions, the formation of the known soil metabolite M555F030cis was confirmed, indicating similar degradation processes but overall metconazole degradation is slightly faster in irradiated samples. No major metabolite and no metabolite specific for one of the labels was detected. The enantiomer ratio of metconazole was about 4:4:1:1 (R,S: S,R: S,S: R,R metconazole (BAS 555 F)) for all analyzed samples. This result suggests that the ratio is stable over time and no isomerization occurs.

Summary: Route of Degradation of metconazole in Soil

The overall understanding of soil degradation of metconazole did not change since the evaluation during the previous Annex I inclusion process (according to EU Directive 91/414/EEC).

Metconazole degraded slowly during incubation under aerobic conditions. The major routes of BAS 555 F degradation in soil was either mineralization or formation of non-extractable residues. Minor amounts of the metabolites M555F030, M555F020 and M555F040 were observed (<5%).

Under anaerobic conditions no significant degradation occurred. Photodegradation on the soil surface does not have a significant impact on the degradation rate or pathway of metconazole in soil. Overall, no major degradation products >5% TAR were detected in any of the laboratory soil degradation studies under various experimental conditions.

A scheme on the proposed route of degradation is given in Figure 7.1.1.3-1

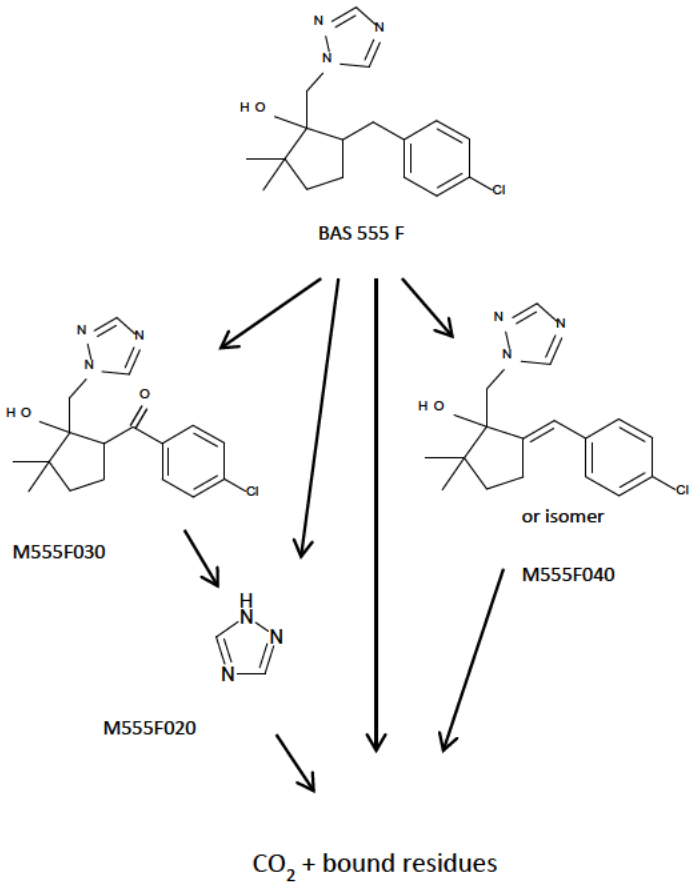


Figure 7.1.1.3-1: Proposed route of degradation of metconazole in soil

CA 7.1.2 Rate of degradation in soil

CA 7.1.2.1 Laboratory studies

CA 7.1.2.1.1 Aerobic degradation of the active substance

In the new soil metabolism study performed with the triazole- and phenyl-labeled metconazole, besides information on the route of degradation also degradation rates were obtained. This study is summarized in chapter M-CA 7.1.1.1 [*CA 7.1.1.1/1, BASF DocID 2014/1000901*].

Further, metconazole degradation rates were re-calculated from the older, already peer-reviewed studies using their experimental data and analysing the kinetic parameters according to the current FOCUS guidance.

A summary table of all obtained laboratory soil degradation values for metconazole can be found at the end of this chapter (best-fit and normalized to 20°C, pF 2).

Report:	CA 7.1.2.1.1/1 Dalkmann P., 2015b Kinetic evaluation of laboratory soil degradation of Metconazole (BAS 555 F) for derivation of trigger and modeling endpoints according to FOCUS 2014/1010790
Guidelines:	FOCUS Kinetics (2006) SANCO/10058/2005 version 1.1 of Dec. 2014
GLP:	no

Executive Summary

The degradation of the fungicide BAS 555 F – metconazole in soil has been investigated in two laboratory degradation studies in a total of six soils. The purpose of this evaluation was to analyze the degradation kinetics of metconazole observed in the studies according to current guidance of the FOCUS workgroup on degradation kinetics.

The best-fit model to derive trigger endpoints was selected based on a visual and statistical assessment. Modeling endpoints were normalized to reference conditions where necessary.

The kinetic evaluation showed that with one exception (soil Ipswich) the bi-phasic models (FOMC, DFOP) provided the best-fit to the measured data for metconazole incubated while the SFO model was appropriate for derivation of modeling endpoints.

Trigger endpoints (best-fit) $DegT_{50}$ values were between 87.7 and >1000 days, and $DegT_{90}$ values were between 291.3 and >1000 days. Modeling $DegT_{50}$ values ranged between 87.7 and 549.9 days, whereas normalized modeling endpoints (20°C, pF2) ranged between 69.0 and 568.6 days.

I. MATERIAL AND METHODS

The degradation of metconazole in six different soils [*old EU dossier, M II A 7.1.1.1.1/1, BASF DocID 2002/7004457; M II A 7.1.1.1.1/2, BASF DocID 1990/7000168*] was analyzed taking into account the current guidance of the FOCUS workgroup on degradation kinetics [*FOCUS (2006)*].

Kinetic modeling strategy

Kinetic evaluation was performed in order to derive degradation parameters as triggers for additional work (trigger endpoints) as well as modeling endpoints. The best-fit model was selected based on visual and statistical assessment and the corresponding $DegT_{50}$ and $DegT_{90}$ values are reported as trigger endpoints. Appropriate $DegT_{50}$ values for use in environmental fate models were derived depending on the kinetic model.

Kinetic models included in the evaluations

For each data set, the kinetic models proposed by FOCUS Kinetics [*FOCUS (2006)*] were tested in order to identify the best-fit model, i.e. single first order (SFO) kinetics, the Gustafson-Holden model (FOMC) and the bi-exponential (DFOP) kinetics. The respective model descriptions and corresponding equations for calculating endpoints ($DegT_{50}$, $DegT_{90}$) are shown in the FOCUS Kinetics guidance [*FOCUS (2006)*].

The goodness-of-fit of the kinetic models was assessed by visual inspection and statistical measures, as recommended by the FOCUS Kinetics guidance [*FOCUS (2006)*].

Data handling and software for kinetic evaluation

The initial value at day zero for metconazole was set to the material balance. For all samplings, measurements from single (*study MK-620-002*) or duplicate (*study MK-620-020*) samplings were considered for the parameter estimation.

The software package KinGUI version 2.2012.224.1704 was used for parameter fitting. The error tolerance and the number of iterations of the optimization tool (IRLS) were set to 10^{-6} and 100, respectively.

Normalization to reference conditions

According to FOCUS *(2006)* the $DegT_{50}$ values obtained from laboratory studies should be normalized for modeling purposes to reference conditions at a temperature of 20°C and soil moisture at pF2 to account for the different soil temperature and moisture conditions during incubation. The temperature and soil moisture normalization was performed according to FOCUS *(2006).*

The moisture content of the soils at pF2 was not available. Therefore, the default value for each soil type given in FOCUS [*FOCUS (2014)*] was used. For consistency, the study soil moisture was also calculated from the default value at MWHC for each soil type given in FOCUS [*FOCUS (2014)*].

Experimental data

In study *MK-620-002* [*BASF DocID 1990/7000168*], the test soils were treated with cyclopentanol-ring-^{14}C-labeled metconazole at a nominal application rate of 0.5 mg kg^{-1}, corresponding to about 500 g a.s. ha^{-1}. The test soils were incubated under dark aerobic conditions at 22 ± 2ºC and 40% of the maximum water holding capacity (MWHC) for 112 days.

The test soil used in study *MK-620-020* [*BASF DocID 2002/7004457*] was treated with triazole-3(5)-^{14}C-labeled metconazole at a nominal application rate of 0.24 mg kg^{-1} dry soil corresponding to about 360 g a.s. ha^{-1}. The test soil was incubated under dark aerobic conditions at 20 ± 2°C and 50% MWHC for 120 days. The soil characteristics for these two studies are summarized in Table 7.1.2.1.1-1.

Table 7.1.2.1.1-1: Soil characteristics

Study	**MK-620-002 [DocID 1990/7000168]**	**MK-620-002 [DocID 1990/7000168]**	**MK-620-002 [DocID 1990/7000168]**
Soil designation	**Bog Farm**	**Chestnut Street**	**Elm Farm**
Origin	Kent, UK	Kent, UK	Kent, UK
Textural class	Sandy loam	Silty clay loam	Sandy loam
Particle size distribution [%] sand silt clay	 68 16 16	 15 51 34	 57 29 14
Organic C [%]	1.98	2.39	1.99
Microbial biomass [mg C 100 g^{-1} dry soil] initial final	 41.8 30.2	 44.7 50.0	 60.3 29.6
CEC [meq 100 g^{-1}]	19.5	25.2	16.3
pH [-]	7.4	8.0	6.7
Study	**MK-620-002 [DocID 1990/7000168]**	**MK-620-002 [DocID 1990/7000168]**	**MK-620-020 [DocID 2002/7004457]**
Soil designation	**Woodstock**	**German standard soil**	**Ipswich**
Origin	Kent, UK	Germany	Ipswich, UK
Textural class	Clay	Sand	Sandy loam
Particle size distribution [%] sand silt clay	 22 38 40	 87 11 2	 66.5 [a] 26.0 [a] 7.5 [a]
Organic C [%]	1.97	2.31	1.3
Microbial biomass [mg C 100 g^{-1} dry soil] initial final	 52.2 42.0	 59.0 18.9	 49.9 62.6
CEC [meq 100 g^{-1}]	26.9	12.2	11.4
pH [-]	6.6	5.3	6.8 [b]

[a] According to USDA classification

[b] Measured in 100 mM KCl

The measured data as well as resulting datasets submitted to kinetic analysis are summarized in Table 7.1.2.1.1-2 to Table 7.1.2.1.1-3.

Table 7.1.2.1.1-2: Experimental data from study MK-620-002 used for kinetic evaluation

DAT	Extractable radioactive residues of metconazole [%TAR]	
	Experimental data	Input data according to FOCUS
Soil Bog Farm, Kent		
0	97.5	101.3 [a]
7	91.9	91.9
14	92.1	92.1
28	86.5	86.5
56	82.1	82.1
112	71.4	71.4
Soil Chestnut Street		
0	96.6	98.6 [a]
7	88.5	88.5
14	87.1	87.1
28	81.3	81.3
56	73.3	73.3
112	62.9	62.9
Soil Elm Farm, Kent		
0	96.2	98.9 [a]
7	86.3	86.3
14	87.4	87.4
28	73.1	73.1
56	70.8	70.8
112	63.1	63.1
Soil Woodstock, Kent		
0	95.5	98.6 [a]
7	90.0	90.0
14	88.6	88.6
28	84.0	84.0
56	79.3	79.3
112	69.1	69.1
Soil German standard soil 2.2		
0	96.3	98.4 [a]
7	92.8	92.8
14	94.6	94.6
28	87.9	87.9
56	86.9	86.9
112	84.2	84.2

DAT Days after treatment
TAR Total Applied Radioactivity
[a] Set to material balance

Table 7.1.2.1.1-3: Experimental data from study MK-620-020 used for kinetic evaluation

DAT	Extractable radioactive residues of metconazole [%TAR]	
	Experimental data	Input data according to FOCUS
Soil Ipswitch, Kent		
0	95.5	97.4 [a]
0	97.2	99.0 [a]
3	91.4	91.4
3	91.7	91.7
7	90.4	90.4
7	91.3	91.3
14	84.3	84.3
14	87.4	87.4
28	79.2	79.2
28	69.5	69.5
56	60.1	60.1
56	62.8	62.8
91	53.1	53.1
91	56.6	56.6
120	29.1	29.1
120	32.3	32.3

DAT Days after treatment
TAR Total Applied Radioactivity
[a] Set to material balance

II. RESULTS AND DISCUSSION

The derived trigger endpoints (best-fit) for metconazole are summarized in Table 7.1.2.1.1-4. The kinetic evaluation showed that the SFO model is appropriate to derive trigger endpoints for additional work in one soil of study MK-620-020, while biphasic kinetic models (DFOP or FOMC) were selected as best-fit models in five soils of study MK-620-002.

Table 7.1.2.1.1-4: Summary of endpoints for use as triggers for additional work of metconazole

BASF DocID	Soil	Soil type (USDA)	Best-fit kinetic model	χ^2 error	$DegT_{50}$ [d]	$DegT_{90}$ [d]
MK-620-002 [DocID 1990/7000168]	Bog Farm	Sandy loam	DFOP	0.9	251.5	906.8
	Chestnut Street	Silty clay loam	DFOP	1.0	184.1	681.8
	Elm Farm	Sandy loam	FOMC	2.7	558.0	>1000
	Woodstock	Clay	FOMC	0.5	249.8	908.2
	German standard soil 2.2	Sand	FOMC	1.3	>1000	>1000
MK-620-020 [DocID 2002/7004457]	Ipswich	Sandy loam	SFO	4.3	87.7	291.3

Modeling endpoints for metconazole were obtained using SFO kinetics in the six trials. The $DegT_{50}$ values suitable for modeling obtained under different incubation conditions were normalized to a reference moisture of pF 2 and a temperature of 20°C. Parameters included in the normalization procedure are shown in Table 7.1.2.1.1-5 while derived modeling endpoints are summarized in Table 7.1.2.1.1-6.

Table 7.1.2.1.1-5: Factors for normalization to reference conditions (20°C, pF 2.0)

Soil, soil type	FOCUS default MWHC	θ_{act} [g/100g]	θ_{ref} [g/100g]	f_{moist} [-]	$Temp_{act}$ [°C]	$Temp_{ref}$ [°C]	f_{temp}
Bog Farm, sandy loam	27	10.8	19	0.673	22	20	1.2
Chestnut Street, silty clay loam	34	13.6	30	0.575	22	20	1.2
Elm Farm, sandy loam	27	10.8	19	0.673	22	20	1.2
Woodstock clay	53	21.2	48	0.564	22	20	1.2
German standard soil 2.2,sand	24	9.6	12	0.855	22	20	1.2
Ipswich, sandy loam	27	13.5	19	0.787	20	20	1.0

θ_{act}	Calculated actual soil moisture (40% of MWHC, soil Ipswich 50%)	[g / 100 g dry soil]
θ_{ref}	Reference soil moisture at field capacity (pF 2) according to FOCUS (2006)	[g / 100 g dry soil]
f_{moist}	Moisture correction factor	[-]
f_{temp}	temperature correction factor	[-]
$Temp_{act}$	Incubation temperature at study conditions	[°C]
$Temp_{ref}$	Incubation temperature at reference conditions	[°C]]

Table 7.1.2.1.1-6: Summary of modeling endpoints of metconazole

Study	Soil	Soil type (USDA)	Kinetic model	χ^2 error	Non-normalized $DegT_{50}$ [d] [a]	Normalized $DegT_{50}$ [d] [b]
MK-620-002 [DocID 1990/7000168]	Bog Farm	Sandy loam	SFO	2.3	243.7	198.4
	Chestnut Street	Silty clay loam	SFO	2.8	179.7	124.8
	Elm Farm	Sandy loam	SFO	5.2	176.6	143.7
	Woodstock	Clay	SFO	2.3	238.1	162.4
	German standard soil 2.2	Sand	SFO	2.1	549.9	568.6
MK-620-020 [DocID 2002/7004457]	Ipswich	Sandy loam	SFO	4.3	87.7	69.0

[a] Actual study conditions
[b] Reference conditions: 20°C, pF 2

III. CONCLUSION

Trigger and modeling endpoints were derived for metconazole in two laboratory degradation studies with six soils. The kinetic evaluation showed that with one exception (soil Ipswich) the bi-phasic models (FOMC, DFOP) provided the best fit to the measured data for metconazole incubated while the SFO model was appropriate for derivation of modeling endpoints.

Trigger endpoints (best-fit) $DegT_{50}$ values were between 87.7 and >1000 days, and $DegT_{90}$ values were between 291.3 and >1000 days. Modeling $DegT_{50}$ values ranged between 87.7 and 549.9 days, whereas normalized modeling endpoints (20°C, pF2) ranged between 69.0 and 568.6 days.

Summary of degradation endpoints for metconazole in various soils under aerobic laboratory conditions

Table 7.1.2.1.1-7: Summary table on best-fit degradation endpoints of metconazole obtained in laboratory soil studies

BASF DocID	Soil / Soil type	pH ($CaCl_2$)	Org. C [%]	Temp. [°C]	Moisture [% MWHC]	Best-fit $DegT_{50}$ / $DegT_{90}$ [d]	Kinetic model	χ^2 error level
1990/7000168 (MK-620-002) 2014/1010790	Bog Farm Sandy loam (c)	7.4 [a]	1.98	22	40	251.5 / 906.8	DFOP	0.9
	Chestnut Street Silty clay loam (c)	8.0 [a]	2.39	22	40	184.1 / 681.8	DFOP	1.0
	Elm Farm Sandy loam (c)	6.7 [a]	1.99	22	40	558.0 / >1000	FOMC	2.7
	Woodstock Clay (c)	6.6 [a]	1.97	22	40	249.8 / 908.2	FOMC	0.5
	German standard soil 2.2 Sand (c)	5.3 [a]	2.31	22	40	>1000 / >1000	FOMC	1.3
2002/7004457 (MK-620-020) 2014/1010790	Ipswich Sandy loam (t)	6.8 [b]	1.3	20	50	87.7 / 291.3	SFO	4.3
2014/1000901	LUFA 5M Sandy loam (t),(p)	7.4	1.98	20	45	128.4 / 426.6	SFO	1.23

(t), (c), (p) – triazole-, cyclopentanol-, or phenyl-labeled test item used
MWHC maximum water holding capacity
[a] no method stated in original study report
[b] Measured in 100 mM KCl

Table 7.1.2.1.1-8: Summary table on degradation endpoints for modeling of metconazole obtained in laboratory soil studies (normalized to 20°C, pF2)

BASF DocID	Soil / Soil type	pH ($CaCl_2$)	Org. C [%]	Temp. [°C]	Moisture [% MWHC]	$DegT_{50}$ at study conditions [d]	$DegT_{50}$ normalized to 20°C, pF2 [d]	Kinetic model	χ^2 error level
1990/7000168 (MK-620-002) 2014/1010790	Bog Farm Sandy loam (c)	7.4 [a]	1.98	22	40	243.7	198.4	SFO	2.3
	Chestnut Street Silty clay loam (c)	8.0 [a]	2.39	22	40	179.7	124.8	SFO	2.8
	Elm Farm Sandy loam (c)	6.7 [a]	1.99	22	40	176.6	143.7	SFO	5.2
	Woodstock Clay (c)	6.6 [a]	1.97	22	40	238.1	162.4	SFO	2.3
	German standard soil 2.2 Sand (c)	5.3 [a]	2.31	22	40	549.9	568.6	SFO	2.1
2002/7004457 (MK-620-020) 2014/1010790	Ipswich Sandy loam (t)	6.8 [b]	1.3	20	50	87.7	69.0	SFO	4.3
2014/1000901	LUFA 5M Sandy loam (t),(p)	7.4	1.98	20	45	128.4	93.9	SFO	1.23

(t), (c), (p) – triazole-, cyclopentanol-, or phenyl-labeled test item used
MWHC maximum water holding capacity
[a] no method stated in original study report
[b] Measured in 100 mM KCl

CA 7.1.2.1.2 Aerobic degradation of metabolites, breakdown and reaction products

No metabolites > 5% occurred in any of the aerobic soil degradation studies. However, M555F020 (1,2,4-triazole) was appointed to an unidentified peak from the already peer-reviewed aerobic metabolism study [*BASF DocID 2002/7004457 (MK-620-020)*]. Therefore, based on data from this study a conservative formation fraction was derived for modeling purposes.

As no data for M555F020 (1,2,4-triazole) were available from the aerobic degradation studies on metconazole, consequently no degradation rates were calculated. Degradation endpoints for 1,2,4-triazole are available and accepted at EU level [*CRD (2014): Triazole Derived Metabolite: 1,2,4-Triazole. Proposed revision to DT_{50} Summary, Scientific Evaluation and Assessment July 2011, revised September 2011 (after comments from MS and EFSA) and further revised January 2013 (minor clarifications added post-commenting) 24 Oct. 2014*].

Report:	CA 7.1.2.1.2/1 Pape L., 2015d Calculation of a formation fraction of 1,2,4-triazole from BAS 555 F – Metconazole for use in environmental fate modeling 2015/1186256
Guidelines:	FOCUS Degradation Kinetics (2006) SANCO/10058/2005 version 2.0 (December 2014)
GLP:	no

Executive Summary

The purpose of this evaluation was the calculation of a formation fraction for 1,2,4-triazole (possible soil degradate of metconazole) for use in environmental fate models according to current guidance of the FOCUS workgroup on degradation kinetics.

The evaluation was based on the results of an aerobic soil degradation study with metconazole where the residues of a polar fraction where assigned to 1,2,4-triazole. The degradation parameters of 1,2,4-triazole were fixed to EU agreed modeling endpoints.

The evaluation resulted in a reliable and conservative formation fraction of 1,2,4-triazole formed from metconazole of 0.2836, which is appropriate for usage in environmental fate models.

I. MATERIAL AND METHODS

The evaluation was based on the findings of a laboratory aerobic soil degradation study with ^{14}C-triazole-labeled metconazole with one soil [peer-reviewed in previous EU evaluation, *BASF DocID 2002/7004457 (MK-620-020)*]. In this study, a polar fraction was detected at a maximum amount of 10.5 % TAR at the end of the study (121 DAT). It was shown that the polar fraction contained at least two components and that the major component represented an average of 9.1% TAR. It is assumed that this polar fraction is 1,2,4-triazole, based on similar HPLC retention times of this polar peak versus 1,2,4-triazole which was observed in a new ^{14}C-triazole-labeled aerobic metabolism study at very low concentrations (maximum of 1.2% TAR) [*CA 7.1.1.1/1, BASF DocID 2014/1000901*]. Individual data points were only available for the total polar fraction. Hence, these residues were used for the derivation of a formation fraction of the assumed 1,2,4-triazole metabolite, representing a conservative approach.

The software package Cake (version 3.1) was used for parameter fitting [*Tessella (2015): Computer Assisted Kinetic Evaluation (CAKE) Application. Version 3.1, March 2015*]. The convergence tolerance and the number of iterations of the optimization tool (IRLS) were set to 10^{-5} and 100, respectively.

For the definitive model run the degradation parameters of metconazole were fixed to the results of the respective kinetic evaluation, which was performed in a separate study [*CA 7.1.2.1.1/1, BASF DocID 2014/1010790*]. The degradation parameters of 1,2,4-triazole were fixed to the EU agreed endpoints recommended for use in environmental fate models [*CRD (2014)*]. The initial value for the formation fraction of 1,2,4-triazole was set to 1.

In addition to the definitive model run with fixed degradation parameters for parent and metabolite, a comparative model run was conducted with released degradation parameters (SFO) for triazole in order to assess the conservativeness of the calculated formation fraction.

II. RESULTS AND DISCUSSION

The results of the visual and statistical assessment are presented in Table 7.1.2.1.2-1.

Table 7.1.2.1.2-1: Visual and statistical assessment for 1,2,4-triazole (formed from metconazole)

Model run	1,2,4-triazole kinetic model	Visual assessment	χ^2 error	Formation fraction	Confidence interval (95%)	$DegT_{50}$ / $DegT_{90}$ [d]
Definitive [a]	DFOP	Acceptable	16.9	0.2836	0.2302 - 0.337	6.93 / 142
Comparative [b]	SFO	Good	11.8	0.1644	0.08795 - 0.241	>10000 [c]

[a] Degradation parameters for 1,2,4-triazole fixed to EU agreed modeling endpoints
[b] Degradation parameters for 1,2,4-triazole free
[c] Estimated not significantly different from zero

The definitive model run results in an acceptable visual fit for 1,2,4-triazole. The χ^2 error value above 15% is acceptable as it results from fixing the degradation parameters for 1,2,4-triazole to EU agreed modeling endpoints. The estimated formation fraction of 0.2836 is reliable indicated by the narrow 95% confidence interval.

The better visual fit and lower χ^2 error value of the comparative model run are attributed to releasing the degradation parameters for 1,2,4-triazole. In contrast to the definitive model run, the estimated formation fraction is considerably lower (0.1644) than for the definitive model run and has a high uncertainty indicated by the wide 95% confidence interval. The degradation rate for 1,2,4-triazole is estimated not significantly different from zero (p(t-test) = 0.5) and results in an extremely high $DegT_{50}$ value.

III. CONCLUSION

The definitive model run, with the degradation parameters of 1,2,4-triazole fixed to EU agreed modeling endpoints, resulted in a reliable and conservative formation fraction of 1,2,4-triazole formed from metconazole of 0.2836, and is appropriate for usage in environmental fate models.

CA 7.1.2.1.3 Anaerobic degradation of the active substance

The degradation rates of metconazole in anaerobic soil are already presented in CA 7.1.1.2. Besides the new anaerobic metabolism study [*CA 7.1.1.2/1, BASF DocID 2014/1000922*], results of the already peer-reviewed anaerobic metabolism study [*BASF DocID 2001/7000226 (MK-620-018)*] were summarized in M-CA 7.1.1.2. No kinetic re-evaluation was required.

CA 7.1.2.1.4 Anaerobic degradation of metabolites, breakdown and reaction products

No metabolites > 5% were observed in any of the anaerobic soil metabolism studies.

CA 7.1.2.2 Field studies

Based on the results of laboratory soil degradation studies, terrestrial field dissipation studies are triggered.

Several already peer-reviewed field soil dissipation studies [*BASF DocID 1993/7000320 (MK-620-010); BASF DocID 1996/7000437 (MK-790-011), BASF DocID 1996/7000438 (MK-790-012)*] as well as residue testing studies [*BASF DocID 1992/7000259 (MK-790-004); BASF DocID 1992/7000261 (MK-790-006); BASF DocID 1992/7000263 (MK-790-008)*] were performed to investigate the degradation and dissipation of metconazole in soil and to determine the DT_{50} and DT_{90}. The studies were conducted in Europe at trial sites in Germany, UK, France and Spain on bare soil with various formulations and application rates. Overall, metconazole did not show a tendency to move into deeper layers of soil and was mostly detected in the top soil layers. The field soil dissipation rates of metconazole were in the range of 1 to 37 weeks (maximum of 259 days).

For the already peer-reviewed studies it was stated by EFSA that for the exposure assessment only the worst-case non-normalized value should be used were applicable. Due to the poor data quality no normalization of the data could be performed. Nevertheless, the study results are considered suitable for derivation of persistence endpoints.

For these reasons and to account for the new requirements of 1107/2009, a new terrestrial field dissipation study, considering current requirements (amongst others exclusion of surface loss processes), was conducted and is submitted under CA 7.1.1.2.1 [*CA 7.1.2.2.1/1, BASF DocID 2015/1000221*]. In line with the results of the previous studies metconazole was observed only in the top soil layer, and it was concluded that metconazole does not show any significant tendency to move into deeper soil layers.

Kinetic evaluation to derive best-fit as well as modeling endpoints was conducted according to current guidance documents and is provided under CA 7.1.2.2.1/2 [*BASF DocID 2015/1137154*] and CA 7.1.2.2.1/3 [*BASF DocID 2015/1137155*].

A summary table of all obtained field soil dissipation and degradation values for metconazole can be found at the end of this chapter (best-fit and normalized to 20°C, pF 2).

Further, in addition to the already peer-reviewed storage stability study [*BASF DocID 1994/7000180 (MK-326-002)*] a storage stability study was carried out under consideration of the two isomers of metconazole as well as its metabolite 1,2,4-(1H)-triazole [*CA 7.1.2.2.1/4, BASF DocID 2015/1204922 ~~2014/1001261~~*].

Note furthermore, that in the *EFSA scientific report (2006) 64*, assignment of half-lives of the old field studies to the respective countries was incorrect, please refer to the corrections as provided in the updating statement as well as in the summary table at the end of this chapter.

In the course of the evaluation of the formulation it had shown up, that the initially assigned formulation type "SL" did not describe the formulation type correctly. It must be exchanged to the formulation type "EC" (see M-CP 1.5). However, as this decision had been taken after most of the studies had been finalized the initially assigned code is used in most of the studies this dossier is referring to.

CA 7.1.2.2.1 Soil dissipation studies

Report:	CA 7.1.2.2.1/1 Bayer H.,Kuhnke G., 2015a Field soil dissipation study of BAS 555 F (Metconazole) in the formulation BAS 555 01 F on bare soil at six sites in Europe, 2012 2015/1000221
Guidelines:	NAFTA Guidance Document for Conducting Terrestrial Field Dissipation Studies Regulatory Directive DIR2006-01 (March 2006), EPA 835.6100, SETAC Procedures for assessing the environmental fate and ecotoxicity for pesticides (March 1995), SANCO/3029/99 rev. 4 (11 July 2000), EFSA Guidance to obtain DegT50 values in soil (2010)
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

The dissipation of metconazole (BAS 555 F) under field conditions was investigated at six sites in Europe representative of Northern, Central and Southern EU conditions (Denmark, Germany (two trials), Italy, France and Spain). All sites represent typical regions of agricultural practice representative for the use of metconazole. The trial sites consisted of an untreated and a treated plot, the latter being subdivided into three subplots that were assigned for replicates.

The product BAS 555 01 F, formulated as an soluble concentrate (SL), was broadcast applied to bare soil in a single application at a nominal rate of 90 g a.s. ha^{-1} using a target water volume of 300 L ha^{-1}. Applications were conducted between mid of April and mid of June 2012 (spring application) and mid of September 2012 (fall application) using a calibrated boom sprayer.

Immediately after application of the test item, the plots were covered with a layer of sand of approximately 4-6 mm depth (average across all trials) to protect the applied product from surface processes like photolysis or volatilization, and to exclude any potential impact on the degradation of the test item caused by any of these processes.

No tillage or fertilization was performed during the course of the study and no crops were grown throughout any of the trials. The plots were kept free of vegetation via the application of herbicides. Rainfall was supplemented with irrigation if needed.

Soil specimens were taken up to 724 days after application and down to a maximum soil depth of 50 cm. Soil cores were cut into 10 cm sections. Soil segments of the same depth and subplot from a defined sampling event were pooled and homogenized and a representative sub-sample of each depth was taken for residue analysis.

Soil specimens were analyzed for residues of metconazole (cis- and trans-isomers) and 1,2,4-(1*H*)-triazole according to BASF method L0203/01. The analytical method involved twice extraction of the soil with acetonitrile/water 70/30 (v/v). The final determination of the analytes was performed by LC-MS/MS with a limit of quantification (LOQ) of 2.0 µg kg^{-1} for each analyte. The limit of detection (LOD) was set at 0.60 µg kg^{-1}.

Field soil specimens from the treated plot were analyzed down to 50 cm depth. Analysis was performed up to a maximum of 724 days after treatment (DAT).

Untreated soil specimens (control samples) were analyzed for residues of metconazole (cis- and trans-isomers) and 1,2,4-(1*H*)-triazole. No residues of metconazole were detected in any of the control samples proving that there were no interferences of the untreated soil material with the analytical procedures used. However, significant interferences (> 30% of LOQ) were observed in some samples at the retention times and mass transitions considered for 1,2,4-(1*H*)-triazole. Therefore, interferences in some control samples were determined and blank correction in the recovery data was needed.

Metconazole degraded well at all six European field sites with no significant change in the cis/trans isomer ratio. The total amount of metconazole residues detected in the soil profiles decreased from an average of 74 g ha^{-1} at day 0 (mean over all trials) to an average of 12 g ha^{-1} (mean over all trials) after two years. In two of the trials (France and Spain) no residues were detectable any longer after two years. In the other four trials the residues left after 710-724 days ranged from 0.7 to 16 g ha^{-1}.

Metconazole residues were exclusively found in the top 0-20 cm layer of the soils in all six trials except one single detect <LOQ at 15 DAT in the 30-40 cm layer of the trial in Germany (West). This single detect was considered to be caused by contamination during sampling or processing rather than by movement of the substance through the soil. Altogether, it was concluded that metconazole does not show any significant tendency to move into deeper soil layers indicating low potential for metconazole residues to leach to groundwater.

1,2,4-(1*H*)-Triazole was detected at all sites. Residues, however, did not appear in a consistent way, but were generally scattered erratically across the whole soil profile without any coherent pattern. Moreover, residues were partly much higher than the amount that could theoretically be formed from the metconazole test item. High amounts of 1,2,4-(1*H*)-triazole were also detected in the untreated control samples of all soils indicating a substantial background contamination. Since no triazole-pesticides had been applied on the trial sites in the previous 3 years, the contamination might have resulted from the potential application of triazole containing nitrogen fertilizers. As a consequence, the analyzed triazole data are not considered valid and therefore, the results of the 1,2,4-(1*H*)-triazole analysis are not summarized. There was no indication that significant amounts of 1,2,4-(1*H*)-triazole were formed.

No calculation of dissipation times is provided in the summarized report. A detailed kinetic evaluation of the degradation behavior of metconazole in the six European field soils is provided in separate modeling reports.

I. MATERIAL AND METHODS

1. Test Material

Test item (formulation):	BAS 555 01 F
Active substance (a.s.):	Metconazole (BAS 555 F, Reg. No. 4056343)
Type of formulation:	SL
Batch No.:	FRE-000698
Content of a.s.:	90.2 g L^{-1} (nominal 90.0 g L^{-1})
Expiration date:	March 31, 2013

2. Test sites

The dissipation of metconazole under field conditions was investigated at six sites in Europe representative of Northern, Central, and Southern EU conditions. Trials were performed in Denmark (L120312), Germany (East - L120313, West – L120314), France (L120315), Italy (L120316), and Spain (L120317). The homogeneity of the upper soil layer was verified prior to the start of the trials. The site characteristics are presented in Table 7.1.2.2.1-1 and Table 7.1.2.2.1-2. Soil parameters were determined from untreated soil samples taken from the fields following segmentation according to the soil horizons. Soil taxonomy was determined on the basis of regional soil maps.

Table 7.1.2.2.1-1: Characteristics of the trial sites L120312 and L120313 used to investigate the field dissipation of metconazole

Trial	**L120312**			**L120313**	
Location	**Bogense, Denmark**			**Brunne, Germany (East)**	
Soil properties	0 - 20 cm	20 - 40 cm	40 - 60 cm	0 - 35 cm	35 – 50 cm
Soil class (DIN 4220)	Medium silty sand (Su3)	Medium silty sand (Su3)	High loamy sand (Sl4)	Poor loamy sand (Sl2)	Poor loamy sand (Sl2)
sand [%]	70.5	67.1	62.3	71.0	71.5
silt [%]	25.6	27.5	23.4	23.7	23.5
clay [%]	3.9	5.3	14.3	5.4	5.1
Soil class (USDA)	Sandy loam	Sandy loam	Sandy loam	Loamy sand	Sandy loam
sand [%]	73.5	69.6	65.8	75.0	74.4
silt [%]	22.3	23.8	21.1	20.8	20.7
clay [%]	4.2	6.5	13.1	4.1	4.9
Total organic C [%]	0.96	0.62	0.30	0.72	0.31
Organic matter [%] [a]	1.66	1.07	0.52	1.24	0.53
pH [$CaCl_2$]	6.29	6.50	6.81	5.01	4.83
pH [H_2O]	6.80	7.21	7.70	5.59	5.57
CEC [mval Ba $100g^{-1}$ dry weight]	8.9	8.8	11.7	5.5	4.1
MWHC [g $100g^{-1}$ dry weight]	47.0	47.5	49.6	40.1	40.3
pF 2.0 [g $100g^{-1}$ dry weight] [b]	16.6	14.6	19.2	11.6	11.0
pF 2.5 [g $100g^{-1}$ dry weight] [b]	8.8	8.3	11.5	6.7	6.0
Dry bulk density [g cm^{-3}] [c]	1.43	-	-	1.51	-

CEC = Cation exchange capacity
MWHC = Maximum water holding capacity
[a] Organic matter = organic carbon x 1.724
[b] Water retention characteristics, soil moisture at 0.1 or 0.33 bar
[c] Mean of 3 replicates taken at 10-20 cm depth (L120312) and 15 cm depth (L120313)

Table 7.1.2.2.1-2: Characteristics of the trial sites L120314, L120315, L120316 and L120317 used to investigate the field dissipation of metconazole

Trial	**L120314**		**L120315**	
Location	**Goch-Nierswalde, Germany (West)**		**Schaeffersheim, France (North)**	
Soil properties	0 - 35 cm	35 - 50 cm	0 - 25 cm	25 – 50 cm
Soil class (DIN 4220)	Sandy silt (Us)	Sandy silt (Us)	Pure silt (Uu)	Medium clay silt (Ut3)
sand [%]	21.7	28.2	9.2	7.0
silt [%]	73.0	71.0	83.3	76.1
clay [%]	5.4	0.9	7.5	16.9
Soil class (USDA)	Silt loam	Silt loam	Silt	Silt loam
sand [%]	30.7	30.2	9.9	9.2
silt [%]	65.5	69.0	80.1	73.1
clay [%]	3.8	0.9	9.9	17.7
Total organic C [%]	1.67	0.53	2.03	1.40
Organic matter [%] [a]	2.88	0.91	3.50	2.41
pH [$CaCl_2$]	6.55	6.16	7.63	7.89
pH [H_2O]	7.04	6.77	8.12	8.41
CEC [mval Ba 100g^{-1} dry weight]	12.3	6.2	18.7	15.8
MWHC [g 100g^{-1} dry weight]	60.7	34.5	60.2	57.6
pF 2.0 [g100g^{-1} dry weight] [b]	24.2	28.4	31.1	29.5
pF 2.5 [g 100g^{-1} dry weight] [b]	16.9	13.0	21.1	20.2
Dry bulk density [g cm^{-3}] [c]	1.39	-	1.12	-

Trial	**L120316**		**L120317**		
Location	**Poggio Renatico, Italy**		**Utrera, Spain**		
Soil properties	0 - 20 cm	20 - 50 cm	0 - 10 cm	10 – 25 cm	25 – 50 cm
Soil class (DIN 4220)	High clay silt (Ut4)	High clay silt (Ut4)	Poor clay sand (St2)	Poor clay sand (St2)	High sandy loam (Ls4)
sand [%]	11.7	10.8	86.6	86.6	60.8
silt [%]	66.4	66.0	8.2	7.7	19.9
clay [%]	21.9	23.3	5.1	5.6	19.3
Soil class (USDA)	Silt loam	Silt loam	Sand	Loamy sand	Sandy clay loam
sand [%]	16.2	13.9	88.0	87.4	62.0
silt [%]	62.3	62.5	6.5	6.5	12.7
clay [%]	21.5	23.6	5.4	6.1	25.3
Total organic C [%]	1.78	1.98	0.57	0.52	0.41
Organic matter [%] [a]	3.07	3.41	0.98	0.90	0.71
pH [$CaCl_2$]	7.75	7.49	7.33	7.19	6.46
pH [H_2O]	8.19	7.92	7.82	7.78	7.01
CEC [mval Ba 100g^{-1} dry weight]	15.7	15.5	6.2	5.8	14.5
MWHC [g 100g^{-1} dry weight]	48.7	44.8	33.7	41.5	48.5
pF 2.0 [g100g^{-1} dry weight] [b]	31.8	37.3	12.8	12.6	31.4
pF 2.5 [g 100g^{-1} dry weight] [b]	27.4	29.9	7.6	7.5	23.7
Dry bulk density [g cm^{-3}] [c]	1.07	-		1.65	-

CEC = Cation exchange capacity
MWHC = Maximum water holding capacity
[a] Organic matter = organic carbon x 1.724
[b] Water retention characteristics, soil moisture at 0.1 or 0.33 bar
[c] Mean of 3 replicates taken at 10-20 cm depth (L120314), 5-10 cm depth (L120315), 15 cm depth (L120316) and 12-15 cm depth (L120317)

The selected fields represented typical regions of agricultural practice with soils representative for growing crops including oilseed rape or cereals, and had been under cultivation for many years. The sites were flat without any significant slope. Before commencement of the first sampling, the soil at each trial site was prepared as for sowing and was rolled or levelled if considered necessary, but then was left fallow.

No product containing the active substance and any triazole fungicide had been used on the test plots in the last three years.

3. Experimental treatments

The trial area at each site was divided into two plots, one untreated control plot (size: 30 - 108 m^2) and one treated plot (size: 306 - 324 m^2). The treated plot consisted of three equal sized subplots A, B, and C that were assigned for replicates.

The product formulated as a soluble concentrate (SL), was broadcast applied to bare soil in a single application at a nominal rate of 90 g a.s. ha^{-1} using a target water volume of 300 L ha^{-1}. Applications were conducted between mid of April 2012 and mid of June 2012 (spring application) and mid of September 2012 (fall application) using a calibrated boom sprayer. Treated plots were three-fold replicated with subplot size ranging from 102 to 108 m^2. For each treated replicate, a separate spray mixture was prepared and the test item was applied to each subplot individually. Each spray mixture was visually checked for homogeneity and small aliquots of the spray mixture were taken before and after application of each individual subplot for later analysis.

The actual application rates determined by quantifying the amount of spray discharged ranged from 87.8 to 93.9 g a.s. ha^{-1} averaged over the three replicates of each treated plot. In addition, the dose was verified by means of sampling Petri dishes filled with fine untreated soil from the trial sites (approximately 50 g per dish, sieved to 2 mm). The petri dishes with an inner diameter of 10.8 cm (10.8-10.9 cm in trial L120312) were placed on the treated plot (ten in each subplot) before application. On completion of the application, the petri dishes were closed with a lid, sealed with adhesive tape, and frozen within 1 hours and 53 minutes. In trials L120314 and L120316 samples were chilled on blue ice after collection and placed in the freezer within 15 and 45 minutes after application. In trial L120315 samples were frozen by means of dry ice immediately after collection and placed in freezer storage within 4 hours and 30 minutes. Further details of application are presented in Table 7.1.2.2.1-3.

Table 7.1.2.2.1-3: Application parameters of field trial sites treated with BAS 555 01 F (SL)

Trial Location	Application method	No. of applications	Subplot (m^2)	Application rate per treatment				Application date
				nominal	Actual [a]	dose verification [b]		
				[g a.s ha^{-1}]	[g a.s. ha^{-1}]	[g a.s ha^{-1}]	% of nominal	
L120312 Denmark	broadcast spray to bare soil	1	A (108)	90	87.5	84	93	23-Sep-2012
			B (108)	90	96.4	96	107	
			C (108)	90	87.2	113	125	
			Average	**90**	**90.4**	**97**	**108**	
L120313 Germany (East)	broadcast spray to bare soil	1	A (102)	90	87	91	102	11-Sep-2012
			B (102)	90	84	87	96	
			C (102)	90	94	88	98	
			Average	**90**	**88.3**	**89**	**98**	
L120314 Germany (West)	broadcast spray to bare soil	1	A (102)	90	95.6	118	131	30-May-2012
			B (102)	90	90.0	101	112	
			C (102)	90	88.5	94	104	
			Average	**90**	**91.4**	**104**	**115**	
L120315 France (North)	broadcast spray to bare soil	1	A (105)	90	94	87	96	15-Jun-2012
			B (105)	90	91	97	108	
			C (105)	90	91	78	87	
			Average	**90**	**92**	**87**	**97**	
L120316 Italy	broadcast spray to bare soil	1	A (102)	90	86.8	61	67	10-May-2012
			B (102)	90	88.2	81	90	
			C (102)	90	88.5	88	98	
			Average	**90**	**87.8**	**76**	**85**	
L120317 Spain	broadcast spray to bare soil	1	A (102)	90	95.9	91	102	18-Apr-2012
			B (102)	90	92.6	89	99	
			C (102)	90	93.2	95	105	
			Average	**90**	**93.9**	**92**	**102**	

[a] Determined by calculation of spray liquid applied
[b] Determined by means of petri dishes filled with soil

Immediately after application of the test item and before subsequent soil sampling, the control plot and the treated replicates were covered with a thin layer of sand to protect the applied product from surface processes like photolysis or volatilization, and to exclude any potential impact on the degradation of the test item caused by any of these processes. The application of sand was conducted using a box- or drop spreader or manually with a spade or by hand until complete coverage of the soil surface. Medium grained sand containing also fine grained material was used. The thickness of the sand layer necessary for complete coverage of the soil was approximately 4-6 mm averaged across all sites. The layer of sand was controlled up to at least 27 days after application and was renewed when needed. It remained intact until at least 27-30 days. Within this time period of 27-30 days, the individual fields received a total precipitation (rain and irrigation) of 119.4 mm (L120312), 73.8 mm (L120313), 125 mm (L120314), 78.6 mm (L120315), 87 mm (L120316), and 59.6 mm (L120317), respectively.

No tillage or fertilization was performed during the course of the study from first to last sampling and no crops were grown throughout any of the trials. The plots were kept free of vegetation via the application of herbicides including glyphosate, MCPA, glufosinate, quinoclamin, and 2,4-D.

Rainfall was supplemented with irrigation at trial sites L120312 (273.3 mm), L120313 (162.1 mm), L120314 (305 mm), L120315 (243.3 mm), L120316 (284.3 mm), and L120317 (488.2 mm).

Actual weather data are based on records of appropriate weather stations located on-site. Monthly summary results on temperature, precipitation and irrigation are presented in Table 7.1.2.2.1-4 and Table 7.1.2.2.1-5.

Table 7.1.2.2.1-4: Summary of climatic conditions at field trial sites used to investigate the dissipation of metconazole (trials L120312, L120313 and L120314)

Trial	**L120312**			**L120313**			**L120314**		
Location	**Bogense**			**Brunne**			**Goch-Nierswalde**		
	Denmark			**Germany (East)**			**Germany (West)**		
Climatic conditions	T_{mean} Air [°C]	Prec. [mm]	Irrigation [mm]	T_{mean} Air [°C]	Prec. [mm]	Irrigation [mm]	T_{mean} Air [°C]	Prec. [mm]	Irrigation [mm]
Month		∑	∑		∑	∑		∑	∑
May 12							15.4 [a]	4.0 [a]	--
Jun 12							14.6	122.2	--
Jul 12							16.9	148.0	10
Aug 12							18.4	45.0	25
Sep 12	11.1 [a]	53.8 [a]	--	12.7 [a]	25.4 [a]	12.4	13.5	48.8	25
Oct 12	9.2	79.7	--	8.4	59.8	--	10.0	84.6	10
Nov 12	6.3	96.4	--	4.7	30.0	--	6.5	34.6	15
Dec 12	0.8	65.4	--	-0.1	33.8	--	4.3	121.0	--
Jan 13	0.8	56.8	--	-0.2	63.2	--	1.8	46.2	--
Feb 13	0.1	29.1	--	-0.5	27.2	--	1.2	37.6	--
Mar 13	-0.5	5.0	--	-1.7	6.8	--	2.8	39.8	--
Apr 13	6.1	29.4	6.1	8.0	17.0	11.5	8.4	45.2	15
May 13	12.2	106.7	26.4	13.3	96.8	9.9	11.8	60.8	15
Jun 13	14.4	85.0	18.7	16.4	95.2	6.3	15.8	64.8	25
Jul 13	18.1	12.9	48.4	19.8	33.4	33.2	19.3	66.2	30
Aug 13	17.5	49.4	20.5	18.6	58.4	27.8	18.1	18.6	50
Sep 13	13.5	108.9	23.5	13.0	62.2	--	14.2	59.4	--
Oct 13	11.2	112.6	38.5	10.8	72.2	--	12.0	39.6	10
Nov 13	6.3	80.9	--	5.0	63.6	--	6.3	39.8	10
Dec 13	5.5	125.8	--	3.9	34.2	--	5.6	22.6	--
Jan 14	2.1	76.3	--	0.2	26.6	--	5.2	47.6	--
Feb 14	4.7	44.6	--	4.3	20.4	--	6.3	46.2	--
Mar 14	6.1	30.1	14.0	6.6	18.2	24.2	8.5	26.6	20
Apr 14	9.0	36.5	8.0	10.8	40.2	6.8	12.2	16.6	45
May 14	12.1	68.8	--	12.7	84.6	--	11.5 [a]	52.6 [a]	--
Jun 14	15.4	62.5	11.0	15.8	93.2	10.5			
Jul 14	19.5	33.3	21.5	20.6	66.2	12.3			
Aug 14	15.9	132	24.4	17.7 [a]	42.4 [a]	7.2			
Sep 14	15.8 [a]	20 [a]	12.3						

-- No irrigation applied

[a] Actual weather data refer to time period from start of trial (day of application) until end of trial (day of last sampling)

Table 7.1.2.2.1-5: Summary of climatic conditions at field trial sites used to investigate the dissipation of metconazole (trials L120315, L120316 and L120317)

Trial	L120315			L120316			L120317		
Location	Schaeffersheim			Poggio Renatico			Utrera		
	France (North)			Italy			Spain		
Climatic conditions / Month	T_{mean} Air [°C]	Prec. [mm] ∑	Irrigation [mm] ∑	T_{mean} Air [°C]	Prec. [mm] ∑	Irrigation [mm] ∑	T_{mean} Air [°C]	Prec. [mm] ∑	Irrigation [mm] ∑
Apr 12							16.8 [a]	23.0 [a]	9.8
May 12				17.6 [a]	76.0 [a]	--	23.6	23.0	23.5
Jun 12	19.3 [a]	51.0 [a]	--	23.6	14.0	48.8	26.6	0.0	44.9
Jul 12	18.4	84.8	9.1	25.6	3.8	33.9	27.4	0.0	45.7
Aug 12	19.5	44.4	17.8	26.0	0.0	40.1	28.1	0.0	45.7
Sep 12	14.2	52.6	32.3	19.6	86.9	23.2	24.6	78.0	27.4
Oct 12	9.2	95.0	--	14.5	97.0	3.8	20.0	127.0	--
Nov 12	6.0	109.6	--	9.9	81.7	--	15.8	129.5	--
Dec 12	3.3	54.6	--	1.7	26.3	--	12.4	37.0	--
Jan 13	1.6	15.8	--	3.1	97.4	--	11.8	49.5	--
Feb 13	0.8	33.6	-	2.8	90.3	--	11.1	55.0	--
Mar 13	2.9	18.4	14.0	7.3	132.9	--	14.6	165.5	--
Apr 13	10.0	84.0	21.5	13.4	55.5	--	18.1	47.0	--
May 13	11.9	154.4	--	16.5	99.2	--	20.2	8.2	37.3
Jun 13	17.4	93.0	--	21.5	31.0	52.6	25.0	0.0	41.6
Jul 13	21.0	21.4	47.2	25.0	4.6	23.4	28.2	0.0	56.8
Aug 13	18.3	75.0	34.2	23.8	63.2	20.7	28.7	0.0	74.5
Sep 13	14.7	94.8	--	19.7	29.7	27.2	25.5	21.5	25.4
Oct 13	11.6	146.4	--	14.9	98.0	--	21.5	88.5	--
Nov 13	5.1	82.6	--	9.4	69.2	--	14.2	3.0	9.8
Dec 13	3.0	51.8	--	3.7	10.8	--	12.0	60.0	25.8
Jan 14	4.1	33.6	--	5.8	94.4	--	12.9	85.0	--
Feb 14	5.2	46.0	--	7.7	87.6	--	13.0	85.3	--
Mar 14	7.9	21.2	28.3	10.4	52.0	--	15.1 [a]	20.8 [a]	20.0
Apr 14	11.9	26.4	25.4	13.9 [a]	32.9 [a]	10.6			
May 14	14.0 [a]	46.4 [a]	13.5						

-- No irrigation applied

[a] Actual weather data refer to time period from start of trial (day of application) until end of trial (day of last sampling)

Historical (long-term) weather data on precipitation and average air temperature from at least 10 years were taken from official weather stations located nearby (1-14.5 km distance to trial site). The historical and actual data each averaged over the complete duration of the individual trials are presented in Table 7.1.2.2.1-6.

The actual air temperature recorded at the six field sites during the study period was slightly higher compared to the historic values, except for the trial L120315 (France), where the average temperature was slightly lower. Five of the trial sites received more rain during the study period compared to the historical values, whereas rainfall was less than the historic value in trial L120314 (Germany (West)). Due to additional irrigation, the total water input at the test sites during the study was at least 104% of the historical average rainfall, which is considered sufficient to allow the cultivation of crops like oilseed rape or cereals.

Table 7.1.2.2.1-6: Summary of historical and actual weather data at field trial sites averaged over entire trial duration

Trial Location	T_{mean} Air [°C] (average over trial period)		Precipitation [mm] (sum over trial period)		Irrigation [mm]	Sum of actual precipitation and irrigation [mm]	% of historic precipitation
	Historic [a]	Actual [b]	Historic [a]	Actual [b]			
L120312 Denmark	8.3	9.3	1336	1602	273	1875	140
L120313 Germany (East)	8.5	9.2	1024	1171	162	1333	130
L120314 Germany (West)	9.9	10.4	1576	1338	305	1643	104
L120315 France (North)	10.9	10.5	1286	1539	243	1780	138
L120316 Italy	13.0	14.1	1224	1434	284	1719	140
L120317 Spain	17.3	19.5	1077	1107	488	1763	164

[a] Historic weather data comprise time from first day in the month of application to last day in the month of last sampling
[b] Actual weather data refer to time from start of trial (day of application) until end of trial (day of last sampling)

4. Sampling

Replicate soil specimens (8 per treated subplot and 10 or 15 per control plot) were taken at intervals up to 724 days and down to a maximum soil depth of 50 cm. At day 0, immediately after application, the treated plots were sampled down to 10 cm only. The detailed sampling intervals are presented in Table 7.1.2.2.1-7.

Table 7.1.2.2.1-7: Summary of sampling intervals at each field trial site

Trial	Location	Sampling intervals [days after treatment]
L120312	Denmark	Control:-3, 362, 723 Treated: 0, 7, 15, 30, 58, 86, 123, 194, 247, 362 , 487, 603, 724
L120313	Germany (East)	Control: -1, 360, 710 Treated: 0, 6, 14, 28, 63, 85, 160, 209, 234, 360, 472, 591, 710
L120314	Germany (West)	Control: -1, 362, 719 Treated: 0, 6, 15, 28, 55, 90, 118, 173, 279, 362, 476, 600, 719
L120315	France (North)	Control: -1, 357, 711 Treated: 0, 6, 13, 27, 63, 89, 125, 174, 248, 357, 483, 594, 711
L120316	Italy	Control: -2, 368, 713 Treated: 0, 6, 14, 29, 61, 90, 120, 180, 244, 368, 476, 602, 713
L120317	Spain	Control:-1, 359, 710 Treated: 0, 7, 14, 30, 61, 91, 126, 180, 231, 359, 470, 594, 710

Untreated specimens were collected from the control plot on three occasions, between one and three days before the application and the day of application down to a depth of 50 cm, and after about one and two years to a depth of 10 cm depth. The specimens were taken randomly from the untreated plot each time and pooled according to soil depth. The 15 cores collected at the first sampling interval were taken using a common soil probe equipped with a plastic/acetate liner of 3.9 to 5.0 cm diameter except for 30-50 cm samples taken at the site in Italy (trial L120316) that were sampled using liners with 2.5 cm diameter. The 10 cores (0-10 cm depth) taken after about one year and two years were collected with a metal tube of 7.2 to 9.8 cm diameter.

Treated soil specimens were taken randomly from eight points of each of the three treated subplots A - C and pooled according to subplot and depth. All soil specimens from 0-10 cm depth collected from the treated plots were taken separately using a metal tube of minimum 7.2 and to maximum 9.8 cm diameter which left a hole contained by a steel collar. Alternatively, samples were taken by pressing the metal tube described above into the ground and collecting the soil with a spoon or similar device. Soil specimens deeper than 10 cm were collected through the center of the excavation hole contained by the guard collar, using a common soil corer fitted with a plastic/acetate liner of diameter 4.4 to 5.0 cm except for 30-50 cm samples taken at the site in Italy that were sampled using liners with 2.5 cm diameter. Sampling of these cores was conducted in one run or in two consecutive steps. All soil cores collected with the soil probe were sectioned into 10 cm segments and pooled by depth.

In addition to the main sampling described above, a second complete sampling (double sampling) was carried out. The reserve samples were not sectioned into 10 cm segments, but directly put into the freezer at the field test sites.

All soil specimens intended for residue analysis were stored frozen through storage, shipping and processing until final analysis. Sample processing was conducted in frozen state in a mill together with dry ice.

At nominal sampling events 0 DAT, 30 DAT and 90 DAT, shipment verification samples were prepared at all test sites to demonstrate stability of the residues in soil during storage and through any shipping processes. Three samples were prepared by fortification of soil with 0.05 mg kg^{-1} metconazole and one sample remained untreated as control sample. The samples were subsequently handled in the same manner as the actual residue samples.

5. Analytical procedure

Field soil specimens were analyzed for cis- and trans-metconazole and 1,2,4-(1*H*)-triazole according to validated BASF method L0203/01 [*CA 4.1.2/2, BASF Doc ID 2013/1377001*]. The analytical method involved twice extraction of the soil with acetonitrile/water (70/30, v/v) and determination of the analytes in the combined extracts by LC-MS/MS. The limit of quantification (LOQ) was 2.0 µg kg^{-1} for each individual analyte. The limit of detection (LOD) was set at 0.6 µg kg^{-1} (30% of LOQ). Analysis of field soil specimens originating from the treated plots was conducted down to 50 cm and was performed until a maximum of 724 days after treatment (DAT).

Petri dish and shipment verification specimens were analyzed for cis- and trans-metconazole according to validated BASF method L0203/01 with minor adaptations to account for the larger quantity of soil to be extracted.

Spray broth specimens were analyzed according to BASF method APL0500/03 [*CA 4.1.2/11*]. Either a spray solution sample aliquot was diluted with 0.1% formic acid in acetonitrile/water (20/80, v/v) or to the whole spray solution sample acetonitrile and acetone was added until a clear solution was obtained. Metconazole was determined using LC-MS/MS.

Generally, for soil samples, a second mass transition was monitored for each analyte.

The validity of the analytical method was proven within the present study by analysis of untreated control and fortified samples within each analytical sample set.

6. Storage stability experiments

Storage stability of metconazole and 1,2,4-(1*H*)-triazole in frozen soil was investigated in a separate study [*CA 7.1.2.2.1/4, BASF DocID 2015/1204922~~2014/1001261~~*] with soils originating from the individual trial sites of the present terrestrial field dissipation study.

7. Calculation of dissipation times

No calculation of dissipation times is provided in the study report. The kinetic evaluation of the degradation behavior of metconazole in the six European field soils is presented in two separate reports [*CA 7.1.2.2.1/2, BASF DocID 2015/1137154; CA 7.1.2.2.1/3, BASF DocID 2015/1137155*].

II. RESULTS AND DISCUSSION

1. Spray broth concentration and application verification

Spray broth homogeneity was confirmed by visual check for all trials. In addition, spray mixtures sampled before and after application of each subplot were analyzed for metconazole. Analyzed concentrations averaged across the individual trial sites were in the range of 0.279 to 0.310 g L^{-1} corresponding to 93-103% of the target concentration of 0.300 g L^{-1}. The analytical results were not corrected for procedural recoveries.

Application verification was conducted by means of petri dish samples from the field. Mean recoveries for the higher fortification levels (1000 and 3000 µg kg^{-1}) for cis-and trans-metconazole ranged from 100-103%.

Residue levels of metconazole achieved on extraction and analysis of the application monitors (Petri dishes filled with soil) were converted into residue rates (in g ha^{-1}) taking into account the area of the Petri dishes (91.6 cm^2). The obtained application rates for the individual trials ranged from 76-104 g ha^{-1} representing 85-115% of the target rate (Table 7.1.2.2.1-12). The applied amount determined via the application monitors in these trials is in good agreement with the nominal value of 90 g ha^{-1}, and the results from spray broth analysis.

2. Residues in field soil samples

Untreated soil specimens (control samples) were analyzed for residues of cis- and trans-metconazole and 1,2,4-(1*H*)-triazole. No residues of metconazole were detected in any of the control samples proving that there were no interferences of the untreated soil material with the analytical procedures used. However, significant interferences (> 30% of LOQ) were observed in some samples at the retention times and mass transitions considered for 1,2,4-(1*H*)-triazole. Therefore, interferences in some control samples were determined and blank correction in the recovery data were needed. Procedural recovery experiments performed with untreated field soil specimens spiked with a mix of the three analytes at concentration levels of 2, 20, 50, 1000 and 3000 µg kg^{-1} yielded overall mean recovery rates for the individual analytes between 97-101%, confirming the validity of the analytical method used in this study. Detailed results are summarized in Table 7.1.2.2.1-8.

Table 7.1.2.2.1-8: Method procedural recoveries

Analyte	Fortification level [µg kg^{-1}]	n	Mean recovery ± RSD [a] [%]
cis-metconazole	2.00	73	99 ± 8
	20.0	79	102 ± 7
	50.0	16	99 ± 2
	1000	73	103 ± 7
	3000	14	101 ± 2
	All fortification levels	**255**	**101 ± 7**
trans-metconazole	2.00	73	99 ± 6
	20.0	79	101 ± 8
	50.0	16	99 ± 3
	1000	73	103 ± 6
	3000	14	100 ± 2
	All fortification levels	**255**	**101 ± 7**
1,2,4-(1*H*)-triazole	2.00	62	95 ± 12
	20.0	79	99 ± 8
	50.0	16	94 ± 7
	1000	73	97 ± 8
	3000	14	98 ± 3
	All fortification levels	**244**	**97 ± 9**

RSD = Relative standard deviation [%]
[a] Mean values are across all soils and soil depths

These data prove that the analytical method applied was suitable to accurately determine residues of cis- and trans-metconazole and 1,2,4-(1*H*)-triazole in soil down to a concentration of 2.0 µg kg^{-1} for each analyte.

Field soil specimens from the treated plots were analyzed down to a depth of 50 cm. All residue values presented are related to the dry weight of the soil and were not corrected for procedural recoveries. Residue levels of the three analytes in µg kg^{-1} dry soil were converted to residue rates in g ha^{-1} taking into account the actual dry soil density of the field samples, and were summed up for all depths between 0 and 50 cm analyzed. Results are presented in Table 7.1.2.2.1-9.

Table 7.1.2.2.1-9: Total residues of metconazole (sum of cis and trans) under field conditions in soil calculated to g ha^{-1} and summed up for all depths analyzed [a]

L120312 (Bogense, Denmark)				L120313 (Brunne, Germany)			
DAT	**Subplot A**	**Subplot B**	**Subplot C**	**DAT**	**Subplot A**	**Subplot B**	**Subplot C**
0	59	89	77	0	78	86	95
7	77	83	63	6	93	65	68
15	85	68	96	14	74	74	71
30	76	64	70	28	90	65	80
58	52	80	66	63	64	64	76
86	68	68	87	85	73	72	74
123	56	85	78	160	76	65	65
194	65	56	67	209	69	55	64
247	33	35	37	234	49	63	49
362	6.3	7.0	11	360	23	15	20
487	4.1	5.6	5.5	472	20	14	14
603	2.6	2.6	3.0	591	15	11	16
724	0.0	1.2	1.0	710	3.2	2.8	3.4
L120314 (Goch-Nierswalde, Germany)				**L120315 (Schaeffersheim, France)**			
DAT	**Subplot A**	**Subplot B**	**Subplot C**	**DAT**	**Subplot A**	**Subplot B**	**Subplot C**
0	80	59	63	0	58	69	69
6	100	90	59	6	53	64	64
15	85	67	68	13	51	55	53
28	88	54	69	27	25	51	39
55	41	48	51	63	15	12	12
90	37	33	37	89	8.7	6.1	8.0
118	54	32	27	125	2.9	8.7	5.3
173	28	20	34	174	3.3	7.3	3.7
279	35	29	26	248	5.3	6.0	2.5
362	28	30	25	357	1.8	1.2	1.1
476	5.8	4.1	1.1	483	0.0	0.0	0.0
600	9.1	10	6.9	594	0.0	0.0	0.0
719	2.6	7.8	5.1	711	0.0	0.0	0.0
L120316 (Poggio Renatico, Italy)				**L120317 (Utrera, Spain)**			
DAT	**Subplot A**	**Subplot B**	**Subplot C**	**DAT**	**Subplot A**	**Subplot B**	**Subplot C**
0	52	62	72	0	75	91	90
6	47	53	73	7	66	68	67
14	62	46	38	14	67	37	66
29	44	47	52	30	41	58	54
61	48	54	53	61	47	48	53
90	24	56	53	91	47	47	23
120	30	41	50	126	36	49	53
180	37	54	39	180	35	26	30
244	42	46	40	231	17	29	26
368	24	29	36	359	5.1	8.1	15
476	27	30	31	470	3.3	7.9	4.8
602	12	36	16	594	1.4	2.3	3.3
713	12	14	22	710	0.0	0.0	0.0

DAT = Days after treatment
a Calculation are based on actual dry soil density for individual soil layers, residue values <LOD were treated as zero

Metconazole degraded well at all six European field sites with no significant change in the cis/trans isomer ratio. The total amount of metconazole residues detected in the soil profiles decreased from an average of 74 g ha^{-1} at day 0 to an average of 12 g ha^{-1} after two years.

Metconazole residues were exclusively found in the top 0-20 cm layer of the soils in all six trials except one single detect <LOQ at 15 DAT in the 30-40 cm layer of trial L120314 (Germany (West)). This single detect was considered to be caused by contamination during sampling or processing rather than by movement of the substance through the soil. Altogether, it can be concluded that metconazole does not show any significant tendency to move into deeper soil layers indicating low potential for metconazole residues to leach to groundwater.

3. Shipment verification specimens

Shipping verification specimens spiked with metconazole were analyzed to check stability of the residues in soil during storage at the test site and through any shipping processes. Concentrations of metconazole were not corrected for procedural recoveries.

The analytical results demonstrated no significant losses from the shipping verification samples. The average amount of metconazole from the spiked field samples was 108% across all trials. It was concluded that metconazole was stable in all soils under the storage and shipment conditions used.

4. Time of storage

The predominant part of the samples was analyzed within one year. The maximum period any soil sample was stored from the time of sampling to extraction was 683 days, except for some samples used for re-analysis. Petri dish specimens were stored for up to 615 days after application. The maximum storage period of the spray broth samples was 317 days. Shipment verification specimens were stored for a maximum of 561 days between spiking and extraction.

III. CONCLUSION

Metconazole degraded well under field conditions in soil at all six European field sites with no significant change in the cis/trans isomer ratio. The total amount of metconazole residues in the soil profiles decreased from an average of 74 g ha^{-1} at day 0 to an average of 12 g ha^{-1} after two years. In two trials (France and Spain) no residues were detectable at the end of the trial after two years. In the other four trials the residues left after 710-724 days ranged from 0.7 to 16 g ha^{-1}.

Metconazole residues were exclusively found in the top 0-20 cm layer of the soils in all six trials except one single detect <LOQ at 15 DAT in the 30-40 cm layer of trial Germany (West). Apart from this single detect which is considered to be caused by contamination during sampling or processing rather than by movement of the substance through the soil, no residues were detected below 20 cm in any sample. Altogether, it can be concluded that metconazole does not show any significant tendency to move into deeper soil layers indicating low potential for metconazole residues to leach to groundwater.

Due to a considerable background contamination of all test soils with 1,2,4-(1*H*)-triazole, no conclusive statement on the potential formation of 1,2,4-(1*H*)-triazole from metconazole can be made. However, there was no indication that significant amounts of 1,2,4-(1H)-triazole were formed.

Residues of cis- and trans-metconazole were analysed separately, but no shift in the isomeric ratio cis/trans was observed. Thus, it is considered adequate to use the sum of cis- and trans-isomer for subsequent kinetic evaluations.

Report:	CA 7.1.2.2.1/2 Pape L.,Studenroth S., 2015b Kinetic evaluation of a field soil dissipation study with BAS 555 F - Metconazole conducted in Europe, 2012 to 2014: Determination of best-fit endpoints according to FOCUS 2015/1137154
Guidelines:	FOCUS Degradation Kinetics (2006) SANCO/10058/2005 version 2.0 (December 2014)
GLP:	no

Executive Summary

Kinetic evaluation of the field dissipation of metconazole (BAS 555 F) was conducted on the data from a field dissipation study, in which six field trials with metconazole were included. The purpose of this evaluation was to analyze the degradation kinetics of metconazole observed in the six soils according to the current guidance of the FOCUS workgroup on degradation kinetics in order to derive reliable best-fit $DegT_{50}$ and $DegT_{90}$ values.

The field trials were situated in different regions of Europe (Denmark, Germany (two trials), France, Italy and Spain), considering a range of different soils and climatic conditions.

For each trial, the best-fit model was selected based on a visual and statistical assessment. Kinetic evaluation showed that the dissipation behavior of metconazole was best described using single first-order (SFO) kinetics for all trials.

The best-fit field half-lives ($DegT_{50}$) for metconazole ranged from 31.0 to 449.2 days. The corresponding $DegT_{90}$ values ranged from 102.9 to >1000 days.

As degradation caused by surface processes like photolysis or volatilization was excluded by covering all plots with a sand layer the reported best-fit endpoints represent a conservative estimate of the dissipation behavior of metconazole in soil. Therefore, the derived best-fit endpoints should not be used as triggers for additional work (trigger endpoints).

I. MATERIAL AND METHODS

The kinetic evaluation was conducted for six field trials with metconazole from the data of one field dissipation study, which can be found in CA 7.1.2.2.1/1 [*BASF DocID 2015/1000221*]. The trials were situated in different regions of Europe (Denmark, Germany (two trials), France, Italy and Spain) considering a range of different soils and climatic conditions. Detailed soil characteristics in each trial are reported in the cited study. Applications were made to bare soil using a calibrated boom sprayer. Immediately after application and before subsequent soil sampling all plots were covered with a thin layer of sand to protect the applied product from surface processes like photolysis or volatilization, and to exclude any potential impact on the degradation of the test item caused by any of these processes. Soil samples were taken at day 0, immediately after application down to 10 cm soil depth and at 12 consecutive sampling dates after application down to a maximum soil depth of 50 cm from three individual subplots.

Metconazole residues were almost exclusively found in the top 0-20 cm soil layer in all six trials, except one single detect <LOQ at 15 days after treatment (DAT) in the 30-40 cm layer of trial L120314 (DE-W). Apart from this single detect which was considered to be caused by contamination during sampling or processing rather than by movement of the substance through the soil, no residues were detected below 20 cm in any sample at any site.

The samples were extracted twice with acetonitrile/water (70/30, v/v) and the combined extracts were analyzed for metconazole (cis- and trans-metconazole) by means of HPLC-MS/MS. The limit of quantification (LOQ) was 0.002 mg kg^{-1}. The limit of detection (LOD) was set at 0.0006 mg kg^{-1} (30% of LOQ).

Kinetic modeling strategy

The kinetic evaluation was performed in order to derive best-fit degradation parameters for metconazole. For the parent substance metconazole, the appropriate kinetic model was identified considering the procedures and kinetic models proposed by the FOCUS kinetics guidance [*FOCUS (2006)*]. The best-fit model was selected based on visual and statistical assessment.

Kinetic models included in the evaluations

For each data set, the kinetic models proposed by FOCUS Kinetics [*FOCUS (2006)*] were tested. The recommended kinetic models, i.e. single-first order (SFO), double first-order in parallel (DFOP) and first-order multi-compartment (FOMC), were applied to the metconazole data. The respective model descriptions and corresponding equations for calculating endpoints ($DegT_{50}$, $DegT_{90}$) are shown in the FOCUS Kinetics guidance [*FOCUS (2006), Box 5-1, Box 5-4 and Box 5-2*].

The goodness-of-fit of the kinetic models was assessed by visual inspection and statistical measures, as recommended by the FOCUS Kinetics guidance. A kinetic model is considered appropriate if the residuals are randomly distributed around zero, the χ^2 error value is ideally <15% and the estimated degradation parameters differ significantly from zero.

Data handling and software for kinetic evaluation

For the evaluation, the sum of cis- and trans-isomer were used. The residue data in g ha^{-1} cumulated over the whole sampling depth were taken from the study report. Values below LOD (0.0006 mg kg^{-1}) were set to 0.5 × LOD according to FOCUS.

The software package KinGUI version 2.2012.224.1704 [*Schäfer et al. (2007); Witt et al. (2014)*]was used for parameter fitting. The error tolerance and the number of iterations of the optimization tool (IRLS) were set to 10^{-6} and 100, respectively.

The datasets submitted to kinetic analysis are provided in the original evaluation report.

II. RESULTS AND DISCUSSION

Kinetic evaluation showed that the dissipation behavior of metconazole was best described using SFO kinetics for all six trials. A summary of the adequate $DegT_{50}$ and $DegT_{90}$ values is given in Table 7.1.2.2.1-10.

Table 7.1.2.2.1-10: Summary of best-fit $DegT_{50}$ and $DegT_{90}$ values of metconazole

Field trial	Soil type (USDA)	Best-fit kinetic model	χ^2 [%]	$DegT_{50}$ [d]	$DegT_{90}$ [d]
L120312 (Bogense, Denmark)	Sandy loam	SFO	18.1	202.8	673.7
L120313 (Brunne, Germany)	Loamy sand	SFO	13.2	258.5	858.6
L120314 (Goch-Nierswalde, Germany)	Silt loam	SFO	17.0	152.4	506.1
L120315 (Schaeffersheim, France)	Silt	SFO	8.3	31.0	102.9
L120316 (Poggio Renatico, Italy)	Silt loam	SFO	7.8	449.2	> 1000
L120317 (Utrera, Spain)	Sand	SFO	14.3	136.3	452.8

III. CONCLUSION

Kinetic evaluation of six field trials with metconazole was conducted in order to derive reliable best-fit $DegT_{50}$ and $DegT_{90}$ values according to the current guidance of the FOCUS workgroup on degradation kinetics.

The best-fit field half-lives ($DegT_{50}$) for metconazole ranged from 31.0 to 449.2 days. The corresponding $DegT_{90}$ values ranged from 102.9 to >1000 days.

Surface processes had been excluded by covering the soil with sand in the field study. Therefore, the derived best-fit endpoints should not be used as trigger endpoints.

Report:	CA 7.1.2.2.1/3 Pape L.,Studenroth S., 2015c Kinetic evaluation of a field soil dissipation study with BAS 555 F - Metconazole conducted in Europe, 2012: Determination of modeling endpoints according to FOCUS 2015/1137155
Guidelines:	FOCUS Kinetics (2006) SANCO/10058/2005 version 1.1 of Dec. 2014, EFSA Guidance to obtain DegT50 values in soil (2014)
GLP:	no

Executive Summary

The dissipation behavior of the metconazole (BAS 555F) in soil has been investigated in a field dissipation study including six field trials located in different regions of Europe. The purpose of this evaluation was to analyze the degradation kinetics of metconazole observed in the six soils according to the current guidance of the FOCUS workgroup on degradation kinetics in order to derive reliable normalized modeling endpoints.

The field trials were situated in different regions of Europe (Denmark, Germany (two trials), France, Italy and Spain), considering a range of different soils and climatic conditions.

As the study design was tailored to exclude surface processes, kinetic evaluation was performed according to FOCUS kinetics as recommended by EFSA (2014).

Prior to kinetic evaluation, sampling intervals of the field studies were normalized to reference conditions (20°C, pF2) by time-step normalization. The kinetic evaluation was performed on the normalized dataset. Modeling endpoints were derived based on a visual and statistical assessment. Kinetic evaluation of the time-step normalized dataset (20°C, pF2) resulted in normalized field half-lives (SFO-DegT_{50}) for metconazole between 26.6 and 368.5 days.

I. MATERIAL AND METHODS

The kinetic evaluation was conducted for six field trials with metconazole from the data of one field dissipation study, which can be found in CA 7.1.2.2.1/1 [*BASF DocID 2015/1000221*].

The data set has already been evaluated for best-fit endpoints; a summary of the material and methods including data handling and kinetic models considered can be found in CA 7.1.2.2.1/2 [*BASF DocID 2015/1137154*].

Surface processes had been excluded by covering the soil with sand in the field study. Therefore, kinetic evaluation was performed according to FOCUS kinetics as recommended by EFSA [*EFSA (2014): Guidance Document for evaluating laboratory and field dissipation studies to obtain $DegT_{50}$ values of active substances of plant protection products and transformation products of these active substances in soil. EFSA Journal 2014;12(5):3662, 38 pp.*].

In order to derive modeling endpoints, kinetic evaluation was performed on the time-step normalized data set.

Normalization procedure

The suitability of field dissipation data for normalization was proven following the evaluation criteria for normalization as compiled by the Dutch regulatory authority (CTGB criteria).

Temperature correction factors (f_{temp}) were determined to account for differences between actual daily soil temperatures as calculated by FOCUS-PEARL 4.4.4 and a reference temperature of 20°C using the Q_{10} approach. The Q_{10} response function was applied for temperatures above 0°C and below field temperatures of 0°C it was assumed that no degradation occurs (Equation 7.1.2.2-2 c). For the evaluation, the EFSA opinion on the default Q_{10} value [*EFSA (2014)*] was followed and a Q_{10} value of 2.58 was included in the assessment.

Moisture correction factors (f_{moist}) were determined to account for differences between actual daily soil moisture as calculated by FOCUS-PEARL 4.4.4 and the reference soil moisture at field capacity (pF 2) (Equation 7.1.2.2-2 d).

The normalized day lengths were derived according to Equation 7.1.2.2.1-1 a. For DAT 0, no normalization was considered and application was assumed to occur at the time point zero. Normalized sampling days (DAT_{norm}) after application were calculated by cumulatively summing up normalized day lengths according to Equation 7.1.2.2.1-1 b.

Equation 7.1.2.2.1-1: Calculation of normalized day length based on combination of soil moisture and soil temperature correction factors

a)
$$D_{norm} = D * f_{temp} * f_{moisture}$$

b)
$$t_i = \sum_{t=1}^{i-1} D_{norm}$$

with:

t_i =	Time from application till sampling at day i	[d]
D_{norm} =	Normalized day length (20°C, pF2)	[d]
i =	Time span between application and sampling	[d]

c)
$$f_{temp} = \begin{cases} Q_{10}^{\frac{Tact-Tref}{10}} & \text{for } T_{act} > 0°C \\ 0 & \text{for } T_{act} \leq 0°C \end{cases}$$

d)
$$f_{moist} = \begin{cases} (\frac{\theta_{act}}{\theta_{ref}})^B & \text{for } \theta_{ref} > \theta_{act} \\ 1 & \text{for } \theta_{ref} \leq \theta_{act} \end{cases}$$

with:

D_{norm} =	normalized day length (temperature and moisture)	
D =	actual day length (1 d)	[days]
f_{temp} =	temperature correction factor	[-]
f_{moist} =	moisture correction factor	[-]
T_{act} =	actual soil temperature (°C)	[C°]
T_{ref} =	reference temperature (20°C)	[C°]
Q_{10}=	factor of increase of degradation rate with an increase in temperature of 10°C (Q_{10} = 2.58 [EFSA 2014])	[-]
θ_{act} =	actual soil moisture (vol. water content)	[m^3 m^{-3}]
θ_{ref} =	reference soil moisture at pF2	[m^3 m^{-3}]
B =	exponent of the moisture response function, B = 0.7	[-]

Table 7.1.2.2.1-11 shows the field sampling dates for the trial locations and the normalized (20°C, pF2) day lengths based on soil moisture and soil temperature data as simulated by FOCUS-PEARL 4.4.4.

Table 7.1.2.2.1-11: Time-step normalized (temperature and moisture) sampling days

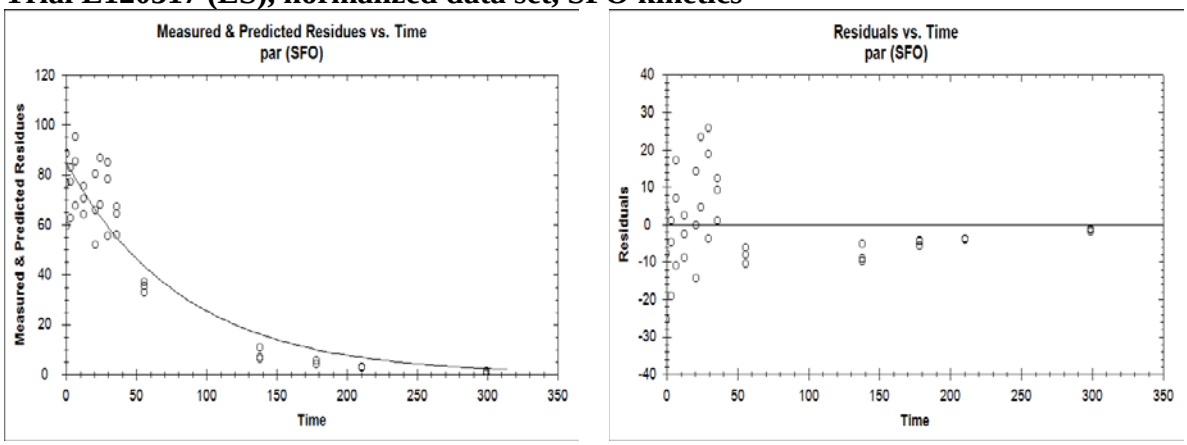
Bogense, Denmark (L120312)		Brunne, Germany (L120313)	
DAT	**D_{norm}**	**DAT**	**D_{norm}**
0	0	0	0
7	3.0	6	3.3
15	6.5	14	6.6
30	12.6	28	13.3
58	20.6	63	24.6
86	24.2	85	28.4
123	29.6	160	35.8
194	35.7	209	38.9
247	55.6	234	48.4
362	137.9	360	145.9
487	178.1	472	185.1
603	210.4	591	214.6
724	299.1	710	304.4
Goch-Nierswalde, Germany (L120314)		**Schaeffersheim, France North (L120315)**	
DAT	**D_{norm}**	**DAT**	**D_{norm}**
0	0	0	0
6	2.7	6	5.2
15	7.6	13	10.5
28	15.4	27	24.1
55	33.6	63	54.9
90	61.9	89	77.1
118	77.5	125	94.4
173	97.6	174	108.1
279	116.2	248	119.5
362	140.4	357	156.6
476	222.4	483	257.0
600	262.3	594	287.0
719	301.4	711	330.4
Poggio Renatico, Italy (L120316)		**Utrera, Spain (L120317)**	
DAT	**D_{norm}**	**DAT**	**D_{norm}**
0	0	0	0
6	5.0	7	5.7
14	10.0	14	10.5
29	25.5	30	35.2
61	74.8	61	81.4
90	119.3	91	136.1
120	163.7	126	212.2
180	210.0	180	299.8
244	226.5	231	340.3
368	273.4	359	412.8
476	406.8	470	586.3
602	478.6	594	779.0
713	520.3	710	847.0

DAT = Days after treatment
D_{norm} = Normalized day (20°C, pF2)

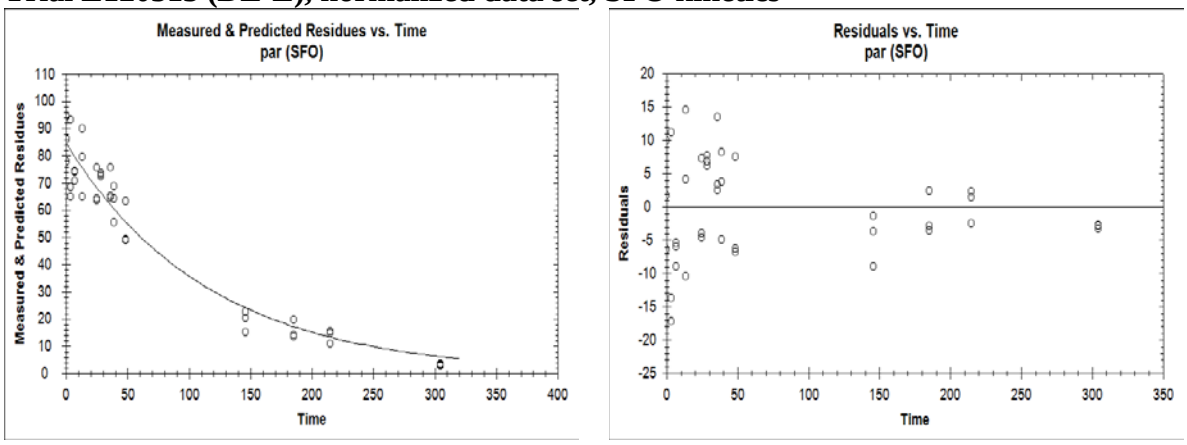
II. RESULTS AND DISCUSSION

The dissipation behavior of metconazole was best described using SFO kinetics for all trials. The plots of the kinetic fits for the normalized datasets are presented in Figure 7.1.2.2.1-1. A summary of the adequate $DegT_{50}$ values, is given in Table 7.1.2.2.1-12.

Trial L120317 (ES), normalized data set, SFO kinetics



Trial L120313 (DE-E), normalized data set, SFO kinetics



Trial L120314 (DE-W), normalized data set, SFO kinetics

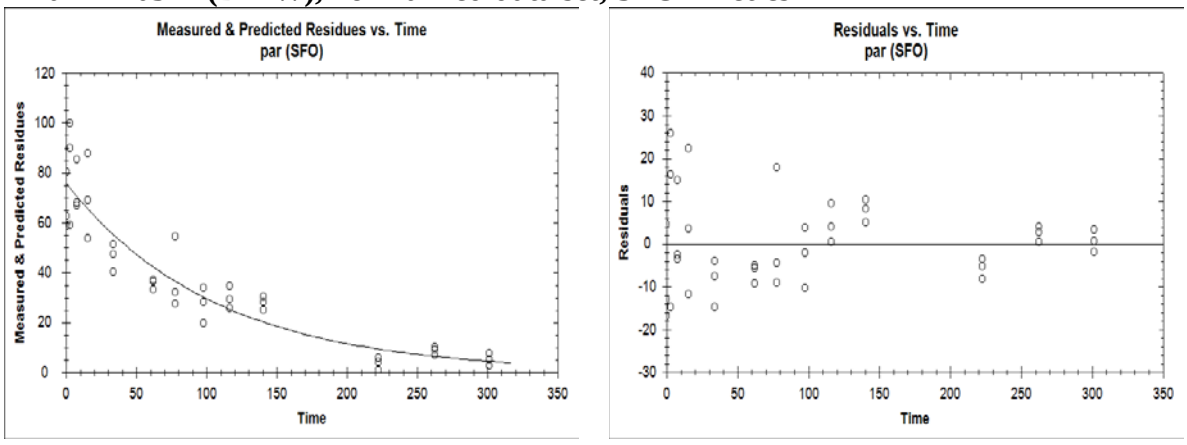
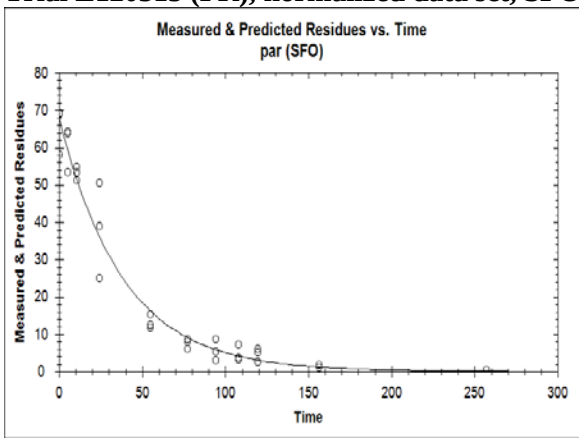
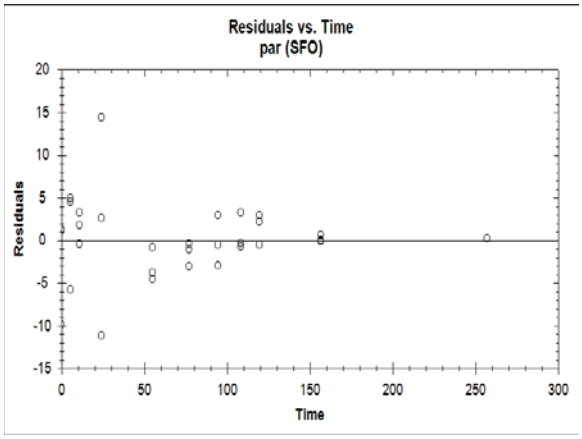


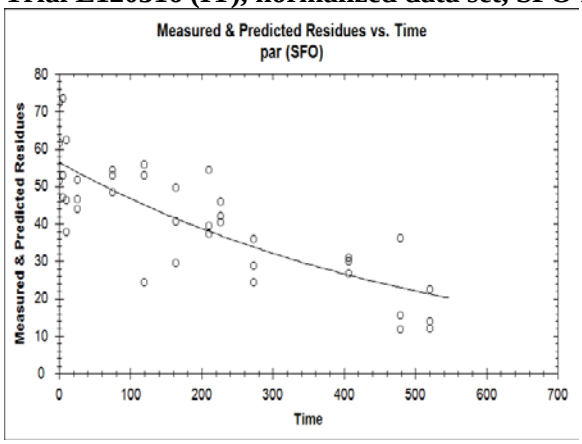
Figure 7.1.2.2.1-1: KinGUI SFO fits for various field trials (normalized data)

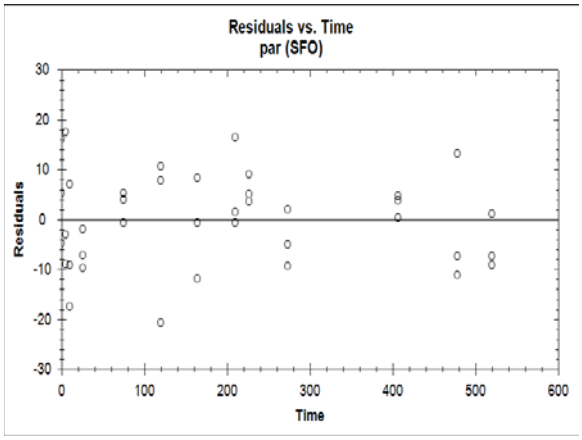
Trial L120315 (FR), normalized data set, SFO kinetics



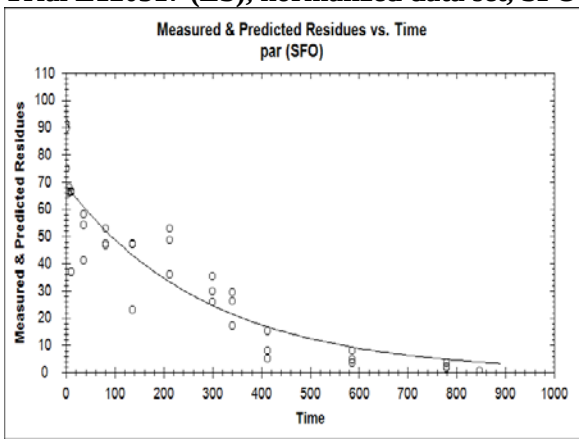


Trial L120316 (IT), normalized data set, SFO kinetics





Trial L120317 (ES), normalized data set, SFO kinetics



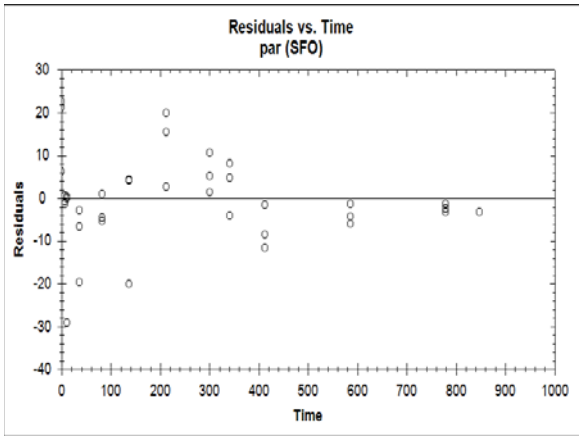


Figure 7.1.2.2.1-1: (cont): KinGUI SFO fits for various field trials (normalized data)

Table 7.1.2.2.1-12: Summary of modeling endpoints of metconazole

Field trial	Soil type (USDA)	Kinetic model	χ^2 [%]	Normalized $DegT_{50}$ [d]
L120312 (Bogense, Denmark)	Sandy loam	SFO	12.5	57.5
L120313 (Brunne, Germany)	Loamy sand	SFO	6.4	80.3
L120314 (Goch-Nierswalde, Germany)	Silt loam	SFO	11.8	73.4
L120315 (Schaeffersheim, France)	Silt	SFO	5.6	26.6
L120316 (Poggio Renatico, Italy)	Silt loam	SFO	8.7	368.5
L120317 (Utrera, Spain)	Sand	SFO	17.5	202.0

III. CONCLUSION

Kinetic evaluation of six field trials with metconazole was conducted in order to derive reliable normalized modeling endpoints according to the current guidance of the FOCUS workgroup on degradation kinetics. The dissipation behavior of metconazole was best described using SFO kinetics for all trials.

Kinetic evaluation of the time-step normalized dataset (20°C, pF2) resulted in normalized field half-lives ($DegT_{50}$) for metconazole between 26.6 and 368.5 days.

Report:	CA 7.1.2.2.1/4 Geschke S., 2015 b Final Report: Determination of storage stability of BAS 555 F (Metconazole) and its metabolite 1,2,4-Triazole in soil 2015/1204922
Guidelines:	EEC 7032/VI/95 rev. 5, OECD 506 (Oct. 2007), EPA 860.1380
GLP:	yes (certified by Landesanstalt fuer Umwelt, Messungen und Naturschutz Baden-Wuerttemberg, Karlsruhe, Germany)

~~**Report:**~~	~~CA 7.1.2.2.1/4~~ ~~Geschke S., 2015a~~ ~~Interim report: Determination of storage stability of BAS 555 F (Metconazole) and its metabolite 1,2,4 triazole in soil~~ ~~2014/1001261~~
~~**Guidelines:**~~	~~EEC 7032/VI/95 rev. 5, OECD 506 (Oct. 2007), EPA 860.1380~~
~~**GLP:**~~	~~yes~~ ~~(certified by Landesanstalt fuer Umwelt, Messungen und Naturschutz Baden Wuerttemberg, Karlsruhe, Germany)~~

Executive Summary

In the present study, the storage stability of metconazole (BAS 555 F) cis-isomer (Reg. No. 4079468) and trans-isomer (Reg. No. 4079654), as well as the metabolite 1,2,4-(1H)-triazole (Reg. No. 87084) in soil was investigated in six soils under deep frozen conditions (≤ -18°C). The soils used for set up the stability samples were derived from six field dissipation trials of a parallel field study [*CA 7.1.2.2.1/1, BASF DocID 2015/1000221*]. Stored samples have been tested for stability up to 720 ~~540~~ days. ~~As the study is still ongoing and further analysis will be performed after 720 days of storage.~~

The stability samples were fortified at a concentration level of 0.02 mg kg^{-1} (10fold LOQ). Each soil aliquot was spiked individually with a single test item, two replicates per field soil type and analyzed time point were prepared. At different intervals (0, 32, 60, 120, 242, 361, 540 and 720 ~~540~~ days) the stability samples were analyzed using BASF method L0203/01. The limit of quantification of the method is 0.002 mg kg^{-1}. The validity of the analytical method was confirmed within the storage stability study.

The storage stability of cis-isomer and trans-isomer of metconazole and its metabolite 1,2,4-(1H)-triazole was proven in all six tested soils over a period of at least 720 ~~540~~ days at ≤ -18°C. The recoveries for cis-metconazole and trans-metconazole in the soil samples after 720 ~~540~~ days of storage under frozen conditions ranged from 89 ~~90~~% to 107 ~~102~~ % of the nominal ~~initial~~ concentration. For 1,2,4-(1H)-triazole, after 720 ~~540~~ days of storage, 72 ~~73~~ % to 92 ~~94~~% of the nominal ~~initial~~ concentration was found in spiked soil samples.

I. MATERIAL AND METHODS

A. MATERIALS

Test materials:	cis-metconazole	trans-metconazole	1,2,4-(1H)-triazole
Reg. No.	4079468	4079654	87084
Batch No.:	AC8879-136A	AC9339-122A	AC10194-134
Purity:	99.3%	99.1%	99.0%
CAS #:	115850-27-6	115850-28-7	288-88-0

B. STUDY DESIGN

1. Experimental Conditions

Six different soil types (sandy loam, loamy sand, silt loam, silt, silt loam and sand), originating from the terrestrial dissipation study [*CA 7.1.2.2.1/1, BASF DocID 2015/1000221*] were used in the storage stability study. The soil samples were untreated and stored refrigerated in the dark (1 – 10°C) until the day of fortification.

5 g of soil were weighed in 50 mL polypropylene-tubes and fortified individually at 0.02 mg kg^{-1} (10fold LOQ) with fortification solutions of cis-metconazole, trans-metconazole and 1,2,4-(1H)-triazole (separate systems for each analyte). The test items in acetonitrile/water (70/30, v/v) respectively demineralized water were distributed drop wise onto the soil, avoiding the formation of concentration “hot-spots”. The solvent was allowed to evaporate for approximately 5 minutes and then the tube was closed and placed into the deep freezer. For each storage interval, soil type and analyte a set of at least four samples (two samples for analysis plus two backup samples) were prepared. Additionally, soil samples without treatment were stored deep-frozen to serve as control samples and for method validation purposes (freshly fortified recovery samples).

The soil samples were stored at approximately -18°C and analyzed after different intervals (0, 32, 60, 120, 242, 361, 540 and 720 ~~540~~ days). Minimum and maximum temperature in the freezer over the entire storage period was continuously recorded and were in the range of -25.3°C to -16.0°C.

2. Description of analytical procedures

For the determination of metconazole (cis- and trans-isomer) and its metabolite 1,2,4-(1H)-triazole, BASF Method L0203/01 was used. Therefore, the soil samples were extracted twice with 40 mL acetonitrile/water (70/30, v/v) by mechanical shaking for 30 minutes at 225 rpm. After centrifugation the extract solution was decanted. Both extracts were combined and analyzed by HPLC-MS/MS. Two characteristic mass transitions per compound (one for quantification, one for confirmation) were monitored. All results from the quantifier mass transition were reported. The limit of quantification (LOQ) of the method was 0.002 mg kg^{-1}.

The validity of the analytical method was analyzed within the storage stability study. The accuracy of the method was proven by simultaneous analysis of two freshly prepared fortified specimens from each trial on each date of analysis. The fortification level was the same as for the storage stability specimens. Significant interferences (> 30% of LOQ) were observed in some samples at the retention times and mass transitions considered for 1,2,4-(1H)-triazole. Therefore, interferences in some control samples were determined and blank correction in the procedural recovery data was needed for the analysis of triazole. Overall mean recoveries ranged from 97% to 103 ~~102~~ % for cis-metconazole, from 97 % to 103 % for trans-metconazole and from 84 ~~86~~ % to 96 ~~97~~ % for 1,2,4-(1H)-triazole with relative standard deviation ranging from 4.6% to 10.1% for all three analytes.

In addition, untreated control samples were analyzed at the date of analysis in duplicate. Residues were below the limit of detection (< 30 % of LOQ) for cis- and trans-isomer of metconazole in all six soil samples. Residues of 1,2,4-(1H)-triazole were detectable in the range of below the limit of detection (< 30 % of LOQ) to below the LOQ (< 0.002 mg/kg). Therefore, interferences in some control samples were determined and blank correction in the recovery data was needed.

Significant matrix effects (> 20%) were not found in HPLC-MS/MS analysis for all six soil types, hence analysis was accomplished using calibration standards prepared in acetonitrile/water (70/30, v/v).

II. RESULTS AND DISCUSSION

The residues in the soil samples were determined immediately after fortification and after storage for up to 720 ~~540~~ days. Two samples were analysed at each defined time point. Significant interferences (> 30% of LOQ) were observed in some samples at the retention time and mass transitions considered for 1,2,4-(1H)-triazole. Therefore, interferences in some control samples were determined and blank correction in the stored sample data was needed.

From the results, recovery rates were calculated which were corrected for the mean recovery rates obtained from the respective freshly fortified specimens (same sampling date and soil). The recovery values in percent of cis-metconazole and trans-metconazole and 1,2,4-(1H)-triazole at the various dates of analysis are shown in Table 7.1.2.2.1-13 to Table 7.1.2.2.1-18.

Table 7.1.2.2.1-13: Results of storage stability samples of cis- and trans-metconazole and its metabolite 1,2,4 (1H)-triazole in soil samples of field trial L1203412

Analyte	Storage Time [d]	Residue [mg kg^{-1}]	Recovery [%]	[%] Mean Recovery uncorrected	[%] Mean Procedural Recovery	Corrected Recovery * [%]	Recovery [% of initial] **
cis-metconazole	0	0.0204	102	**101**	101	101	100
		0.0200	100			99	
	32	0.0206	103	**101**	98	105	100
		0.0196	98			100	
	60	0.0197	99	**99**	90	110	98
		0.0195	98			109	
	120	0.0187	94	**95**	86	109	94
		0.0190	95			110	
	242	0.0199	100	**99**	103	97	98
		0.0196	98			95	
	361	0.0211	106	**107**	105	101	105
		0.0214	107			102	
	540	0.0186	93	**94**	101	92	93
		0.0189	95			94	
	720	0.0201	101	**99**	103	98	98
		0.0193	97			94	
trans-metconazole	0	0.0206	103	**103**	101	102	100
		0.0206	103			102	
	32	0.0208	104	**102**	99	105	99
		0.0198	99			100	
	60	0.0190	95	**97**	90	106	94
		0.0195	98			109	
	120	0.0179	90	**92**	86	105	89
		0.0187	94			109	
	242	0.0175	88	**86**	104	85	83
		0.0165	83			80	
	361	0.0204	102	**100**	105	97	97
		0.0193	97			92	
	540	0.0182	91	**90**	98	93	87
		0.0178	89			91	
	720	0.0185	93	**94**	102	91	91
		0.0188	94			92	
1,2,4-(1H)-triazole	0	0.0194	97	**97**	94	103	100
		0.0193	97			103	
	32	0.0160	80	**80**	82	98	82
		0.0159	80			98	
	60	0.0161	81	**80**	85	95	82
		0.0156	78			92	
	120	0.0154	77	**76**	74	104	78
		0.0150	75			101	
	242	0.0133	67	**72**	98	68	74
		0.0151	76			78	
	361	0.0140	70	**71**	75	93	73
		0.0143	72			96	
	540	0.0142	71	**73**	94	76	75
		0.0148	74			79	
	720	0.0142	71	**72**	72	99	74
		0.0146	73			101	

* Mean recovery of storage stability sample corrected by procedural recovery sample at same time point
** Percent of initial was calculated from two **uncorrected** samples. The mean recovery at 0 days was set to 100 % of initial.

Table 7.1.2.2.1-14: Results of storage stability samples of cis- and trans-metconazole and its metabolite 1,2,4 (1H)-triazole in soil samples of field trial L120313

Analyte	Storage Time [d]	Residue [mg kg^{-1}]	Recovery [%]	[%] Mean Recovery uncorrected	[%] Mean Procedural Recovery	Corrected Recovery * [%]	Recovery [% of initial] **
cis-metconazole	0	0.0213	107	**107**	100	107	100
		0.0213	107			107	
	32	0.0212	106	**106**	100	106	99
		0.0212	106			106	
	60	0.0185	93	**94**	97	96	88
		0.0190	95			98	
	120	0.0206	103	**102**	95	108	95
		0.0201	101			106	
	242	0.0214	107	**105**	101	106	98
		0.0203	102			101	
	361	0.0202	101	**102**	106	95	95
		0.0206	103			97	
	540	0.0191	96	**96**	92	104	89
		0.0189	95			103	
	720	0.0186	93	**94**	105	89	87
		0.0188	94			90	
trans-metconazole	0	0.0202	101	**101**	102	99	100
		0.0202	101			99	
	32	0.0213	107	**103**	97	110	102
		0.0198	99			102	
	60	0.0189	95	**97**	95	100	96
		0.0195	98			103	
	120	0.0192	96	**94**	94	102	93
		0.0182	91			97	
	242	0.0176	88	**87**	100	88	86
		0.0171	86			86	
	361	0.0208	104	**104**	105	99	102
		0.0205	103			98	
	540	0.0184	92	**93**	89	103	92
		0.0186	93			104	
	720	0.0177	89	**89**	107	83	88
		0.0178	89			83	
1,2,4-(1H)-triazole	0	0.0181	91	**99**	96	95	100
		0.0214	107			111	
	32	0.0197	99	**93**	95	104	93
		0.0172	86			91	
	60	0.0159	80	**78**	90	89	78
		0.0150	75			83	
	120	0.0154	77	**77**	92	84	77
		0.0151	76			83	
	242	0.0157	79	**79**	90	88	79
		0.0156	78			87	
	361	0.0155	78	**75**	86	91	76
		0.0143	72			84	
	540	0.0153	77	**75**	82	94	75
		0.0143	72			88	
	720	0.0163	82	**76**	86	95	77
		0.0139	70			81	

* Mean recovery of storage stability sample corrected by procedural recovery sample at same time point
** Percent of initial was calculated from two **uncorrected** samples. The mean recovery at 0 days was set to 100 % of initial.

Table 7.1.2.2.1-15: Results of storage stability samples of cis- and trans-metconazole and its metabolite 1,2,4 (1H)-triazole in soil samples of field trial L120314

Analyte	Storage Time [d]	Residue [mg kg^{-1}]	Recovery [%]	[%] Mean Recovery uncorrected	[%] Mean Procedural Recovery	Corrected Recovery * [%]	Recovery [% of initial] **
cis-metconazole	0	0.0204	102	**103**	103	99	100
		0.0205	103			100	
	32	0.0203	102	**102**	100	102	99
		0.0202	101			101	
	60	0.0184	92	**92**	93	99	90
		0.0183	92			99	
	120	0.0183	92	**92**	95	97	89
		0.0181	91			96	
	242	0.0206	103	**103**	102	101	100
		0.0203	102			100	
	361	0.0197	99	**102**	100	99	99
		0.0208	104			104	
	540	0.0206	103	**97**	92	112	95
		0.0181	91			99	
	720	0.0191	96	**94**	94	102	92
		0.0184	92			98	
trans-metconazole	0	0.0206	103	**104**	103	100	100
		0.0207	104			101	
	32	0.0214	107	**108**	98	109	104
		0.0216	108			110	
	60	0.0190	95	**92**	94	101	88
		0.0176	88			94	
	120	0.0182	91	**91**	95	96	88
		0.0181	91			96	
	242	0.0174	87	**87**	104	84	84
		0.0172	86			83	
	361	0.0192	96	**96**	103	93	93
		0.0191	96			93	
	540	0.0178	89	**90**	89	100	86
		0.0179	90			101	
	720	0.0182	91	**90**	93	98	86
		0.0175	88			95	
1,2,4-(1H)-triazole	0	0.0175	88	**88**	98	90	100
		0.0176	88			90	
	32	0.0188	94	**94**	100	94	107
		0.0187	94			94	
	60	0.0169	85	**83**	90	94	94
		0.0162	81			90	
	120	0.0159	80	**84**	85	94	95
		0.0176	88			104	
	242	0.0174	87	**80**	102	85	90
		0.0143	72			71	
	361	0.0140	70	**71**	80	88	80
		0.0142	71			89	
	540	0.0236	118	**94**	86	137	106
		0.0137	69			80	
	720	0.0165	83	**77**	88	94	88
		0.0141	71			81	

* Mean recovery of storage stability sample corrected by procedural recovery sample at same time point
** Percent of initial was calculated from two **uncorrected** samples. The mean recovery at 0 days was set to 100 % of initial.

Table 7.1.2.2.1-16: Results of storage stability samples of cis- and trans-metconazole and its metabolite 1,2,4 (1H)-triazole in soil samples of field trial L120315

Analyte	Storage Time [d]	Residue [mg kg^{-1}]	Recovery [%]	[%] Mean Recovery uncorrected	[%] Mean Procedural Recovery	Corrected Recovery * [%]	Recovery [% of initial] **
cis-metconazole	0	0.0201	101	**103**	101	100	100
		0.0207	104			103	
	32	0.0209	105	**107**	101	104	104
		0.0215	108			107	
	60	0.0207	104	**103**	102	102	100
		0.0204	102			100	
	120	0.0202	101	**101**	96	105	99
		0.0202	101			105	
	242	0.0204	102	**101**	104	98	98
		0.0197	99			95	
	361	0.0232	116	**112**	111	105	109
		0.0216	108			97	
	540	0.0205	103	**102**	99	104	100
		0.0201	101			102	
	720	0.0203	102	**104**	102	100	101
		0.0210	105			103	
trans-metconazole	0	0.0201	101	**100**	101	100	100
		0.0198	99			98	
	32	0.0213	107	**109**	100	107	109
		0.0219	110			110	
	60	0.0185	93	**94**	101	92	94
		0.0190	95			94	
	120	0.0183	92	**94**	94	98	94
		0.0190	95			101	
	242	0.0178	89	**87**	104	86	87
		0.0167	84			81	
	361	0.0203	102	**104**	108	94	104
		0.0210	105			97	
	540	0.0198	99	**99**	96	103	99
		0.0195	98			102	
	720	0.0182	91	**90**	98	93	90
		0.0178	89			91	
1,2,4-(1H)-triazole	0	0.0198	99	**99**	101	98	100
		0.0196	98			97	
	32	0.0161	81	**82**	86	94	83
		0.0163	82			95	
	60	0.0156	78	**81**	83	94	82
		0.0166	83			100	
	120	0.0146	73	**75**	80	91	76
		0.0151	76			95	
	242	0.0145	73	**76**	89	82	77
		0.0156	78			88	
	361	0.0153	77	**75**	79	97	76
		0.0144	72			91	
	540	0.0164	82	**84**	91	90	85
		0.0170	85			93	
	720	0.0160	80	**81**	82	98	82
		0.0162	81			99	

* Mean recovery of storage stability sample corrected by procedural recovery sample at same time point
** Percent of initial was calculated from two **uncorrected** samples. The mean recovery at 0 days was set to 100 % of initial.

Table 7.1.2.2.1-17: Results of storage stability samples of cis- and trans-metconazole and its metabolite 1,2,4 (1H)-triazole in soil samples of field trial L120316

Analyte	Storage Time [d]	Residue [mg kg^{-1}]	Recovery [%]	[%] Mean Recovery uncorrected	[%] Mean Procedural Recovery	Corrected Recovery * [%]	Recovery [% of initial] **
cis-metconazole	0	0.0202	101	**102**	104	97	100
		0.0203	102			98	
	32	0.0224	112	**113**	110	102	111
		0.0228	114			104	
	60	0.0200	100	**101**	100	100	99
		0.0201	101			101	
	120	0.0190	95	**93**	102	93	92
		0.0182	91			89	
	242	0.0216	108	**106**	103	105	104
		0.0206	103			100	
	361	0.0184	92	**96**	101	91	95
		0.0200	100			99	
	540	0.0198	99	**99**	94	105	97
		0.0195	98			104	
	720	0.0204	102	**102**	110	93	100
		0.0203	102			93	
trans-metconazole	0	0.0214	107	**109**	101	106	100
		0.0220	110			109	
	32	0.0222	111	**113**	109	102	104
		0.0229	115			106	
	60	0.0217	109	**108**	100	109	99
		0.0211	106			106	
	120	0.0199	100	**97**	100	100	89
		0.0188	94			94	
	242	0.0171	86	**85**	105	82	78
		0.0168	84			80	
	361	0.0187	94	**94**	106	89	87
		0.0187	94			89	
	540	0.0187	94	**94**	93	101	86
		0.0186	93			100	
	720	0.0186	93	**95**	107	87	87
		0.0192	96			90	
1,2,4-(1H)-triazole	0	0.0179	90	**92**	100	90	100
		0.0185	93			93	
	32	0.0192	96	**95**	100	96	104
		0.0188	94			94	
	60	0.0176	88	**88**	86	102	96
		0.0176	88			102	
	120	0.0157	79	**79**	83	95	86
		0.0156	78			94	
	242	0.0148	74	**76**	86	86	83
		0.0155	78			91	
	361	0.0140	70	**71**	72	97	77
		0.0142	71			99	
	540	0.0162	81	**83**	91	89	90
		0.0168	84			92	
	720	0.0180	90	**90**	97	93	98
		0.0177	89			92	

* Mean recovery of storage stability sample corrected by procedural recovery sample at same time point
** Percent of initial was calculated from two **uncorrected** samples. The mean recovery at 0 days was set to 100 % of initial.

Table 7.1.2.2.1-18: Results of storage stability samples of cis- and trans-metconazole and its metabolite 1,2,4 (1H)-triazole in soil samples of field trial L120317

Analyte	Storage Time [d]	Residue [mg kg^{-1}]	Recovery [%]	[%] Mean Recovery uncorrected	[%] Mean Procedural Recovery	Corrected Recovery * [%]	Recovery [% of initial] **
cis-metconazole	0	0.0200	100	**100**	93	108	100
		0.0200	100			108	
	32	0.0229	115	**112**	109	106	112
		0.0218	109			100	
	60	0.0207	104	**103**	102	102	103
		0.0202	101			99	
	120	0.0189	95	**93**	97	98	93
		0.0182	91			94	
	242	0.0207	104	**104**	103	101	104
		0.0206	103			100	
	361	0.0228	114	**115**	106	108	115
		0.0232	116			109	
	540	0.0194	97	**97**	106	92	97
		0.0192	96			91	
	720	0.0215	108	**107**	108	100	107
		0.0211	106			98	
trans-metconazole	0	0.0203	102	**102**	97	105	100
		0.0201	101			104	
	32	0.0214	107	**110**	108	99	108
		0.0223	112			104	
	60	0.0207	104	**104**	102	102	102
		0.0206	103			101	
	120	0.0181	91	**91**	97	94	89
		0.0180	90			93	
	242	0.0169	85	**86**	106	80	84
		0.0171	86			81	
	361	0.0221	111	**111**	107	104	109
		0.0220	110			103	
	540	0.0193	97	**98**	103	94	97
		0.0198	99			96	
	720	0.0199	100	**97**	107	93	96
		0.0187	94			88	
1,2,4-(1H)-triazole	0	0.0190	95	**94**	97	98	100
		0.0183	92			95	
	32	0.0210	105	**105**	108	97	112
		0.0209	105			97	
	60	0.0198	99	**95**	102	97	102
		0.0182	91			89	
	120	0.0181	91	**87**	94	97	93
		0.0166	83			88	
	242	0.0151	76	**78**	97	78	83
		0.0157	79			81	
	361	0.0156	78	**77**	79	99	82
		0.0151	76			96	
	540	0.0179	90	**89**	103	87	95
		0.0175	88			85	
	720	0.0182	91	**92**	94	97	98
		0.0186	93			99	

* Mean recovery of storage stability sample corrected by procedural recovery sample at same time point
** Percent of initial was calculated from two **uncorrected** samples. The mean recovery at 0 days was set to 100 % of initial.

The storage stability of metconazole (cis-isomer and trans-isomer) and its metabolite 1,2,4-(1H)-triazole was proven in all six tested soils over the tested period of 720 ~~540~~ days at ≤ -18°C. ~~Further analysis will be performed after 720 days of storage.~~

For cis-metconazole and trans-metconazole, the uncorrected recoveries in the six different soil samples after 720 ~~540~~ days of storage ranged from 94 % to 107 ~~102~~ % and from 89 ~~90~~ % to 97 ~~99~~ % of the fortified concentration, respectively. The uncorrected recovery values for 1,2,4-(1H)-triazole, after 720 ~~540~~ days of storage, ranged from 72 ~~73~~ % to 92 ~~94~~ % of the fortified ~~initial~~ concentration.

III. CONCLUSION

Residues can be regarded as stable if the mean recovery at a given storage period is in the range of 70 % up to 120 % of the fortified value. The results of the storage stability study show that the two isomers of metconazole, cis-metconazole (Reg. No. 4079468) and trans-metconazole (Reg. No. 4079654), as well as its metabolite 1,2,4-(1H)-triazole (Reg. No. 87084) are stable under deep-frozen conditions (≤ -18°C) in soil for at least 720 ~~540~~ days of storage.

Summary of degradation rates for metconazole in field dissipation studies

Table 7.1.2.2.1-19: Summary of best-fit field half-lives of metconazole obtained in terrestrial field dissipation studies

BASF DocID	Trial / Location	Soil type	pH ($CaCl_2$)	Org. C [%]	Best-fit $DisT_{50}$ / $DisT_{90}$ [d] [b]	Kinetic model	χ^2 error
1996/7000437 (MK-790-011)	95-096-01 Schwabenheim, Germany	Silty loam	5.7	0.96	40 / 442	Square root 1st-order	-
	95-096-02 Kloppenheim, Germany	Silty clay loam	6.1	1.11	33 / 363	Square root 1st-order	-
1996/7000438 (MK-790-012)	95-097-01 Biscester, Oxfordshire, UK	Sandy clay loam	6.1 [a]	1.83	34 / 370	Square root 1st-order	-
	95-097-02 Horncastle, Lincolnshire, UK	Sandy loam	7.2 [a]	1.78	138 / 457	1st-order	-
1993/7000320 (MK-620-010)	Hoath, Kent, UK	Clay loam	8 [a]	1.6 [c]	133 / 434 [d]	1st-order	-
					182 / 609 [e]	1st-order	-
					161 / 539 [f]	1st-order	-
					147 / 483 [g]	1st-order	-
	Reculver, Kent, UK	Sandy loam	4.1 [a]	0.5 [c]	112 / 371 [d]	1st-order	-
					203 / 665 [e]	1st-order	-
					231 / 770 [f]	1st-order	-
					259 / 854 [g]	1st-order	-
	Quincieux, France	Sandy silt loam	-	-	28 / 98 [d]	1st-order	-
					28 / 98 [e]	1st-order	-
					7 / 28 [f]	1st-order	-
					14 / 42 [g]	1st-order	-
	Espiet, France	Silty clay loam	-	-	70 / 238 [d]	1st-order	-
					70 / 238 [e]	1st-order	-
					70 / 231 [f]	1st-order	-
					49 / 168 [g]	1st-order	-
	Schwabenheim, Germany	Sandy loam	7.5 [a]	1.0 [c]	77 / 259 [d]	1st-order	-
					154 / 504 [e]	1st-order	-
					133 / 441 [f]	1st-order	-
					112 / 371 [g]	1st-order	-
	Schonau, Germany	Silty clay loam	7.0 [a]	1.3 [c]	70 / 224 [d]	1st-order	-
					91 / 287 [e]	1st-order	-
					56 / 189 [f]	1st-order	-
					56 / 189 [g]	1st-order	-

[a] No information on method available
[b] All degradation half-lives for DocID 1993/7000320 are given in weeks in original study report; multiplied by 7 for $DisT_{50}$/$DisT_{90}$ in days
[c] Calculated from organic matter (OM) content by OC=OM/1.724
[d] Formulation 60g/L SL, dose rate 175 g a.s./ha
[e] Formulation 60 g/L SL, dose rate 350 g a.s./ha
[f] Formulation 100g/L SL, dose rate 250 g a.s./ha
[g] Formulation 100 g/L SL, dose rate 350 g a.s./ha
Bold: worst-case $DisT_{50}$ for modeling

Table 7.1.2.2.1-20: Summary of best-fit field half-lives of metconazole obtained in covered terrestrial field dissipation studies (not adequate for trigger endpoints)

BASF DocID	Trial / Location	Soil type	pH [a] ($CaCl_2$)	Org. C [a] [%]	Best-fit [b] DT_{50} / DT_{90} [d]	Kinetic model	χ^2 error
2015/1137154	L120312 Bogense, Denmark	Sandy loam	6.29	0.96	202.8 / 673.7	SFO	18.1
	L120313 Brunne, Germany	Loamy sand	5.01	0.72	258.5 / 858.6	SFO	13.2
	L120314 Goch-Nierswalde, Germany	Silt loam	6.55	1.67	152.4 / 506.1	SFO	17.0
	L120315 Schaeffersheim, France	Silt	7.63	2.03	31.0 / 102.9	SFO	8.3
	L120316 Poggio Renatico, Italy	Silt loam	7.75	1.78	449.2 / >1000	SFO	7.8
	L120317 Utrera, Spain	Sand	7.33	0.57	136.3 / 452.8	SFO	14.3

[a] Data given for the respective top soil layer
[b] Covered field dissipation study; only to derive modeling endpoints in the soil matrix, excluding surface loss processes [EFSA (2010): EFSA Panel on Plant Protection Products; Guidance for evaluating laboratory and field dissipation studies to obtain DegT50 values of plant protection products in soil. EFSA Journal 2010;8(12):1936, 67 pp.]. Best-fit endpoints should not be used as triggers for additional work.

Table 7.1.2.2.1-21: Summary of normalized field half-lives of metconazole suitable for modeling

BASF DocID	Trial / Location	Soil type	pH [a] ($CaCl_2$)	Org. C [a] [%]	$DegT_{50}$ (20°C, pF2) [d]	Kinetic model	χ^2 error
2015/1137154	L120312 Bogense, Denmark	Sandy loam	6.29	0.96	57.5	SFO	12.5
	L120313 Brunne, Germany	Loamy sand	5.01	0.72	80.3	SFO	6.4
	L120314 Goch-Nierswalde, Germany	Silt loam	6.55	1.67	73.4	SFO	11.8
	L120315 Schaeffersheim, France	Silt	7.63	2.03	26.6	SFO	5.6
	L120316 Poggio Renatico, Italy	Silt loam	7.75	1.78	368.5	SFO	8.7
	L120317 Utrera, Spain	Sand	7.33	0.57	202.0	SFO	17.5
Geometric mean					**93.6**		

[a] Data given for the respective top soil layer

Summary of degradation rates for the metabolite 1,2,4-triazole in field dissipation studies for use in environmental fate modeling

As no data for M555F020 (1,2,4-triazole) were available from the aerobic degradation studies on metconazole, consequently no degradation rates were calculated. Degradation endpoints for 1,2,4-triazole are available and accepted at EU level [*CRD (2014)*]. According to the available information, field derived DT_{50} and DT_{90} values should be used. Relevant data are summarized in Table 7.1.2.2.1-22 and Table 7.1.2.2.1-23.

Table 7.1.2.2.1-22: Summary of best-fit field half-lives of 1,2,4-triazole obtained in terrestrial field dissipation studies

Reference	Trial / Location	Soil type	pH [b] ($CaCl_2$)	Org. C [b] [%]	Best-fit DT_{50} / DT_{90} [d]	Kinetic model	χ^2 error
CRD (2014) [a]	Burscheid, Germany	Silt loam	6.36	0.89	7.8 / 366.7	FOMC	15.2
	Albaro, Italy	Silty clay loam	7.56	1.37	21.2 / 207.4	DFOP	10.7
	Little Shelford, UK	Sandy loam	7.37	1.14	6.8 / 109.3	DFOP	17.8
	Vilobi d'Onyar, Spain	Loam	5.81	1.21	28.1 / 717.6	DFOP	13.3

[a] CRD (2014): Triazole Derived Metabolite: 1,2,4-Triazole. Proposed revision to DT_{50} Summary, Scientific Evaluation and Assessment July 2011, revised September 2011 (after comments from MS and EFSA) and further revised January 2013 (minor clarifications added post-commenting), 24 Oct. 2014
[b] Data given for the respective top soil layer

Table 7.1.2.2.1-23: Summary of normalized field half-lives of 1,2,4-triazole suitable for modeling

Reference	Trial / Location	Soil type	pH [b] ($CaCl_2$)	Org. C [b] [%]	DT_{50} (20°C, pF2) [d]			Kinetic model	χ^2 error
					Fast phase	Slow phase	g		
CRD (2014) [a]	Burscheid, Germany	Silt loam	6.36	0.89	2.5	70.7	0.656	DFOP	18.8
	Albaro, Italy	Silty clay loam	7.56	1.37	1.4	59.8	0.364	DFOP	10.6
	Little Shelford, UK	Sandy loam	7.37	1.14	0.5	25.1	0.458	DFOP	18.1
	Vilobi d'Onyar, Spain	Loam	5.81	1.21	4.6	126.0	0.489	DFOP	12.7
Geometric mean (g value is arithmetic mean)					1.68	60.5	0.489	DFOP	

[a] CRD (2014): Triazole Derived Metabolite: 1,2,4-Triazole. Proposed revision to DT_{50} Summary, Scientific Evaluation and Assessment July 2011, revised September 2011 (after comments from MS and EFSA) and further revised January 2013 (minor clarifications added post-commenting), 24 Oct. 2014
[b] Data given for the respective top soil layer

CA 7.1.2.2.2 Soil accumulation studies

An experimental study on the potential accumulation of residues of metconazole following typical applications of BAS 555 00 F CARAMBA (metconazole 60 g a.s./L SL) to cereals was submitted for the previous Annex I listing [*BASF DocID 2002/7004410 (MK-790-015)*]. The observed very low residue values indicate that significant accumulation of residues of metconazole in the soil is unlikely to occur following applications of the formulated product to cereals according to recommended agricultural practice. No additional study was conducted. The accumulation of metconazole under consideration of the relevant GAP is addressed via PEC_{soil} calculations.

CA 7.1.3 Absorption and desorption in soil

CA 7.1.3.1 Adsorption and desorption

CA 7.1.3.1.1 Adsorption and desorption of the active substance

No new studies on adsorption/desorption behavior of the active substance metconazole were performed. The studies peer-reviewed during the previous Annex I listing process [*BASF DocID 1991/7000232 (MK-620-003); BASF DocID 1995/7000252 (MK-620-012)*] are considered still valid. However, in the *EFSA scientific report (2006) 64* an incorrect listing of the Freundlich 1/n parameter was noted. Therein, it ranged from 0.666 – 1.02; however, based on the study data it should range from 0.887 to 0.983. Data can be found in the original study reports [*BASF DocID 1991/7000232 (MK-620-003),* p.18, Table 3: X Coefficient(s)*; BASF DocID 1995/7000252 (MK-620-012),* p.26/27, Table 10 a)-e)]. The adsorption values are listed in Table 7.1.3.1.1-1.

Table 7.1.3.1.1-1: Adsorption of metconazole in various soil types

Soil	Soil type	OC [%]	pH [a] [-]	K_f [mL g^{-1}]	K_{foc} [mL g^{-1}]	1/n [-]
BASF DocID 1991/7000232 (MK-620-003)						
Godstone	Sand	0.6	6.7	9.6	1592	0.942
Elm Farm	Sandy loam	1.8	7.1	21.9	1217	0.895
Woodstock	Silty clay	3.1	6.8	31.6	1019	0.910
Keyol	Loamy sand	1.4	6.7	17.0	1214	0.887
BASF DocID 1995/7000252 (MK-620-012)						
Engelstadt Benz	Silt loam	2.24	7.4	18.72	836	0.917
Ingelheim / Moers	Sandy loam	1.33	7.6	10.80	812	0.928
Nieder-Ingelheim	Loamy sand	0.74	7.1	6.74	911	0.983
Schwabenheim III/B	Silt loam	1.09	5.9	7.91	726	0.911
Speyer 2.2	Loamy sand	2.29	5.8	39.35	1718	0.950
Geometric mean (n = 9)					**1071**	-
Arithmetic mean (n = 9)					-	**0.925**

[a] pH in water (DocID 1991/7000232), method not stated (DocID 1995/7000252)

CA 7.1.3.1.2 Adsorption and desorption of metabolites, breakdown and reaction products

As laid out before 1,2,4-triazole was attributed a relevant metabolite. No other major metabolites in soil were detected. Adsorption data for 1,2,4-triazole had been accepted at EU level [*CRD (2014)*]. Relevant data are summarized in Table 7.1.3.1.2-1.

Table 7.1.3.1.2-1: Adsorption of 1,2,4-triazole in various soil types

Soil	Soil type USDA	pH $CaCl_2$	OC [%]	K_f [mL g^{-1}]	$K_{f,oc}$ [mL g^{-1}]	(1/n) [-]
Alpaugh	Silty clay	8.8	0.70	0.833	120	0.897
Hollister	Clay loam	6.9	1.74	0.748	43	0.827
Lawrenceville	Silty clay loam	7.0	0.70	0.722	104	0.922
Pachappa	Sandy loam	6.9	0.81	0.720	89	1.016
Arithmetic mean (n = 4) [a]				**0.756**	**89**	**0.916**

[a] Endpoints accepted at EU level

CA 7.1.3.2 Aged sorption

No experimental data are available. Due to the high soil adsorption values no aged sorption experiments are considered necessary.

CA 7.1.4 Mobility in soil

No suitable annex point is available for the reporting of studies that deal with dissipation on plant leaves. Although these studies do not describe mobility in soil, this annex point is deemed most suitable.

In fact, the target in the studies below is to derive an overall foliar dissipation rate based on the mass on the leave available over time. This mass is subject to volatilisation, transformation and degradation. The overall foliar dissipation rate is then used for refinement in PECsw, where it enters the models PRZM and MACRO, and finally refines the amount of mass washed-off from plant leaves. It can directly be used without a normalisation procedure or endangering double counting of plant processes, please refer to MACRO and PRZM manual for more details.

Report:	CA 7.1.4/1 Sandt H.J. van de, 2014a Determination of dislodgeable foliar residues of Metconazole (BAS 555 F) and determination of foliar DT50 after application of BAS 555 01 F to wheat, 2013-2014 2014/1306965
Guidelines:	EPA 875.2100, EEC 1607/VI/97 rev. 2 10.06.1999, SANCO/3029/99 rev. 4 (11 July 2000)
GLP:	yes (certified by Ministry of Health, Welfare and Sport, The Hague, The Netherlands)

Executive Summary

The objective of this study was to determine the magnitude of dislodgeable foliar residues of metconazole (BAS 555 F) resulting from a single ground boom application of BAS 555 01 F to wheat and to determine the foliar $DisT_{50}$ by mathematical processing of the analytical results. The test item was applied to wheat grown in pots in a greenhouse.

The actual application rate of the active substance metconazole was 89.95 g a.s. ha^{-1}. The crop growth stage at the application time was BBCH 39. Untreated samples were taken before application, treated samples were taken at and at 2, 6, 24 and 48 hours after application and at 4 and 7 days after application. Dislodging of leaf residues was performed by shaking 20 g of leaf material with 200 mL dislodging solution (water / aerosol OT-B (0.01%)) twice for 10 min and subsequent analysis of the dislodging solutions by HPLC-MS/MS. Each dislodging event was performed on five replicate leaf specimens.

The analytical results of field fortification experiments (105% overall recovery) showed that frozen storage and transport of the dislodging solution did not influence the analytical results of this study.

Foliar $DisT_{50}$ and $DisT_{90}$ values were estimated from residues measurements of the a.s. metconazole on the leaf surfaces of wheat. Metconazole dissipates rapidly following SFO kinetics from the leaves with $DisT_{50}$ of 2 days.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test material

Test item code:	BAS 555 01 F
Active substance:	Metconazole
Content of a.s.:	Nominal: 90 g L^{-1}, actual: 87.5 g L^{-1}
CAS No.:	125116-23-6
Formulation type:	EC (emulsifiable concentrate)
Batch No.:	FRE-000986

2. Test System

The foliar dislodging study was carried out in raw agricultural commodity of wheat in a greenhouse in The Netherlands (trial no. R13-272-01).

B. STUDY DESIGN

1. Experimental conditions

The wheat plants were subjected to a residue program that simulated the proposed use of BAS 555 01 F. The seeds were sown on 31st March 2014. At application (22nd May 2014), the wheat plants were at BBCH 39. The application was carried out with a hand carried compressed air sprayer with 1.5 m spraying boom fitted with three flat fan nozzles XR 11003 VS at 1.5 bar. The intended spray volume was 200 L ha^{-1}. The trial was designed with one non-treated plot (15 pots) and one treated plot (60 pots). Each plot consisted of pots with ± 35 plants each.

During the trial no additional pesticides were applied. The plants were cultivated in a greenhouse at defined environmental conditions (temperature between 16.4 to 34.8°C and humidity between 22.1 to 89.1% during trial period from 22nd May 2014 to 29th May 2014).
The application rate was verified by sprayer calibration and confirmed by measuring the remaining volume. The actual application rates of the product and of the active substance is given in Table 7.1.4-1.

Table 7.1.4-1: Actual application rates of product and active substance

Trial No.	Test item	Application date	Growth stage (BBCH)	Actual product rate [L ha^{-1}]	Actual rate of a.s. [g a.s. ha^{-1}]
R13-272-01	BAS 555 01 F	22/05/2014	39	1.028	89.95

2. Sampling

Before application, untreated samples were taken. Treated samples were taken at 2, 6, 24 and 48 HAA (hours after application), 4 and 7 DAA (days after application).

Each specimen collected from the untreated or treated plant pots consisted of the flag leaves F and F-1 leaves selected randomly from the entire of plants in the respective pots to give a total amount of ≥ 20 g. The leaves were cut from the haulms at their base using scissors.

For leaf area determination, a total of 40 leaves were collected from randomly selected plants of control plant pots.

The total weight of 40 leaves was determined and the leaf area of each of these 40 leaves was measured by use of a leaf area meter. Beside the individual leaf areas the total weight of these 40 leaves was documented.

3. Leaf dislodging and analytical procedure

Control specimen and specimen of treated plants

Dislodging of the leaf specimens was performed as soon as possible after collection of the leaves. 200 mL of the dislodging solution (water / aerosol OT-B (0.01%)) was added to the bottle containing about 20 g of leaf material and the bottle was subsequently shaken at 250 rpm for 10 min on a reciprocating table shaker. Then, the dislodging solution was decanted into a beaker and the dislodging procedure was repeated with the remaining leaves with another 200 mL of fresh dislodging solution. After shaking, the dislodging solutions were combined and thoroughly mixed.

Fortification samples

Two fortification solutions at different concentrations containing metconazole, as outlined in the table below, were prepared at the analytical test site to investigate the stability of the test item in the dislodging solution. The field fortification experiments were performed on the first day of collecting leaves. Field fortifications were made in triplicate at both fortification levels and one control sample as shown in Table 7.1.4-2.

Table 7.1.4-2: Field fortification samples

Dislodging solution volume [mL] [a]	Number of replicates	LOQ (proposed) [µg L^{-1}]	Fortification level	Volume of solution [mL]	Conc. of solution [µg mL^{-1}]
20	1	5	control	-	-
20	3	5	10 x LOQ	0.5	2
20	3	5	1000 x LOQ	0.5	200

DFR = Dislodgeable foliar residue
LOQ = Limit of quantification
[a] Dislodging solution originated from dislodging of control leaf material.

The specimens from dislodging solution and field fortification were stored deep-frozen at ≤ -18 °C. All samples were stored frozen until they were shipped frozen to the analytical laboratory. The analysis of metconazole in the dislodging solutions was performed by LC-MS/MS. The limit of quantification (LOQ) was 5 µg L^{-1}.

4. Calculation of the dissipation rate

Kinetic evaluation was performed in order to derive dissipation rates of metconazole on the leaf surfaces of wheat (Foliar $DisT_{50}$/$DisT_{90}$). All measurements per sampling point were considered as replicates. Despite the fact, that no guidance on how to derive dissipation rates from such studies is available, the kinetic analysis and calculations of $DisT_{50}$ and $DisT_{90}$ values were performed following the recommendations of the FOCUS Kinetics workgroup [*FOCUS (2006)*]. The software package KinGUI [*Schmitt, W., Gao, Z., Meyer, H. (2011 KinGUII, Version 2.2012.320.1629 Bayer CropScience AG.)*] was used for parameter fitting. The error tolerance and the number of iterations of the optimization tool were set to 0.00001 and 100, respectively.

II. RESULTS AND DISCUSSION

Residues of metconazole in dislodging (DFR) solution specimens ranged between 80 µg L^{-1} and 506 µg L^{-1}. None of the analyzed control specimens collected before the application had any residues exceeding the LOQ of 5 µg L^{-1}. An overview of the results is given in Table 7.1.4-3.

Table 7.1.4-3: Summarized results of metconazole in dislodging solution samples

Sampling Occasion	C_{End} [ng mL^{-1}]	Amount a.s. per leaf weight [µg L^{-1}]
2 HAA	4.75	475
	4.74	474
	4.85	485
	4.89	489
	5.06	506
6 HAA	4.53	453
	4.61	461
	4.38	438
	4.77	477
	4.96	496
24 HAA	2.29	229
	2.23	223
	2.19	219
	1.89	189
	2.57	257
48 HAA	2.41	241
	2.72	272
	2.49	249
	2.29	229
	2.54	254
4 DAA	1.28	128
	1.74	174
	1.29	129
	1.41	141
	1.30	130
7 DAA	0.96	96
	0.86	86
	0.80	80
	0.87	87
	0.98	98

HAA = Hours after application
DAA = Days after application
RSD = Relative standard deviation
Average amount a.s. per leaf weight = C_{end} x DF (dilution factor), DF = 100

The analytical results of the field fortifications (10 x LOQ and 1000 x LOQ) show that average recoveries were in the acceptable range of 70% and 110% (Table 7.1.4-4). Blank field controls were below the limit of quantification (LOQ = 5 µg L^{-1}). The results revealed that frozen storage and transport did not influence the analytical results of the study.

Table 7.1.4-4: Summarized results of the field fortification experiments with metconazole

MS/MS transition	Fortification Level [µg L^{-1}]	Number of samples	Mean average recovery [%]	RSD [%]	Overall average recovery [%]	RSD [%]
320 m/z → 70 m/z	50	3	106	2	105	3
	5000	3	104	3		
320 m/z → 125 m/z	50	3	104	1	104	3
	5000	3	105	4		

RSD = Relative standard deviation

Foliar $DisT_{50}$ Results

Foliar $DisT_{50}$ and $DisT_{90}$ values were estimated from residues measurements of the a.s. metconazole on the leaf surfaces of wheat. Metconazole dissipates rapidly following SFO kinetics from the leaves with $DisT_{50}$ of 2 days (Table 7.1.4-5).

Table 7.1.4-5: Statistical and visual assessment of the SFO kinetic model and the DisT50 and DisT90 values for metconazole

Model	χ^2-error	type I error rate (Prob. > t)	Visual fit	$DisT_{50}$ [d]	$DisT_{90}$ [d]
SFO	15.0	k: < 0.001	good	2.04	6.77

III. CONCLUSION

The objective of this study was to determine the magnitude of dislodgeable foliar residues of metconazole resulting from a single application of BAS 555 01 F to wheat.

The analytical results of field fortification experiments (105% overall recovery) showed that frozen storage and transport of the dislodging solution did not influence the analytical results of this study.

Foliar $DisT_{50}$ and $DisT_{90}$ values were estimated from residue measurements of the a.s. metconazole on the leaf surfaces of wheat. Metconazole dissipates rapidly following SFO kinetics from the leaves with a $DisT_{50}$ of 2 days.

Report:	CA 7.1.4/2 Roussel C.H., 2015a Determination of dislodgeable foliar residues of Metconazole (BAS 555 F) and determination of foliar DT50 after application of BAS 555 01 F to oil seed rape, 2013 2013/1386123
Guidelines:	EC 1107/2009 of the European Parliament and of the Council of 21 Oct 2009, European Commission Regulation No 283/2013, European Commission Regulation No 284/2013, SANCO/3029/99 rev. 4 (11 July 2000), OECD (2011) Guidance Document on Crop Field Trials (Series on Testing and Assessment No. 164 and Series On Pesticides No. 66)
GLP:	yes (certified by Groupe Interministeriel des Produits Chimiques, France)

Executive Summary

The objective of this study was to determine the magnitude of dislodgeable foliar residues of metconazole (BAS 555 F) resulting from a single ground boom application of BAS 555 01 F to oilseed rape and to determine the foliar $DisT_{50}$ by mathematical processing of the analytical results. The test item was applied to oilseed rape grown in pots in a greenhouse.

The actual application rate of the active substance metconazole was 93.8 g a.s. ha^{-1}. The crop growth stage at the application time was BBCH 18. Untreated samples were taken before application, treated samples were taken at 2, 6, 24 and 48 hours after application and at 4 and 7 days after application. Dislodging of leaf residues was performed by shaking 20 leaf discs of 5 cm^{-3} with 100 mL dislodging solution (aerosol OT (0.01%) in water) twice for 10 min and subsequent analysis of the dislodging solutions by LC-MS/MS. Each dislodging event was performed on five replicate leaf specimens.

The analytical results of field fortification experiments (96% overall recovery) showed that frozen storage and transport of the dislodging solution did not influence the analytical results of this study.

Foliar $DisT_{50}$ and $DisT_{90}$ values were estimated from residues measurements of the active substance metconazole on the leaf surfaces of oilseed rape. Metconazole dissipates rapidly following SFO kinetics from the leaves with $DisT_{50}$ of 8.7 days.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test material

Test item code: BAS 555 01 F
Active substance: metconazole
Content of a.s.: Nominal: 90 g L^{-1}, actual: 87.5 g L^{-1}
CAS No.: 125116-23-6
Formulation type: EC (emulsifiable concentrate)
Batch No.: FRE-000986

2. Test System

The foliar dislodging study was carried out in a greenhouse in Dame Marie les Bois, France (trial no. ChR 14 16768).

B. STUDY DESIGN

1. Experimental conditions

The oilseed rape plants were subjected to a residue program that simulated the proposed use of BAS 555 01 F. The seeds were sown on 13th September 2013 and left outside for emergence and then placed in a greenhouse. At application (25th November 2013), the oilseed rape plants were at growth stage BBCH 18. The application was carried out in a spraying chamber. The sprayer was checked with water prior to the application. The target spray volume was 200 L ha^{-1}. The trial was designed with one non-treated plot (30 pots) and one treated plot (70 pots) with 5 seeds per pot.

The pots were maintained in good conditions without any application of products containing metconazole. The plants were cultivated in a greenhouse at controlled conditions. During the trial slug repellent and an insecticide (esfenvalerate) were applied.

The actual amounts of the formulated product and active substance were calculated with the actual concentration (87.5 g L^{-1}) and actual density (1.045 g cm^{-3}). The actual application rates of the product and of the active substance is given in Table 7.1.4-6.

Table 7.1.4-6: Actual application rates of product and active substance

Trial No.	Test item	Application date	Growth stage (BBCH)	Actual product rate [L ha^{-1}]	Actual rate of a.s. [g a.s. ha^{-1}]
ChR 14 16768	BAS 555 01 F	25/11/2013	18	1.072	93.8

2. Sampling

Before application, untreated samples were taken. Treated samples were taken at 2, 6, 24 and 48 HAA (hours after application), 4 and 7 DAA (days after application).

Each sample consisted in 20 leaf discs of 5 cm^{-3}, taken from 2 different pots. The selected pots were randomly taken over the remaining pots. After each sampling, the sampled pots were removed from the trial. On each sampling event, 5 different samples were taken.

3. Leaf dislodging and analytical procedure

Specimen of treated plants

After sampling, dislodging of the leaf specimens was performed by adding 100 mL of dislodging solution (Aerosol OT (0.01%) in Evian™ water) on the leaves and shaking on an orbital shaker for 10 minutes at 200 rpm. The dislodging procedure was repeated with another 100 mL of dislodging solution. Afterwards, both dislodging solutions were pooled in a glass bottle resulting in a volume of 200 mL. After homogenization, a 20 mL aliquot was taken for analysis.

Control and fortification samples

At one occasion on the application day, specimens (20 mL) were prepared with blank dislodging solution. One single control and three replicates at two fortification levels, as outlined in the table below, were prepared at the analytical test site to investigate the stability of the test item in the dislodging solution. Field fortifications were made in triplicate at both fortification levels and one control sample as shown in Table 7.1.4-7

Table 7.1.4-7: Field fortification samples

Dislodging solution volume [mL] [a]	Number of replicates	LOQ (proposed) [µg L^{-1}]	Fortification level	Volume of solution [mL]	Conc. of solution [µg mL^{-1}]
20	1	5	control	-	-
20	3	5	10 x LOQ	0.5	2
20	3	5	1000 x LOQ	0.5	200

DFR = Dislodgeable foliar residue
LOQ = Limit of quantification
[a] Dislodging solution originated from dislodging of control leaf material

The specimens from dislodging solution and field fortification were stored frozen at < -18°C immediately after the dislodging process. All samples were stored frozen until they were shipped frozen to the analytical laboratory. The analysis of metconazole in the dislodging solutions was performed by LC-MS/MS. The limit of quantification (LOQ) was 5 µg L^{-1}. Limit of detection (LOD) was defined as 20% of LOQ (1 µg L^{-1}).

4. Calculation of the dissipation rate

Kinetic evaluation was performed in order to derive dissipation rates of metconazole on the leaf surfaces of oilseed rape (Foliar $DisT_{50}$/$DisT_{90}$). All measurements per sampling point were considered as replicates. Despite the fact, that no guidance on how to derive dissipation rates from such studies is available, the kinetic analysis and calculations of $DisT_{50}$ and $DisT_{90}$ values were performed following the recommendations of the FOCUS Kinetics workgroup [*FOCUS (2006)*]. The software package KinGUI [*Schmitt, W., Gao, Z., Meyer, H. (2011 KinGUI2, Version 2.2014.224.1704 Bayer CropScience AG.)*] was used for parameter fitting. The error tolerance and the number of iterations of the optimization tool were set to 0.00001 and 100, respectively.

II. RESULTS AND DISCUSSION

Residues of metconazole in dislodging (DFR) solution specimens ranged between 66 µg L^{-1} and 140 µg L^{-1}. None of the analyzed control specimens collected before the application had any residues exceeding the LOQ of 5 µg L^{-1}. An overview of the results is given in Table 7.1.4-8.

Table 7.1.4-8: Summarized results of metconazole in dislodging solution samples

Sampling Occasion	C_{End} [ng mL^{-1}]	Amount a.s. per leaf weight [µg L^{-1}]
2 HAA	6.23	125
	5.79	116
	5.29	106
	6.48	130
	6.07	121
6 HAA	5.51	110
	5.70	114
	5.88	118
	5.48	110
	7.00	140
24 HAA	5.00	100
	4.40	88
	5.54	111
	5.85	117
	5.84	117
48 HAA	5.00	100
	4.78	96
	4.48	90
	5.09	102
	5.35	107
4 DAA	8.00	80
	7.08	71
	7.26	73
	6.61	66
	7.03	70
7 DAA	7.13	71
	7.31	73
	7.44	74
	8.42	84
	8.36	84

HAA = Hours after application
DAA = Days after application
RSD = Relative standard deviation
Average amount a.s. per leaf weight = C_{end} x DF, DF = 20 (2, 6, 24, 48 HAA; DF = 10 (4, 7 DAA))

The analytical results of the field fortifications (10 x LOQ and 1000 x LOQ) show that average recoveries were in the acceptable range of 70% and 110% (Table 7.1.4-9). Blank field controls were below the limit of quantification (LOQ = 5 µg L^{-1}). The results revealed that frozen storage and transport did not influence the analytical results of the study.

Table 7.1.4-9: Summarized results of the field fortification experiments with metconazole

MS/MS transition	Fortification Level [µg L^{-1}]	Number of samples	Mean average recovery [%]	RSD [%]	Overall average recovery [%]	RSD [%]
320 m/z → 70 m/z	50	3	100	0.3	96	5
	5000	3	92	3		
320 m/z → 125 m/z	50	3	99	0.6	95	5
	5000	3	91	3		

RSD = Relative standard deviation

Foliar $DisT_{50}$ Results

Foliar $DisT_{50}$ and $DisT_{90}$ values were estimated from residues measurements of the a.s. metconazole on the leaf surfaces of oilseed rape. Metconazole dissipates rapidly following SFO kinetics from the leaves with $DisT_{50}$ of 8.7 days (Table 7.1.4-10).

Table 7.1.4-10: Statistical and visual assessment of the SFO kinetic model and the $DisT_{50}$ and $DisT_{90}$ values for metconazole

Model	χ^2-error	type I error rate (Prob. > t)	Visual fit	$DisT_{50}$ [d]	$DisT_{90}$ [d]
SFO	5.7	k: < 0.001	good	8.7	29.0

III. CONCLUSION

The objective of this study was to determine the magnitude of dislodgeable foliar residues of metconazole resulting from a single application of BAS 555 01 F to oilseed rape.

The analytical results of field fortification experiments (96% overall recovery) showed that frozen storage and transport of the dislodging solution did not influence the analytical results of this study.

Foliar $DisT_{50}$ and $DisT_{90}$ values were estimated from residue measurements of the a.s. metconazole on the leaf surfaces of oilseed rape. Metconazole dissipates rapidly following SFO kinetics from the leaves with a $DisT_{50}$ of 8.7 days.

Determination of dislodgeable foliar residues of metconazole after application to wheat or oil seed rape – method validation

The methods for the determination of metconazole (BAS 555 F) in dislodgeable foliar residues, resulting from one application of BAS 555 01 F to wheat or oilseed rape were validated at PTRL Europe GmbH, Ulm, Germany as given in the Analytical Phase Reports of the respective study reports [see *CA 7.1.4/1, BASF DocID 2014/1306965; CA 7.1.4/2, BASF DocID 2013/1386123*]. The method validation is briefly described below for each study.

Determination of dislodgeable foliar residues of metconazole after application of BAS 555 01 F to wheat [*CA 7.1.4/1, BASF DocID 2014/1306965]* – method validation

Principle of the method

The method for the determination of metconazole (BAS 555 F) in dislodgeable foliar residues, resulting from one application of BAS 555 01 F to wheat, was validated at PTRL Europe GmbH, Ulm, Germany. The limit of quantification (LOQ) is defined by the lowest fortification level successfully tested. It is 5 µg L^{-1} for metconazole.

A 20 mL dislodging solution (0.01% Aerosol OT-B in water) is diluted with a mixture of methanol/water (1/1, v/v) prior to LC-MS/MS analysis, monitoring two mass transitions of metconazole (m/z 320→70 for quantification and m/z 320→125 for confirmation).

Recovery findings

The method proved to be suitable to determine metconazole in dislodgeable foliar residues, resulting from one application of BAS 555 01 F to wheat. Fresh dislodging solutions (lab fortification) and field quality control samples (field fortification) were fortified with the analyte at the limit of quantification of 5 µg L-^{1}, 50 µg L^{-1} (10 x LOQ) and 5000 µg L^{-1} (1000 x LOQ). None of the analyzed control specimens had any residues exceeding the LOQ of 5 µg L^{-1}. Mean recovery values (mean of three/five replicates per fortification level and analyte) were between 70% and 110% (Table 7.1.4-11), which fulfils the legal requirements.

Table 7.1.4-11: Summary of the residue data in dislodgeable solutions of field and lab fortification samples

Matrix	Analyte	m/z	Forti-fication level [µg L^{-1}]	Number of replicates	Mean recovery [%]	RSD [%]	Overall recovery [%]	RSD [%]
DFR solution – field fortification	Metconazole	320 → 70	50	3	106	2	105	3
			5000	3	104	3		
		320 → 125	50	3	104	1	104	3
			5000	3	105	4		
DFR solution – lab fortification		320 → 70	5	5	97	2	102	6
			5000	5	108	1		
		320 → 125	5	5	94	5	102	9
			5000	5	109	1		

DFR = Dislodgeable foliar residues
RSD = Relative standard deviations

Linearity

Good linearity ($r \geq 0.999$) was observed in the range of 0.1 ng mL^{-1} to 10 ng mL^{-1} for both mass transitions of metconazole. At least five standards, prepared in methanol/water (1/1, v/v), were used for calibration.

Specificity

No significant interferences (> 20% of LOQ) were observed at the retention times and mass transitions of metconazole.

Due to the high selectivity and specificity of LC-MS/MS, monitoring two mass transitions, an additional confirmatory technique was not necessary.

Matrix Effects

The influence of the matrix load on the analysis of metconazole was tested by preparation of matrix-matched standards. Final extracts of control samples were fortified with metconazole. No significant effects of matrix (enhancement or suppression) on the LC-MS/MS responses were observed.

Limit of Quantification

The limit of quantification (LOQ) is defined by the lowest fortification level successfully tested. It is 5 µg L^{-1} for metconazole.

Limit of Detection

The limit of detection (LOD) was set to 20% of LOQ or 1.0 µg L^{-1}.

Repeatability

The relative standard deviations (RSD, %) for all fortification levels were below 20%.

Stability of Working Solutions

The recoveries in the fortified samples (matrix-matched standards) prepared in methanol/water (1/1, v/v) were within an acceptable range of 70% and 110%, thus stability was sufficiently proven.

Reproducibility

The method was validated concurrently by processing laboratory fortifications and field fortifications, thus demonstrating transport and storage stability.

Conclusion

The method for analysis of metconazole in dislodgeable foliar residues, resulting from one application of BAS 555 01 F to wheat, uses LC-MS/MS for final determination, with a limit of quantification of 5 µg L^{-1}.

It could be demonstrated that the method fulfils the requirements with regard to specificity, repeatability, limit of quantification and recoveries and is therefore applicable to correctly determine metconazole in dislodgeable foliar residues, resulting from one application of BAS 555 01 F to wheat.

Determination of dislodgeable foliar residues of metconazole after application of BAS 555 01 F to oilseed rape [*CA 7.1.4/2, BASF DocID 2013/1386123]* – method validation

Principle of the method

The method for the determination of metconazole (BAS 555 F) in dislodgeable foliar residues, resulting from one application of BAS 555 01 F to oilseed rape, was validated at PTRL Europe GmbH, Ulm, Germany. The limit of quantification (LOQ) is defined by the lowest fortification level successfully tested. It is 5 µg L^{-1} for metconazole.

A 20 mL dislodging solution (0.01% Aerosol OT in water) is diluted with a mixture of methanol/water (1/1, v/v) prior to LC-MS/MS analysis, monitoring two mass transitions of metconazole (m/z 320→70 for quantification and m/z 320→125 for confirmation).

Recovery findings

The method proved to be suitable to determine metconazole in dislodgeable foliar residues, resulting from one application of BAS 555 01 F to oilseed rape. Fresh dislodging solutions (lab fortification) and field quality control samples (field fortification) were fortified with the analyte at the LOQ of 5 µg L-^{1}, 50 µg L^{-1} (10 x LOQ) and 5000 µg L^{-1} (1000 x LOQ). None of the analyzed control specimens had any residues exceeding the LOQ of 5 µg L^{-1}. Mean recovery values (mean of three/five replicates per fortification level and analyte) were between 70% and 110% (Table 7.1.4-12), which fulfils the legal requirements.

Table 7.1.4-12: Summary of the residue data in dislodgeable solutions of field and lab fortification samples

Matrix	Analyte	m/z	Forti-fication level [µg L^{-1}]	Number of replicates	Mean recovery [%]	RSD [%]	Overall recovery [%]	RSD [%]
DFR solution – field fortification	Metconazole	320 → 70	50	3	100	0.3	96	5
			5000	3	92	3		
		320 → 125	50	3	99	0.6	95	5
			5000	3	91	3		
DFR Solution – lab fortification		320 → 70	5	5	96	1	93	4
			5000	5	91	5		
		320 → 125	5	5	95	3	92	5
			5000	5	90	6		

DFR = dislodgeable foliar residues
RSD = relative standard deviations

Linearity

Good linearity ($r \geq 0.99$) was observed in the range of 0.1 ng mL^{-1} to 10 ng mL^{-1} for both mass transitions of metconazole. At least five standards, prepared in methanol/water (1/1, v/v), were used for calibration.

Specificity

No significant interferences (> 20% of LOQ) were observed at the retention times and mass transitions of metconazole.

Due to the high selectivity and specificity of LC-MS/MS, monitoring two mass transitions, an additional confirmatory technique was not necessary.

Matrix Effects

The influence of the matrix load on the analysis of metconazole was tested by preparation of matrix-matched standards. Final extracts of control samples (diluted with methanol/water, 1/1, v/v) were fortified with metconazole. No significant effects of matrix (enhancement or suppression) on the LC-MS/MS responses were observed at the tested concentration levels 1.0 and 10 ng mL^{-1}.

Limit of Quantification

The limit of quantification (LOQ) is defined by the lowest fortification level successfully tested. It is 5 µg L^{-1} for metconazole.

Limit of Detection

The limit of detection (LOD) of the method was set to 20% of LOQ or 1.0 µg L^{-1}.

Repeatability

The relative standard deviations (RSD, %) for all fortification levels were below 20%.

Stability of Working Solutions

The recoveries in the fortified samples (matrix-matched standards) prepared in methanol/water (1/1, v/v) were within an acceptable range of 70% and 110%, thus stability was sufficiently proven.

Reproducibility

The method was validated concurrently by processing laboratory fortifications and field fortifications, thus demonstrating transport and storage stability.

Conclusion

The method for analysis of metconazole in dislodgeable foliar residues, resulting from one application of BAS 555 01 F to oilseed rape, uses LC-MS/MS for final determination, with a limit of quantification of 5 µg L^{-1}.

It could be demonstrated that the method fulfils the requirements with regard to specificity, repeatability, limit of quantification and recoveries and is therefore applicable to correctly determine metconazole in dislodgeable foliar residues, resulting from one application BAS 555 01 F to oilseed rape.

CA 7.1.4.1 Column leaching studies

CA 7.1.4.1.1 Column leaching of the active substance

The soil mobility of metconazole and its metabolites was evaluated during the previous Annex I inclusion process based on soil-thin layer chromatography [*BASF DocID 1998/7000279 (MK-620-001)*] and aged column leaching studies [*BASF DocID 1992/7000231 (MK-620-007*)]. TLC analysis of the soil extracts showed only the presence of metconazole indicating the test substance was stable throughout the duration of the study. The retention time of metconazole compared to the reference substances indicate metconazole has little to no tendency for mobility within these soils and therefore is unlikely to contaminate groundwater by simply leaching through the soil profile. No significant amount of radioactivity was observed in the leachate of the aged soil columns. No new study was deemed necessary.

CA 7.1.4.1.2 Column leaching of metabolites, breakdown and reaction products

Please see justification in M-CA 7.1.4.1.1 above.

CA 7.1.4.2 Lysimeter studies

The leaching risk of metconazole and its metabolites is addressed by PEC_{gw} calculations using results from degradation rate and adsorption/desorption studies. Neither the active substance nor its metabolites reveal any risk for groundwater contamination. Lysimeter studies are therefore considered not necessary.

CA 7.1.4.3 Field leaching studies

Neither the active substance nor its metabolites reveal any risk for groundwater contamination. Field leaching studies are therefore considered not necessary.

CA 7.2 Fate and behaviour in water and sediment

CA 7.2.1 Route and rate of degradation in aquatic systems (chemical and photochemical degradation)

CA 7.2.1.1 Hydrolytic degradation

No new experimental data on hydrolytic degradation of metconazole were produced. The already peer-reviewed study [*BASF DocID 1991/7000188 (MK-322-001)*] is considered to be still valid. Metconazole was hydrolytically stable at 50°C at pH 4, 5, 7 and 9.

CA 7.2.1.2 Direct photochemical degradation

For the previous Annex I listing three studies were submitted, a direct aquatic photolysis study performed with [triazole-^{14}C]-labeled metconazole at pH 5, 7 and 9 [*BASF DocID 1996/7000340 (MK-324-001)*] and a natural aquatic photolysis study with [triazole-^{14}C]-labeled metconazole [*BASF DocID 2002/7004456 (MK-630-005)*]. Further, the quantum yield in buffered medium at pH 7 was determined in a study with [chlorophenyl-^{14}C]-labeled metconazole [*BASF DocID 1999/7000164 (MK-324-003)*].

In the direct aquatic photolysis study, photodegradation half-life values (first order) for metconazole were 27.5, 36.3, and 35.8 days of continuous irradiation at pH 5, 7, and 9, respectively. Numerous minor metabolites were observed in all three pH systems, each accounting for <8% total applied radioactivity (TAR). One metabolite reached 14.5% TAR after 30 days of irradiation at pH7 and was identified by mass spectrometry as the hydroxylated parent (CL395834). In the natural water photolysis study, metconazole degraded slowly under test conditions (temperature 22°C, light intensity 0.35 W/m^2) in natural water with a DT_{50} of 58 days of continuous irradiation. The major degradation product was a polar fraction, which accounted for 17% TAR at day 15. Further analysis of this fraction indicated multiple components, none of which exceeded 10% TAR. The mineralization of metconazole in natural water was <0.1% TAR. In the study on quantum yield the DT_{50} of metconazole was calculated to be 83 days of continuous irradiation at pH 7 and 20°C.

The results obtained in the two aquatic photolysis studies are not conclusive with regard to the degradation of metconazole and identification of degradation products. Further, the identity of major degradation products was reported only for those exceeding 10% of the applied radioactivity, accounting for the trigger then in force. Thus, for further elucidation of the photochemical degradation behavior of metconazole in water an additional aqueous photolysis study was conducted. The study was performed according to guideline OECD 316 considering two radiolabels, enantiomeric separation and determination of quantum yield. It is submitted under CA 7.2.1.2/1.

In the new study it was demonstrated that metconazole (BAS 555 F) is stable in water at pH 7 with and without the influence of light. Hence, no half-life and no quantum yield were calculated. Furthermore, no degradation products >5% AR were observed. The enantiomer ratio

was about 4:4:1:1 (R,S: S,R: S,S: R,R metconazole), demonstrating that the ratio of isomers remained stable during the course of the study.

Report:	CA 7.2.1.2/1 Knight L., 2015a Metconazole: Photodegradation in water and determination of the quantum yield 2014/1000925
Guidelines:	OECD 316 (Photodegradation in Water), SETAC Procedures for assessing the environmental fate and ecotoxicity for pesticides (March 1995), JMAFF No 12 Nosan No 8147
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Executive Summary

The study was conducted to determine the rate of photolytic degradation and quantum yield of phenyl-U-^{14}C- and triazole-3(5)-^{14}C-labeled metconazole (BAS 555 F).

Metconazole was applied to pH 7 sterile aqueous phosphate buffer solutions at a nominal application rate of 15 mg L^{-1} (which is approximately one-half of the reported water solubility at 20 C). Treated samples were irradiated with a xenon arc simulated sunlight source continuously for up to 15 days. The samples were irradiated (and control samples incubated in darkness) at 22 ± 1°C. Duplicate samples were taken for analysis at time zero and at intervals of 2, 5, 8, 10, 13 and 15 days. Total radioactivity in test solutions and trapping solutions was measured directly by liquid scintillation counting (LSC). Test solutions were analyzed directly by radio-HPLC, which separated cis- and trans-isomers, and confirmation of parent by TLC and LC-MS.

p-nitroacetophenone (PNAP)/pyridine actinometer solutions were irradiated and incubated alongside test solutions and sub-sampled at the same intervals as test solutions. Analysis was performed by radio-HPLC.

Total recoveries of radioactivity from irradiated and dark control test solutions were in the range 93.5 – 100.8% of the total applied radioactivity (TAR). Only in the irradiated samples of the phenyl-label, low amounts of volatile radioactivity ($^{14}CO_2$) were detected (≤0.4% TAR).

The amount of metconazole present in the irradiated samples of both labels (sum of cis and trans metconazole) was in the range 92.6 – 97.6% TAR throughout the irradiation period and accounted for 94.6 and 97.6% TAR at day 15 for phenyl-^{14}C- and triazole-^{14}C-metconazole, respectively. Two transformation products were detected in low amounts (≤1.3% TAR). The enantiomer ratio was about 4:4:1:1 (R,S: S,R: S,S: R,R metconazole), demonstrating that the ratio of isomers remained stable during the course of the study.

The results for dark control samples were similar. Hence, it was demonstrated that metconazole was stable with and without the influence of light. No degradation half-life and no quantum yield were calculated.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

The test item metconazole (BAS 555 F) was used in two ^{14}C-labeled forms.

BAS code:	BAS 555 F
Reg. No.:	4056343
CAS No.:	125116-23-6
Chemical name (IUPAC):	(1*RS*,5*RS*;1*RS*,5*SR*)-5-(4-chlorobenzyl)-2,2-dimethyl-1-(1*H*-1,2,4-triazol-1-ylmethyl)cyclopentanol
Molecular mass:	319.8 g mol^{-1}
Molecular formula:	$C_{17}H_{22}ClN_3O$

1. Phenyl-U-^{14}C-label

Batch No.:	1065-1029
Cis/trans isomer ratio:	81.4 : 18.6
Specific radioactivity of a.s.:	9.26 MBq mg^{-1}
Radiochemical purity:	99.4%, see certificate of analysis attached to the final report (measurement prior to application: 100%)
Purity:	92.7%

2. Triazole-3(5)-^{14}C-label

Batch No.:	811-1101
Cis/trans isomer ratio:	82 : 18
Specific radioactivity of a.s.:	4.84 MBq mg^{-1}
Radiochemical purity:	97.0%, see certificate of analysis attached to the final report (not measurement prior to application, but was 100% in T_0 samples)
Purity:	92.5%

Unlabeled

Batch No.:	AC12140-17
Cis/trans isomer ratio:	85 : 15
Purity:	98.1%

2. Test solutions

A buffer solution at a concentration of 0.01 M and pH 7.0 was prepared by dissolving potassium dihydrogen orthophosphate in water. The pH was adjusted to pH 7 with 1 M sodium hydroxide solution.

A *p*-nitroacetophenone (PNAP)/pyridine actinometer solution was prepared by adding an aliquot of a p-nitroacetophenone (PNAP) solution in acetonitrile to pyridine which was made up to volume with sterilized ultra high purity water. The concentration of PNAP in the actinometer solution was approximately 1.65 mg L^{-1}.

B. STUDY DESIGN

1. Experimental conditions

Test vessels and buffer solution were sterilized. Aseptic techniques were used throughout for preparing, treating, and sampling of the test solutions.

Portions of the sterile buffer solution were treated at a nominal concentration of 15 mg a.s. L^{-1}. The amount of organic solvent present in the treated test solutions was less than 1% (v/v).

The study was conducted using a Suntest accelerated exposure unit (Heraeus Equipment Ltd) fitted with a xenon arc light source and filters to cut off light of less than 290 nm wavelength. The emission spectrum produced was similar to that of natural sunlight.

Solutions for irradiation were contained in cylindrical borosilicate glass fitted with a quartz window on its upper face and inlet and outlet ports for the collection of volatiles. The irradiated vessels were placed in sockets in a water-cooled block, within the photolysis apparatus, which incorporated magnetic stirrers such that the temperature of the test solutions was 22 ± 1°C. For volatile collection, test vessels were incorporated into flow-through systems. Air was drawn through a water trap (for humidification), a microbial filter (0.2 µm), the test vessel headspace, another microbial filter, and finally through a sequence of traps containing: 1. Ethyl digol; 2. 1 M KOH (+ phenolphthalein); 3. 1 M H_2SO_4.

Dark control solutions were incubated in the dark in a temperature-controlled environment at 22 ± 1°C and incorporated into an airflow system with traps as described for the irradiated samples.

Portions of the actinometer solution were dispensed into photolysis vessels. Duplicate vessels for irradiation were maintained at 22 ± 1°C. Irradiation was continuous except for short periods during sampling.

Control vessels were incubated in the dark at 22 ± 1°C and were shaken continuously.

2. Sampling

For each label, two test solution samples were taken for analysis immediately after application, and after 2, 5, 8, 10, 13 and 15 days irradiation or dark control incubation.

Aliquots of the irradiated and dark control actinometer solutions were aseptically removed at the same dates. The remaining solutions were returned to the Suntest apparatus or dark control incubation.

3. Description of analytical procedures

Each sampled test vessel was weighed and aliquots of the solution were analyzed by liquid scintillation counting (LSC) and radio-HPLC. A portion was transferred aseptically for pH and sterility testing at each sampling interval. At each sampling interval, the associated trapping solutions were taken and analyzed by LSC.

For each radiolabeled treatment group at time zero and day 15, the aqueous solutions were combined and the total volume recorded. Aliquots were measured by LSC. Samples for chiral analysis had a portion of each combined sample removed and were concentrated by centrifugal evaporation. The concentrated samples were resuspended in methanol. The remaining combined samples, which were used for TLC and LC-MS analysis, were concentrated to dryness and resuspended in methanol : water (1:1, v/v). The concentrated samples were sonicated and aliquots were removed for radioassay to confirm the recovery, which was quantitative.

Aliquots of each sampled actinometer solution were analyzed by HPLC-UV.

4. Calculation of the degradation/dissipation rates

No degradation time for metconazole was calculated since stability in aqueous solution under the influence of light was demonstrated.

II. RESULTS AND DISCUSSION

A. MATERIAL BALANCE

Total recoveries of radioactivity extracted from soil are summarized in Table 7.2.1.2-1 to Table 7.2.1.2-2. The overall mean values for the material balance in the photolysis and in the dark control were in the range of 93.5 – 100.8% TAR.

Table 7.2.1.2-1: Recovery and distribution of radioactivity in pH 7 buffer after treatment with phenyl-U-^{14}C-labeled metconazole [% TAR]

Days after treatment	Irradiated					Dark control
	Water	Volatiles			Material balance	Material balance [1]
		KOH	Ethyl digol	H_2SO_4		
0/I	-	-	-	-	-	95.7
0/II	-	-	-	-	-	95.2
0 mean	-	-	-	-	-	**95.5**
2/I	94.1	n.d.	n.d.	n.d.	94.1	94.6
2/II	95.0	0.1	n.d.	n.d.	95.1	95.3
2 mean	**94.6**	**0.1**	**n.d.**	**n.d.**	**94.6**	**95.0**
5/I	94.7	0.1	n.d.	n.d.	94.8	95.7
5/II	97.4	0.2	n.d.	n.d.	97.6	94.4
5 mean	**96.1**	**0.2**	**n.d.**	**n.d.**	**96.2**	**95.1**
8/I	95.4	0.2	n.d.	n.d.	95.6	93.2
8/II	96.3	0.1	n.d.	n.d.	96.4	96.9
8 mean	**95.9**	**0.2**	**n.d.**	**n.d.**	**96.0**	**95.1**
10/I	96.0	0.3	n.d.	n.d.	96.3	99.8
10/II	96.7	0.3	n.d.	n.d.	97.0	101.8
10 mean	**96.4**	**0.3**	**n.d.**	**n.d.**	**96.7**	**100.8**
13/I	93.4	0.2	n.d.	n.d.	93.6	96.1
13/II	94.0	0.4	n.d.	n.d.	94.4	95.8
13 mean	**93.7**	**0.3**	**n.d.**	**n.d.**	**94.0**	**96.0**
15/I	93.8	0.5	n.d.	n.d.	94.3	94.8
15/II	95.3	0.3	n.d.	n.d.	95.6	97.4
15 mean	**94.6**	**0.4**	**n.d.**	**n.d.**	**95.0**	**96.1**

- = no sample
n.d. = not detected
[1] = no volatiles were formed from dark control samples

Table 7.2.1.2-2: Recovery and distribution of radioactivity in pH 7 buffer after treatment with triazole-3(5)-14C-labeled metconazole [% TAR]

Days after treatment	Irradiated					Dark control
	Water	Volatiles			Material balance	Material balance [1]
		KOH	Ethyl digol	H_2SO_4		
0/I	-	-	-	-	-	99.5
0/II	-	-	-	-	-	101.1
0 mean	-	-	-	-	-	**100.3**
2/I	98.9	n.d.	n.d.	n.d.	98.9	98.2
2/II	98.2	n.d.	n.d.	n.d.	98.2	98.6
2 mean	**98.6**	**n.d.**	**n.d.**	**n.d.**	**98.6**	**98.4**
5/I	98.8	n.d.	n.d.	n.d.	98.8	99.1
5/II	98.3	n.d.	n.d.	n.d.	98.3	99.6
5 mean	**98.6**	**n.d.**	**n.d.**	**n.d.**	**98.6**	**99.4**
8/I	98.1	n.d.	n.d.	n.d.	98.1	98.6
8/II	97.1	n.d.	n.d.	n.d.	97.1	98.7
8 mean	**97.6**	**n.d.**	**n.d.**	**n.d.**	**97.6**	**98.7**
10/I	90.8	n.d.	n.d.	n.d.	90.8	96.8
10/II	96.1	n.d.	n.d.	n.d.	96.1	98.5
10 mean	**93.5**	**n.d.**	**n.d.**	**n.d.**	**93.5**	**97.7**
13/I	95.3	n.d.	n.d.	n.d.	95.3	97.1
13/II	97.9	n.d.	n.d.	n.d.	97.9	98.8
13 mean	**96.6**	**n.d.**	**n.d.**	**n.d.**	**96.6**	**98.0**
15/I	97.8	n.d.	n.d.	n.d.	97.8	99.0
15/II	99.2	n.d.	n.d.	n.d.	99.2	99.2
15 mean	**98.5**	**n.d.**	**n.d.**	**n.d.**	**98.5**	**99.1**

- = no sample
n.d. = not detected
[1] = no volatiles were formed from dark control samples

B. TRANSFORMATION OF PARENT COMPOUND

Results of radio-HPLC analyses are presented in Table 7.2.1.2-3 to Table 7.2.1.2-6.

The amount of metconazole remaining in irradiated solutions at the end of the study for both label treatments (mean values of 94.6 - 97.6% TAR) showed that no significant degradation occurred over the 15 day incubation period. Metconazole degraded to two unidentified degradates, however, occurring in low amounts (≤ 1.3% TAR).

Dark control solutions revealed also no significant degradation of metconazole. The mean amount of metconazole remaining in solution after 15 days was in the range 96.1 – 96.6% TAR for both label treatments. Two unidentified minor degradates were present at levels of ≤ 1.8% TAR.

Table 7.2.1.2-3: Radio-HPLC analysis of pH 7 buffer samples after treatment with phenyl-U-14C-labeled metconazole and incubation under irradiated conditions [% TAR]

Days after treatment	trans-metconazole (Rt 40 mins)	cis-metconazole (Rt 41 mins)	Total metconazole
2/I	16.8	77.3	94.1
2/II	16.6	78.4	95.0
2 mean	**16.7**	**77.9**	**94.6**
5/I	17.2	77.5	94.7
5/II	17.2	80.2	97.4
5 mean	**17.2**	**78.9**	**96.1**
8/I	16.2	79.2	95.4
8/II	17.3	79.0	96.3
8 mean	**16.8**	**79.1**	**95.9**
10/I	16.5	79.5	96.0
10/II	17.7	79.0	96.7
10 mean	**17.1**	**79.3**	**96.4**
13/I	17.4	76.0	93.4
13/II	16.4	77.6	94.0
13 mean	**16.9**	**76.8**	**93.7**
15/I	16.0	77.8	93.8
15/II	17.3	78.0	95.3
15 mean	**16.7**	**77.9**	**94.6**

Rt = retention time [min]

Table 7.2.1.2-4: Radio-HPLC analysis of pH 7 buffer samples after treatment with phenyl-U-14C-labeled metconazole and incubation under dark conditions [% TAR]

Days after treatment	trans-metconazole (Rt 40 mins)	cis-metconazole (Rt 41 mins)	Total metconazole	Unknown (Rt 38 mins)	Sum
0/I	16.6	79.1	95.7	-	95.7
0/II	17.5	77.7	95.2	-	95.2
0 mean	**17.1**	**78.4**	**95.5**	**-**	**95.5**
2/I	16.7	77.9	94.6	-	94.6
2/II	17.2	78.1	95.3	-	95.3
2 mean	**17.0**	**78.0**	**95.0**	**-**	**95.0**
5/I	16.7	79.0	95.7	-	95.7
5/II	18.0	76.4	94.4	-	94.4
5 mean	**17.4**	**77.7**	**95.1**	**-**	**95.1**
8/I	16.7	76.1	92.8	0.4	93.2
8/II	18.7	78.2	96.9	-	96.9
8 mean	**17.7**	**77.2**	**94.9**	**0.2**	**95.1**
10/I	18.5	81.3	99.8	-	99.8
10/II	17.4	84.4	101.8	-	101.8
10 mean	**18.0**	**82.9**	**100.8**	**-**	**100.8**
13/I	17.5	78.6	96.1	-	96.1
13/II	17.1	78.7	95.8	-	95.8
13 mean	**17.3**	**78.7**	**96.0**	**-**	**96.0**
15/I	16.8	78.0	94.8	-	94.8
15/II	18.5	78.9	97.4	-	97.4
15 mean	**17.7**	**78.5**	**96.1**	**-**	**96.1**

Rt = retention time [min]
- = not apparent

Table 7.2.1.2-5: Radio-HPLC analysis of pH 7 buffer samples after treatment with triazole-3,(5)-14C-labeled metconazole and incubation under irradiated conditions [% TAR]

Days after treatment	trans-metconazole (Rt 40 mins)	cis-metconazole (Rt 41 mins)	Total metconazole	Unknown (Rt 37 mins)	Unknown (Rt 38 mins)	Sum
2/I	16.9	80.9	97.8	0.8	0.3	98.9
2/II	16.5	80.9	97.4	0.8	-	98.2
2 mean	**16.7**	**80.9**	**97.6**	**0.8**	**0.2**	**98.6**
5/I	17.5	79.8	97.3	1.1	0.4	98.8
5/II	16.7	80.6	97.3	1.0	-	98.3
5 mean	**17.1**	**80.2**	**97.3**	**1.1**	**0.2**	**98.6**
8/I	16.6	80.7	97.3	0.8	-	98.1
8/II	16.4	79.6	96.0	-	1.1	97.1
8 mean	**16.5**	**80.2**	**96.7**	**0.4**	**0.6**	**97.6**
10/I	15.1	74.9	90.0	-	0.8	90.8
10/II	17.6	77.6	95.2	1.0	-	96.2
10 mean	**16.4**	**76.3**	**92.6**	**0.5**	**0.4**	**93.5**
13/I	16.1	77.1	93.2	1.3	0.8	95.3
13/II	17.9	78.8	96.7	1.2	-	97.9
13 mean	**17.0**	**78.0**	**95.0**	**1.3**	**0.4**	**96.6**
15/I	17.5	79.4	96.9	0.9	-	97.8
15/II	17.7	80.6	98.3	-	0.9	99.2
15 mean	**17.6**	**80.0**	**97.6**	**0.5**	**0.5**	**98.5**

Rt = retention time [min]
- = not apparent

Table 7.2.1.2-6: Radio-HPLC analysis of pH 7 buffer samples after treatment with triazole-3,(5)-14C-labeled metconazole and incubation under dark conditions [% TAR]

Days after treatment	trans-metconazole (Rt 40 mins)	cis-metconazole (Rt 41 mins)	Total metconazole	Unknown (Rt 37 mins)	Unknown (Rt 38 mins)	Sum
0/I	17.2	82.3	99.5	-	-	99.5
0/II	17.4	83.7	101.1	-	-	101.1
0 mean	**17.3**	**83.0**	**100.3**	**-**	**-**	**100.3**
2/I	17.2	80.3	97.5	-	0.7	98.2
2/II	16.7	81.2	97.9	-	0.7	98.6
2 mean	**17.0**	**80.8**	**97.7**	**-**	**0.7**	**98.4**
5/I	16.6	81.5	98.1	0.8	0.2	99.1
5/II	18.3	81.3	99.6	-	-	99.6
5 mean	**17.5**	**81.4**	**98.9**	**0.4**	**0.1**	**99.4**
8/I	17.5	78.8	96.3	1.1	1.3	98.7
8/II	18.1	79.6	97.7	1.1	-	98.8
8 mean	**17.8**	**79.2**	**97.0**	**1.1**	**0.7**	**98.8**
10/I	17.3	78.0	95.3	1.5	-	96.8
10/II	16.8	80.5	97.3	1.2	-	98.5
10 mean	**17.1**	**79.3**	**96.3**	**1.4**	**-**	**97.7**
13/I	16.3	80.8	97.1	-	-	97.1
13/II	15.6	80.7	96.3	1.5	1.0	98.8
13 mean	**16.0**	**80.8**	**96.7**	**0.8**	**0.5**	**98.0**
15/I	16.4	80.1	96.5	1.8	0.7	99.0
15/II	19.1	77.5	96.6	1.7	0.9	99.2
15 mean	**17.8**	**78.8**	**96.6**	**1.8**	**0.8**	**99.1**

TAR = total applied radioactivity
Rt = retention time [min]

Chiral HPLC analysis on samples at time zero and irradiated and dark control samples at day 15 of both label treatments was performed. The enantiomer ratio was about 4:4:1:1 (R,S: S,R: S,S: R,R metconazole) for all analyzed samples. This shows that the ratio of isomers remains stable during the course of the study.

The quantum yield was not determined as no photolysis was observed for metconazole.

III. CONCLUSION

It was demonstrated that metconazole (BAS 555 F) is stable in water at pH 7 with and without the influence of light. Hence, no half-life and no quantum yield were calculated. The enantiomer ratio was about 4:4:1:1 (R,S: S,R: S,S: R,R metconazole), demonstrating that the ratio of isomers remained stable during the course of the study.

CA 7.2.1.3 Indirect photochemical degradation

No data were generated.

CA 7.2.2 Route and rate of biological degradation in aquatic systems

CA 7.2.2.1 "Ready biodegradability"

The aerobic ready biodegradability of metconazole was evaluated using the "Modified Sturm Test"; further, a separate microbial inhibition study was conducted with *Pseudomonas fluorescens* [*BASF DocID 1990/7000169 (MK-690-001); BASF DocID 1991/7000237 (MK-690-002)*]. The already peer-reviewed studies are considered to be still valid. Therefore, no new experimental data are provided. According to the previous EU evaluation metconazole has to be considered as "*not readily biodegradable"*.

CA 7.2.2.2 Aerobic mineralisation in surface water

Report: CA 7.2.2.2/1
Jones A., 2013a
Metconazole: Aerobic mineralisation in surface water (Pelagic Test)
2014/1000924
Guidelines: OECD 309 (April 2004)
GLP: yes
(certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Executive Summary

The purpose of this study was to determine the mineralization and degradation rate of the fungicidal active substance metconazole in a natural aquatic system under dark conditions. The study was performed according to OECD guideline 309 (Aerobic mineralization in surface water – Simulation biodegradation test). The pelagic test system was chosen for this study. Additionally, it was investigated if a potential shift between the four enantiomers occurred under the applied test conditions.

The test was performed at two different concentrations (10 µg L^{-1} and 100 µg L^{-1}). The test item radiolabeled in two different positions ([phenyl-U-^{14}C] and [3,(5)-triazole-^{14}C] labels) were applied, respectively. The test vessels were attached to a flow-through system for continuous aeration and incubated at a temperature of 20 ± 2°C in the dark. Samples were taken at 0, 3, 7, 14, 21, 38 and 60 days after treatment.

The amount and nature of radioactivity in the water samples was determined by liquid scintillation counting (LSC) and radio-HPLC. Insoluble precipitates (biofilms) were formed during the incubation and where appropriate radioactivity associated with this material was quantified by extraction/LSC and combustion/LSC of the residue. The enantiomer ratio was determined by using a chiral HPLC method. Volatiles were trapped and analyzed by LSC.

The total material balance was generally in the range of 91.3 – 102.4% total applied radioactivity (TAR) (both labels, both concentrations). From the results obtained it can be concluded that metconazole is not significantly degraded under the applied test conditions. After 60 days the majority of the recovered radioactivity (≥ 85.5% TAR) was unchanged test item. The results were comparable to the sterile test vessels.

Two minor peaks could be detected in the chromatograms (both labels), however, they did not exceed 1.8% TAR at any sampling time. The enantiomer ratio of test item was about 4:4:1:1 (R,S: S,R: S,S: R,R metconazole) in all analyzed samples indicating that no enantiomer shift took place during incubation.

Radioactivity in the volatile $^{14}CO_2$ traps accounted for up to 3.7% TAR with the phenyl-labeled metconazole and up to 0.4% TAR with the triazole-labeled metconazole indicating an overall low rate of mineralization.

Since metconazole was relatively stable in the test system, degradation kinetics were not calculated.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test material

The test item metconazole (BAS 555 F) was used in two ^{14}C-labelled forms.

Internal code:	BAS 555 F
Reg. No.:	4056343
Common name:	Metconazole
CAS No.:	125116-23-6
Chemical name (IUPAC):	(1RS,5RS;1RS,5SR)-5-(4-chlorobenzyl)-2,2-dimethyl-1-(1H-1,2,4-triazol-1-ylmethyl)cyclopentanol
Molecular mass:	319.8 g mol^{-1}
Molecular formula:	$C_{17}H_{22}ClN_3O$

1. Phenyl-U-^{14}C-label (in the following referred to as "phenyl"-label)

Batch No.:	1065-1029
Cis/trans isomer ratio:	81.4 : 18.6
Specific radioactivity of a.s.:	9.26 MBq mg^{-1}
Radiochemical purity:	99.4%, see certificate of analysis (measurement prior to application: 100%)
Purity:	92.7%

2. 3,(5)-triazole-^{14}C-label (in the following referred to as "triazole"-label)

Batch No.:	811-1101
Cis/trans isomer ratio:	82 : 18
Specific radioactivity of a.s.:	4.84 MBq mg^{-1}
Radiochemical purity:	97.0%, see certificate of analysis (measurement prior to application: 97.9%)
Purity:	92.5%

Unlabeled

Batch No.:	AC12140-17
Cis/trans isomer ratio:	85 : 15
Purity:	98.1%

2. Test system

Surface water and sediment were collected from the River Great Ouse (United Kingdom). The physico-chemical properties of the system are summarized in Table 7.2.2.2-1.

Upon receipt at the testing facility the surface water was passed through a 0.2 mm sieve and a coarse filter paper (GF/A) and the sediment was passed through a 2 mm sieve. The concentration of sediment in the water was adjusted to an approximate concentration of 0.01 g sediment L^{-1}. The test system can still be considered as pelagic.

Table 7.2.2.2-1: Characterization of the water/sediment system

Designation **Origin**		**River Great Ouse** **United Kingdom**
Water		
Temperature [a]	[°C]	21.0
pH water [a]	-	8.04
Oxygen saturation [a]	[%]	75
Total organic carbon	[mg L^{-1}]	5.1
Total P	[mg L^{-1}]	0.3
Total N	[mg L^{-1}]	7.0
Hardness as $CaCO_3$	[mg L^{-1}]	406
Sediment		
Textural class		UK Particle Size Distribution
Sand	[%]	47
Silt	[%]	20
Clay	[%]	33
pH (H_2O)	-	8.0
pH ($CaCl_2$)	-	7.1
Organic matter	[%]	5.3

[a] measured directly at sampling site

B. STUDY DESIGN

1. Experimental conditions

A total of 51 test vessels was prepared: 9 test vessels for each radiolabel (phenyl and triazole) and each nominal concentration (10 and 100 µg L^{-1}), 9 vessels for the sterile incubation (both labels; 100 µg L^{-1}), 2 vessels as system control with radiolabeled benzoic acid and 2 vessels with benzoic acid plus treatment solvent.

The test vessels were filled with about 100 mL water. Appropriate amounts of the respective application solutions were pipetted to the water surface to achieve a nominal application rate of 10 µg L^{-1} or 100 µg L^{-1}, respectively.

The systems were incubated at 20 ± 2°C in a metabolism apparatus (incubator) with a gas flow system. Each test vessel was connected to a volatile trapping system of three gas washing bottles containing trapping solutions (1x ethyl digol, 2x 1 M KOH + phenolphthalein indicator) for the ^{14}C-volatiles to be expected. Test vessels containing sterile water were also aerated, however, the air stream was led through sterile filters to avoid contamination of the test system by airborne germs.

2. Sampling

Samples, including the sterile groups, were taken at 0, 3, 7, 14, 21, 38 and 60 days after treatment (DAT). For sampling, the flasks were removed from the airflow system and the temperature, O_2 content, and pH of the water was measured.

3. Description of analytical procedures

Water

Aliquots of the water phases were measured directly by Liquid Scintillation Counting (LSC) and radio-HPLC without further workup.

Volatiles

Aliquots of the trapping solutions were analyzed directly by LSC.

Biofilm and test vessels

For some test vessels it was not possible to achieve a full material balance (>90% of the total applied radioactivity). It was assumed that the missing radioactivity was associated with the observed biofilm or had adhered to the glass vessel. Attempts were made to recover the missing radioactivity from the biofilm and glass test vessel.

The biofilm material was either separated from the surface water by centrifugation the precipitate was allowed to settle prior to carefully decanting the surface water from the vessel. Following removal of the surface water, the glass test vessel was rinsed with the solvent. The solvent rinse was then used to extract the separated biofilm by ultrasonication followed by centrifugation.

The following series of solvents was used:

(i) Acetonitrile, Acetonitrile : 0.05 M HCl, Acetone (Vessel TR15 only),
(ii) Acetonitrile : 1 M HCl (1:1 v/v), Acetonitrile : 1 M NaOH (1:1, v/v), Acetonitrile : water (1:1, v/v), Tetrahydrofuran, Acetone (Vessel PH16 only)
(iii) Acetonitrile : water (1:1, v/v), Acetone (all vessels except PH16 and TR15).

Aliquots of the supernatant taken for LSC. The extracts were pooled and subsamples concentrated to dryness under nitrogen. The samples were resuspended in a small volume of acetonitrile : water 1:1 (v/v) and analyzed by HPLC. The tetrahydrofuran extract (vessel pH16) was analyzed directly by TLC. The proportion of components in these samples was derived by adding the results from the extracts analysis to those from the water analysis. The biofilm residue was quantified by combustion followed by LSC.

For selected samples, portions of the surface water were concentrated to dryness using a centrifugal evaporator for chiral analysis/TLC or LC/MS analysis. The dry residues were then either resuspended in methanol for chiral HPLC or TLC analysis or in acetonitrile : water (1:1, v/v) for structure elucidation by LC/MS analysis.

4. Calculation of the degradation/dissipation rates

Since metconazole was relatively stable in the test system, degradation kinetics were not calculated.

II. RESULTS AND DISCUSSION

A. MASS BALANCE

The distribution of radioactivity to the different fractions of the water system treated with ^{14}C-metconazole (phenyl- and triazole-label) is presented in Table 7.2.2.2-2 and Table 7.2.2.2-3.

The applied mass of test item per test vessel containing 100 mL of water was 10 µg and 9 µg (low concentration) and 94 µg and 95 µg (high concentration) for the phenyl- and the triazole-labeled test item, respectively.

The material balance was for the low test concentration (both labels) in the range of 91 to 108% TAR and from 94 to 109% TAR for the high test concentration (except for two test vessels [phenyl-label]). Due to the higher concentration, the adsorption effect to the glass walls and/or biofilm was even more pronounced and reached up to 48 and 54% TAR in the phenyl- and triazole-label experiments, respectively. Despite all attempts to recover the radioactivity from the glass wall, in two of the phenyl-label test vessels, the material balance only amounted to 74 and 89% TAR.

In the sterile vessels, the material balance ranged from 93.5 to 106.8 TAR. All radioactivity was recovered from the water phase and no adsorption to the test vessels surface occurred. It can therefore be concluded that the adsorption phenomenon observed in the non-sterilized vessels is indeed attributed to the formation of a biofilm.

The CO_2-formation (mineralization) was very low, reaching 3.1 and 3.7% TAR (low/high test concentration) after 60 days with the phenyl-label and only 0.4 and 0.2% TAR with the triazole-label. No radioactivity was measured in the ethyl digol traps.

Table 7.2.2.2-2: Material balance and distribution of radioactivity after application of [phenyl-U-^{14}C] metconazole to river water (non-sterile and sterile) [% TAR]

Days after treatment	Vessel number	Surface water	Vessel/ residue extracts	Non-extractable residues	Volatile radioactivity		Total recovery
					Ethyl digol	1 M KOH	
Low concentration (10 µg L^{-1})							
0	PH1	102.4	n.s.	n.s.	n.s.	n.s.	102.4
3	PH2	96.1	n.s.	n.s.	<0.1	0.2	96.3
7	PH3	101.4	n.s.	n.s.	<0.1	0.2	101.6
14	PH4	87.6	3.3	<0.1	<0.1	0.4	91.3
21	PH5	95.4	n.s.	n.s.	<0.1	0.4	95.8
38	PH6	95.6	n.s.	n.s.	<0.1	0.9	96.5
60	PH7	72.9	14.1	1.4	<0.1	3.1	91.5
High concentration (100 µg L^{-1})							
0	PH11	106.9	n.s.	n.s.	n.s.	n.s.	106.9
3	PH12	101.7	n.s.	n.s.	<0.1	0.1	101.8
7	PH13	97.1	n.s.	n.s.	<0.1	0.2	97.3
14	PH14	93.7	n.s.	n.s.	<0.1	0.3	93.7
21	PH15	42.1	30.7	0.1	<0.1	0.3	74.3
38	PH16	38.4	48.0	n.a.	<0.1	0.3	86.7
60	PH17	63.3	29.3	0.9	0.4	3.7	97.6
Sterilized water (100 µg L^{-1})							
0	S-1	106.8	n.s.	n.s.	n.s.	n.s.	106.8
3	S-2	102.8	n.s.	n.s.	<0.1	<0.1	102.8
7	S-3	93.5	n.s.	n.s.	<0.1	<0.1	93.5
14	S-4	105.5	n.s.	n.s.	<0.1	<0.1	105.5
21	S-5	97.4	n.s.	n.s.	<0.1	<0.1	97.4
38	S-6	106.0	n.s.	n.s.	<0.1	<0.1	106.0
60	S-7	103.6	n.s.	n.s.	<0.1	<0.1	103.6

TAR = total applied radioactivity
n.s. = no sample
n.a. = not analyzed, insufficient sample remained for analysis

Table 7.2.2.2-3: Material balance and distribution of radioactivity after application of [triazole-3,(5)-^{14}C] metconazole to river water (non-sterile) [% TAR]

Days after treatment	Vessel number	Surface water	Vessel/ residue extracts	Non-extractable residues	Volatile radioactivity		Total recovery
					Ethyl digol	1 M KOH	
Low concentration (10 µg L^{-1})							
0	TR1	105.6	n.s.	n.s.	ns	ns	105.6
3	TR2	103.9	n.s.	n.s.	<0.1	<0.1	103.9
7	TR3	77.2	30.2	0.3	<0.1	<0.1	107.7
14	TR4	97.4	n.s.	n.s.	<0.1	<0.1	97.4
21	TR5	62.3	31.5	0.1	<0.1	<0.1	93.9
38	TR6	91.9	n.s.	n.s.	<0.1	<0.1	91.9
60	TR7	84.2	15.4	1.3	<0.1	0.4	101.3
High concentration (100 µg L^{-1})							
0	TR11	104.5	n.s.	n.s.	n.s.	n.s.	104.5
3	TR12	101.2	n.s.	n.s.	<0.1	<0.1	101.2
7	TR13	72.0	37.0	n.a.	<0.1	<0.1	109.0
14	TR14	96.6	n.s.	n.s.	<0.1	0.1	96.7
21	TR15	41.6	54.4	n.a.	<0.1	<0.1	96.0
38	TR16	93.2	3.9	n.a.	<0.1	0.1	97.2
60	TR17	95.5	n.s.	N.s.	0.1	0.2	95.8

TAR = total applied radioactivity
n.s. = no sample
n.a. = not analyzed, insufficient sample remained for analysis

B. TRANSFORMATION OF PARENT COMPOUND

The summary of radio-HPLC analysis of the water phases is presented in Table 7.2.2.2-4 and Table 7.2.2.2-5.

Unchanged metconazole was the major component in all samples. HPLC analysis of the surface water and extracts resolved up to two low level unknown components in addition to metconazole. Unknown 1 (3-4 minutes) accounted for a maximum of 1.6% TAR at 3 days. This component was also detected in the application solutions and is therefore considered to be an impurity of application solution and not the product of biological degradation of the test substance.

Unknown 2 (38 minutes) was detected only in the low concentration samples and reached a maximum of 1.8% TAR after 7 days. In the HPLC chromatograms of the high concentration water samples, no corresponding peaks became visible. It may be assumed that due to the very low test concentration single peaks (near the limit of detection) which were identified as real peaks in chromatograms of the low concentration samples may be artefacts and thus are not observed in the high test concentration.

Results obtained for the sterilized samples revealed similar results to the non-sterilized samples.

Table 7.2.2.2-4: Metabolite overview for the different fractions after application of [phenyl-U-^{14}C] metconazole to river water [% TAR]

	Time after application (days)						
Component	0	3	7	14	21	38	60
Low concentration (10 µg L^{-1})							
Surface Water							
Metconazole	101.2	95.4	100.0	86.1	93.7	94.9	71.7
Trans-metconazole	17.6	12.3	13.8	14.6	14.8	11.9	6.2
Cis-metconazole	83.6	83.1	86.2	71.5	78.9	83.0	65.5
Unknown (4 minutes)	0.5	-	0.8	-	-	-	-
Unknown (38 minutes)	0.7	0.7	0.6	1.5	1.7	0.8	1.2
Vessel Extracts							
Metconazole	n.s.	n.s.	n.s.	3.3	n.s.	n.s.	13.9
Trans-metconazole				0.4			2.6
Cis-metconazole				2.9			11.3
Unknown (4 minutes)				-			-
Unknown (38 minutes)				-			0.3
Total							
Metconazole	101.2	95.4	100.0	89.4	93.7	94.9	85.6
Trans-metconazole	17.6	12.3	13.8	15.0	14.8	11.8	8.8
Cis-metconazole	83.6	83.1	86.2	74.4	78.9	83.0	76.8
Unknown (4 minutes)	0.5	-	0.8	-	-	-	-
Unknown (38 minutes)	0.7	0.7	0.6	1.5	1.7	0.8	1.5

High concentration (100 µg L^{-1})							
Surface Water							
Metconazole	106.2	101.0	96.5	93.7	42.1	38.4	63.3
Trans-metconazole	18.1	18.5	17.5	17.0	7.6	7.6	7.6
Cis-metconazole	88.1	82.5	79.0	76.7	34.5	30.8	55.7
Unknown (4 minutes)	0.7	0.7	0.6	-	-	-	-
Unknown (38 minutes)	-	-	-	-	-	-	-
Pooled Extracts							
Metconazole	n.s.	n.s.	n.s.	n.s.	30.7	27.1	29.3
Trans-metconazole					5.1	3.3	6.0
Cis-metconazole					25.6	23.8	23.3
Unknown (4 minutes)					-	-	-
Unknown (38 minutes)					-	-	-
THF Extracts							
Metconazole [a]	n.s.	n.s.	n.s.	n.s.	n.s.	20.9	n.s.
Unknown (4 minutes)						-	
Unknown (38 minutes)						-	
Total							
Metconazole	106.2	101.0	96.5	93.7	72.8	86.4	92.6
Trans-metconazole	18.1	18.5	17.5	17.0	12.7	a	13.6
Cis-metconazole	88.1	82.5	79.0	76.7	60.1	a	79.0
Unknown (4 minutes)	0.7	0.7	0.6	-	-	-	-
Unknown (38 minutes)	-	-	-	-	-	-	-
Sterilized water (100 µg L^{-1})							
Surface Water							
Metconazole	106.5	102.3	92.8	105.5	97.4	106.0	103.6
Trans-metconazole	19.1	18.4	17.2	17.6	17.6	19.0	19.0
Cis-metconazole	87.4	83.9	75.6	87.9	79.8	87.0	84.6
Unknown (4 minutes)	0.4	0.6	0.7	-	-	-	-
Unknown (38 minutes)	-	-	-	-	-	-	-

Values calculated from HPLC analysis except those from the THF extracts, which were derived from TLC analysis.
TAR = total applied radioactivity
- = not detected
n.s. = no sample
[a] Values not calculated as Trans/cis isomers could not be determined in the THF extracts by TLC.

Table 7.2.2.2-5: Metabolite overview for the different fractions after application of [triazole-3,(5)-^{14}C] metconazole to river water [% TAR]

	Time after application (days)						
Component	0	3	7	14	21	38	60
Low concentration (10 µg L^{-1})							
Surface Water							
Metconazole	104.2	102.1	75.8	95.9	62.3	90.5	82.9
Trans-metconazole	14.8	10.0	10.3	14.5	6.0	20.4	5.2
Cis-metconazole	89.4	92.1	65.5	81.4	56.3	70.1	77.7
Unknown (4 minutes)	1.4	1.9	1.4	-	-	-	-
Unknown (38 minutes)	-	-	-	1.5	-	1.4	1.3
Vessel Extracts							
Metconazole	n.s.	n.s.	28.4	n.s.	31.0	n.s.	15.1
Trans-metconazole			4.5		4.5		2.0
Cis-metconazole			23.9		26.5		13.1
Unknown (4 minutes)			-		-		-
Unknown (38 minutes)			1.8		0.5		0.3
Total							
Metconazole	104.2	102.1	104.2	95.9	93.3	90.5	98.0
Trans-metconazole	14.8	10.0	14.8	14.5	10.5	20.4	7.2
Cis-metconazole	89.4	92.1	89.4	81.4	82.8	70.1	90.8
Unknown (4 minutes)	1.4	-	1.4	-	-	-	-
Unknown (38 minutes)	-	1.6	1.8	1.5	0.5	1.4	1.6
High concentration (100 µg L^{-1})							
Surface Water							
Metconazole	103.9	98.6	70.6	95.4	41.6	93.2	95.5
Trans-metconazole	16.2	18.6	12.1	16.0	8.1	16.0	15.8
Cis-metconazole	87.7	80.0	58.5	79.4	33.5	77.2	79.7
Unknown (4 minutes)	0.6	1.6	1.4	1.3	-	-	-
Unknown (38 minutes)	-	-	-	-	-	-	-
Pooled Extracts							
Metconazole	n.s.	n.s.	37.0	n.s.	54.4	3.9	n.s.
Trans-metconazole			6.5		9.8	0.6	
Cis-metconazole			30.5		44.6	3.3	
Unknown (4 minutes)			-		-	-	
Unknown (38 minutes)			-		-	-	
Total							
Metconazole	103.9	98.6	107.6	95.4	96.0	97.1	95.5
Trans-metconazole	16.2	18.6	18.6	16.0	17.9	16.6	15.8
Cis-metconazole	87.7	80.0	89.0	79.4	78.1	80.5	79.7
Unknown (4 minutes)	0.6	1.6	1.4	1.3	-	-	-
Unknown (38 minutes)	-	-		-	-	-	-

Values calculated from HPLC analysis except those from the THF extracts which were dervired from TLC analysis.
TAR = total applied radioactivity
- = not detected
n.s. = no sample

Enantiomer specific analysis

In addition to the quantification of the parent, enantiomer-specific analyses were performed on selected water samples at the high concentration. The obtained enantiomer ratios are shown in Table 7.2.2.2-6.

The enantiomer ratio was about 4:4:1:1 (R,S: S,R: S,S: R,R metconazole) for all analyzed samples of both radiolabels (high concentration). No shift between enantiomers was observed.

Table 7.2.2.2-6: Isomer overview for the water phase after application of ^{14}C-metconazole to river water

Radiolabel	Vessel number	R,S metconazole	S,R metconazole	S,S metconazole	R,R metconazole
phenyl-^{14}C	PH13 (7 DAT)	44	42	8	7
	PH14 (14DAT)	40	41	11	8
triazole-^{14}C	TR14 (14DAT)	42	41	8	9
	TR17 (60 DAT)	43	41	8	8

Results expressed as % sample radioactivity

Control samples

The total recoveries of radioactivity in the surface water treated at 100 µg L^{-1} concentrations of reference substance [^{14}C]-sodium benzoate in the main experiment were 87.7 – 99.2% TAR after 60 days. Direct volatile radioactivity (carbon dioxide) accounted for a mean of 80.6 – 95.7% TAR after 60 days. Therefore, the test confirmed that the test system was viable.

III. CONCLUSION

The obtained results show that metconazole is not significantly degraded in natural water under conditions as provided in the OECD 309 pelagic test. After 60 days 85.5 - 98.0% TAR was unchanged active substance.

Some trace amounts of two unknown metabolites were detected, never exceeding 1.8% TAR. The enantiomer ratio of test item was about 4:4:1:1 (R,S: S,R: S,S: R,R metconazole) in all analyzed samples indicating that no enantiomer shift took place during incubation.

Mineralization was overall very low, reaching a maximum of 3.1 and 3.7% TAR with the phenyl-label and 0.4 and 0.2% TAR with the triazole-label (low and high concentration, respectively). No differences in the test item behavior were observed between radiolabel or concentration levels.

Overall, the compound was stable in the test system. Degradation kinetics were not calculated as no significant degradation was observed.

CA 7.2.2.3 Water/sediment studies

For the previous Annex I listing a water/sediment study was submitted [*BASF DocID 1996/7000413 (MK-630-002)*] which was performed with [triazole-^{14}C]-labeled metconazole in two natural water/sediment systems in the dark at 20 ± 2°C for 182 days. The kinetic evaluation was separately calculated for the previous submission [*BASF DocID 2002/7004607 (MK-630-006)*]. The results showed that metconazole dissipated from the water phase in both the natural water-sediment systems studied with DT_{50} values of 1 and 13 days for the Hellersberger Weiher pond and Glan river, respectively. Degradation of metconazole occurred in the total system (water and sediment) with estimated DT_{50} values ranging from 116 to 814 days.

The majority of total applied radioactivity (TAR) found in the water and the sediment extracts consisted of unchanged metconazole. Six individual degradation products were formed in small amounts. Beside one unknown metabolite, five of the metabolites were identified by MS analysis M555F021 (M11, Reg.No. 4558878), M555F013 (M13, CL359139), M555F015 (M15, Reg.No. 4111796), M555F011 (M21, Reg.No. 4111112) and M555F030 (=M555F030cis, M30, Reg.No. 4110625). M555F013 accounted for a maximum of 9% in the water phase of one test system. None of the other single degradation products accounted for >5% TAR in water or sediment at more than a single sampling, regardless of the system or sampling time.

The already peer-reviewed water/sediment study is considered to be still valid. However, only one label was investigated. Therefore, an additional water/sediment study was conducted. The study was performed in two natural water/sediment systems in the dark according to guideline OECD 308, considering two radiolabels and enantiomeric separation. It is submitted under CA 7.2.2.3/1.

Overall, the results of the new study confirm the behavior of metconazole in water/sediment systems under dark conditions observed in the old study, i.e. metconazole dissipates at a fast rate from the water phase with DT_{50} values of 2.9 and 3.9 days and degrades at a rather slow rate in the sediment. For the whole system DT_{50} values of 307 and 660 days were calculated.

Up to five metabolites were formed only sporadically in low amounts (<5%). One of them was identified as metabolite M555F030 (Reg. No. 4110625), accounting for up to 1.0% TAR in water phase and 0.7% in sediment extracts.

The enantiomer ratio was about 4:4:1:1 (R,S: S,R: S,S: R,R metconazole), demonstrating that the ratio of isomers remained stable during the course of the study.

A scheme on the proposed route of degradation was derived under consideration of the results of both studies and is given in Figure 7.2.2.3-1.

Further, the evaluation of degradation kinetics of metconazole in the two water/sediment systems of the old water/sediment study was reassessed. Based on the derived endpoints of the previous calculation [*BASF DocID 2002/7004607 (MK-630-006)*] no deviating conclusion on the resulting trigger endpoints was expected, i.e. metconazole has to be considered persistent in sediment. Thus, a re-calculation of best-fit endpoints used as trigger values was omitted. However, kinetic re-evaluation of the old study data was performed to derive modeling endpoints following the recommendations of the FOCUS workgroup on degradation [*FOCUS (2006) "Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration" Report of the FOCUS Work Group on Degradation Kinetics, EC Document Reference Sanco/10058/2005 version 1.1 (December 2014), 440 pp.*]. The calculation is submitted under CA 7.2.2.3/2.

A summary of degradation and dissipation endpoints for metconazole in various water/sediment systems is given in Table 7.2.2.3-14 and Table 7.2.2.3-15 at the end of this section.

Report:	CA 7.2.2.3/1 Knight L., 2015c Metconazole: Aerobic transformation in aquatic sediment systems 2014/1000921
Guidelines:	OECD 308, 2004/10/EC of 11 February 2004
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Executive Summary

The fate of metconazole was studied in two natural water/sediment systems under dark laboratory conditions. The sediment from Swiss Lake was a sand with an acidic pH and low organic carbon while that from Calwich Abbey Lake was a neutral sandy silt loam with a higher organic carbon content. Water/sediment systems were allowed to acclimatize before being treated with [phenyl-U-^{14}C]-metconazole at a rate of 0.1 mg L^{-1} based on the amount of water in the test vessel. The influence of microbial activity was tested during the experiment by applying the test substance to sterilized vessels.

The test vessels were attached to a flow-through system for continuous aeration and incubated at a temperature of 20 ± 2°C in the dark.

Samples for the experiments were taken at 0, 1, 3, 7, 14, 29, 60 and 99 days after treatment (DAT). The sterile vessels were taken after 0, 60 and 99 days.

Water and sediment were worked up separately. Water samples and sediment extracts were analyzed by liquid scintillation counting (LSC) and radio-HPLC. The amount of non-extractable residues was determined by combustion and LSC. Volatiles were trapped in appropriate trapping solutions and also analyzed by LSC. Metabolites were identified by comparison of retention times to those reference items by HPLC and TLC.

The total radioactivity in the water layer declined from a mean of 88.4% - 91.7% total applied radioactivity (TAR) at time zero to 5.3% - 6.2% after 99 days in both systems. Correspondingly, the total radioactivity in sediment increased in both systems to a mean of 97.5% - 98.6% TAR after 99 days. About 78.7% - 84.6% TAR in sediment was still extractable with acetonitrile.

After 99 days, metconazole was found in the water phase at a mean of 4.6% TAR in Calwich Abbey Lake system and 2.4% TAR in Swiss Lake system. Besides up to four unknown degradation products (all a mean of ≤ 1.9% TAR at any sampling date), the known metabolite Reg. No. 4110625 (M555F030cis) was detected, reaching a maximum mean concentration of ≤ 1.0% TAR in both test systems.

The analyses of sediment extracts show that metconazole reached its highest amount (single replicates) after 60 days with a mean of 84.1% TAR in the Calwich Abbey Lake sediment extracts and 29 days with a mean of 79.7% TAR in the Swiss Lake sediment extracts. After 99 days, it declined to means of 83.6 and 76.6% TAR in the Calwich Abbey Lake and Swiss Lake systems, respectively. Up to two unknown degradation products were detected, however, never exceeding a mean of 2.1% TAR. The metabolite M555F030cis was detected in a maximum mean concentration of 0.7% TAR after 29 days (single replicate) in system Calwich Abbey Lake only.

In addition to the quantification of the parent, enantiomer-specific analyses were performed on selected samples for Calwich Abbey Lake and Swiss Lake. The enantiomer ratio was about 4:4:1:1 (R,S : S,R : S,S : R,R metconazole) for all analyzed samples. There was no change in enantiomer ratio of metconazole during the 99 day incubation period.

The non-extractable residues slowly increased to mean amounts of 12.9% TAR in system Calwich Abbey Lake and 19.9% TAR in system Swiss Lake.

The non-extractable residues of selected sediment samples were further characterized by humic substance fractionation. Non-extractable radioactivity in the Calwich Abbey Lake and Swiss Lake sediments was mainly associated with the humin fraction. After 99 days, 7.7% and 11.1% TAR was associated with the humin fraction in the Calwich Abbey and Swiss Lake sediments, respectively. Up to 3.7% was associated with the fulvic acid and up to 1.8% with the humic acid fractions. The distribution of radioactivity over the different humic fractions was very similar in both systems.

In both water/sediment systems, evolution of volatile radioactivity was minimal, reaching a maximum mean of 1.2% TAR after 99 days, all associated with $^{14}CO_2$.

The sterilized test vessels showed slightly higher metconazole concentrations in the water phase (mean values of 11.2 - 12.2% TAR) at the end of incubation than the viable vessels (mean values of 2.4% - 4.6% TAR). All radioactivity recovered in the water phases or sediment extracts consisted of unchanged parent. The non-extractable residues in the sterilized test vessels were significantly lower (mean values of 4.0 - 6.6% TAR) than those of the biological active incubations (mean values of 12.9% - 19.9% TAR) indicating that degradation of metconazole in sediment by incorporation into the humic substance matrix is enhanced in the presence of an active microbial population.

Kinetic analysis and calculations of DT_{50} and DT_{90} values was performed following the recommendations of the FOCUS Kinetics workgroup in order to derive persistence and modeling aquatic degradation endpoints. For the parent substance, the analysis at P-I level (one-compartment approach) was performed for degradation in the whole system as well as for the respective dissipations from the water and sediment phases of the test systems. At the P-II level (two compartments approach), the kinetic analysis considered the degradation in water and sediment taking into account the partitioning between the two phases. However, the P-II evaluation could not be accepted according to FOCUS guidance because of the poor visual fit for the water phase and failure of the degradation parameter k in both compartments regarding the t-test. An overview of the estimated kinetic endpoints for metconazole is given below.

Table 7.2.2.3-1: Trigger and modeling endpoints for metconazole (P-I)

	Test system	Trigger endpoints				Modeling endpoints
		Model	χ^2 error	DT_{50} [d]	DT_{90} [d]	DT_{50} [d]
Whole system [a]	Calwich Abbey Lake	SFO	2.1	660.4	>1000	660.4
	Swiss Lake	SFO	1.6	306.7	>1000	306.7
Water [b]	Calwich Abbey Lake	DFOP	1.0	2.9	55.6	16.7 [c]
	Swiss Lake	HS	6.6	3.9	52.1	15.7 [c]
Sediment [b]	Calwich Abbey Lake	No reliable endpoints could be derived.				
	Swiss Lake					

[a] degradation rate
[b] dissipation rate
[c] back-calculated from $DisT_{90}$ ($DisT_{50}$ = $DisT_{90}$ / 3.32) as required by FOCUS kinetics

Overall, the results of this study showed that metconazole dissipates at a fast rate from the water phase and then degrades at a moderate to slow rate in the sediment when incubated in water/sediment systems under dark conditions.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test material

Internal code:	BAS 555 F
Reg. No.:	4056343
Common name:	Metconazole
CAS No.:	125116-23-6
Chemical name (IUPAC):	(1RS,5RS;1RS,5SR)-5-(4-chlorobenzyl)-2,2-dimethyl-1-(1*H*-1,2,4-triazol-1-ylmethyl)cyclopentanol
Molecular mass:	319.8 g mol^{-1}
Molecular formula:	$C_{17}H_{22}ClN_3O$

Phenyl-U-^{14}C-label (in the following referred to as "phenyl"-label)

Batch No.:	1065-1029
Cis/trans isomer ratio:	81.4 : 18.6
Specific radioactivity of a.s.:	9.26 MBq mg^{-1}
Radiochemical purity:	99.4%, see certificate of analysis (measurement prior to application: 98.4%)
Purity:	92.7%

Unlabeled

Batch No.:	AC12140-17
Cis/trans isomer ratio:	85 : 15
Purity:	98.1%

2. Test system

Two natural water/sediment systems were collected on September 02, 2013 (Swiss Lake) and on September 03, 2013 (Calwich Abbey Lake). Both sampling sites are located in the UK.

Prior to use the sediment was passed through a 2 mm sieve and the water was passed through a 0.2 mm sieve at the sampling site. Sediment and water were transported to the laboratory and stored at 4°C for less than three weeks prior to initiation of the equilibration period. The physico-chemical properties of the systems are summarized in Table 7.2.2.3-2.

Table 7.2.2.3-2: Characterization of the water/sediment system

Designation **Origin**		**Calwich Abbey Lake** **UK**			**Swiss Lake** **UK**		
Water							
pH		8.51			7.06		
Hardness	[mg $CaCO_3$ L^{-1}]	278			24.7		
Dissolved organic C	[mg L^{-1}]	52.0			11.4		
Total N	[mg L^{-1}]	3.3			0.6		
Total P	[mg L^{-1}]	<0.1			<0.1		
		Beginning	End (solvent)	End (no solvent)	Beginning	End (solvent)	End (no solvent)
Aerobic bacteria	[cfu g^{-1}]	5.00×10^4	1.31×10^3	2.80×10^2	90	1.54×10^3	6.75×10^2
Aerobic bacterial spores	[cfu mL^{-1}]	<10	35	35	<10	10	15
Fungi	[cfu mL^{-1}]	<10	25	<10	<10	<10	<10
Actinomycetes	[cfu mL^{-1}]	<10	<10	<10	<10	<10	<10
Sediment							
Textural class		UK	USDA		UK	USDA	
Sand	[%]	32	32		94	92	
Silt	[%]	54	50		4	2	
Clay	[%]	14	18		2	6	
Soil type		Sandy silt loam	Loam/silt loam		Sand	Sand	
pH (H_2O) pH ($CaCl_2$)		7.6 7.2			6.0 5.2		
CEC	[$cmol^+$ kg^{-1}]	14.7			2.5		
Organic C	[%]	5.1			0.8		
Total N	[%]	0.42			0.06		
Total P	[mg kg^{-1}]	872			150		
		Beginning	End (solvent)	End (no solvent)	Beginning	End (solvent)	End (no solvent)
Aerobic bacteria	[cfu g^{-1}]	7.70×10^5	3.80×10^5	3.40×10^5	1.30×10^5	5.35×10^5	3.35×10^5
Aerobic bacterial spores	[cfu g^{-1}]	2.90×10^5	3.75×10^4	5.05×10^4	6.40×10^3	5.05×10^3	3.20×10^4
Anaerobic bacteria	[cfu g^{-1}]	3.50×10^5	4.80×10^4	6.95×10^4	9.60×10^3	1.19×10^4	9.7×10^4
Anaerobic bacterial spores	[cfu g^{-1}]	4.30×10^4	4.20×10^4	3.95×10^4	3.95×10^3	5.15×10^3	4.35×10^4
Fungi	[cfu g^{-1}]	2.15×10^3	4.10×10^3	3.40×10^2	7.15×10^2	2.25×10^2	2.40×10^2
Actinomycetes	[cfu g^{-1}]	1.60×10^5	3.10×10^2	5.35×10^3	5.20×10^2	2.30×10^2	85

CEC = cation exchange capacity

B. STUDY DESIGN

1. Experimental conditions

For system Calwich Abbey Lake, 36 vessels were filled with about 220 g of wet sediment (86.5 g dry weight equivalent) and about 525 mL of water. For system Swiss Lake, the vessels were filled with about 275 g of wet sediment (207 g dry weight equivalent) and about 550 mL of water. This corresponded to a sediment layer of about 2.5 cm and a water depth of about 7.5 cm for both systems. After being filled with sediment and water, the vessels were allowed to equilibrate for 31 - 35 days before treatment. Equilibration was reached after 31 – 35 days. Nine vessels per system were heat sterilized (115°C, 45 min on two consecutive days) prior to the application of the test item.

Aliquots of the application solution were applied to the surface of the water phase of the water/sediment system samples. The actual application rate was 0.095 mg L^{-1} in water based on the volume of water in the vessel including water contained within the sediment layer. This rather high application rate was chosen to ensure acceptable limits of detection and to allow reliable quantitative and qualitative analytics of metabolites potentially formed in the systems and is not directly related to the currently recommended field application rate.

Equilibration and subsequent incubation was carried out in a temperature-controlled room. The test vessels were incorporated into individual flow-through systems. A trapping system consisting of flasks of ethyl digol, 1 M KOH (+ phenolphthalein indicator), and 1 M NaOH (+ phenolphthalein indicator) were connected in series to flow-through system. Humidified air was drawn through each system. Sterile filters were incorporated to the heads of sterilized test vessels to maintain the sterility of the water/sediment system. The test systems were incubated at a temperature of 20 ± 2°C in the dark.

2. Sampling

Duplicate samples of each water/sediment system were taken for analysis immediately after application, after 1, 3, 7, 14, 29, 60 and 99 days of incubation (viable samples). Sterile samples were taken after 60 and 99 days of incubation using aseptic techniques.

For sampling, the respective flasks were removed from the growth room. The parameters temperature, O_2 content, pH and redox potential of the water and the redox potential of the sediment were measured. To separate the water and sediment phases, the water was decanted into a bottle. Trapping solutions were taken for analysis when the associated sample was taken for analysis.

3. Description of analytical procedures

Water

The water was decanted and aliquots were measured by liquid scintillation counting (LSC). The water was analyzed directly by HPLC. For chiral analysis, portions of selected water samples were evaporated to dryness, reconstituted in methanol, and analyzed by radio-HPLC. For TLC and LC-MS analysis, portions of selected water samples were evaporated to dryness and reconstituted in methanol : water (1:1, v:v). Samples were measured by radio-TLC or LC-MS.

Sediment

Sediment samples were transferred into centrifuge pots, extracted with acetonitrile by shaking followed by sonication at room temperature, and subsequent centrifugation. The procedure was repeated three times for all samples except at 99 days where the procedure was repeated four times. Aliquots were analyzed by LSC.

Sediment extracts for each individual sample were combined and taken for radioassay. A portion of the combined extract was concentrated by rotary film evaporation. The recovery of radioactivity on concentration was quantitative. The concentrated extracts were measured by radio-HPLC. For TLC and chiral analysis, portions of selected pooled extracts were evaporated to dryness and reconstituted in methanol. Samples were analyzed by radio-TLC and radio-HPLC. For LC-MS analysis, portions of selected pooled extracts were concentrated and afterwards diluted with acetonitrile. Samples were analyzed by LC-MS.

The sediment debris remaining after extraction was air-dried, weighed, and triplicate aliquots taken for combustion and radioassay.

Characterization of bound residues

The non-extractable radioactivity in the samples taken at 99 days was further characterized using an acid/base fractionation procedure for separation into fulvic acids, humic acids and humins.

For each of the selected samples, a sub-sample of the dried sediment debris was extracted with 0.5 M NaOH by shaking. The debris was separated by centrifugation and the supernatant removed. The debris was washed with 0.5 M NaOH and the supernatant combined with these washings.

The supernatant was adjusted to pH 1 with concentrated HCl and left to stand to precipitate the acid-insoluble humic acids. The sample was centrifuged and the supernatant removed. The precipitate was washed with 1 M HCl and the supernatant combined with these washings. This solution, the fulvic acid fraction, was measured by LSC. The precipitate, the humic acid fraction, was dissolved in 0.5 M NaOH and analyzed by LSC.

The remaining non-extractable radioactivity in the sediment debris (humins) was determined by combustion after air drying of the sediments and subsequent LSC measurement.

Volatiles

Subsamples of the trapping solutions were taken and analyzed by LSC.

4. Kinetic modeling

Kinetic evaluation was performed in order to derive degradation parameters as triggers for additional work (trigger endpoints) as well as endpoints for modeling (modeling endpoints). Kinetic analysis and calculation of $DegT_{50}$ and $DegT_{90}$ values was performed following the recommendations of the FOCUS Kinetics workgroup [*FOCUS (2006)*]. The software package KinGUII (version 2.2014.224.1704) was used for parameter fitting.

Kinetic evaluation at Level P-I (one-compartment approach) was performed for degradation in the total system as well as dissipation from the water and sediment phase of the test systems. At Level P-II (two-compartment approach: water and sediment), the kinetic analysis considered the degradation in water and sediment and the partitioning between both phases.

The appropriateness of the distinct kinetic model to describe soil degradation was tested by visual assessment, estimation of the error percentage at which the χ^2 test was passed, and the t-test to evaluate whether estimated degradation parameters differ from zero.

The trigger endpoints were derived from the kinetic models that provided the best-fit to the measured data, generally indicated by the lowest χ^2 – error and a visual assessment of the fit.

II. RESULTS AND DISCUSSION

A. MASS BALANCE

The distribution of radioactivity in the different compartments of the two water/sediment systems treated with ^{14}C-labeled metconazole are presented in Table 7.2.2.3-3 and Table 7.2.2.3-4.

The mass balances of the viable test vessels were in the range 93.6% to 106.5% of the total applied radioactivity (TAR) (mean values of 95.2 - 104.8% TAR). One test vessel exhibited a higher recovery of 114.3% TAR. In the sterile vessels, the material balance ranged from 94.4 – 104.7% TAR (mean values of 98.2 - 103.9% TAR).

Water

Total radioactivity in the water layer declined relatively fast from a mean of 91.7% (Calwich Abbey Lake) and 88.4% TAR (Swiss Lake) at time zero to 6.2% and 5.3% TAR after 99 days.

Sediment

Corresponding to the decline in the water phase, total radioactivity in the sediment phase increased from a mean of 3.8% (Calwich Abbey Lake) and 7.0% TAR (Swiss Lake) at time zero to 97.5% and 98.6% TAR at 99 days. Non-extractable radioactivity in the sediment (bound residues) accounted for 12.9% and 19.9% TAR after 99 days in the respective test system.

The non-extractable residues in the sterilized test vessels were significantly lower (mean values of 4.0 – 6.6% TAR) than those of the biological active incubations (12.9 – 19.9% TAR) indicating that degradation of metconazole in sediment by incorporation into the humic substance matrix is enhanced in the presence of an active microbial population.

Volatiles

Volatile radioactivity recovered from trapping solutions was all $^{14}CO_2$ and accounted for a maximum mean of 1.2% TAR for system Calwich Abbey Lake after 99 days and 0.9% TAR for system Swiss Lake after 60 days.

Table 7.2.2.3-3: Material balance and distribution of radioactivity after application of ^{14}C-metconazole to water/sediment system Calwich Abbey Lake and incubation under dark conditions [% TAR]

DAT	Sample ID	Water	Sediment							Volatiles radioactivity		Mass balance
			Acetonitrile extracts					NER	Total			
			1	2	3	4	Total extr.			Ethyl digol	1 M KOH	
Non-sterile conditions												
0	CA01	91.2	2.6	0.6	0.2	n.p.	3.4	0.2	3.6	n.p.	n.p.	94.8
	CA02	92.1	2.9	0.7	0.2	n.p.	3.8	0.2	4.0	n.p.	n.p.	96.1
	Mean	**91.7**	**2.8**	**0.7**	**0.2**	**n.p.**	**3.6**	**0.2**	**3.8**	**n.p.**	**n.p.**	**95.5**
1	CA03	68.5	20.9	1.3	1.1	n.p.	23.3	1.8	25.1	n.d.	n.d.	93.6
	CA04	76.9	16.5	1.9	1.5	n.p.	19.9	1.2	21.1	n.d.	n.d.	98.0
	Mean	**72.7**	**18.7**	**1.6**	**1.3**	**n.p.**	**21.6**	**1.5**	**23.1**	**n.d.**	**n.d.**	**95.8**
3	CA05	44.4	40.2	11.2	3.4	n.p.	54.8	2.7	57.5	n.d.	n.d.	101.9
	CA06	49.4	36.2	10.1	3.4	n.p.	49.7	2.7	52.4	n.d.	0.1	101.9
	Mean	**46.9**	**38.2**	**10.7**	**3.4**	**n.p.**	**52.3**	**2.7**	**55.0**	**n.d.**	**0.1**	**101.9**
7	CA07	26.7	65.5	13.2	4.7	n.p.	83.4	4.2	87.6	n.d.	0.1	114.3
	CA08	30.9	50.4	11.3	3.7	n.p.	65.4	3.5	68.9	n.d.	0.2	100.0
	Mean	**28.8**	**58.0**	**12.3**	**4.2**	**n.p.**	**74.4**	**3.9**	**78.3**	**n.d.**	**0.2**	**107.2**
14	CA09	20.1	59.0	13.9	4.9	n.p.	77.8	4.5	82.3	n.d.	0.2	102.6
	CA10	22.3	58.0	13.4	4.7	n.p.	76.1	4.6	80.7	n.d.	0.2	103.2
	Mean	**21.2**	**58.5**	**13.7**	**4.8**	**n.p.**	**77.0**	**4.6**	**81.5**	**n.d.**	**0.2**	**102.9**
29	CA11	19.8	57.9	13.5	4.4	n.p.	75.8	6.2	82.0	n.d.	1.1	102.8
	CA12	15.1	61.4	14.5	5.0	n.p.	80.9	6.1	87.0	n.d.	0.5	102.6
	Mean	**17.5**	**59.7**	**14.0**	**4.7**	**n.p.**	**78.4**	**6.2**	**84.5**	**n.d.**	**0.8**	**102.7**
60	CA13	9.8	62.0	16.7	5.9	n.p.	84.6	8.5	93.1	n.d.	0.7	103.6
	CA14	9.0	60.7	17.9	6.3	n.p.	84.9	8.8	93.7	n.d.	0.5	103.2
	Mean	**9.4**	**61.4**	**17.3**	**6.1**	**n.p.**	**84.8**	**8.7**	**93.4**	**n.d.**	**0.6**	**103.4**
99	CA15	6.3	59.5	16.6	5.7	2.4	84.2	13.7	97.9	n.d.	1.4	105.5
	CA16	6.0	58.3	17.8	6.1	2.8	85.0	12.0	97.0	n.d.	1.0	104.0
	Mean	**6.2**	**58.9**	**17.2**	**5.9**	**2.6**	**84.6**	**12.9**	**97.5**	**n.d.**	**1.2**	**104.8**
Sterile conditions												
0	CA21	88.3	13.8	1.3	0.5	n.p.	15.6	0.4	16.0	n.p.	n.p.	104.3
	CA22	92.7	9.1	1.0	0.3	n.p.	10.4	0.3	10.7	n.p.	n.p.	103.4
	Mean	**90.5**	**11.5**	**1.2**	**0.4**	**n.p.**	**13.0**	**0.4**	**13.4**	**n.p.**	**n.p.**	**103.9**
60	CA23	13.6	61.5	14.7	5.4	n.p.	81.6	4.9	86.5	n.d.	n.d.	100.1
	CA24	17.4	62.6	13.1	4.7	n.p.	80.4	2.9	83.3	n.d.	0.1	100.7
	Mean	**15.5**	**62.1**	**13.9**	**5.1**	**n.p.**	**81.0**	**3.9**	**84.9**	**n.d.**	**0.1**	**100.4**
99	CA25	11.5	62.3	14.2	4.8	1.6	82.9	3.3	86.2	n.d.	n.d.	97.7
	CA26	10.8	63.6	15.3	5.7	2.0	86.6	4.7	91.3	n.d.	n.d.	102.1
	Mean	**11.2**	**63.0**	**14.8**	**5.3**	**1.8**	**84.8**	**4.0**	**88.8**	**n.d.**	**n.d.**	**99.9**

TAR = total applied radioactivity
DAT = days after treatment
NER = non-extractable residues
n.p. = not performed
n.d. = not detected

Table 7.2.2.3-4: Material balance and distribution of radioactivity after application of ^{14}C-metconazole to water/sediment system Swiss Lake and incubation under dark conditions [% TAR]

DAT	Sample ID	Water	Sediment							Volatiles radioactivity		Mass balance
			Acetonitrile extracts					NER	Total			
			1	2	3	4	Total extr.			Ethyl digol	1 M KOH	
Non-sterile conditions												
0	SL01	88.5	4.1	1.1	0.3	n.p.	5.5	0.2	5.7	n.p.	n.p.	94.2
	SL02	88.2	5.6	1.6	0.4	n.p.	7.8	0.4	8.2	n.p.	n.p.	96.1
	Mean	**88.4**	**4.9**	**1.4**	**0.4**	**n.p.**	**6.7**	**0.3**	**7.0**	**n.p.**	**n.p.**	**95.2**
1	SL03	75.1	16.9	4.7	4.5	n.p.	26.1	0.6	26.7	n.d.	0.1	101.8
	SL04	75.8	15.8	6.2	4.6	n.p.	26.5	0.4	26.9	n.d.	n.d.	102.7
	Mean	**75.5**	**16.4**	**5.5**	**4.6**	**n.p.**	**26.3**	**0.5**	**26.8**	**n.d.**	**0.1**	**102.3**
3	SL05	54.6	32.8	9.6	2.5	n.p.	44.9	4.1	49.0	n.d.	0.2	103.7
	SL06	58.2	29.7	8.7	2.3	n.p.	40.6	2.1	42.7	n.d.	0.1	101.0
	Mean	**56.4**	**31.3**	**9.2**	**2.4**	**n.p.**	**42.8**	**3.1**	**45.9**	**n.d.**	**0.2**	**102.4**
7	SL07	33.1	49.5	13.1	3.5	n.p.	66.1	2.4	68.5	n.d.	0.2	101.8
	SL08	35.3	49.7	13.3	4.0	n.p.	67.0	4.1	71.1	n.d.	0.2	106.5
	Mean	**34.2**	**49.6**	**13.2**	**3.8**	**n.p.**	**66.6**	**3.3**	**69.8**	**n.d.**	**0.2**	**104.2**
14	SL09	42.0	41.1	11.4	3.2	n.p.	55.7	3.3	59.0	n.d.	0.4	101.3
	SL10	27.7	50.1	14.1	3.9	n.p.	68.1	4.1	72.2	n.d.	0.3	100.2
	Mean	**34.9**	**45.6**	**12.8**	**3.6**	**n.p.**	**61.9**	**3.7**	**65.6**	**n.d.**	**0.4**	**100.8**
29	SL11	23.9	52.3	15.4	4.1	n.p.	71.8	5.2	77.0	n.d.	1.0	101.8
	SL12	7.0	64.3	19.3	5.4	n.p.	89.0	7.5	96.5	n.d.	0.6	104.0
	Mean	**15.5**	**58.3**	**17.4**	**4.8**	**n.p.**	**80.4**	**6.4**	**86.8**	**n.d.**	**0.8**	**102.9**
60	SL13	11.4	54.4	18.7	5.8	n.p.	78.9	9.6	88.5	n.d.	0.9	100.8
	SL14	11.3	56.6	17.8	5.6	n.p.	80.0	5.5	85.5	n.d.	0.8	97.6
	Mean	**11.4**	**55.5**	**18.3**	**5.7**	**n.p.**	**79.5**	**7.6**	**87.0**	**n.d.**	**0.9**	**99.2**
99	SL15	5.4	53.2	17.5	6.3	1.9	78.9	20.5	99.4	n.d.	0.9	105.6
	SL16	5.1	51.8	17.6	7.0	2.0	78.5	19.2	97.7	n.d.	0.9	103.7
	Mean	**5.3**	**52.5**	**17.6**	**6.7**	**2.0**	**78.7**	**19.9**	**98.6**	**n.d.**	**0.9**	**104.7**
Sterile conditions												
0	SL21	89.7	6.2	1.4	0.4	n.p.	8.0	0.3	8.3	n.p.	n.p.	98.0
	SL22	88.9	7.1	1.6	0.4	n.p.	9.1	0.3	9.4	n.p.	n.p.	98.3
	Mean	**89.3**	**6.7**	**1.5**	**0.4**	**n.p.**	**8.6**	**0.3**	**8.9**	**n.p.**	**n.p.**	**98.2**
60	SL23	15.2	53.2	16.1	4.8	n.p.	74.1	5.0	79.1	n.d.	0.1	94.4
	SL24	17.2	58.3	16.1	4.9	n.p.	79.3	7.2	86.5	n.d.	n.d.	103.7
	Mean	**16.2**	**55.8**	**16.1**	**4.9**	**n.p.**	**76.7**	**6.1**	**82.8**	**n.d.**	**0.1**	**99.1**
99	SL25	13.6	52.5	16.2	5.0	1.4	75.1	5.8	80.9	n.d.	n.d.	94.5
	SL26	10.7	61.0	19.0	5.4	1.2	86.6	7.4	94.0	n.d.	n.d.	104.7
	Mean	**12.2**	**56.8**	**17.6**	**5.2**	**1.3**	**80.9**	**6.6**	**87.5**	**n.d.**	**n.d.**	**99.6**

TAR = total applied radioactivity
DAT = days after treatment
NER = non-extractable residues
n.p. = not performed
n.d. = not detected

B. TRANSFORMATION OF PARENT COMPOUND

Characterization and identification of residues in water and sediment extracts

An overview of metconazole and metabolites for the water samples and sediment extracts is presented in Table 7.2.2.3-5 to Table 7.2.2.3-8. Results of the enantiomeric investigation are shown in Table 7.2.2.3-9.

A proposed route of degradation of metconazole in water/sediment systems is given in Figure 7.2.2.3-1.

Water

The proportion of metconazole declined from a mean of 91.7% TAR at time zero to 4.6% at 99 days and from a mean of 88.4% at time zero to 2.4% TAR at 99 days in the Calwich Abbey Lake and Swiss Lake systems respectively.

Up to five minor components were detected. One could be assigned to the known metabolite Reg. No. 4110625 (M555F030cis) by co-chromatography with reference item, reaching a maximum mean concentration of 1.0% TAR. Four degradates were not assigned to known metabolites Most of them occurred only sporadically. None of the unknown metabolites exceeded a mean of 1.9% TAR at any sampling time.

The sterilized test vessels showed slightly higher metconazole concentrations in the water phase (mean values of 11.2 - 12.2% TAR) at the end of incubation than the viable vessels (2.4% - 4.6% TAR). All radioactivity recovered in the water phases consisted of unchanged parent.

Table 7.2.2.3-5: Metabolite overview for water after application of ^{14}C-metconazole to system Calwich Abbey Lake and incubation under dark conditions [% TAR]

DAT	Sample identity	metconazole			M555F030cis (Reg. No. 4110625) (Rt 37 min)	unknown 1 (Rt 24 min)	unknown 2 (Rt 27 min)	unknown 3 (Rt 30 min)	unknown 4 (Rt 33 min)
		trans- (Rt 40 min)	cis- (Rt 41 min)	Total					
Non-sterile conditions									
0	CA01	15.6	75.6	91.2	-	-	-	-	-
0	CA02	11.7	80.4	92.1	-	-	-	-	-
0	**Mean**	**13.7**	**78.0**	**91.7**	**-**	**-**	**-**	**-**	**-**
1	CA03	9.7	58.8	68.5	-	-	-	-	-
1	CA04	13.0	63.9	76.9	-	-	-	-	-
1	**Mean**	**11.4**	**61.4**	**72.7**	**-**	**-**	**-**	**-**	**-**
3	CA05	7.0	37.4	44.4	-	-	-	-	-
3	CA06	8.4	41.0	49.4	-	-	-	-	-
3	**Mean**	**7.7**	**39.2**	**46.9**	**-**	**-**	**-**	**-**	**-**
7	CA07	2.6	24.1	26.7	-	-	-	-	-
7	CA08	5.0	24.7	29.7	1.2	-	-	-	-
7	**Mean**	**3.8**	**24.4**	**28.2**	**0.6**	**-**	**-**	**-**	**-**
14	CA09	1.5	18.6	20.1	-	-	-	-	-
14	CA10	2.2	20.1	22.3	-	-	-	-	-
14	**Mean**	**1.9**	**19.4**	**21.2**	**-**	**-**	**-**	**-**	**-**
29	CA11	1.6	16.2	17.8	1.4	-	-	-	0.5
29	CA12	1.7	13.4	15.1	-	-	-	-	-
29	**Mean**	**1.7**	**14.8**	**16.5**	**0.7**	**-**	**-**	**-**	**0.3**
60	CA13	-	7.9	7.9	0.2	0.3	-	-	1.5
60	CA14	1.0	7.1	8.1	-	-	-	-	0.8
60	**Mean**	**0.5**	**7.5**	**8.0**	**0.1**	**0.2**	**-**	**-**	**1.2**
99	CA15	0.5	4.3	4.8	0.4	0.3	0.3	-	0.5
99	CA16	-	4.4	4.4	0.5	0.2	0.2	0.2	0.5
99	**Mean**	**0.3**	**4.4**	**4.6**	**0.5**	**0.3**	**0.3**	**0.1**	**0.5**
Sterile conditions									
0	CA21	12.4	75.9	88.3	-	-	-	-	-
0	CA22	15.9	76.8	92.7	-	-	-	-	-
0	**Mean**	**14.2**	**76.4**	**90.5**	**-**	**-**	**-**	**-**	**-**
60	CA23	1.6	12.0	13.6	-	-	-	-	-
60	CA24	2.3	15.1	17.4	-	-	-	-	-
60	**Mean**	**2.0**	**13.6**	**15.5**	**-**	**-**	**-**	**-**	**-**
99	CA25	1.4	10.1	11.5	-	-	-	-	-
99	CA26	0.7	10.1	10.8	-	-	-	-	-
99	**Mean**	**1.1**	**10.1**	**11.2**	**-**	**-**	**-**	**-**	**-**

DAT = days after treatment
TAR = total applied radioactivity
Rt = retention time [min], approx. value
- = not apparent or below limit of detection

Table 7.2.2.3-6: Metabolite overview for water after application of ^{14}C-metconazole to system Swiss Lake and incubation under dark conditions [% TAR]

DAT	Sample identity	metconazole			M555F030cis (Reg. No. 4110625) (Rt 37 min)	unknown 1 (Rt 24 min)	unknown 2 (Rt 27 min)	unknown 3 (Rt 30 min)	unknown 4 (Rt 33 min)
		trans- (Rt 40 min)	cis- (Rt 41 min)	Total					
Non-sterile conditions									
0	SL01	13.9	74.6	88.5	-	-	-	-	-
0	SL02	16.2	72.0	88.2	-	-	-	-	-
0	**Mean**	**15.1**	**73.3**	**88.4**	**-**	**-**	**-**	**-**	**-**
1	SL03	11.6	63.5	75.1	-	-	-	-	-
1	SL04	12.6	63.2	75.8	-	-	-	-	-
1	**Mean**	**12.1**	**63.4**	**75.5**	**-**	**-**	**-**	**-**	**-**
3	SL05	7.3	47.3	54.6	-	-	-	-	-
3	SL06	7.5	50.8	58.3	-	-	-	-	-
3	**Mean**	**7.4**	**49.1**	**56.5**	**-**	**-**	**-**	**-**	**-**
7	SL07	3.0	27.3	30.3	1.4	-	-	-	1.4
7	SL08	4.9	29.2	34.1	-	-	-	-	1.2
7	**Mean**	**4.0**	**28.3**	**32.2**	**0.7**	**-**	**-**	**-**	**1.3**
14	SL09	7.2	34.8	42.0	-	-	-	-	-
14	SL10	3.8	22.4	26.2	1.4	-	-	-	-
14	**Mean**	**5.5**	**28.6**	**34.1**	**0.7**	**-**	**-**	**-**	**-**
29	SL11	3.1	20.8	23.9	-	-	-	-	-
29	SL12	1.0	4.6	5.6	0.5	-	-	-	0.9
29	**Mean**	**2.1**	**12.7**	**14.8**	**0.3**	**-**	**-**	**-**	**0.5**
60	SL13	-	8.0	8.0	0.9	-	-	0.4	2.1
60	SL14	1.3	7.4	8.7	1.0	-	-	-	1.7
60	**Mean**	**0.7**	**7.7**	**8.4**	**1.0**	**-**	**-**	**0.2**	**1.9**
99	SL15	-	2.4	2.4	0.6	0.9	-	-	1.5
99	SL16	-	2.3	2.3	0.7	0.2	-	0.3	1.5
99	**Mean**	**-**	**2.4**	**2.4**	**0.7**	**0.6**	**-**	**0.2**	**1.5**
Sterile conditions									
0	SL21	13.6	76.1	89.7	-	-	-	-	-
0	SL22	13.6	75.3	88.9	-	-	-	-	-
0	**Mean**	**13.6**	**75.7**	**89.3**	**-**	**-**	**-**	**-**	**-**
60	SL23	2.0	11.4	13.4	-	-	-	-	-
60	SL24	2.2	14.9	17.1	-	-	-	-	-
60	**Mean**	**2.1**	**13.2**	**15.3**	**-**	**-**	**-**	**-**	**-**
99	SL25	1.6	12.0	13.6	-	-	-	-	-
99	SL26	1.0	9.7	10.7	-	-	-	-	-
99	**Mean**	**1.3**	**10.9**	**12.2**	**-**	**-**	**-**	**-**	**-**

DAT = days after treatment
TAR = total applied radioactivity
Rt = retention time [min], approx. value
- = not apparent or below limit of detection

Sediment

The proportion of metconazole extracted from the sediment phase reached its highest amount after 60 days with a mean of 84.1% TAR in the Calwich Abbey Lake sediment extracts and 29 days with a mean of 79.7% TAR in the Swiss Lake sediment extracts. After 99 days, it declined to a mean of 83.6% TAR and 76.6% TAR in the Calwich Abbey Lake and Swiss Lake systems, respectively.

Up to three minor components were detected. The known metabolite Reg. No. 4110625 (M555F030cis) was detected in a maximum mean concentration of 0.7% TAR after 29 days in system Calwich Abbey Lake only. Up to two unknown degradation products were detected which were not assigned to known metabolites by co-chromatography with reference items. They occurred only sporadically and never exceeded a mean of 2.1% TAR.

Results from the sterilized assays were similar to the viable assays. All radioactivity recovered in the sediment extracts consisted of unchanged parent.

Table 7.2.2.3-7: Metabolite overview for sediment after application of ^{14}C-metconazole to system Swiss Lake and incubation under dark conditions [% TAR]

DAT	Sample identity	metconazole			M555F030cis (Reg. No. 4110625) (Rt 37 min)	unknown 1 (Rt 24 min)	unknown 2 (Rt 27 min)	unknown 3 (Rt 30 min)	unknown 4 (Rt 33 min)
		trans- (Rt 40 min)	cis- (Rt 41 min)	Total					
Non-sterile conditions									
0	CA01	-	3.4	3.4	-	-	-	-	-
0	CA02	0.6	3.3	3.9	-	-	-	-	-
0	**Mean**	**0.3**	**3.4**	**3.7**	**-**	**-**	**-**	**-**	**-**
1	CA03	4.5	18.8	23.3	-	-	-	-	-
1	CA04	2.9	16.9	19.8	-	-	-	-	-
1	**Mean**	**3.7**	**17.9**	**21.6**	**-**	**-**	**-**	**-**	**-**
3	CA05	10.1	44.7	54.8	-	-	-	-	-
3	CA06	9.9	39.9	49.8	-	-	-	-	-
3	**Mean**	**10.0**	**42.3**	**52.3**	**-**	**-**	**-**	**-**	**-**
7	CA07	12.3	70.1	82.4	-	-	-	-	1.0
7	CA08	11.9	53.5	65.4	-	-	-	-	-
7	**Mean**	**12.1**	**61.8**	**73.9**	-	**-**	**-**	**-**	**0.5**
14	CA09	12.7	65.1	77.8	-	-	-	-	-
14	CA10	13.5	62.6	76.1	-	-	-	-	-
14	**Mean**	**13.1**	**63.9**	**77.0**	-	**-**	**-**	**-**	**-**
29	CA11	12.9	60.5	73.4	0.8	-	-	-	1.6
29	CA12	13.6	66.7	80.3	0.6	-	-	-	-
29	**Mean**	**13.3**	**63.6**	**76.9**	**0.7**	**-**	**-**	**-**	**0.8**
60	CA13	15.0	68.8	83.8	0.3	-	-	-	0.5
60	CA14	14.6	69.8	84.4	-	-	-	-	0.4
60	**Mean**	**14.8**	**69.3**	**84.1**	**0.2**	-	**-**	**-**	**0.5**
99	CA15	14.1	68.8	82.9	0.5	-	-	-	0.7
99	CA16	14.5	69.7	84.2	-	-	-	-	0.8
99	**Mean**	**14.3**	**69.3**	**83.6**	**0.3**	-	-	-	**0.8**
Sterile conditions									
0	CA21	2.2	12.9	15.1	-	-	-	-	-
0	CA22	1.3	8.8	10.1	-	-	-	-	-
0	**Mean**	**1.8**	**10.9**	**12.6**	**-**	**-**	**-**	**-**	**-**
60	CA23	14.0	67.6	81.6	-	-	-	-	-
60	CA24	13.7	66.6	80.3	-	-	-	-	-
60	**Mean**	**13.9**	**67.1**	**81.0**	**-**	**-**	**-**	**-**	**-**
99	CA25	13.7	68.9	82.6	-	-	-	-	-
99	CA26	16.1	70.1	86.2	-	-	-	-	-
99	**Mean**	**14.9**	**69.5**	**84.4**	**-**	**-**	**-**	**-**	**-**

DAT = days after treatment
TAR = total applied radioactivity
Rt = retention time [min], approx. value
- = not apparent or below limit of detection

Table 7.2.2.3-8: Metabolite overview for sediment after application of ^{14}C-metconazole to system Swiss Lake and incubation under dark conditions [% TAR]

DAT	Sample identity	metconazole			M555F030cis (Reg. No. 4110625) (Rt 37 min)	unknown 1 (Rt 24 min)	unknown 2 (Rt 27 min)	unknown 3 (Rt 30 min)	unknown 4 (Rt 33 min)
		trans- (Rt 40 min)	cis- (Rt 41 min)	Total					
Non-sterile conditions									
0	SL01	1.0	4.2	5.2	-	-	-	-	-
0	SL02	1.2	5.9	7.1	-	-	-	-	-
0	**Mean**	**1.1**	**5.1**	**6.2**	**-**	**-**	**-**	**-**	**-**
1	SL03	4.5	21.6	26.1	-	-	-	-	-
1	SL04	4.2	22.4	26.6	-	-	-	-	-
1	**Mean**	**4.4**	**22.0**	**26.4**	**-**	**-**	**-**	**-**	**-**
3	SL05	6.6	38.3	44.9	-	-	-	-	-
3	SL06	7.8	32.9	40.7	-	-	-	-	-
3	**Mean**	**7.2**	**35.6**	**42.8**	**-**	**-**	**-**	**-**	**-**
7	SL07	12.2	53.9	66.1	-	-	-	-	-
7	SL08	11.2	55.7	66.9	-	-	-	-	-
7	**Mean**	**11.7**	**54.8**	**66.5**	-	-	-	-	-
14	SL09	10.4	45.2	55.6	-	-	-	-	-
14	SL10	11.0	57.2	68.2	-	-	-	-	-
14	**Mean**	**10.7**	**51.2**	**61.9**	**-**	**-**	**-**	**-**	**-**
29	SL11	12.9	57.6	70.5	-	-	1.3	-	-
29	SL12	14.9	74.0	88.9	-	-	-	-	-
29	**Mean**	**13.9**	**65.8**	**79.7**	-	-	**0.7**	-	-
60	SL13	12.6	65.7	78.3	-	-	-	-	0.6
60	SL14	13.4	66.0	79.4	-	-	-	-	0.6
60	**Mean**	**13.0**	**65.9**	**78.9**	**-**	**-**	**-**	**-**	**0.6**
99	SL15	12.4	65.4	77.8	-	-	-	-	1.0
99	SL16	12.1	63.2	75.3	-	-	-	-	3.1
99	**Mean**	**12.3**	**64.3**	**76.6**	-	-	-	-	**2.1**
Sterile conditions									
0	SL21	1.0	6.6	7.6	-	-	-	-	-
0	SL22	1.6	7.1	8.7	-	-	-	-	-
0	**Mean**	**1.3**	**6.9**	**8.2**	**-**	**-**	**-**	**-**	**-**
60	SL23	13.2	60.9	74.1	-	-	-	-	-
60	SL24	13.1	66.2	79.3	-	-	-	-	-
60	**Mean**	**13.2**	**63.6**	**76.7**	**-**	**-**	**-**	**-**	**-**
99	SL25	13.1	61.7	74.8	-	-	-	-	-
99	SL26	14.1	72.3	86.4	-	-	-	-	-
99	**Mean**	**13.6**	**67.0**	**80.6**	**-**	**-**	**-**	**-**	**-**

DAT = days after treatment
TAR = total applied radioactivity
Rt = retention time [min], approx. value
- = not apparent or below limit of detection

In addition to the quantification of the parent, enantiomer-specific analyses were performed on the water and sediment extracts of day 0 and day 99 samples for both systems. No shift in the enantiomeric ratio of metconazole in water or in sediment extracts was detected. The enantiomer ratio was about 4:4:1:1 (R,S: S,R: S,S: R,R metconazole) for all analyzed samples.

Table 7.2.2.3-9: Enantiomer ratio in water and sediment extracts after application of ^{14}C-metconazole to water/sediment systems Calwich Abbey Lake and Swiss Lake [% TAR]

Water/ Sediment System	Sample Type	DAT	R,S metconazole	S,R metconazole	S,S metconazole	R,R metconazole
Calwich Abbey	Water	0	41	41	9	9
	Sediment Extract	99	41	41	9	9
Swiss Lake	Water	0	40	41	10	9
	Sediment Extract	99	44	40	9	8

DAT = days after treatment

C. CHARACTERIZATION OF NON-EXTRACTABLE RESIDUES (NER)

The overall distribution of radioactivity was rather similar for both systems (see Table 7.2.2.3-10). Most of the radioactivity was associated with the humin fraction (7.7 – 11.1% TAR after 99 days), whereas the least radioactivity was found in the fulvic acid fraction (1.5%, Calwich Abbey) or humic acid fraction (1.8% TAR, Swiss Lake).

Table 7.2.2.3-10: Distribution of radioactivity between fulvic acids, humic acids, and humins after application of ^{14}C-metconazole to water/sediment systems [% TAR]

Test system	DAT	NER	Fulvic acids	Humic acids	Humins
Calwich Abbey	99	13.7	1.5	1.8	7.7
Swiss Lake	99	20.5	3.7	1.8	11.1

DAT = days after treatment
NER = non-extractable radioactivity
TAR = total applied radioactivity

D. KINETIC MODELING RESULTS

The evaluation of the two test systems at Level P-I showed that degradation of metconazole in the total system was best described by SFO kinetics while the dissipation in the water phase was best described with biphasic models. For the sediment phase, no reliable dissipation endpoints could be calculated. The evaluation at Level P-II did not result in reliable degradation endpoints.

Table 7.2.2.3-11: Trigger and modeling endpoints for metconazole (P-I)

	Test system	Trigger endpoints				Modeling endpoints
		Model	χ^2 error	DT_{50} [d]	DT_{90} [d]	DT_{50} [d]
Whole system [a]	Calwich Abbey Lake	SFO	2.1	660.4	>1000	660.4
	Swiss Lake	SFO	1.6	306.7	>1000	306.7
Water [b]	Calwich Abbey Lake	DFOP	1.0	2.9	55.6	16.7 [c]
	Swiss Lake	HS	6.6	3.9	52.1	15.7 [c]
Sediment [b]	Calwich Abbey Lake	No reliable endpoints could be derived.				
	Swiss Lake					

[a] degradation rate
[b] dissipation rate
[c] back-calculated from $DisT_{90}$ ($DisT_{50}$ = $DisT_{90}$ / 3.32) as required by FOCUS kinetics

III. CONCLUSION

Overall, the results of this study showed that metconazole dissipates at a fast rate from the water phase and then degrades at a rather slow rate in the sediment when incubated in water/sediment systems under dark conditions. The major route of degradation was the incorporation into the organic sediment matrix.

Up to five metabolites were visible in the chromatograms. Four unidentified degradates were formed only sporadically in low amounts never exceeding a mean of 1.9% TAR in water and 2.1% TAR in sediment (for a single metabolite). The known metabolite Reg. No. 4110625 (M555F030cis) was identified at up to a mean of 1.0% TAR in water phase and 0.7% in sediment extracts.

The DT_{50} values for the parent compound in the water phase were 2.9 – 3.9 days. For the whole system DT_{50} values of 307 to 660 days were calculated.

Results of chiral analysis showed that the enantiomer ratio was about 4:4:1:1 (R,S: S,R: S,S: R,R metconazole) for all analysed samples. No shift in the enantiomeric ratio of metconazole in water or in sediment extracts was detected.

Report:	CA 7.2.2.3/2 Pape L., 2015a Kinetic evaluation of degradation of BAS 555 F - Metconazole in a water/sediment systems: Determination of modeling endpoints according to FOCUS 2014/1010791
Guidelines:	FOCUS Kinetics (2006) SANCO/10058/2005 version 1.1 of Dec. 2014
GLP:	no

Executive Summary

The aim of the study was to evaluate the dissipation and degradation kinetics of metconazole in two aerobic water/sediment systems [*BASF DocID 1996/7000413 (MK-630-002)*] treated with triazole-^{14}C-labeled test substance and to derive modeling endpoints according to the recommendations of FOCUS kinetics.

The degradation of metconazole was investigated over a period of up to 182 days in two water/sediment systems (Hellersberger Weiher Pond and Glan River). The experimental data were evaluated using single first order (SFO), first-order multi-compartment (FOMC), double first-order in parallel (DFOP) and hockey stick (HS) kinetic models at the evaluation levels P-I and P-II.

The evaluation at Level P-I resulted in reliable modeling half-lives for all compartments of both systems. The modeling $DegT_{50}$ for the whole system ranged from 138.0 to 777.5 days. The modeling $DisT_{50}$ ranged from 1.4 to 28.5 days in the water compartment and from 213.0 to 369.4 days in the sediment compartment. The kinetic evaluation at Level P-II revealed no reliable fit for any of the evaluated water/sediment systems.

I. MATERIAL AND METHODS

The kinetic evaluation of metconazole was conducted for an aerobic water/sediment study in the dark with two natural aerobic aquatic systems [*old EU Dossier, M II A 7.2.1.3.2 / 1, - BASF DocID 1996/7000413 (MK-630-002)*]. The water-sediment systems were taken from a pond and a small river ("Hellersberger Weiher" and "Glan", district of Bad Kreuznach, Rhineland-Palatinate, Germany). Kinetic evaluation was performed in order to derive degradation and dissipation parameters for modeling purposes (modeling endpoints) according to the recommendation of the FOCUS workgroup on degradation kinetics [*FOCUS (2006)*].

The triazole-^{14}C labeled metconazole was applied to the water at a rate of 15 µg a.s. per test vessel which corresponded to a maximum recommended application rate of 90 g as ha^{-1} when related to a 30 cm deep water body. Afterwards, the test systems were incubated in the dark at 20°C for up to 182 days.

Kinetic modeling

The appropriate kinetic model was identified considering the procedures and kinetic models proposed by the FOCUS workgroup on degradation kinetics [*FOCUS (2006)*]. Appropriate $DegT_{50}$ and $DisT_{50}$ values for use in environmental fate models were derived depending on the kinetic model and are reported as *modeling endpoints*.

Kinetic models included in the assessment

The kinetic models employed for this evaluation were described by the FOCUS workgroup on degradation kinetics [*FOCUS (2006)*]. The analysis at P-I level (one-compartment approach) was done for degradation in the whole system as well as the respective dissipations from the water and sediment phases of the test system. At the P-II level (two-compartment approach) the kinetic analysis considered the degradation in water and sediment taking into account the partitioning between the two phases.

The purpose of the study was to derive modeling endpoints, for Level P-I all four kinetic models proposed by FOCUS were used during the evaluation (SFO, FOMC, DFOP and HS) and for Level P-II the kinetic model SFO was used. Details on the models are given in the FOCUS Kinetics guidance [*FOCUS (2006)*].

The appropriateness of a distinct kinetic model to describe degradation was tested with the following checks recommended by FOCUS [*FOCUS (2006)*]:

- Visual assessment of goodness-of-fit
- Estimation of the error percentage at which the χ^2 test is passed
- t-test to evaluate whether estimated degradation parameters differ from zero

A kinetic model is considered appropriate for deriving modeling endpoints in water/sediment systems if the χ^2 - error value was low (ideally below 15%) and the t-test for the degradation parameters was passed at 10% error level.

Data handling

In general, measurements from duplicate samples were considered for the parameter estimation. For the sampling at 182 DAT, four replicates were considered.

At Level P-I and P-II of the analysis, the kinetic evaluation started on the day of treatment (i.e. 0 DAT). The initial concentration of the parent substance in the total system or in the water phase was set to the material balance recovered at 0 DAT as recommended by FOCUS [*FOCUS (2006)*]. As the measurement at 0 DAT for the Glan River system was considered as outlier, the average material balance of 1 DAT to 182 DAT was used for the initial concentration. For the evaluation at Level P-II, the initial concentration in the sediment phase was assumed to be zero.

The assessment of dissipation in sediment at Level P-I only requires kinetics to be fitted to the corresponding decline data, starting from the maximum observed concentration in the compartment. The dissipation of the parent substance was thus evaluated starting at the day of maximum occurrence and all later time points were adjusted accordingly.

Software for kinetic evaluation

The software package KinGUI2 (version 2.2014.224.1704) was used for parameter fitting [*Schäfer, D., Mikolasch, M., Rainbird, P., Harvey, B. (2007) KinGUII: A new kinetic software tool for evaluations according to FOCUS Degradation Kinetics. In: Del Re, A.A.M. et al. (Eds.): Proceedings of the XIII Symposium on Pesticide Chemistry, Piacenza, 2007, p. 916-923. - BASF DocID 2007/1062781; Witt, J., Gao, Z., Meyer, H. (2014) KinGUII, Version 2.2014.224.1704 Bayer CropScience AG*].

Experimental data

The experimental data of metconazole used as model input values for the kinetic evaluations are given in Table 7.2.2.3-12.

Table 7.2.2.3-12: Experimental data of metconazole in Hellersberger Weiher Pond and Glan River system used for kinetic evaluation

	Metconazole residues in Hellersberger Weiher Pond system [% TAR]			Metconazole residues in Glan River system [% TAR]		
DAT [d]	Total system	Water	Sediment	Total system	Water	Sediment
0	95.3 [a]	95.3 [a]	0.0 [b]			
0	96.8 [a]	96.8 [a]	0.0 [b]	93.9 [c]	93.9 [c]	0.0 [b]
1	82.7	42.0	40.7	91.2	69.2	22.0
1	75.6	31.0	44.6	90.6	70.5	20.1
2	80.9	2.8	78.0	77.4	57.9	19.5
2	81.7	24.1	57.6	88.0	66.9	21.2
7	78.1	6.3	71.7	81.4	45.1	36.3
7	85.3	10.1	75.2	81.4	44.6	36.8
14	58.4	2.0	56.4	82.0	32.1	49.8
14	68.5	1.4	67.1	85.1	36.1	49.0
30	63.1	0.6	62.5	77.0	22.1	**54.9**
30	67.6	0.6	67.0	66.3	19.3	**47.0**
62	75.9	2.0	74.0	64.8	15.6	49.3
62	70.9	0.9	70.0	64.9	15.4	49.5
100	80.4	1.3	**79.1**	48.5	8.0	40.5
100	78.6	0.9	**77.7**	57.5	10.4	47.0
152	66.8	1.6	67.2	39.4	5.3	34.1
152	69.7	1.5	68.3	33.5	4.9	28.5
182	66.3	NA	66.3	37.9	3.3	34.7
182	66.5	NA	66.5	42.7	6.4	36.3
182	74.6	1.8	72.7	28.0	4.9	23.2
182	63.9	NA	63.9	41.0	3.2	37.9

DAT Days after treatment
Bold numbers: peak concentration considered for sediment phase evaluation; previous values were omitted; sampling dates were re-calculated for kinetic evaluation to start at zero.
[a] Set to material balance at 0 DAT
[b] Set to zero
[c] Set to average material balance of 1 DAT to 182 DAT as original value at 0 DAT was considered as outlier in study report

II. RESULTS AND DISCUSSION

The datasets for each water/sediment system were analyzed considering the procedures and kinetic models proposed by the FOCUS workgroup on degradation kinetics [*FOCUS (2006)*]. The complete fitting procedure is given in the original study report.

Level P-I

The evaluation of both test systems at Level P-I resulted in reliable endpoints for metconazole for degradation in the total system as well as for dissipation in the water and sediment phase.

An overview of the estimated modeling endpoints for metconazole in both water/sediment systems is given in Table 7.2.2.3-13.

Table 7.2.2.3-13: Summary of modeling endpoints for metconazole, Level P-I

Compartment	Test system	Kinetic model	χ^2 error	Modeling $DegT_{50}/DisT_{50}$ [d] [a]
Total system	Hellersberger Weiher Pond	SFO	8.9	777.5
	Glan River	SFO	4.0	138.0
Water	Hellersberger Weiher Pond	HS	5.9	1.4 [b]
	Glan River	DFOP	7.0	28.5 [b]
Sediment	Hellersberger Weiher Pond	SFO	3.3	369.4
	Glan River	SFO	4.5	213.0

[a] $DegT_{50}$: total system; $DisT_{50}$: water or sediment phase.
[b] Calculated as $DisT_{50} = DisT_{90}/3.32$ (less than 10% of initial concentration at end of study).

Level P-II

The kinetic evaluation at Level P-II revealed no reliable fit for any of the evaluated water/sediment systems. Consequently, no modeling endpoints were calculated.

III. CONCLUSION

The dissipation and degradation kinetics of metconazole in two water/sediment systems were evaluated according to the recommendations of the FOCUS workgroup on degradation kinetics [*FOCUS (2006)*]. The visual assessment and goodness-of-fit statistics of the respective models indicate plausible fit. Therefore, the resulting endpoints can be considered reliable.

The experimental data of metconazole in both test systems (Hellersberger Weiher Pond and Glan River) and for triazole-^{14}C-labeled test substance were evaluated at Level P-I and Level P-II.

The evaluation at Level P-I resulted in reliable modeling half-lives for all compartments of both systems. The modeling $DegT_{50}$ for the whole system ranged from 138.0 to 777.5 days. The modeling $DisT_{50}$ ranged from 1.4 to 28.5 days in the water compartment and from 213.0 to 369.4 days in the sediment compartment. The kinetic evaluation at Level P-II revealed no reliable fit for any of the evaluated water/sediment systems.

Summary: Route of degradation of metconazole in water/sediment studies

In the old water/sediment study [*BASF DocID 1996/7000413 (MK-630-002)*] performed with [triazole-^{14}C]-labeled metconazole the following metconazole degradation pathway was proposed: Metconazole was slowly degraded by oxidation at different positions of the molecule. The methylene group in the chlorobenzyl moiety was a preferred position for oxidation. At first, the isomeric hydroxyl metabolites M555F021 and M555F011 were formed and then converted into the keto metabolite M555F30. Further, oxidation took place at the methyl group in position 2 of the cyclopentyl ring and resulted in the known acid metabolite M555F013. The m-position of the chlorobenzyl ring was also oxidized, forming metabolite M555F015.

In the new water/sediment study [*BASF DocID 2014/1000921*] only M555F030 was identified at low levels (maximum of 1% TAR). As was shown by co-chromatography with reference substances, the metabolite M555F021 (and thus also its isomer M555F011) was not observed.

Based on these findings, an overall degradation pathway for metconazole in water is proposed as given in Figure 7.2.2.3-1.

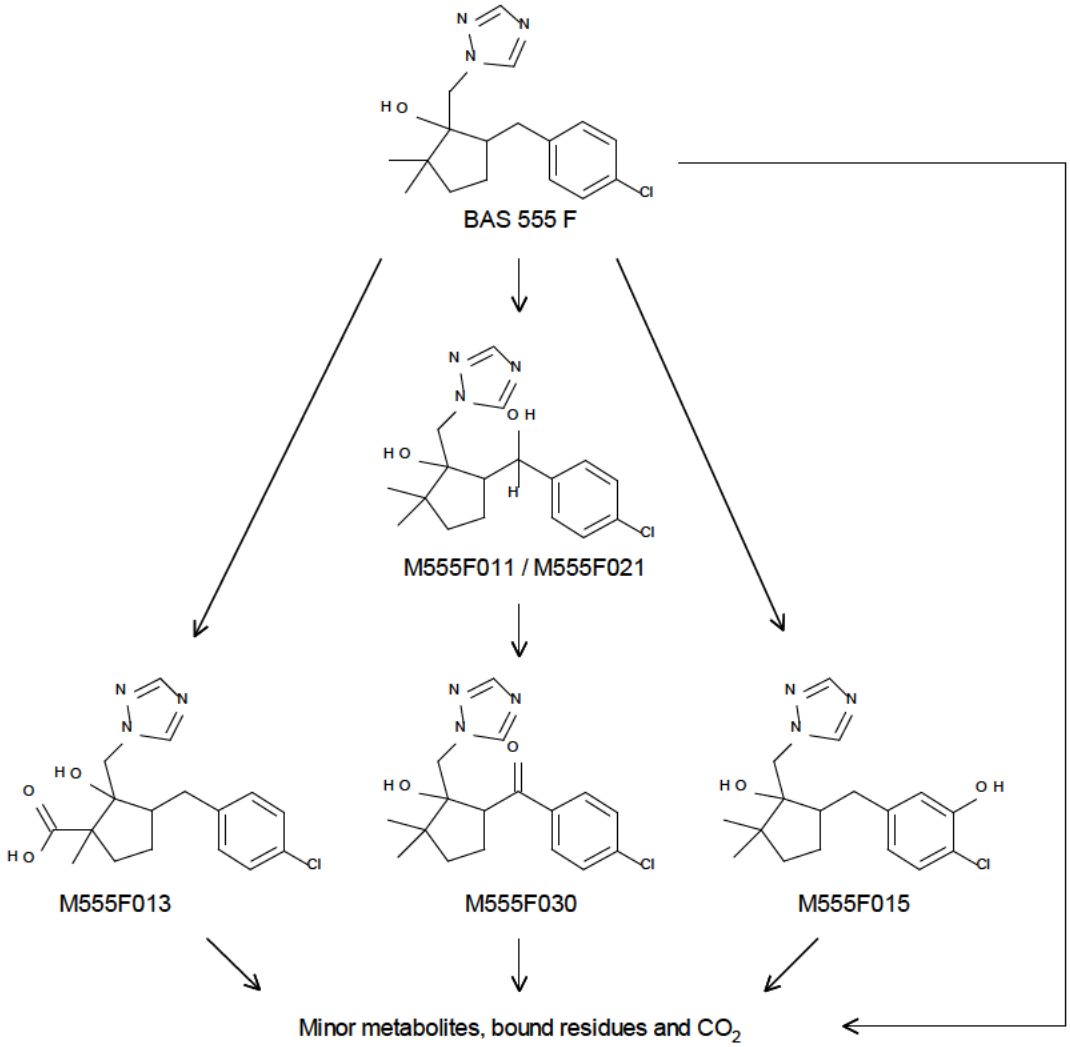


Figure 7.2.2.3-1: Proposed degradation pathway of metconazole in water/sediment systems

Summary of degradation and dissipation endpoints for metconazole in various water/sediment systems

Table 7.2.2.3-14: Summary table on best-fit endpoints of metconazole obtained in water/sediment studies (Level P-I)

Water/ sediment system	pH water	pH sed ($CaCl_2$)	T [°C]	Best-fit $DegT_{50}/DegT_{90}$ whole system [a] [d]	Kinetic model	Best-fit $DisT_{50}/DT_{90}$ water [b] [d]	Kinetic model	Best-fit $DisT_{50}/DT_{90}$ sediment [b] [d]	Kinetic model
BASF DocID 2014/1000921									
Calwich Abbey Lake (p)	8.51	7.2	20	660.4 / >1000	SFO	2.9 / 55.6	DFOP	n.c. [1]	-
Swiss Lake (p)	7.06	5.2	20	306.7 / >1000	SFO	3.9 / 52.1	HS	n.c. [1]	-
BASF DocID 1996/7000413 (MK-630-002) and 2002/7004607 (MK-630-006) [c]									
Hellersberger Weiher Pond (t)	7.7	7.4	20	814 / >1000	first-order	1 / 3	first-order	n.c. [1]	-
Glan River (t)	7.8	6.8	20	116 / 384	first-order	13 / 112	biphasic first-order	n.c. [1]	-

(p), (t) - phenyl or triazole-labeled test item used
[a] degradation rate
[b] dissipation rate
[c] based on the already peer-reviewed derived endpoints metconazole has to be considered persistent in sediment. A re-calculation of best-fit endpoints used as trigger values would not result in anything different and was therefore omitted in framework of Annex I renewal.
n.c. not calculated
[1] no reliable endpoints derived in kinetic evaluation

Table 7.2.2.3-15: Summary table on modeling endpoints of metconazole obtained in water/sediment studies (Level P-I)

Water/ sediment system	pH water	pH sed ($CaCl_2$)	T [°C]	Modeling $DegT_{50}$ whole system [a] [d]	Kinetic model	Modeling $DisT_{50}$ water [b] [d]	Kinetic model	Modeling $DisT_{50}$ sediment [b] [d]	Kinetic model
BASF DocID 2014/1000921									
Calwich Abbey Lake (p)	8.51	7.2	20	660.4	SFO	16.7 [c]	DFOP	n.c. [1]	-
Swiss Lake (p)	7.06	5.2	20	306.7	SFO	15.7 [c]	HS	n.c. [1]	-
BASF DocID 1996/7000413 (MK-630-002) and 2014/1010791									
Hellersberger Weiher Pond (t)	7.7	7.4	20	777.5	SFO	1.4 [b]	HS	369.4	SFO
Glan River (t)	7.8	6.8	20	138.0	SFO	28.5 [b]	DFOP	213.0	SFO

(p), (t) - phenyl or triazole-labeled test item used
[a] degradation rate
[b] dissipation rate
[c] calculated as $DisT_{90}$ / 3.32 as required by FOCUS kinetics (less than 10% of initial concentration at end of study)
n.c. not calculated
[1] no reliable endpoints derived in kinetic evaluation

Table 7.2.2.3-16: Maximum occurrence of metconazole metabolites in water/sediment studies[1]

Metabolite	Matrix	BASF DocID	System	Incubation	Parent label	Maximum % AR
M555F013 (M555F013cis, M13, Reg.No. 4543816)	water	1996/7000413 [2] (MK-630-002)	Hellersberger Weiher Pond	dark	triazole	n.d.
			Glan River	dark	triazole	9.0
	sediment	1996/7000413 [2] (MK-630-002)	Hellersberger Weiher Pond	dark	triazole	0.5
			Glan River	dark	triazole	1.9

[1] only metabolites listed which occur in a compartment at > 5% AR.
[2] already peer-reviewed during previous EU evaluation

CA 7.2.2.4 Irradiated water/sediment study

No irradiated water/sediment study was performed.

CA 7.2.3 Degradation in the saturated zone

Due to its low leaching potential, metconazole is not expected to reach deeper soil layers or saturated zones. Therefore, investigations on the degradation in the saturated zone are considered to be not necessary.

CA 7.3 Fate and behaviour in air

CA 7.3.1 Route and rate of degradation in air

No new experimental data on fate and behavior of metconazole in air are provided. The already peer-reviewed data are considered to be still valid and are summarized as follows.

The vapor pressure was determined to be 2.1 x 10^{-8} Pa at 20ºC [*BASF DocID 2000/7000057 (MK-306-002)*].

The Henry's Law Constant for metconazole was calculated to be 2.21 x 10^{-7} Pa m^3/mol [*BASF DocID 2002/7004361 (MK-336-001)*].

The volatilization behavior from soil and plant surface during the first 24 hours after application was experimentally investigated [*BASF DocID 1996/7000414 (MK-640-005)*]. Since >85% of the applied metconazole remained on the substrate after 24 hours, no significant losses of metconazole due to volatilization would be expected during the first 24 hours after application.

Further, the photochemical oxidation rate of metconazole in the atmosphere was calculated to be $t_{1/2}$ = 6.5 hours (hydroxyl radical concentration of 1.5 x 10^6 molecules/cm^3 over a 12 hour day) [*BASF DocID 1996/7000365 (MK-324-002)*].

In conclusion, metconazole has no relevant tendency to enter the air. Further, based on the results of the atmospheric degradation half-life the substance, if reaching the air, will be rapidly degraded by photochemical processes in the troposphere.

CA 7.3.2 Transport via air

Metconazole has a very low volatilization potential and is degraded very fast by photochemical processes. Consequently, there is no risk of long-range transport of metconazole.

CA 7.3.3 Local and global effects

No effects are expected since transport via air is very unlikely (for details see above).

CA 7.4 Definition of the residue

CA 7.4.1 Definition of the residue for risk assessment

According to the results presented in M-CA 7.1 – 7.4 the following compounds have to be considered for the environmental risk assessment:

Soil:
Metconazole (sum cis/trans) and its aerobic soil metabolite M555F020 (1,2,4-triazole).

Groundwater:
Metconazole (sum cis/trans) and its aerobic soil metabolite M555F020 (1,2,4-triazole).

Surface Water:
Metconazole (sum cis/trans) and its metabolites M555F013 cis, M555F020 (1,2,4-triazole)

Sediment:
Metconazole (sum cis/trans)

Air:
Metconazole (sum cis/trans)

CA 7.4.2 Definition of the residue for monitoring

According to the results of the risk assessment the following compounds should be considered for environmental monitoring:

Soil:	Metconazole (sum cis/trans)
Ground Water:	Metconazole (sum cis/trans)
Surface Water:	Metconazole (sum cis/trans)
Sediment:	Metconazole (sum cis/trans)
Air:	Metconazole (sum cis/trans)

CA 7.5 Monitoring data

According to the knowledge of the applicant, there are currently no published environmental monitoring data available for metconazole or its metabolites, which would provide knowledge on the environmental behaviour not covered by this dossier.

During literature search a few publications were found dealing with environmental monitoring of various pesticides in surface water (Poland, USA), groundwater and/or sediment (USA) mentioning also metconazole. Metconazole was found only once <0.01 µg/L in surface water (USA) [*Battaglin et al. (2011), Occurrence of Azoxystrobin, Propiconazole, and Selected Other Fungicides in US Streams (2005-2006), Water, Air, and Soil Pollution, 218(1-4), 307 - 322*] in periods around occurrence of soybean rust , it was not detected in any other publications.

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Metconazole

Document M-CA, Section 8

ECOTOXICOLOGICAL STUDIES ON THE ACTIVE SUBSTANCE

Compiled by:

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Version history[1]

Date	Data points containing amendments or additions and brief description	Document identifier and version number
27Jan/2016	CA 8.1: ECx calculations for chronic toxicity studies: Reference to BASF Document 2016/1002152 was included Typo correction in Table 8.1/1	Document MCA Section 8 Version 2 (BASF DocID 2016/1030847)
	CA 8.2: ECx calculations for chronic toxicity studies: Reference to BASF Document 2016/1002152 was included	
	CA 8.4: ECx calculations for chronic toxicity studies: Reference to BASF Document 2016/1002152 was included	
	Typo correction in CA 8.5 / Table 8.5-1 Old: BASF DocID 2001/1021861 New: BASF DocID 2000/1021861	

[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 How to revise an Assessment Report

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CA 8.ECOTOXICOLOGICAL STUDIES ON THE ACTIVE SUBSTANCE

CA 8.1. Effects on birds and other terrestrial vertebrates

Studies conducted for the use in the risk assessments for birds and mammals

Two new field studies investigating the residue behavior of metconazole were conducted to support evaluation of its potential risks to birds and mammals. According to these studies a kinetic report, calculating DT50 values, was issued. The summaries of these aforementioned studies and documents (overview see Table 8.1-1) are given further down from M-CA 8.1/1 to 8.1/3. The studies have not been peer-reviewed on EU level, which is indicated prior to the respective summary.

Results of ECx calculations for chronic toxicity studies are provided in a separate document for new and old studies*[see KCA 8.1/4 2016/1002152]*

For studies following guidelines/protocols that were designed to derive a NOEC/NOAEL and have significant limitations for deriving an ECx like limited number of dose groups and/or large dose spacing (e.g. reproductive studies on birds and mammals), no ECx values were calculated.

Table 8.1-1: Overview of study reports and documents whose summaries are given further down

Data point	Author(s)	Year	Title Reference (BASF DocID)
CA 8.1/1	Martin T.	2015	Study on the residue behavior of BAS 555 F (Metconazole) on wheat (young plants) after the application of BAS 555 01 F under field conditions in Germany, Netherlands, Italy and Spain, 2013/2014 (2014/1018040)
CA 8.1/2	Moreno S., Galvez O.	2015	Study on the residue behaviour of Metconazole (BAS 555 F) on pea after treatment with BAS 555 01 F under field conditions in North and South Europe, season 2013-2014 (2014/1001973)
CA 8.1/3	Delgado Cartay	2015	Dissipation of BAS 555 F (Metconazole) on young plants (wheat and peas) from field trials conducted in the Northern and Southern Zones of Europe - Calculation of DT50 / DT90 dissipation times (2015/1040887)

The following study on the residue behaviour of metconazole on young wheat plants has not been evaluated previously on EU level.

Report:	CA 8.1/1 Martin T., 2015a Study on the residue behavior of BAS 555 F (Metconazole) on wheat (young plants) after the application of BAS 555 01 F under field conditions in Germany, Netherlands, Italy and Spain, 2013/2014 2014/1018040
Guidelines:	EC 1107/2009 of the European Parliament and of the Council of 21 Oct 2009, EEC 7029/VI/95 rev. 5 (July 22 1997), EEC 7525/VI/95 rev. 9 (March 2011)
GLP:	yes (certified by ENAC, Entidad Nacional de Acreditación, Madrid Spain)

Executive Summary

The objective of the study was to determine the residue behaviour of metconazole (BAS 555 F) on young wheat (Martin 2015, BASF DocID 2014/1018040) plants after one application of BAS 555 01 F. Samplings were carried out directly after and at subsequent time intervals after the application. The selected application rate corresponds to the maximum single rate of the Good Agricultural Practice (critical GAP) of BAS 555 01 F.

I. MATERIAL AND METHODS

A. MATERIALS

Test Material:	BAS 555 01 F
Description:	BAS 555 01 F (90.0 g/L metconazole (nominal))
Lot/Batch #:	BAS 555 01 F: FRE-000947
Purity:	BAS 555 F: 98.1%
CAS#:	BAS 555 F: 125116-23-6
Crop parts(s) or processed commodity:	wheat (young plants without roots; BBCH 11-22)
Sample size:	22.22-127.02g

B. STUDY DESIGN

Study site

During the growing seasons of 2013 and 2014, a total of eight trials were conducted in representative wheat growing areas in Germany, the Netherlands, Spain and Italy.

Test item and application

The trials consisted of a control plot (untreated, plot 1) and one treated plot (plot 2) without replication. No product containing the test item was used on the test plots during the season.
The test item BAS 555 01 F was foliar applied on plot 2 at a nominal rate of 0.09 kg metconazole/ha in a nominal spray volume of 200 L/ha at growth stage (BBCH) 11-13 according to Good Laboratory Practice.

Sampling information

For this study treated specimens were collected as wheat whole plants without roots 1 hour after the last application as well as 1, 2, 3, 4, 5, 7, 10, 12, and 14 days thereafter. Untreated specimens were collected also as whole plants without roots at the day of application as well as 5 and 14 days thereafter. Each specimen was collected in duplicate ("ship" and "retain"), so that reserve specimens were available in case of potential problems during sample shipment, storage or analysis.
Damaged plants were not harvested. Each specimen was collected randomly from a minimum of 12 different places within each plot.
Untreated specimens were obtained prior to treated specimens when sampling times coincided to avoid contamination. Each specimen was placed into an individual plastic bag and was subsequently double bagged with a second plastic bag. Specimen labels were fixed to the inner plastic bags detailing the specimen type, specimen number, trial and study number. The retain specimens were harvested at the same time of the original ones and were destroyed in the facilities of each test site or the Test Facility by agreement of the Study Director and Sponsor after analysis of the original samples.
All specimens were stored at ≤ -18ºC and were sent to Specimen Management in BASF SE Agricultural Center Limburgerhof.

Description of analytical methods

All specimens were analysed for BAS 555 F (metconazole) using BASF method no. L0076/01. The method has a limit of quantitation (LOQ) of 0.01 mg/kg.
For further details on the analytical methods, please consult the consumer safety part (M-CA 6.3).
The results of procedural recovery experiments averaged 88.4% for wheat plants for BAS 555 F at fortification levels between 0.01 and 15 mg/kg.

II RESULTS AND DISCUSSION

Metconazole

The metconazole residues in the wheat specimens taken 0 DALA (1 HALA) ranged from 7.2 - 12.0 mg/kg. They changed to 4.6-10.0 mg/kg in the specimens taken 1 DALA and further to 4.8 – 9.6 mg/kg at 2 DALA. In the specimens taken 3 DALA 3.6 – 9.3 mg/kg were determined. The residue level in the specimens taken 4 DALA was 3.2 – 6.1 mg/kg, whereas in those taken 5 DALA 3.2 - 6.7 mg/kg were found. Afterwards a decline was observed in the specimens taken 7 DALA (2.4 – 4.2 mg/kg), 10 DALA (1.1 - 5.4 mg/kg) and 12 DALA (0.68 – 2.7 mg/kg). At the last sampling (14 DALA) they remained at this level (0.29 – 2.6 mg/kg).

No residues of metconazole above the limit of quantitation were found in any of the analysed untreated specimens.

Table 8.1-2: Summary of residues of metconazole in wheat (whole plant without roots) (Martin 2015, BASF DocID 2014/1018040)

Trial details	Sampling timing	Sampling no.	Date	Crop growth stage (BBCH)	Residues (mg/kg)
					Metconazole
Trial no. L130737 Study site: Germany	1 HALA	1	21.10.2013	11	7.2
	1 DALA	2	22.10.2013	11	8.7
	2 DALA	3	23.10.2013	11-12	7.0
	3 DALA	4	24.10.2013	11-12	6.6
	4 DALA	5	25.10.2013	11-12	5.6
	5 DALA	6	26.10.2013	12-13	4.4
	7 DALA	7	28.10.2013	12-13	2.8
	10 DALA	8	31.10.2013	13	2.2
	12 DALA	9	02.11.2013	13-21	1.6
	14 DALA	10	04.11.2013	13-21	1.3
Trial no. L130738 Study site: Germany	1 HALA	1	06.03.2014	13	7.8
	1 DALA	2	07.03.2014	13	5.3
	2 DALA	3	08.03.2014	13	5.3
	3 DALA	4	09.03.2014	13-14	5.8
	4 DALA	5	10.03.2014	13-21	4.8
	5 DALA	6	11.03.2014	13-21	4.6
	7 DALA	7	13.03.2014	21-22	3.0
	10 DALA	8	16.03.2014	21-22	1.7
	12 DALA	9	18.03.2014	21-22	1.6
	14 DALA	10	20.03.2014	21-22	1.4
Trial no. L130739 Study site: Netherlands	1 HALA	1	10.03.2014	13	9.9
	1 DALA	2	11.03.2014	13	8.3
	2 DALA	3	12.03.2014	13	8.4
	3 DALA	4	13.03.2014	14	8.9
	4 DALA	5	14.03.2014	14	5.2
	5 DALA	6	15.03.2014	14	4.2
	7 DALA	7	17.03.2014	15	2.5
	10 DALA	8	20.03.2014	18	1.5
	12 DALA	9	22.03.2014	21	0.72
	14 DALA	10	24.03.2014	21	0.70

Trial details	Sampling timing	Sampling no.	Date	Crop growth stage (BBCH)	Residues (mg/kg)
					Metconazole
Trial no. L130740 Study site: Netherlands	1 HALA	1	10.03.2014	13	12
	1 DALA	2	11.03.2014	13	9.1
	2 DALA	3	12.03.2014	13	9.0
	3 DALA	4	13.03.2014	14	7.7
	4 DALA	5	14.03.2014	14	5.7
	5 DALA	6	15.03.2014	14	4.2
	7 DALA	7	17.03.2014	15	2.6
	10 DALA	8	20.03.2014	18	1.5
	12 DALA	9	22.03.2014	21	0.93
	14 DALA	10	24.03.2014	21	0.95
Trial no. L130741 Study site: Spain	1 HALA	1	31.10.2013	12	12
	1 DALA	2	01.11.2013	12	9.2
	2 DALA	3	02.11.2013	12	9.6
	3 DALA	4	03.11.2013	12-13	9.3
	4 DALA	5	04.11.2013	13	6.1
	5 DALA	6	05.11.2013	13	4.7
	7 DALA	7	07.11.2013	13	2.4
	10 DALA	8	10.11.2013	13	1.1
	12 DALA	9	12.11.2013	14	0.68
	14 DALA	10	14.11.2013	14	0.29
Trial no. L130742 Study site: Spain	1 HALA	1	18.02.2014	12	8.7
	1 DALA	2	19.02.2014	12	4.6
	2 DALA	3	20.02.2014	12-13	4.8
	3 DALA	4	21.02.2014	13	3.6
	4 DALA	5	22.02.2014	13	4.7
	5 DALA	6	23.02.2014	13	3.2
	7 DALA	7	25.02.2014	13	2.7
	10 DALA	8	28.02.2014	14	1.6
	12 DALA	9	02.03.2014	14	1.0
	14 DALA	10	04.03.2014	14	0.83
Trial no. L130743 Study site: Italy	1 HALA	1	06.12.2013	13	7.5
	1 DALA	2	07.12.2013	13	10
	2 DALA	3	08.12.2013	13	7.3
	3 DALA	4	09.12.2013	13	7.7
	4 DALA	5	10.12.2013	13	5.8
	5 DALA	6	11.12.2013	13	6.7
	7 DALA	7	13.12.2013	13	4.2
	10 DALA	8	16.12.2013	14	5.4
	12 DALA	9	18.12..2013	14	2.7
	14 DALA	10	20.12.2013	14	2.6
Trial no. L130744 Study site: Italy	1 HALA	1	11.11.2013	12-13	8.0
	1 DALA	2	12.11.2013	12-13	5.6
	2 DALA	3	13.11.2013	13	6.1
	3 DALA	4	14.11.2013	13	4.9
	4 DALA	5	15.11.2013	13	3.2
	5 DALA	6	16.11.2013	13	3.4
	7 DALA	7	18.11.2013	13-14	3.1
	10 DALA	8	21.11.2013	13-14	1.5
	12 DALA	9	23.11.2013	14	1.3
	14 DALA	10	25.11.2013	14-15	1.2

HALA: hours after last application; DALA: days after last application

III. CONCLUSION

In wheat plant samples collected directly after the application (BBCH 11-13) of BAS 555 01 F, the residues of BAS 555 F ranged between 7.2 to 12.0 mg/kg. At the last sampling at 14 DALA (BBCH 13-22) residues of BAS 555 F decreased to a range of 0.29 to 2.6 mg/kg.

Residues of BAS 555 F were below 0.01 mg/kg in all control specimens.

The following study on the residue behaviour of metconazole on young pea plants has not been evaluated previously on EU level.

Report:	CA 8.1/2 Moreno S.,Galvez O., 2015a Study on the residue behaviour of Metconazole (BAS 555 F) on pea after treatment with BAS 555 01 F under field conditions in North and South Europe, season 2013-2014 2014/1001973
Guidelines:	EC 1107/2009 of the European Parliament and of the Council of 21 Oct 2009, EEC 7029/VI/95 rev. 5 Appendix B, OECD 509 Crop Field Trial (2009), EEC 7525/VI/95 rev. 9 (March 2011)
GLP:	yes (certified by ENAC, Entidad Nacional de Acreditación, Madrid Spain)

Executive Summary

The objective of the study was to determine the residue behaviour of metconazole (BAS 555 F) on young pea (Gálvez & Moreno 2015, BASF DocID 2014/1001973) plants after one application of BAS 555 01 F. Samplings were carried out directly after and at subsequent time intervals after the application. The selected application rate corresponds to the maximum single rate of the Good Agricultural Practice (critical GAP) of BAS 555 01 F.

I. MATERIAL AND METHODS

A. MATERIALS

Test Material:	BAS 555 01 F
Description:	BAS 555 01 F (90.0 g/L metconazole (nominal))
Lot/Batch #:	BAS 555 01 F: FRE-000947, only trial L130770: FRE-000986
Purity:	BAS 555 F: 98.1%
CAS#:	BAS 555 F: 125116-23-6
Crop parts(s) or processed commodity:	pea (young plants without roots; BBCH 12-30)
Sample size:	50.3 – 193 g

B. STUDY DESIGN

Study site

During the growing seasons of 2013 and 2014, a total of eight trials were conducted in representative wheat growing areas in Germany, the Netherlands, Spain and Italy.

Test item and application

The trials consisted of a control plot (untreated, plot 1) and one treated plot (plot 2) without replication. No product containing the test item was used on the test plots during the season.
The test item BAS 555 01 F was foliar applied on plot 2 at a nominal rate of 0.09 kg metconazole/ha in a nominal spray volume of 200 L/ha at growth stage (BBCH) 12-13 according to Good Laboratory Practice.

Sampling information

For this study treated specimens were collected as pea whole plants without roots 1 hour after the last application as well as 1, 2, 3, 4, 5, 7, 10, 12, and 14-15 days thereafter. Untreated specimens were collected also as whole plants without roots 1 hour before application as well as 5 and 14-15 days thereafter. Each specimen was collected in duplicate ("ship" and "retain"), so that reserve specimens were available in case of potential problems during sample shipment, storage or analysis.
Damaged plants were not harvested. Each specimen was collected randomly from a minimum of 12 different places within each plot.
Untreated specimens were obtained prior to treated specimens when sampling times coincided to avoid contamination. Each specimen was placed into an individual plastic bag and was subsequently double bagged with a second plastic bag. Specimen labels were fixed to the inner plastic bags detailing the specimen type, specimen number, trial and study number. The retain specimens were harvested at the same time of the original ones and were destroyed in the facilities of each test site or the Test Facility by agreement of the Study Director and Sponsor.
All specimens were stored at $\leq$ -18ºC and were sent to Specimen Management in BASF SE Agricultural Center Limburgerhof.

Description of analytical methods

All specimens were analysed for BAS 555 F (metconazole) using BASF method no. L0076/01. The method has a limit of quantitation (LOQ) of 0.01 mg/kg.
For further details on the analytical methods, please consult the consumer safety part (M-CA 6.3).
The results of procedural recovery experiments averaged 91.7% for pea plants for BAS 555 F at fortification levels between 0.01 and 15 mg/kg.

II RESULTS AND DISCUSSION

Metconazole

The metconazole residues in the pea specimens taken 0 DALA (1 HALA) ranged from 5.3 - 11.0 mg/kg. They changed to 5.5-11.0 mg/kg in the specimens taken 1 DALA and further to 3.1 – 9.7 mg/kg at 2 DALA. In the specimens taken 3 DALA 2.3 – 9.0 mg/kg were determined. The residue level in the specimens taken 4 DALA was 2.1 – 7.5 mg/kg, whereas in those taken 5DALA 0.92 - 6.9 mg/kg were found. Afterwards a decline was observed in the specimens taken 7 DALA (0.55 – 5.5 mg/kg), 10 DALA (0.23 – 3.2 mg/kg), 12 DALA (0.22 – 3.3 mg/kg) and the last sampling 14 - 15 DALA (0.21 – 1.9 mg/kg).

No residues of metconazole above the limit of quantitation were found in any of the analysed untreated specimens.

Table 8.1-3: Summary of residues of metconazole in pea (whole plant without roots) (Gálvez & Moreno 2015, BASF DocID 2014/1001973)

Trial details	Sampling timing	Sampling no.	Date	Crop growth stage (BBCH)	Residues (mg/kg)
					Metconazole
Trial no. L130765 Study site: Germany	1 HALA	1	25.04.2013	13	11
	1 DALA	2	26.04.2013	13	8.2
	2 DALA	3	27.04.2013	14	6.8
	3 DALA	4	28.04.2013	14	6.5
	4 DALA	5	29.04.2013	15	5.2
	5 DALA	6	30.04.2013	15	4.0
	7 DALA	7	02.05.2013	16	3.0
	10 DALA	8	05.05.2013	17	1.7
	12 DALA	9	07.05.2013	19	1.1
	14 DALA	10	09.05.2013	30	1.4
Trial no. L130766 Study site: Germany	1 HALA	1	25.04.2013	13	11
	1 DALA	2	26.04.2013	13	8.1
	2 DALA	3	27.04.2013	14	7.5
	3 DALA	4	28.04.2013	14	5.9
	4 DALA	5	29.04.2013	15	5.8
	5 DALA	6	30.04.2013	15	4.0
	7 DALA	7	02.05.2013	16	3.0
	10 DALA	8	05.05.2013	17	2.2
	12 DALA	9	07.05.2013	19	0.74
	14 DALA	10	09.05.2013	30	1.4
Trial no. L130767 Study site: Netherlands	1 HALA	1	05.05.2014	13	6.9
	1 DALA	2	06.05.2014	13	5.9
	2 DALA	3	07.05.2014	14	4.4
	3 DALA	4	08.05.2014	14	3.9
	4 DALA	5	09.05.2014	15	4.5
	5 DALA	6	10.05.2014	15	2.2
	7 DALA	7	12.05.2014	16	2.0
	10 DALA	8	15.05.2014	17	1.3
	12 DALA	9	17.05.2014	18	1.2
	14 DALA	10	19.05.2013	19	0.79

Trial details	Sampling timing	Sampling no.	Date	Crop growth stage (BBCH)	Residues (mg/kg)
					Metconazole
Trial no. L130768 Study site: Netherlands	1 HALA	1	05.05.2014	13	7.4
	1 DALA	2	06.05.2014	13	5.5
	2 DALA	3	07.05.2014	14	3.7
	3 DALA	4	08.05.2014	14	3.8
	4 DALA	5	09.05.2014	15	3.1
	5 DALA	6	10.05.2014	15	2.1
	7 DALA	7	12.05.2014	16	2.7
	10 DALA	8	15.05.2014	17	1.2
	12 DALA	9	17.05.2014	18	1.2
	14 DALA	10	19.05.2013	19	0.83
Trial no. L130769 Study site: Spain	1 HALA	1	23.01.2014	12	11
	1 DALA	2	24.01.2014	12	11
	2 DALA	3	25.01.2014	12	9.7
	3 DALA	4	26.01.2014	12	9.0
	4 DALA	5	27.01.2014	12	7.5
	5 DALA	6	28.01.2014	13	6.9
	7 DALA	7	30.01.2014	13	5.5
	10 DALA	8	02.02.2014	14	3.2
	12 DALA	9	04.02.2014	14	3.3
	15 DALA [1)]	10	07.02.2014	14-15	1.9
Trial no. L130770 Study site: Spain	1 HALA	1	23.04.2014	12	5.3
	1 DALA	2	24.04.2014	12	5.9
	2 DALA	3	25.04.2014	12-13	3.1
	3 DALA	4	26.04.2014	12-13	2.3
	4 DALA	5	27.04.2014	12-13	2.1
	5 DALA	6	28.04.2014	13-14	0.92
	7 DALA	7	30.04.2014	14	0.55
	10 DALA	8	03.05.2014	14-15	0.23
	12 DALA	9	05.05.2014	15	0.22
	14 DALA	10	07.05.2014	16	0.21
Trial no. L130771 Study site: Italy	1 HALA	1	18.11.2013	12-13	9.1
	1 DALA	2	19.11.2013	12-13	7.9
	2 DALA	3	20.11.2013	13-14	6.6
	3 DALA	4	21.11.2013	14	6.7
	4 DALA	5	22.11.2013	14-15	3.7
	5 DALA	6	23.11.2013	14-15	3.4
	7 DALA	7	25.11.2013	15	1.8
	10 DALA	8	28.11.2013	15-16	1.8
	12 DALA	9	30.11.2013	15-16	0.72
	14 DALA	10	02.12.2013	15-16	0.74
Trial no. L130772 Study site: Italy	1 HALA	1	14.05.2013	12-13	8.8
	1 DALA	2	15.05.2013	12-13	6.9
	2 DALA	3	16.05.2013	13-14	7.2
	3 DALA	4	17.05.2013	14	5.1
	4 DALA	5	18.05.2013	14-15	5.4
	5 DALA	6	19.05.2013	14-15	3.8
	7 DALA	7	21.05.2013	16-17	2.3
	10 DALA	8	24.05.2013	17-18	1.2
	12 DALA	9	26.05.2013	18-19	0.94
	14 DALA	10	28.05.2013	19	0.69

HALA: hours after last application; DALA: days after last application

[1)] Last sampling was performed at 15 DALA instead of 14 DALA as required in the study plan. Due to heavy rain at 14 DALA it was decided to delay the sampling one day in order to take dry plants.

III. CONCLUSION

In pea plant samples collected directly after the application (BBCH 12-13) of BAS 555 01 F, the residues of BAS 555 F ranged between 5.3 to 11.0 mg/kg. At the last sampling at 14-15 DALA (BBCH 14-30) residues of BAS 555 F decreased to a range of 0.21 to 1.9 mg/kg.

Residues of BAS 555 F were below 0.01 mg/kg in all control specimens.

The following kinetics assessment on the residue behaviour of metconazole on young plants has not been evaluated previously on EU level.

Report:	CA 8.1/3 Delgado Cartay M.D., 2015a Dissipation of BAS 555 F (Metconazole) on young plants (wheat and peas) from field trials conducted in the Northern and Southern Zones of Europe - Calculation of DT50 / DT90 dissipation times 2015/1040887
Guidelines:	none
GLP:	no

This modelling report provides kinetic analysis and estimation of dissipation times (DT_{50} and DT_{90} values) for metconazole on young wheat and pea plants.

MATERIAL AND METHODS

Calculation of DT_{50}

The residue decline of BAS 555 F (metconazole) on young wheat and young pea plants has been studied at a range of field trials at different sites in Europe (northern and southern zone) during the growing season of the year 2013-2014. The modelling report provides kinetic analyses and estimations of the dissipation times (DT_{50}, DT_{90} values) for metconazole for each field data set. Individual dissipation parameter estimations were conducted for wheat and peas at the different field trials.

Guidance of the FOCUS workgroup on degradation kinetics was used in order to derive DT_{50} endpoints for modelling purposes. This means the selected DT_{50} values are suitable input parameters for models that require single first order (SFO) DT_{50} values or conservative substitutes. The software package KinGUI version 2.1 was used for parameter fitting.

The goodness-of-fit was evaluated by means of visual and statistical assessment as proposed by the FOCUS kinetics guidance. The fits are visually and statistically acceptable for all datasets.

The decline of metconazole residues on young plants was well described by the SFO kinetic model. The resulting DT_{50} values for metconazole in young wheat and young pea plants and the respective statistical indices are presented in the following table.

RESULTS

Table 8.1-4: DT_{50} values of metconazole in young wheat and pea plants

Plant	Trial	Zone	DT50 [d]	Kinetic model	$\chi 2$ error
Wheat	L130737	North	5.4	SFO	11.67
Wheat	L130738	North	5.8	SFO	10.72
Wheat	L130739	North	4.1	SFO	15.48
Wheat	L130740	North	3.6	SFO	7.97
Wheat	L130741	South	3.7	SFO	14.25
Wheat	L130742	South	4.2	SFO	18.08
Wheat	L130743	South	8.8	SFO	13.41
Wheat	L130744	South	4.6	SFO	10.43
Peas	L130765	North	3.8	SFO	6.12
Peas	L130766	North	3.9	SFO	7.30
Peas	L130767	North	4.1	SFO	10.86
Peas	L130768	North	3.8	SFO	13.42
Peas	L130769	South	6.2	SFO	4.98
Peas	L130770	South	2.4	SFO	20.96
Peas	L130771	South	3.6	SFO	10.82
Peas	L130772	South	4.0	SFO	9.82

CONCLUSION

The decline of metconazole residues on young plants was well described by single first order kinetics.

CA 8.1.1 Effect on birds

Toxicity studies for the use in the risk assessments for birds

For the convenience of the reviewer Table 8.1.1-1 provides information on the EU-reviewed and agreed avian toxicity studies for the active substance metconazole (BAS 555 F) as reviewed in the draft monograph of the Rapporteur Member State Belgium, Vol.3, B.9 (January, 2004).

Table 8.1.1-1: Summary of EU-reviewed and agreed avian toxicity studies relevant for AIR3 for the active substance metconazole (BAS 555 F) for assessing the risk to birds[1)]

Test system	Test species	Reference [Author, year, BASF DocID / Name]	EU reviewed and agreed
Acute oral toxicity	*Colinus virginianus*	[redacted] 1992, 1992/7004775 / MK-505-001	Yes (still valid for AIR 3 according to EFSA/2009/1438)
Acute oral toxicity	*Colinus virginianus*	[redacted] 1998, 1998/7001838 / MK-505-007	Yes (still valid for AIR 3 according to EFSA/2009/1438)
Short-term dietary toxicity	Colinus virginianus	[redacted] 1991, 1991/7000222 / MK-505-002	Yes (but no longer part of core data package according to EFSA/2009/1438)
Short-term dietary toxicity	Anas platyrhynchos	[redacted] 1991, 1991/7000223 / MK-505-003	Yes (but no longer part of core data package according to EFSA/2009/1438)
Sub-chronic toxicity and reproduction	*Colinus virginianus*	[redacted] 1999, 1999/7001942 / MK 505-009	Yes (still valid for AIR 3 according to EFSA/2009/1438)
Sub-chronic toxicity and reproduction	*Anas platyrhynchos*	[redacted] 1992, 1992/7000220 / MK 505-004	Yes (still valid for AIR 3 according to EFSA/2009/1438)

[1)] EU agreed means assessed during the previous EU evaluation process, but not necessarily listed in the list of endpoint as in the review report for the active substance metconazole (EFSA Scientific Report (2006) 64, 1-71, 13 January 2006).

For the convenience of the reviewer Table 8.1.1-2 lists the available toxicity studies in birds for the metabolites identified to be relevant for the birds and mammal risk assessment. These studies were evaluated on EU level before as reviewed in the Draft Assessment Report for the active substance epoxiconazole by the rapporteur member state Germany, Vol. 3, B.9 (March 2006).

Table 8.1.1-2: Summary of avian toxicity studies with metabolites of the active substance metconazole (BAS 555 F) relevant for the bird risk assessment

Test substance	Test system	Test species	Reference [Author, year, BASF DocID]
M555F034 (triazolyl acetic acid)	Acute oral toxicity	Colinus virginianus	[redacted], 2003 2003/1004105
M555F035 (triazolyl alanine)	Short-term dietary toxicity	Colinus virginianus	[redacted], 1983 1983/1000462
M555F035 (triazolyl alanine)	Short-term dietary toxicity	Anas platyrhynchos	[redacted], 1983 1983/1000463

CA 8.1.1.1. Acute oral toxicity to birds

No new study available (see Table 8.1.1-1).

CA 8.1.1.2. Short-term dietary toxicity to birds

No new study available (see Table 8.1.1-1).

CA 8.1.1.3. Sub-chronic and reproductive toxicity to birds

No new study available (see Table 8.1.1-1).

CA 8.1.2 Effects on terrestrial vertebrates other than birds

Toxicity studies for the use in the risk assessments for mammals

For the convenience of the reviewer, Table 8.1.2-1 provides information on the EU-reviewed and agreed mammalian toxicity studies, as well as additional toxicity studies relevant for the active substance metconazole (BAS 555 F) for assessing the risk to mammals in the AIR 3 process. Note, that only the critical studies relevant for the risk assessment in M-CP 10.1 are listed. Additional studies like preliminary studies are not included here, but are summarized in the draft monograph of the Rapporteur Member State Belgium, Vol. 3, B.9 (January, 2004).

Table 8.1.2-1: Summary of EU-reviewed and agreed, as well as additional mammalian toxicity studies relevant for AIR3 for the active substance metconazole (BAS 555 F) for assessing the risk to mammals[1)]

Test system	Test species	Reference [Author, year, BASF DocID / Name]	EU reviewed and agreed
Acute oral toxicity	*Mouse*	[redacted] r 1990 [2002/7004728 / MK-411-001]	Yes (still valid for AIR 3 according to EFSA/2009/1438)
Acute oral toxicity	*Rat*	[redacted] r 1990 [2002/7004728 / MK-411-001]	Yes (still valid for AIR 3 according to EFSA/2009/1438)
Acute oral toxicity	*Rat*	[redacted] 1991 [2002/7004729 / MK-410-001]	Yes (still valid for AIR 3 according to EFSA/2009/1438)
Acute oral toxicity	*Rat*	[redacted] r 2005 [2005/1005772]	No (new study)
2-Generation reproductive toxicity	*Rat*	[redacted] 1992 [1992/7000194 / MK-430-003]	Yes (still valid for AIR 3 according to EFSA/2009/1438)
2-Generation reproductive toxicity	*Rat*	[redacted] 2015 [2015/1087913]	No (new study)
Prenatal Development toxicity	*Rat*	[redacted] s 1991 [1991/7000207 / MK-432-005]	Yes (still valid for AIR 3 according to EFSA/2009/1438)
Prenatal Development toxicity	*Rat*	[redacted] 1992 [1992/7000345 / MK-432-009]	Yes (still valid for AIR 3 according to EFSA/2009/1438)
Prenatal Development toxicity	*Rat*	[redacted] 2015 [2015/1087909]	No (new study)
Prenatal Development toxicity	*Rabbit*	[redacted] 1997 [1996/7000384 / MK-432-011]	Yes (still valid for AIR 3 according to EFSA/2009/1438)
Prenatal Development toxicity	*Rabbit*	[redacted] 1991 [1991/7000205 / MK-432-003]	Yes (still valid for AIR 3 according to EFSA/2009/1438)

Test system	Test species	Reference [Author, year, BASF DocID / Name]	EU reviewed and agreed
Prenatal Development toxicity	*Rabbit*	[redacted] s 1992 [1992/7000196 / MK-432-007]	Yes (still valid for AIR 3 according to EFSA/2009/1438)
Prenatal Development toxicity	*Rabbit*	[redacted] 1992 [1992/7000198 / MK-432-010]	Yes (still valid for AIR 3 according to EFSA/2009/1438)

[1)] EU agreed means assessed during the previous EU evaluation process, but not necessarily listed in the list of endpoint as in the review report for the active substance metconazole (EFSA Scientific Report (2006) 64, 1-71, 13 January 2006).

Mammalian toxicity studies for metabolites that were identified to be relevant for the birds and mammal risk assessment are listed in Table 8.1.2-2. Only studies that were considered relevant for the mammalian metabolite assessment are listed below. These studies were evaluated on EU level before as reviewed in the draft assessment report for epoxiconazole of the rapporteur member state Germany, Vol. 3, B.9 (March 2006) or are under evaluation by CRD (UK) as the RMS under COP No 2011/00502 and COP 2012/01449, submitted as part of the data packages of the triazole derivative metabolites by the Triazole Derivative Metabolite Group (TDMG). For details consult the stated documents.

Table 8.1.2-2: Summary of critical mammalian toxicity studies with metabolites of the active substance metconazole (BAS 555 F) relevant for the mammalian risk assessment

Test substance	Test system	Test species	Reference [Author, year, BASF DocID]
M555F034 (triazolyl acetic acid)	Acute oral toxicity	rat	[redacted] z, 1984; 1984/1000342
M555F034 (triazolyl acetic acid)	1-generation reproduction toxicity	rat	[redacted] r, 2010; 2010/1225534
M555F035 (triazolyl alanine)	Acute oral toxicity	rat	[redacted], 1980; 1980/1000167
M555F035 (triazolyl alanine)	Acute oral toxicity	rat and mouse	[redacted] 1982; 1986/1000485
M555F035 (triazolyl alanine)	2-generation reproduction toxicity	rat	[redacted], 1986; 1986/1000486

CA 8.1.2.1 Acute oral toxicity to mammals

A new study is available (see Table 8.1.2-1). See M-CA 5.2 for the study summary of Gamer & Leibold (2005): BAS 555 F – Acute oral toxicity study in rats. BASF DocID 2005/1005772.

CA 8.1.2.2 Long-term and reproductive toxicity to mammals

Two new studies are available. See M-CA 5.6 for the study summaries for the new rat reproductive toxicity study (2015, BASF DocID 2015/1087913) and the new rat teratology study 2015, BASF DocID 2015/1087909).

CA 8.1.3 Effects of active substance bioconcentration in prey of birds and mammals

No new study available.

CA 8.1.4 Effects on terrestrial vertebrate wildlife (birds, mammals, reptiles and amphibians)

According to the revised data requirements under regulation 1107/2009 (Commission Regulations (EU) 283/2013 and 284/2013 for the active ingredient and the plant protection products, respectively), the risk to amphibians and reptiles shall be addressed. Nevertheless, unlike birds and mammals, toxicity tests for amphibian and reptile species are not requested. In the EU there is no guidance or validated regulatory protocols yet available neither on the type of regulatory testing necessary nor how to conduct a risk assessment for amphibian and reptiles. In the case of metconazole, there are no studies in the literature on the toxicity of metconazole on amphibians and reptiles.

According to the new aquatic guidance document (EFSA, 2013) amphibian should be included in the aquatic and terrestrial risk assessment. In absence of GLP studies the assessment should be based on any existing relevant information (testing of amphibian is not recommended at first instance due to animal welfare reasons and the absence of standard guidelines for amphibian testing). With regard to the aquatic risk assessment several data analyses indicate that the risk assessment for aquatic organisms (and fish in particular) covers the risk assessment for aquatic phases of amphibians (Fryday and Thompson, 2012; Weltje et al., 2013).

Compared to aquatic studies, regulatory ecotoxicological information on amphibians based on dosing studies (LD50) is rather scarce. However, in the few cases where terrestrial stages of amphibians were tested in this kind of study as birds and mammals, the general pattern is that amphibians are less sensitive than the latter two taxa (see Table 12 and 13 in Fryday and Thompson, 2012).

In the case of reptiles there is even less information available than for amphibians (see the revision by Fryday and Thompson, 2009). The risk from dietary exposure can be assumed to be lower for reptiles than for birds and mammals (Fryday and Thompson 2009). This is because reptiles are poikilotherm (i.e., do not maintain a constant body temperature) and as a result feeding activity will peak at warm days and will be zero during hibernation or at cold days. In contrast, birds and mammals will have to maintain a constant body temperature and, hence, will need to feed every day (Fryday & Thompson 2009). Uncertainties remain on the contribution of dermal exposure to the overall exposure to reptiles. However, in contrast to amphibians the skin of reptiles is much less permeable; its functions is in general protection and barrier and not an organ used for respiration or water/mineral exchange with the environment. Accordingly, reptiles are considered less vulnerable to dermal exposure as compared to amphibians. Nevertheless, some uncertainty with respect to the risk to reptiles, i.e. whether they are sufficiently covered by other (more standard) ecotoxicological data will remain and further research is needed.

However, metconazole has been used for many years in many countries worldwide. So far, there are no publications indicating a potential risk of this compound to amphibians / reptiles and despite the long term use worldwide we are not aware of a single findings or (incidence) reports that amphibians / reptiles were harmfully affected by applications of this substance.

References:

Commission Regulation (EU) No 283/2013 setting out data requirements for active substances, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market. Official Journal of the European Union: 1st March 2013.

Commission Regulation (EU) No 284/2013: setting out the data requirements for plant protection products, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market. Official Journal of the European Union: 1st March 2013.

Fryday S and Thompson H (2009a): Literature reviews on ecotoxicology of chemicals with a special focus on plant protection products. Lot 1. Exposure of reptiles to plant protection products. EFSA (CFT/EFSA/PPR/2008/01).

Fryday S and Thompson, H (2012): Toxicity of pesticides to aquatic and terrestrial life stages of amphibians and occurrence, habitat use and exposure of amphibian species in agricultural; Food and Environment research agency, UK

Weltje L., Simpson P., Gross M., Crane M., Wheeler J.R. (2013): Comparative acute and chronic sensitivity of fish and amphibians: a critical review of data. Environmental Toxicology and Chemistry, Vol. 32, No. 5, pp. 984-994

CA 8.1.5 Endocrine disrupting properties

Mammals

For mammals, the endocrine disruption potential of metconazole is discussed in detail in chapter M-CA 5.8.3.

Birds

Until now, there are no formal criteria available in the EU of what constitutes an endocrine disruptor under Regulation 1107/2009. For birds, there is also no internationally validated regulatory testing guideline available. Effects on avian reproduction are covered by the avian reproduction study, which is part of the standard data package for an active ingredient.

For metconazole, two avian reproduction studies are available, one in bobwhite quail and one in mallard duck (see M-CA 8.1.1). As guideline regulatory studies, the focus of these studies was on the general and reproductive toxicity of metconazole to birds. Therefore, and similar for the overwhelming majority of active substances, no specific assessments were included with regard to endocrine effects, since standard guideline studies are not designed to specifically investigate those parameters. Still, the bird reproduction study does cover reproductive endpoints that are under endocrine control. With their long-term exposure (≥ 21 weeks) and detailed assessment of fitness and reproductive parameters, and gross necropsy assessment, the studies provide adequate information on the overall effect pattern of the active substance metconazole in birds. Hence, the studies are considered suitable to allow for a full evaluation of the reproductive toxicity, including any endocrine potential of metconazole that might impact the reproductive performance in birds.

This is in line with a recent conclusion of the US EPA within its Endocrine Disruptor Screening Program (EDSP)[1]. In June 2015, US EPA released its review[2] of the Tier 1 screening assay results for the list 1 chemicals (in total 52 chemicals) in the EDSP. EPA clearly states that data obtained from the "avian reproduction studies (OCSPP 850.2300) are considered sufficient for evaluating potential reproductive effects to birds" and "additional testing is not recommended" (e.g. propiconazole, p. 33[3]).

[1] The Endocrine Disruptor Screening Program (EDSP) of US EPA is a program to screen chemicals for their potential to affect the estrogen, androgen and thyroid hormone systems using a two-tiered screening and testing process. The results of the screening are evaluated in a weight of evidence (WoE) approach by EPA to determine whether a chemical has the potential to interact with the endocrine system and whether more thorough testing is required. The WoE conclusion on the tier 1 screening assays for list 1 chemicals was published in June 2015.

[2] United States Environmental Protection Agency Washington, D.C. 20460, Memorandum, June 29, 2015, EDSP Weight of Evidence Conclusions on the Tier 1 Screening Assays for the List 1 Chemicals.

[3] United States Environmental Protection Agency. EDSP: Weight of Evidence Analysis of Potential Interaction with Estrogen, Androgen or Thyroid Pathways. Chemical: Propiconazole. Washington, D.C. 20460, Memorandum, June 29, 2015.

Many plant protection products were amongst the chemicals screened in the EDSP including four triazoles, namely myclobutanil, propiconazole, tebuconazole and triadimefon. All these compounds belong to the same chemical group as metconazole, i.e. triazoles in the fungicidal group of demethylase inhibitors (DMI) (FRAC group G1).

For all evaluated triazoles, the US EPA concluded that further bird testing is not recommended. Specifically note that for all the four triazoles the standard guideline avian reproduction toxicity studies were the only available data on chronic bird toxicity, and hence were considered sufficient for the evaluation of potential reproductive effect to birds. Based on the US EPA assessment for the EDSP, it is considered valid to conclude for the case of metconazole that the available data from the avian reproduction toxicity studies in bobwhite quail and mallard duck are suitable for the evaluation of a potential reproductive effect to birds, and to derive an endpoint that is suitable to cover the endocrine potential of metconazole in the avian reproductive risk assessment.

The standard data requirements for registration in the EU for reproductive testing is with one bird species; however, for metconazole avian reproduction studies are available for two species, hence reducing the risk linked to potential variations in species sensitivity. Under the conservative assumptions in both the study design (constant long-term exposure to high concentrations) and the long-term bird risk assessment (which demonstrated that even under the very conservative assumptions of tier 1 the long-term TER values are above the trigger value of 5), sufficient data is available to conclude that the risk to birds from the proposed uses of metconazole is acceptable.

In summary, based on i) the availability of two avian reproduction studies for metconazole, ii) the conclusions of the US EPA for triazoles regarding the sufficiency of the avian reproductive studies for evaluating potential reproductive effects on birds, and iii) the conservative nature of the bird reproductive risk assessment, it can be reasoned for the case of metconazole that the standard endpoints from the avian reproduction studies are sufficiently protective to address the potential endocrine activity and are suitable for their use in the avian reproductive risk assessment for metconazole.

CA 8.2 Effects on aquatic organisms

Since Annex I inclusion of the active substance metconazole (BAS 555 F), new toxicity studies on the active substance and it's metabolites have been performed and as a result there are new endpoints which are now used in the aquatic risk assessment. Summaries of these new studies are provided below. For completeness this includes some older studies, which have not been submitted during the previous Annex I inclusion process (*e.g.* because there is no respective data requirement in the EU). In addition, a summary is provided for peer-reviewed scientific literature that was considered to be of relevance for the aquatic risk assessment of metconazole.

Details on the EU agreed studies which have been already evaluated within the Annex I inclusion of metconazole are provided in the EU Review documents of metconazole (Draft Assessment Report (DAR), Vol. 3, B.9, 2004; EFSA Scientific Report 64 (2006)). Studies of the metconazole metabolite 1,2,4-triazole have been evaluated and EU agreed within the Annex I inclusion of epoxiconazole (EFSA Scientific Report (2008) 138, 1-80, Conclusion on the peer review of epoxiconazole).

The metconazole metabolite M13 (=CL 359139; M555F013) was found in water-sediment system close to a level of 10% of AR at DAT 152 (for details see DAR, Vol. 3, Annex B.8.4.4, 2004). Because of the very similar structure a higher toxicity of the metabolite compared to the parent is considered to be unlikely (the only difference is the formation of a carboxylic acid group). No studies are available for the metabolite, however regarding the worst-case $PEC_{sw,\ ini}$ values (please refer to M-CP 10.2) of M13 a risk to aquatic organisms is expected to be low.

For better transparency and traceability of the active substance history, the results of all studies are summarized in Table 8.2-1.

Results of ECx calculations for chronic toxicity studies are provided in a separate document for new and old studies *[see KCA 8.2/1 2016/1002152]*

For studies following guidelines/protocols that were designed to derive a NOEC/NOAEL and have significant limitations for deriving an ECx like limited number of dose groups and/or large dose spacing (e.g. reproductive studies on birds and mammals), no ECx values were calculated.

Full references used within the following chapters are given at the end M-CA 8.2.

Table 8.2-1: List of studies and endpoints for aquatic organisms exposed to the active substance metconazole (BAS 555 F)

Organism	Endpoint	Value [mg a.s./L]	Reference (BASF DocID / Name)	EU agreed
Fish				
Oncorhynchus mykiss	96 h LC_{50}	**2.1** [1)]	1990/7000167 / MK-510-001	yes
Oncorhynchus mykiss [2), 3)]	96 h LC_{50}	4.0	1991/7000224 / MK-510-002	no (supplemental data)
Cyprinodon variegatus [3)]	96 h LC_{50}	6.3	2005/1042060	no (new study conducted for refined risk assessment)
Cyprinus carpio	96 h LC_{50}	3.99	1996/7000400 / MK-511-002	yes
Danio rerio [3)]	96 h LC_{50}	6.8	2008/1080979	no (new study conducted for refined risk assessment)
Gasterosteus aculeatus [3)]	96 h LC_{50}	4.2	2010/1007158	no (new study conducted for refined risk assessment)
Lepomis macrochirus [3)]	96 h LC_{50}	4.9	2008/1084069	no (new study conducted for refined risk assessment)
Pimephales promelas	96 h LC_{50}	3.9	1991/7000225 / MK-511-001	yes
Oncorhynchus mykiss	28 d NOEC	1.14	1996/7000401 / MK-513-001	yes
Oncorhynchus mykiss (ELS)	95 d NOEC	**0.00291**	2001/1015080	yes
Cyprinodon variegatus [3)] (ELS)	33 d NOEC	0.011	2009/1129142	no (new study conducted for refined risk assessment)
Pimephales promelas [2), 3)] (ELS)	35 d NOEC	0.01	1992/7000222 / MK-512-001	no (supplemental data)
Pimephales promelas [3)] (FLC)	5 months NOEC	0.00358	2008/1064993	no (new study conducted for refined risk assessment)

Organism	Endpoint	Value [mg a.s./L]	Reference (BASF DocID / Name)	EU agreed
Aquatic invertebrates				
Daphnia magna	48 h EC_{50}	**4.2**	1990/7000167 / MK-510-001	yes
Daphnia magna [2), 3)]	48 h EC_{50}	3.6	1991/7000224 / MK-510-002	no (supplemental data)
Daphnia magna	21 d NOEC	**0.16**	2002/1004678	yes
Daphnia magna [2), 3)]	21 d NOEC	0.21	1991/7000226 / MK-523-001	no (supplemental data)
Sediment dwelling aquatic invertebrates				
Chironomus riparius (spiked water study)	28 d NOEC	**2.12**	1997/7000363 / MK-549-006	yes
Algae [4)]				
Selenastrum capricornutum (Syn. *Pseudokirchneriella subcapitata*)	72 h E_rC_{50}	**2.2**	1990/7000167 / MK-510-001	yes (study submitted in previous Annex I process; however, the growth rate endpoint is now used)
Selenastrum capricornutum [2), 3)]	72 h E_rC_{50}	2.6	1991/7000224 / MK-510-002	no (supplemental data)
Macrophytes				
Lemna gibba [3)]	7 d E_rC_{50}	0.527	2014/1093918	no (new study to address requirements for plant growth regulators)
Bioconcentration				
Lepomis macrochirus	BCF_{SS} (parent) (28 days uptake, 14 days depuration)	87.9	1996/7000402 / MK-519-002	yes
Lepomis macrochirus	BCF_{SS} (parent) (28 days uptake, 14 days depuration)	78.9	2002/7004691 / MK-519-003	yes
Peer-reviewed literature studies				
Oncorhynchus mykiss [5)]	BMF_{SS} (8 days uptake, 16 days depuration)	0.019	2006/1051433	no (peer-reviewed scientific study; used for risk refinement)

Bold figures: Where several endpoints are available for the same species or group, only the relevant endpoint(s) for the most sensitive species is used in the risk assessment presented in chapter 10.2 of the MCP dossier part for Annex I renewal.
Abbreviations: FLC = Full Life Cycle; ELS = Early Life Stage

1) Lowest EC_{50} value after 72 h in the 96 h test.
2) Study was conducted with metconazole cis-isomer (95 : 5 - cis : trans).
3) Study has not been submitted during Annex I inclusion process of metconazole; a study summary is provided in M-CA 8.2.
4) In accordance with the new EFSA Aquatic Guidance Document (2013) and OECD Guidelines for aquatic primary producers (OECD 201 and 221), the endpoint growth rate (E_rC_{50}) is now used for the risk assessment for algae and macrophytes; the E_rC_{50} values obtained in the studies already submitted during Annex I inclusion process are thus included as new information.
5) Study was conducted with a mixture of triazole fungicides and α-HCH containing 28.14 µg metconazole/g wet weight (mean measured)

Table 8.2-2: List of studies and endpoints for aquatic organisms exposed to the metabolite of metconazole 1,2,4-triazole (= M555F020)

Organism	Endpoint	Value [mg/L]	Reference	EU agreed*
Fish				
Oncorhynchus mykiss	96 h LC_{50}	760	1983/1000494	yes
	28 d NOEC	3.2	2002/1007850	yes
Aquatic invertebrates				
Daphnia magna	48 h EC_{50}	> 100	1995/1001851	yes
Algae				
Pseudokirchneriella subcapitata	72 h E_rC_{50} 72 h E_yC_{50}	> 31 14	2001/1022266	yes

* EFSA Scientific Report (2008) 138, 1-80, Conclusion on the peer review of epoxiconazole

CA 8.2.1 Acute toxicity to fish

An acute toxicity study with rainbow trout (*Oncorhynchus mykiss*) conducted with metconazole was already evaluated during the previous Annex I inclusion process. The following additional acute toxicity study with rainbow trout performed with the active substance metconazole has not been evaluated previously on EU level. The study was conducted with test material consisting of a different isomeric ratio (cis:trans - 95:5). It confirms the toxicity endpoint of the standard study used for the risk assessment (MK-510-001) and is submitted here only for completeness and as supplemental data.

Report:	CA 8.2.1/1 [redacted] 1991a WL136184 (KNF-S-474c): Acute toxicity to Oncorhynchus mykiss, Daphnia magna and Selenastrum capricornutum MK-510-002
Guidelines:	OECD 203, OECD 202, OECD 201
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

I. MATERIAL AND METHODS

In a 96-hour semi-static acute toxicity laboratory study, juvenile rainbow trout were exposed to a dilution water control and to nominal concentrations of 0.16, 0.34, 0.75, 1.7, 1.7, 3.6 and 8.0 mg metconazole/L in groups of 10 animals in glass aquaria containing 10 L water. Fish were observed for survival and symptoms of toxicity after 3, 24, 48, 72 and 96 hours after start of exposure.

The biological results are based on nominal concentrations of the test item. After 96 hours of exposure, no mortality was observed in the dilution water control and at test item concentrations of up to and including 0.34 mg a.s./L and at 1.7 mg a.s./L. In the test vessel of 0.75 mg a.s./L, 100% mortality was observed. However, mortality in this test vessel did not conform to the dose response curve and suggested contamination of the test vessel. Therefore, a second test vessel was set up at this test item concentration. As no mortality occurred in this second vessel the mortality seen in the original vessel has been excluded from data analysis. At the test item concentrations of 3.6 and 8.0 mg a.s./L, 20% and 100% mortality were observed, respectively. Sub-lethal effects (*i.e.* increased cough frequency / hyperventilation, abnormal swimming, immobilization) were found at 1.7 and 3.6 mg a.s./L after 96 hours.

In a semi-static acute toxicity study with rainbow trout the LC_{50} (96 h) of metconazole was 4.0 mg a.s./L based on nominal concentrations. The NOEC (96 h) was determined to be 0.75 mg a.s./L (nominal).

A. MATERIALS

Test item: Metconazole (BAS 555 F), batch no. F900250, purity: metconazole cis-isomer: 95.2%, metconazole trans-isomer: 0.1% and 1,3,4-triazole analogue: 4.2%.

B. STUDY DESIGN

Test species: Rainbow trout (*Oncorhynchus mykiss* WALBAUM), juveniles; mean length: 3.8 cm (range: 3.3 to 4.3 cm; mean wet weight: 0.51 g (range 0.35 to 0.67 g); supplied by "Beckettswell Trout Farm", Otford, Kent, Great Britain.

Test design: Semi-static system (96 h); 6 test item concentrations plus a dilution water control, 1 replicate per treatment; 10 fish per aquarium; assessment of mortality and sub-lethal effects after 3, 24, 48, 72 and 96 hours after start of exposure.

Endpoints: LC_{50}, NOEC, mortality and sub-lethal effects.

Test concentrations: Control (dilution water), 0.16, 0.34, 0.75, 1.7, 3.6 and 8.0 mg metconazole/L (nominal).

Test conditions: Glass aquaria, test volume: 10 L; dilution water: dechlorinated mains water; total hardness: 268 - 278 mg $CaCO_3$/L; temperature: 14 - 15°C; pH 7.1 - 8.5; oxygen content: 9.7 mg/L - 10.5 mg/L; daily renewal of dilution water; photoperiod: not reported; light intensity: not reported; gentle aeration; no feeding.

Analytics: Analytical verification of test item concentrations was conducted using a GC-method with nitrogen selective detection.

Statistics: Descriptive statistics; moving average angle method for calculation of LC_{50}.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of metconazole concentrations was conducted for all test item concentrations in samples of fresh solutions and spent solutions on days 1, 2, 3 and 4. The mean analysed contents of metconazole ranged from 100% to 112% of nominal in samples of fresh solutions and from 97% to 106% in samples of spent solutions. The value measured in the sample of the fresh solution on day 1 at the lowest test item concentration was excluded because the measured value of 338% of nominal indicated contamination from the analysis phase.

Biological results: After 96 hours of exposure, no mortality was observed in the dilution water control and at test item concentrations of up to and including 0.34 mg a.s./L and at 1.7 mg a.s./L. In the test vessel of 0.75 mg a.s./L, 100% mortality was observed. However, mortality in this test vessel did not conform to the dose response curve and suggested contamination of the test vessel. Therefore, a second test vessel was set up at this test item concentration. As no mortality occurred in this second vessel the mortality seen in the original vessel has been excluded from data analysis. At the test item concentrations of 3.6 and 8.0 mg a.s./L, 20% and 100% mortality were observed, respectively. Sub-lethal effects (*i.e.* increased cough frequency / hyperventilation, abnormal swimming, immobilization) were found at 1.7 and 3.6 mg a.s./L after 96 hours. The results are summarized in Table 8.2.1-1.

Table 8.2.1-1: Acute toxicity (96 h) of metconazole to rainbow trout (*Oncorhynchus mykiss*)

Concentration [mg a.s./L] (nominal)	**Control**	**0.16**	**0.34**	**0.75**		**1.7**	**3.6**	**8.0**
				1st test vessel	**2nd test vessel**			
Mortality [%] (96 h)	0	0	0	100 [a)]	0	0	20	100
Symptoms (after 96 h) *	none	none	none	n.d.	none [b)]	F	S, I	n.d.
Endpoints [mg metconazole/L] (nominal)								
LC_{50} (96 h)	4.0 (95% confidence limits: 3.3- 5.1)							
NOEC (96 h)	0.75							

n.d. = not determined; all fish dead

* Symptoms after 96 h: F = increased cough frequency / hyperventilation; S = abnormal swimming *e.g.* on side or back; I = immobilization (fish lying on bottom of tank or floating at surface, but still alive)

a) Mortality in this test vessel did not conform to the dose response curve and suggested contamination of the test vessel. A second test vessel was therefore set up at this test item concentration. As no mortality occurred in this second vessel the mortality seen in the original vessel has been excluded from data analysis.

b) The second test vessel was set up 24 h after the others. As no mortality occurred at the test item concentration above this one, no observation was made in the second test vessel at 96 h.

III. CONCLUSION

In a semi-static acute toxicity study with rainbow trout the LC_{50} (96 h) of metconazole was 4.0 mg a.s./L based on nominal concentrations. The NOEC (96 h) was determined to be 0.75 mg a.s./L (nominal). This endpoint is not used in the risk assessment.

The following acute toxicity study with sheepshead minnow (*Cyprinodon variegatus*) performed with the active substance metconazole is not required for registration in the EU and it has not been evaluated previously on EU level. The study was conducted to due to U.S. data requirements and is used for the refined risk assessment.

Report:	CA 8.2.1/2 [redacted] 2005a Metconazole (KNF-S-474m) - Acute toxicity to sheepshead minnow (Cyprinodon variegatus) under static conditions 2005/1042060
Guidelines:	EPA 850.1075, EPA 850.1000
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary

In a static acute toxicity laboratory study, sheepshead minnow (body weight: 0.14 g (0.05 - 0.25 g), body length: 20 mm (16 - 24 cm)) were exposed to mean measured concentrations of 0.97, 0.22, 4.6, 8.5 and 13 mg/L metconazole in groups of 10 animals in glass aquaria containing 15 L water. Fish were observed for survival and symptoms of toxicity at 0, 6, 24, 48, 72 and 96 hours exposure.

Biological results are based on mean measured concentrations. After 96 h of exposure metconazole caused no mortality or sub-lethal effects up to and including the concentration of 4.6 mg/L. 100% mortality were observed at the two highest test concentrations of 8.5 mg/L and 13 mg/L.

The 96-hour LC_{50} value for metconazole during a static acute toxicity test with sheepshead minnow was determined to be 6.3 mg metconazole/L based on mean measured concentrations. The no-observed effect concentration (NOEC) was 4.6 mg metconazole/L (mean measured).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Metconazole (CAS No. 125116-23-6), Lot No. AS2122a; purity: 98.7%.

B. STUDY DESIGN

Test species: Sheepshead minnow (*Cyprinodon variegatus)*; weight: 0.14 g (0.05 - 0.25 g); length: 20 mm (16 - 24 mm); source: Aquatic Biosystems Inc., Fort Collins, Colorado.

Test design: Static (96 h); 10 fish per treatment and aquarium; 2 replicate aquaria per treatment and control; Assessment of mortality and toxic signs was done at 0, 6, 24, 48, 72 and 96 hours of exposure.

Endpoints: Mortality, sub-lethal effects.

Test conditions: Glass aquaria (test volume: 15 L); natural filtered seawater; temperature: 21°C; pH: 7.3 - 7.9; dissolved oxygen: 3.1 to 7.9 mg/L; salinity: 19 – 20%; photoperiod: 16 h light, 8 h dark, light intensity: approx. 320 - 540 lux; no feeding; gentle aeration from 72 h onwards.

Test concentrations: Control, 0.94, 1.9, 3.8, 7.5 and 15 mg/L metconazole (nominal). Mean measured concentrations: control, 0.97, 0.22, 4.6, 8.5 and 13 mg/L.

Analytics: The concentrations of *cis*- and *trans*-metconazole in the test solutions were determined using a GC method with nitrogen phosphorus detection.

Statistics: The LC_{50} values were determined using binomial probability.

II. RESULTS AND DISCUSSION

Analytical measurements: The analytically detected concentrations at test initiation for metconazole in comparison to the nominal value generally ranged from 93% - 129%. At test termination the analytically detected concentrations for Metconazole ranged from 87% - 121%. However, the 1.9 mg/L treatment level resulted in very low recoveries (mean measured 11% of nominal). The low recovery might be due to an error made during preparation of the dosing stock solution. This is believed to have no impact on the results of the study since this treatment level is below the NOEC and LC_{50} values determined for this study. The biological results are based on mean measured concentrations.

Biological results: After 96 h of exposure metconazole caused no mortality or sublethal effects up to and including the concentration of 4.6 mg/L. 100% mortality were observed at the two highest test concentrations of 8.5 mg/L and 13 mg/L. The results are summarized in Table 8.2.1-2.

Table 8.2.1-2: Acute toxicity (96 h) of metconazole on Sheepshead Minnow (*Cyprinodon variegatus*)

Concentration [mg/L] mean measured	**Control**	**0.97**	**0.22**	**4.6**	**8.5**	**13**
Mortality [%]	0	0	0	0	100	100
Symptoms	none	none	none	none	--	--
Endpoints [mg/L]						
LC_{50} (96 h)	6.3					
NOEC (96 h)	4.6					

-- not observed, all animals dead

III. CONCLUSION

The 96-hour LC_{50} value for metconazole during a static acute toxicity test with sheepshead minnow was determined to be 6.3 mg metconazole/L based on mean measured concentrations. The no-observed effect concentration (NOEC) was 4.6 mg metconazole/L (mean measured).

The following acute toxicity study with bluegill sunfish (*Lepomis macrochirus*) performed with the active substance metconazole is not required for registration in the EU and it has not been evaluated previously on EU level. The study was conducted to generate data for the refined risk assessment.

Report: CA 8.2.1/3
[redacted] 2008a
Acute toxicity study with the bluegill sunfish (Lepomis macrochirus)
2008/1084069
Guidelines: (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH, EEC 1907/2006, OECD 203, EPA 72-1, EPA 850.1075
GLP: yes
(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

In a static acute toxicity laboratory study, bluegill sunfish (body weight: 0.32 g (0.28 - 0.37 g), body length: 3.2 cm (3.1 - 3.3 cm)) were exposed to 0.46, 1.0, 2.2, 4.6 and 10 mg/L metconazole in groups of 10 animals in glass aquaria containing 10 L water. Fish were observed for survival and symptoms of toxicity after 1, 6, 24, 48, 72 and 96 hours of test start.

Biological results are based on nominal concentrations. Metconazole caused no mortality up to and including the concentration of 2.2 mg/L. 100% mortality were observed at the highest test rate of 10 mg/L.

The 96-hour LC_{50} value for metconazole during a static acute toxicity test with bluegill sunfish was determined to be 4.9 mg metconazole/L based on nominal concentrations. The no-observed effect concentration (NOEC) was 2.2 mg metconazole/L (nominal).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Metconazole (BAS 555 F, Reg.No. 4056343), Batch number COD-000779; purity: 98.8%.

B. STUDY DESIGN

Test species: Bluegill Sunfish (*Lepomis macrochirus*); age: approx. 3 months; weight: 0.32 g (0.28 - 0.37 g); length: 3.2 cm (3.1 - 3.3 cm); source: Osage Catfisheries Inc., Osage Beach, MO, USA.

Test design: Static (96 h); 10 fish per treatment and aquarium; Assessment of mortality and toxic signs was done within 1 hour after start of exposure and after 6, 24, 48, 72 and 96 hours.

Endpoints: Mortality, sub-lethal effects.

Test conditions: Glass aquaria (test volume: 10 L); loading: 0.32 g fish/L; non-chlorinated, filtered tap water; total hardness: approx. 100 mg/L ($CaCO_3$); conductivity: approx. 250 µS/cm; acid capacity: approx. 2.5 mmol/L; temperature: 23°C; pH: 8.0 to 8.3; dissolved oxygen: 6.1 to 8.5 mg/L; photoperiod: 16 h light, 8 h dark, light intensity: approx. 100 - 490 lux; no feeding; aeration after 24 h for 30 min due to declining dissolved oxygen levels.

Test concentrations: Control, 0.46, 1.0, 2.2, 4.6 and 10 mg/L metconazole (BAS 555 F, Reg.No. 4056343)

Analytics: The concentrations of metconazole in the test solutions were determined using a HPLC method with MS-detection.

Statistics: The LC_{50} values were estimated using Probit analysis.

II. RESULTS AND DISCUSSION

Analytical measurements: The analytically detected concentrations at test initiation for metconazole in comparison to the nominal value ranged from 102% - 103%. At test termination the analytically detected concentrations for metconazole ranged from 97% - 98%. As the analytically determined concentrations confirmed the nominal concentrations, the biological results are based on nominal concentrations.

Biological results: Metconazole caused no mortality up to and including the concentration of 2.2 mg/L. 100% mortality were observed at the highest test rate of 10 mg/L. The results are summarized in Table 8.2.1-3.

Table 8.2.1-3: Acute toxicity (96 h) of metconazole on bluegill sunfish (*Lepomis macrochirus*)

Concentration [mg/L] nominal	**Control**	**0.46**	**1.0**	**2.2**	**4.6**	**10**
Mortality [%]	0	0	0	0	30	100
Symptoms*	none	none	none	none	A, T	--
Endpoints [mg/L]						
LC_{50} (96 h)	4.9					
NOEC (96 h)	2.2					

* toxic symptoms: A: apathy, T: tottering
-- not observed, all animals dead

III. CONCLUSION

The 96-hour LC_{50} value for metconazole during a static acute toxicity test with bluegill sunfish was determined to be 4.9 mg metconazole/L based on nominal concentrations. The no-observed effect concentration (NOEC) was 2.2 mg metconazole/L (nominal).
The following acute toxicity study with the zebrafish (*Danio rerio*) performed with the active substance metconazole is not required for registration in the EU and it has not been evaluated previously on EU level. The study was conducted to generate data for the refined risk assessment.

Report:	CA 8.2.1/4 [redacted] 2008b BAS 555 F - Acute toxicity study with the zebrafish (Danio rerio) 2008/1080979
Guidelines:	OECD 203, EPA 850.1075, (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH, EEC 1907/2006
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

In a static acute toxicity laboratory study, zebrafish (body weight: 0.34 g (0.22 - 0.62 g), body length: 2.9 cm (2.5 - 3.4 cm)) were exposed to 0.46, 1.0, 2.2, 4.6 and 10 mg/L Metconazole in groups of 10 animals in glass aquaria containing 10 L water. Fish were observed for survival and symptoms of toxicity after 1, 6, 24, 48, 72 and 96 hours of test start.

Biological results are based on nominal concentrations. Metconazole caused no mortality up to and including the concentration of 4.6 mg/L. 100% mortality were observed at the highest test rate of 10 mg/L.

The 96-hour LC_{50} value for metconazole during a static acute toxicity test with zebrafish was determined to be 6.8 mg metconazole/L based on nominal concentrations. The no-observed effect concentration (NOEC) was 2.2 mg metconazole/L (nominal).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Metconazole (BAS 555 F, Reg.No. 4056343), Batch number COD-000779; purity: 98.8%.

B. STUDY DESIGN

Test species: Zebrafish (*Danio rerio)*; age: approx. 5 months; weight: 0.34 g (0.22 - 0.62 g); length: 2.9 cm (2.5 - 3.4 cm); source: Zierfisch-Center Kloeckner, Ludwigshafen, Germany.

Test design: Static (96 h); 10 fish per treatment and aquarium; Assessment of mortality and toxic signs was done within 1 hour after start of exposure and after 6, 24, 48, 72 and 96 hours.

Endpoints: Mortality, sub-lethal effects.

Test conditions: Glass aquaria (test volume: 10 L); loading: 0.34 g fish/L; non-chlorinated, filtered tap water; total hardness: approx. 100 mg/L ($CaCO_3$); conductivity: approx. 250 µS/cm; acid capacity: approx. 2.5 mmol/L; temperature: 23 - 24 °C; pH: 7.9 - 8.3; dissolved oxygen: 5.2 to 8.5 mg/L; photoperiod: 16 h light, 8 h dark, light intensity: approx. 100 - 490 lux; no feeding; aeration after 48 h for 1 h due to low dissolved oxygen levels.

Test concentrations: Control, 0.46, 1.0, 2.2, 4.6 and 10 mg/L metconazole (BAS 555 F, Reg.No. 4056343)

Analytics: The concentrations of metconazole in the test solutions were determined using a HPLC method with MS-detection.

Statistics: The LC_{50} values were determined using the geometric mean of the LC_0 and LC_{100}.

II. RESULTS AND DISCUSSION

Analytical measurements: The analytically detected concentrations at test initiation for metconazole in comparison to the nominal value ranged from 100% - 106%. At test termination the analytically detected concentrations for Metconazole ranged from 92% - 101%. As the analytically determined concentrations confirmed the nominal concentrations, the biological results are based on nominal concentrations.

Biological results: Metconazole caused no mortality up to and including the concentration of 4.6 mg/L. 100% mortality were observed at the highest test rate of 10 mg/L. The results are summarized in Table 8.2.1-4.

Table 8.2.1-4: Acute toxicity (96 h) of metconazole on zebrafish (*Danio rerio*)

Concentration [mg/L] nominal	**Control**	**0.46**	**1.0**	**2.2**	**4.6**	**10**
Mortality [%]	0	0	0	0	0	100
Symptoms*	none	none	none	none	A, T	--
Endpoints [mg/L]						
LC_{50} (96 h)	6.8					
NOEC (96 h)	2.2					

* toxic symptoms: A: apathy, T: tottering
-- not observed, all animals dead

III. CONCLUSION

The 96-hour LC_{50} value for metconazole during a static acute toxicity test with zebrafish was determined to be 6.8 mg metconazole/L based on nominal concentrations. The no-observed effect concentration (NOEC) was 2.2 mg metconazole/L (nominal).

The following acute toxicity study with threespine stickleback (*Gasterosteus aculeatus*) performed with the active substance metconazole is not required for registration in the EU and it has not been evaluated previously on EU level. The study was conducted to generate data for the refined risk assessment.

Report:	CA 8.2.1/5 [redacted] 2010a BAS 555 F (Metconazole): Acute toxicity study in the Threespine Stickleback (Gasterosteus aculeatus) 2010/1007158
Guidelines:	(EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part C.1, OECD 203 (1992), EPA 72-1, EPA 712-C-96-118, EPA 540/9-82-024, EPA 850.1075
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

In a static acute toxicity laboratory study, threespine stickleback (*Gasterosteus aculeatus*) were exposed to nominal concentrations of 0 (control), 1.0, 1.8, 3.2, 5.6 and 10 mg metconazole/L in groups of 10 animals in glass aquaria containing 10 L water. Fish were observed for survival and symptoms of toxicity within 1 hour after start of exposure and 6, 24, 48, 72 and 96 hours after start of exposure.

The biological results are based on nominal concentrations. After 96 hours of exposure no mortality was observed in the control and at concentrations of up to 3.2 mg metconazole/L, whereas 100% mortality was observed at the two highest tested concentrations of 5.6 and 10 mg a.s./L. After 96 hours of exposure no symptoms of toxicity were observed for surviving fish at any of the test item concentrations.

In a static acute toxicity study with threespine stickleback the LC_{50} (96 h) of metconazole was 4.2 mg a.s./L based on nominal concentrations. The NOEC (96 h) was determined to be 3.2 mg a.s./L (nominal).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Metconazole (BAS 555 F, Reg. No. 4056343), batch no. AC12140-17, purity: 98.1% (tolerance ± 1%).

B. STUDY DESIGN

Test species: Threespine stickleback (*Gasterosteus aculeatus*), approx. 18 months old at test initiation; mean body length 4.1 cm (3.8 - 4.5 cm); mean body weight 0.51 g (0.37 - 0.71 g); supplied by "Fischzucht Rhönforelle", Rendelmühle, Germany.

Test design: Static system (96 hours); 10 fish per aquarium (loading 0.51 g fish/L) and per concentration; assessment of mortality and symptoms of toxicity within 1 hour after start of exposure and 6, 24, 48, 72 and 96 hours after start of exposure.

Endpoints: LC_{50}, NOEC, mortality and sublethal effects.

Test concentrations: Control, 1.0, 1.8, 3.2, 5.6 and 10 mg a.s./L (nominal).

Test conditions: Glass aquaria (30 x 22 x 24 cm), test volume: 10 L, non-chlorinated, filtered tap water mixed with deionized water; temperature: 12.0°C; pH 8.0 - 8.2; oxygen content: 7.6 mg/l - 10.4 mg/L; total hardness: approx. 100 mg $CaCO_3$/L; conductivity: approx. 250 µS/cm; photoperiod: 16 h light : 8 h dark; light intensity: approx. 70 lux - 570 lux; no aeration over the first 24 hours, afterwards slight aeration due to decreased oxygen content; no feeding.

Analytics: Analytical verification of test item concentrations was conducted using a HPLC-method with MS detection.

Statistics: Descriptive statistics; probit analysis for calculation of LC_{50}.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each concentration at the beginning and the end of the test. The analyzed contents of metconazole ranged from 92.0% to 98.9% of the nominal concentrations at test initiation and from 80.3% to 84.8% of the nominal concentrations at test termination As measured concentrations confirmed correct application of the test substance, the following biological results are based on nominal concentrations.

Biological results: After 96 hours of exposure no mortality was observed in the control and at concentrations of up to and including 3.2 mg metconazole/L, whereas 100% mortality was observed at the two highest tested concentrations of 5.6 and 10 mg a.s./L. After 96 hours of exposure no symptoms of toxicity were observed for surviving fish at any of the test item concentrations. The results are summarized in Table 8.2.1-5.

Table 8.2.1-5: Acute toxicity (96 hours) of metconazole on threespine stickleback (*Gasterosteus aculeatus*)

Concentration [mg /L] (nominal)	**Control**	**1.0**	**1.8**	**3.2**	**5.6**	**10**
Mortality [%]	0	0	0	0	100	100
Symptoms	none	none	none	none	n.d.	n.d.
Endpoints [mg a.s./L]						
LC_{50}	4.2					
NOEC	3.2					

n.d. = not determined; all fish dead

III. CONCLUSION

In a static acute toxicity study with threespine stickleback the LC_{50} (96 h) of metconazole was 4.2 mg a.s./L based on nominal concentrations. The NOEC (96 h) was determined to be 3.2 mg a.s./L (nominal).

CA 8.2.2 Long-term and chronic toxicity to fish

CA 8.2.2.1 Fish early life stage toxicity test

The following early life-stage test on sheepshead minnow (*Cyprinodon variegatus*) performed with the active substance metconazole is not required for registration in the EU and it has not been evaluated previously on EU level. The study was conducted due to U.S. data requirements and is used for the refined risk assessment.

Report:	CA 8.2.2.1/1 [redacted] 2009a Metconazole - Early life-stage toxicity test with sheepshead minnow (Cyprinodon variegatus) following OPPTS draft guideline 850.1400 2009/1129142
Guidelines:	EPA 850.1400
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary

The chronic toxicity of metconazole to sheepshead minnow (*Cyprinodon variegatus*) was evaluated in a 33 d (28 d post-hatch) early life stage test under flow-through conditions. The test was started with the exposure of embryos < 30 hours old. Each test concentration was replicated 4 times and was started with 30 embryos per replicate. On test day 5, the surviving larvae present in each incubation cup were thinned to 10 organisms per replicate/40 organisms per treatment level or control and placed into their respective exposure aquaria. Mean measured concentrations were (control) 0.95, 1.5, 3.0, 5.4, 11 and 24 µg a.s./L. In this study survival, appearance and behavior, total length and dry weight in sheepshead minnow were observed.

The following results are based on mean measured concentrations. No statistically significant effects compared to the control were observed in any of the treatment levels regarding hatching success, appearance and survival.

At test termination, total length of larvae in the control group averaged 25.6 mm. Mean total length of larvae exposed to 0.95, 1.5, 3.0, 5.4, 11 and 24 µg a.i./L were 25.7, 25.8, 25.4, 25.5, 25.4 and 24.9 mm, respectively. Statistical analysis (Williams' Test) determined a significant reduction in total length among fish exposed to 24 µg a.i./L compared to the control.

Dry weight of the larvae in the control group averaged 0.0687 g. Mean dry weight of larvae exposed to 0.95, 1.5, 3.0, 5.4, 11 and 24 µg a.i./L was 0.0699, 0.0716, 0.0707, 0.0728, 0.0684, and 0.0627 g, respectively. Statistical analysis (Williams' Test) determined a significant reduction in dry weight among fish exposed to 24 µg a.i./L compared to the control.

In an early life stage test conducted under flow through conditions, fathead minnow were exposed to metconazole over a period of 33 days (28 days post-hatch). The overall NOEC identified in this study was 11 µg a.s./L (mean measured concentration).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Metconazole (CAS No. 125116-23-6), Lot No. AS2122a, purity: 99.4%

B. STUDY DESIGN

Test species: Sheepshead minnow (*Cyprinodon variegatus*), embryos < 30 hours old, source: Aquatic Biosystems, Fort Collins, Colorado.

Test design: Flow-through system (33 days; 28 days post-hatch); 4 replicates per treatment with 30 embryos each. On test day 5, the surviving larvae present in each incubation cup were thinned to 10 organisms per replicate/40 organisms per treatment level or control and placed into their respective exposure aquaria. Survival, appearance and behavior were monitored daily. Length and dry weight were determined at study termination (28 days post-hatch).

Endpoints: NOEC values based on survival, appearance and behavior, growth.

Test concentrations: Control, 0.94, 1.9, 3.8, 7.5, 15 and 30 µg a.s./L (nominal). Mean measured concentrations: control, 0.95, 1.5, 3.0, 5.4, 11 and 24 µg a.s./L.

Test conditions: Test vessels: glass aquaria (test volume 6.5 L); embryo incubation cups: round glass jars.
Dilution water: natural filtered seawater; temperature 24 - 26°C; pH 7.6 - 8.0; salinity: 20 – 21%; oxygen content 5.6 - 7.8 mg/L.
Flow rate: 51 L/day/test vessel
Photoperiod: 16 hours light: 8 hours darkness, 550 - 950 Lux.
Feeding: Nauplii of *Artemia salina* three times daily *ad libitum*.

Analytics: The test item concentrations were analyzed using a LC/MS/MS method.

Statistics: Descriptive statistics, Shapiro-Wilks´ Test for normality; Bartlett´s Test; Kruskal-Wallis´ Test; Williams´ Test.

II. RESULTS AND DISCUSSION

Analytical measurements: The mean analytically determined concentrations of the test substance in the test water were in the range 72 - 101% of the nominal concentration. Based on mean measured concentrations, treatment levels were defined as 0.95, 1.5, 3.0, 5.4, 11 and 24 µg metconazole/L. The results of the study are based on mean measured concentrations.

Biological results: No statistically significant effects compared to the control were observed in any of the treatment levels regarding hatching success, appearance and survival.

At test termination, total length of larvae in the control group averaged 25.6 mm. Mean total length of larvae exposed to 0.95, 1.5, 3.0, 5.4, 11 and 24 µg a.i./L were 25.7, 25.8, 25.4, 25.5, 25.4 and 24.9 mm, respectively. Statistical analysis (Williams' Test) determined a significant reduction in total length among fish exposed to 24 µg a.i./L compared to the control.

Dry weight of the larvae in the control group averaged 0.0687 g. Mean dry weight of larvae exposed to 0.95, 1.5, 3.0, 5.4, 11 and 24 µg a.i./L was 0.0699, 0.0716, 0.0707, 0.0728, 0.0684, and 0.0627 g, respectively. Statistical analysis (Williams' Test) determined a significant reduction in dry weight among fish exposed to 24 µg a.i./L compared to the control.
The results are summarized below (see Table 8.2.2.1-1).

Table 8.2.2.1-1: Chronic toxicity (ELS, 33 days (28 days post-hatch)) of metconazole on sheepshead minnow (*Cyprinodon variegatus*)

Concentration [µg a.s./L] nominal	**Control**	**0.94**	**1.9**	**3.8**	**7.5**	**15**	**30**
Concentration [µg a.s./L] mean measured	**Control**	**0.95**	**1.5**	**3.0**	**5.4**	**11**	**24**
Embryo hatching success [%]	99	99	98	99	97	99	99
Normal fry at hatch [%]	99	100	100	100	100	100	100
Larval survival [%]	100	98	95	98	95	100	98
Total length [mm]	25.6	25.7	25.8	25.4	25.5	25.4	24.9*
Dry weight [g]	0.0687	0.0699	0.0716	0.0707	0.0728	0.0684	0.0627*
	Endpoint [µg a.s./L]						
	mean measured						
Overall NOEC	**11**						

* Statistically significant differences compared to the control (Williams´ Test)

III. CONCLUSION

In an early life stage test conducted under flow through conditions, fathead minnow were exposed to metconazole over a period of 33 days (28 days post-hatch). The overall NOEC identified in this study was 11 µg a.s./L (mean measured concentration).

The following fish early life-stage test on fathead minnow, (*Pimephales promelas*) performed with the active substance metconazole is not required for registration in the EU and it has not been evaluated previously on EU level. The study was conducted due to U.S. data requirements and is used for the refined risk assessment.

Report:	CA 8.2.2.1/2 [redacted] 1992a Metconazole (WL136184, KNF-S-474c): An early life stage test with the fathead minnow, Pimephales promelas MK-512-001
Guidelines:	EPA 40 CFR Part 796, EPA 40 CFR Part 797, TSCA 40 CFR 798, OECD Guidelines for Testing of Chemicals (Draft Nov. 1988), American Society for Testing and Materials. Standard Guide for Conducting Early Life Stage Toxicity Tests with Fishes (E1241-88 1988)
GLP:	yes (certified by Department of Health and Social Security of the Government of the United Kingdom, United Kingdom)

Executive Summary

The chronic toxicity of metconazole to fathead minnow (*Pimephales promelas*) was evaluated in a 35 d early life stage test under flow-through conditions. Each test concentration was replicated 3 times and the test was started with the exposure of 30 embryos per replicate.
Nominal concentrations were: 0 (control), 0.01, 0.031, 0.093, 0.28, 0.83 and 2.5 mg a.s./L (corresponding to time weighted average concentrations of 0.011, 0.032, 0.098, 0.27, 0.78 and 2.4 mg a.s./L). In this study survival, appearance and behavior, length, wet weight and dry weight in fathead minnow were recorded daily.

The following results are based on nominal concentrations. Survival of embryos over the first 48 h of the test was 99% to 100% in all test vessels. The percentage of embryos that failed to hatch was 0% to 10% and was not dose-related. The mean day on which hatch started (day 3) and finished (day 6) was the same in all treatments. The highest concentration in which fry survival after 35 days of exposure was not statistically different from that in the control was 0.031 mg a.s./L.
At the end of the experiment the highest NOEC for fry length and wet weight was 0.031 mg a.s./L and that for fry dry weight was 0.010 mg a.s./L. The lowest observed effect concentration for fry length has been taken to be the highest concentration causing a reduction in fry length (0.093 mg a.s./L), although at the lowest test concentration (0.01 mg a.s./L) the fry were significantly longer than the control fish. At concentrations greater than the NOECs, all three measures of fish size showed a monotonic concentration/ response with fry size decreasing as metconazole concentration increased.
Abnormalities observed during the study were either a failure to develop or immobility. At the end of the experiment there were two fish with deformed spines but no other sub-lethal effects were observed from days 28-35. Thus, it is probable that the fish with damaged spines at the end of the test were injured as they died or as they were removed from the test vessels.

In an early life stage test conducted under flow through conditions, fathead minnow were exposed to metconazole over a period of 35 days. The overall NOEC identified in this study was 0.01 mg a.s./L (nominal).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Metconazole (BAS 555 F), batch no. F900250, purity: metconazole cis-isomer: 95.2% - 95.5%, metconazole trans-isomer: 0.1% and 1,3,4-triazole analogue: 4.0% - 4.2%.

B. STUDY DESIGN

Test species: Fathead minnow (*Pimephales promelas*); embryos at the stage between the blastoderm enveloping one third of the yolk sphere and the closure of the blastopore; source: "Aquatic Bioassay Laboratories Inc.", Baton, USA.

Test design: Flow-through system (35 days); 3 replicates per treatment with 30 embryos each. The number of dead and live embryos in each test vessel was recorded daily and after hatching commenced the numbers of live normal, live abnormal and dead fry were also recorded daily.

Endpoints: NOEC values based on survival, appearance, behavior and growth.

Test concentrations: 0 (control), 0.01, 0.031, 0.093, 0.28, 0.83 and 2.5 mg a.s./L (nominal) (corresponding to time weighted average concentrations of 0.011, 0.032, 0.098, 0.27, 0.78 and 2.4 mg a.s./L).

Test conditions: Test vessels: glass aquaria (290 x 190 x 140 mm); test volume either 2.8 L (day 0 - day 15) or 5.9 L (day 15 - day 35); embryo cups: glass tube (180 mm length, 63 mm diameter) fitted with nylon mesh base.
Dilution water: UV-filtered mixture of filtered dechlorinated water and deionised water (mixture ratio: 1:5), pH 6.5 - 7.6; total hardness: 56 - 84 mg $CaCO_3$/L; conductivity: 90 - 140 µS/cm.
Flow rate: 25 mL/min (± 8%); day 0 - day 15: 13 volume additions per 24 h/test vessel; day 15 - day 35: 6.1 volume additions per 24 h/test vessel.
Photoperiod: not reported.
Feeding: days 3 - 9: unoiled "Pruteen" (commercially produced protein feed material), days 5 - 34: brine shrimp (*Artemia salina*) < 48 h old, days 16 - 33: proprietary flaked food ("Tetramin®"); food was provided as *ad libitum*.
Temperature: 24 - 25.5°C.
Oxygen content: 6.9 - 8.5 mg/L (83% - 103%).

Analytics: The test item concentrations were analyzed using a GC method with nitrogen/phosphorus detection.

Statistics: Descriptive statistics, Cochran-Armitage trend test and Dunnett's test for determination of the NOEC values ($\alpha = 0.05$).

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical measurements of metconazole concentrations were conducted in the four lower test item concentrations at the beginning of the test, on days 5, 7, 12 and 13, and from day 19 on every day until test termination on day 35. In the two highest test item concentrations, analytical concentrations were only determined at the beginning of the test and on days 5, 7, 12 and 13. The time-weighted mean measured concentrations of the test item in the test media over the whole study period were in the range of 94% to 110% of the nominal concentrations. The following biological results are based on nominal concentrations.

Biological results: Survival of embryos over the first 48 h of the test was 99% to 100% in all test vessels. The percentage of embryos that failed to hatch was 0% to 10% and was not dose-related. The mean day on which hatch started (day 3) and finished (day 6) was the same in all treatments. The highest concentration in which fry survival after 35 days of exposure was not statistically different from that in the control was 0.031 mg a.s./L (Cochran-Armitage trend test, $\alpha = 0.05$ (one-sided)).
At the end of the experiment the highest NOEC for fry length and wet weight was 0.031 mg a.s./L and that for fry dry weight was 0.010 mg a.s./L. The lowest observed effect concentration for fry length has been taken to be the highest concentration causing a reduction in fry length (0.093 mg a.s./L), although at the lowest test concentration (0.01 mg a.s./L) the fry were significantly longer than the control fish. At concentrations greater than the NOECs, all three measures of fish size showed a monotonic concentration/ response with fry size decreasing as metconazole concentration increased.
Abnormalities observed during the study were either a failure to develop or immobility. At the end of the experiment there were two fish with deformed spines but no other sub-lethal effects were observed from days 28-35. Thus, it is probable that the fish with damaged spines at the end of the test were injured as they died or as they were removed from the test vessels. The results are summarized below (see Table 8.2.2.1-2).

Table 8.2.2.1-2: Chronic toxicity (ELS, 35 days) of metconazole on fathead minnow (*Pimephales promelas*)

Concentration [mg a.s./L] (nominal)	**Control**	**0.01**	**0.031**	**0.093**	**0.28**	**0.83**	**2.5**
Concentration [mg a.s./L] (time weighted average)	**--**	**0.011**	**0.032**	**0.098**	**0.27**	**0.78**	**2.4**
Survival of embryos on day 2 [%]	99	100	99	99	99	100	100
Mean start of hatch [day]	3	3	3	3	3	3	3
Mean end of hatch [day]	6	6	6	6	6	6	6
Number of embryos that produced fry	85	88	86	89	87	88	89
Number of live fry on day 35	75	71	72	34 ***	3 ***	0 ***	0 ***
Mean wet weight on day 35 [mg]	56	65	44	19 ++	5.0 ++	-- a)	-- a)
Mean dry weight on day 35 [mg]	11	11	7.7 +	2.3 ++	1.1 ++	-- a)	-- a)
Mean length on day [mm]	16	17 +	15	9.8 ++	8.0 ++	-- a)	-- a)
	Endpoint [mg metconazole/L] (nominal)						
Overall NOEC (35 d)	0.01						

*** Statistically significant differences compared to the control (Cochran-Armitage trend test, $p < 0.001$ (one-sided)).
+ Statistically significant differences compared to the control (Dunnett's test $p < 0.05$).
++ Statistically significant differences compared to the control (Dunnett's test $p < 0.01$).
a) At the test item concentrations of 0.83 and 2.5 mg a.s./L, fish did not survive to the end of the test.

III. CONCLUSION

In an early life stage test conducted under flow through conditions, fathead minnow were exposed to metconazole over a period of 35 days. The overall NOEC identified in this study was 0.01 mg a.s./L (nominal).

CA 8.2.2.2 Fish full life cycle test

The following fish full life cycle test on fathead minnow (*Pimephales promelas*) performed with the active substance metconazole is not required for registration in the EU and it has not been evaluated previously on EU level. The study was conducted to generate data for the refined risk assessment and to clarify potential endocrine effects.

Report:	CA 8.2.2.2/1 [redacted] 2008a BAS 555 F (Metconazole) - Life cycle test on the fathead minnow (Pimephales promelas) in a flow through system 2008/1064993
Guidelines:	EPA 72-5, EPA 850.1500, EPA 712-C-96-122
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary
The chronic toxicity of metconazole to fathead minnow (*Pimephales promelas*) was evaluated in a 5-month full life cycle test under flow-through conditions. The test was started with the exposure of freshly fertilized eggs derived from 7 egg clutches. Each test concentration was replicated 4 times and was started with 25 eggs per replicate. On day 36 the number of individuals per replicate was reduced to 15. The observation of egg-laying started on day 60 and a pair wise exposure was started on day 93. The reproduction of 12 pairs per test group was monitored over 14 days. Observation of the F2-generation started on days 112 - 116 with 4 replicates per test group, each consisting of 25 eggs from a specific pair of this test group. The F2-generation was exposed for 42 days after insertion of eggs. Nominal concentrations were: 0 (Control), 0.32, 1.0, 3.2 and 10 µg a.s./L. In this study survival, appearance and behavior, growth, sexual development and sex ratio in fathead minnow were observed over two generations. The reproduction of the F1-generation was monitored by recording egg-laying and fertility. A histological examination of the gonads of both generations was performed as well as a determination of vitellogenin in the blood plasma of 6 pairs per test group (F1-generation) and in whole body homogenate of 12 fish per test group (F2-generation).

The following results are based on nominal and mean measured concentrations. In the test concentrations up to and including 3.2 µg a.s./L (3.58 µg a.s./L mean measured) no treatment related effects on the test organisms were observed.
In the tests group exposed to a concentration of 10 µg a.s./L (10.43 µg a.s./L mean measured concentration) the survival in the F2-generation was statistically significantly reduced.
An inconclusive effect on growth (body length) was observed in the F1- generation on day 36. In the F2-generation the body weight and body length of female fish was markedly reduced.
During reproduction the fertility of the 10 µg a.s./L group was statistically significantly decreased and a marked, but not statistically significant reduction of the egg-production was recorded as well as a delay in the time to maturity (time to first spawn).

No test substance effect on the vitellogenin level in the F1- or F2-generation was found, however, the determination of vitellogenin in the F1-generation was considered invalid due to a technical failure. This does not impair the study conclusion, since vitellogenin only serves as an indicative biomarker and the population relevant parameters (i.e. reproduction and sex ratio) are of more relevance.

The sex ratio was not influenced by the test substance. The histological examination revealed no treatment related effect on the development of the gonads. In the 10 µg a.s./L concentration group indications for a toxic effect on the liver were found in the F1-generation.

In a full life cycle test conducted under flow-through conditions, fathead minnow were exposed to metconazole over a period of 5 months. The overall NOEC identified in this study was 3.2 µg a.s./L (nominal concentration) and 3.58 µg a.s./L (mean measured concentration).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Metconazole (BAS 555 F, Reg. No. 405 6343), batch no. 43707, purity: 99.1%

B. STUDY DESIGN

Test species: Fathead minnow (*Pimephales promelas*), freshly fertilized eggs, source: parental from Osage catfisheries, Osage Beach (MO), USA.

Test design: Flow-through system (5 months); 4 replicates per treatment with 25 fertilized eggs each. On day 36 no. of individuals/replicate was reduced to 15. Pairwise exposure from day 93. F2-generation was started on days 112 - 116 with 4 replicates/test group, with 25 eggs each. F2-generation was exposed for 42 days after insertion of eggs. Survival, appearance and behavior, growth, sexual development and sex ratio were observed over two generations. Reproduction of the F1-generation was monitored by recording egg-laying and fertility from day 60 on. A histological examination of the gonads of both generations was performed as well as a determination of vitellogenin in the blood plasma of 6 pairs per test group (F1-generation) and in whole body homogenate of 12 fish per test group (F2-generation).

Endpoints: NOEC values based on survival, appearance and behavior, growth, sexual development, sex ratio, histology, vitellogenin content in blood plasma.

Test concentrations:	Control, 0.32, 1.0, 3.2 and 10 µg a.s./L (nominal).
Test conditions:	Test vessels: 1.7 L cylindrical glass vessels for eggs, larvae and juveniles; 30 L stainless steel aquaria from day 17 on; 9 L stainless steel aquaria for spawning pairs Dilution water: non-chlorinated, filtered drinking water; temperature 25 ± 1°C; pH 7.5 - 7.9; oxygen content 6.4 - 8.9 mg/L; total hardness: 100 - 104 mg $CaCO_3$/L; conductivity 256 - 268 µSi; acid capacity 2.16 - 2.28 mmol/L Flow rate: 5 L/hour/test vessel (early life stages, juveniles and adults), 1.9 L/hour for each test vessel (paired groups) Photoperiod: 16 hours light: 8 hours darkness F1: 1.7 L test vessels: 222 - 528 Lux, stainless steel aquaria (24 L): 86 - 280 Lux; F1 pair groups: stainless steel aquaria (9 L): 58 - 164 Lux; F2: 1.7 L vessels: 323 - 452 Lux, stainless steel aquaria (24 L): 176 - 248 Lux Feeding: Freshly hatched nauplii of *Artemia salina* for newly hatched fish larvae, and commercial fish diet and larvae of *Artemia salina* for juveniles and adults Aeration: from day 28 (F1), from day 112 (F1 pair groups) or from day 143 (F2).
Analytics:	The test item concentrations were analyzed using a HPLC/MS method.
Statistics:	Descriptive statistics, one-sided Fisher´s exact test and one-sided Wilcoxon-Test for survival; two-sided Dunnett´s test for weight and length data; two-sided Fisher´s exact test and two-sided Wilcoxon test for sex ratio; two-sided Wilcoxon-Test for vitellogenin content.

II. RESULTS AND DISCUSSION

Analytical measurements: The mean analytically determined concentrations of the test substance in the test water were generally in the range of ±20% of the nominal concentration during exposure of the F1- generation until the pairwise exposure started. During pairwise exposure a decreased concentration (37% of the nominal value) was observed on one occasion for 6 out of 12 pairs of the test group exposed to 3.2 µg a.s./L (nominal). Since no obvious difference was observed between these pairs and the other 6 pairs, which were correctly exposed, it was concluded that the transient deviation had no influence on the test results. The F2-generation originated from pairs that were exposed to the correct concentration. Furthermore a part of the pairs (6 out of 12) of the test group 10 µg a.s./L (nominal) were exposed transiently to 188% of the nominal concentration during the reproduction phase. In this case the F2-generation was derived also from pairs that were correctly exposed. On two additional occasions the pair groups of the F1-generation and the F2-generation of all concentration groups were transiently exposed to elevated concentrations.

In one case for 42 h up to 220% of the nominal value, in the other case for in maximum 6 h up to 201% of the nominal value. In the concentration groups up to and including 3.2 µg a.s./L the transiently increased concentrations did not lead to statistically significant deviations in comparison to the control group and had thus no effect on the study results.

In the test concentration 10 µg a.s./L (nominal) the maximum concentrations measured during the three incidences were 188%, 165% and 145% in the pair groups and 169% in the F2-generation (the third deviation over 6 h had no influence on the F2-generation of this test group). The test substance effects in this test group may therefore be increased by the deviations in the exposure concentration. However, since indications for effects were already observed during the time period before the first deviation occurred (inconclusive effects on growth on day 36, delayed time to maturity), it was concluded, that effects in this concentration would have been observed also if the concentrations would have been completely in the range of the nominal concentration. Mean measured concentrations from day 0 - 100 (F1 generation) were between 91 and 104%, from day 93 - 136 (F1 pair groups) between 109 and 120% and for F2 generation 104 - 121%. Considering the fact that nominal test concentrations were not met during some test periods, emphasis should be given to the results based on mean measured test concentrations.

Biological results:

In the test concentrations up to and including 3.2 µg a.s./L (3.58 µg a.s./L mean measured) no treatment related effects on the test organisms were observed. In the tests group exposed to a concentration of 10 µg a.s./L (10.43 µg a.s./L mean measured concentration) the survival in the F2-generation was statistically significantly reduced.

An inconclusive effect on growth (body length) was observed in the F1- generation on day 36. In the F2-generation the body weight and body length of female fish was markedly reduced.

During reproduction the fertility of the 10 µg a.s./L group (10.43 µg a.s./L mean measured concentration) was statistically significantly decreased and a marked, but not statistically significant reduction of the egg-production was recorded as well as a delay in the time to maturity (time to first spawn).

No test substance effect on the vitellogenin level in the F1- or F2-generation was found, however, the determination of vitellogenin in the F1-generation was considered invalid due to a technical failure. This does not impair the study conclusion, since vitellogenin only serves as an indicative biomarker and the population relevant parameters (i.e. reproduction and sex ratio) are of more relevance. In this study the sex ratio was not influenced by the test substance.

The histological examination revealed no effect of the test substance on the development of the gonads. In the 10 µg a.s./L group (10.43 µg a.s./L mean measured concentration) indications for a toxic effect on the liver were found in the F1-generation. The results are summarized below Table 8.2.2.2-1.

Table 8.2.2.2-1: Chronic toxicity (FLC, 5 months) of Metconazole on fathead minnow (*Pimephales promelas*)

Concentration [µg a.s./L] nominal		**Control**	**0.32**	**1.0**	**3.2**	**10**
Concentration [µg a.s./L] mean measured over all study parts		**Control**	**0.32**	**1.14**	**3.58**	**10.43**
F1 generation	survival [%]: start - hatch	94	95	95	92	93
	survival [%]: hatch - swim up	100	99	99	99	100
	survival [%]: swim up - reduction	88	89	90	92	91
	survival [%]: reduction - start reproduction	100	98	95	100	98
	survival [%]: start reproduction - sacrifice	96	100	100	96	92
	length on day 36 [cm]	2.57	2.64	2.66	2.63	2.41**[a]
	length on day 66 [cm]	4.44	4.54	4.67*[b]	4.65*[b]	4.45
	male length at sacrifice (day 140) [cm]	6.85	7.00	6.73	6.67	6.75
	female length at sacrifice (day 140) [cm]	5.27	5.67*[b]	5.41	5.36	5.37
	male weight at sacrifice (day 140) [g]	4.58	4.60	4.35	4.46	4.12
	female weight at sacrifice (day 140) [g]	1.56	2.04**[b]	1.74	1.72	1.73
	% fertility	99.2	98.4	99.2	98.5	**73.3***
	eggs/female/day	11.86	12.81	21.11	20.69	**6.93**
	clutches/female/day	0.141	0.173	0.202	0.247*[b]	**0.083**
	mean VTG pair males [ng/mL] [c]	37684	41475	1989674	2138820	176111
	mean VTG pair females [ng/mL] [c]	5979676	7171244	11232927	12131155	8816437
	sex ratio [% males]	58	59	56	55	69
F2 generation	survival [%]: start - hatch	93	94	92	93	92
	survival [%]: hatch - swim up	90	90	96	85*[d]	91
	survival]%]: swim up - end	89	87	95	92	**75***
	male length at sacrifice (day 42) [cm]	2.92	3.32**[b]	3.28**[b]	3.32**[b]	3.11
	female length at sacrifice (day 42) [cm]	3.13	3.19	3.21	3.27	**2.84****
	male weight at sacrifice (day 42) [g]	0.24	0.37**[b]	0.33** [b]	0.35** [b]	0.31*
	female weight at sacrifice (day 42) [g]	0.29	0.31	0.31	0.34** [b]	**0.23****
	mean VTG pair males [ng/mL]	< 2.19	< 1.43	375 [e]	1.25	707 [e]
	mean VTG pair females [ng/mL]	337	1812	75	< 1.21	< 2.14
	sex ratio [% males]	32	54	54	53	40

	Endpoints [µg a.s./L]	
	nominal	**mean measured**
Overall NOEC	3.2	**3.58**

* Statistically significant differences compared to the control ($p \leq 0.05$)
** Statistically significant differences compared to the control ($p \leq 0.01$)
Deviations which are considered to be substance-related are printed **bold**.
[a] Inconclusive effect, since the deviation was less than 10% and on day 66 no reduction of growth was observed.
[b] Value better than control, not regarded as adverse effect.
[c] Values invalid, due to low reliability of the measurement of the samples.
[d] Clearly no effect of the test substance
[e] Increased value caused by value of one fish

III. CONCLUSION

In a full life cycle test conducted under flow through conditions, fathead minnow were exposed to Metconazole over a period of 5 months. The overall NOEC identified in this study was 3.2 µg a.s./L (nominal concentration) and 3.58 µg a.s./L (mean measured concentration).

CA 8.2.2.3 Bioconcentration in fish

A bioconcentration study performed with rainbow trout has already been evaluated during the previous Annex I inclusion process of metconazole. No additional bioconcentration studies are required and no (new) study has been conducted.

A biomagnification study with rainbow trout was found in peer-reviewed literature and summarized in M-CA 8.2.8. No biomagnification in fish was seen due to dietary intake of metconazole.

CA 8.2.3 Endocrine disrupting properties

Metconazole belongs to the group of DMI fungicides, which inhibit ergosterol biosynthesis in the fungal cell. Due to the structural analogy of the target enzyme with aromatase, this group of active substances is under suspicion of having endocrine potential. Aromatase has a decisive role in sexual differentiation by catalyzing the transformation of testosterone to 17β-estradiole.

A fish full life cycle test with fathead minnow (*Pimephales promelas*) was performed to clarify if metconazole had any relevant endocrine potential (DocID 2008/1064993). Population relevant parameters like reproductive performance and sex ratio (including histological examination of the gonads of both generations) did not show an effect of metconazole up to and including 3.58 µg a.s./L (mean measured). At 10 µg a.s./L, survival and growth (body weight and body length of female fish) in the F2 generation was reduced and fertility in the F1 generation was statistically significantly decreased.

Expectation for an endocrine mediated effect with this substance class would be a shift in sex ratio towards male fish. However, up to the highest test concentration no effect on sex ratio (including histological examination of the gonads of both generations) could be detected. In contrast, the effects seen at 10 µg a.s./L, which determine the NOEC, reflect general toxicity of the compound under long-term exposure as already seen in the fish early life stage studies, which yielded similar NOEC values.

In conclusion, based on results of available long-term fish studies (and studies on terrestrial vertebrates; see chapter M-CA 8.1.5) there is no indication for a relevant endocrine potential of metconazole. This is supported by several impact assessments conducted by different organizations (see M-CA 5.8.3).

CA 8.2.4 Acute toxicity to aquatic invertebrates

CA 8.2.4.1 Acute toxicity to Daphnia magna

The following acute toxicity study on *Daphnia magna* performed with the active substance metconazole has not been evaluated previously on EU level. The study was conducted with test material consisting of a different isomeric ratio (cis:trans - 95:5). It confirms the toxicity endpoint of the standard study used for the risk assessment (MK-510-001), although it shows some deficiencies (lack of analytical verification and inconclusive reporting for first trial). The study is submitted here only for completeness and as supplemental data.

Report:	CA 8.2.4.1/1 Toy R., 1991a WL136184 (KNF-S-474c): Acute toxicity to Oncorhynchus mykiss, Daphnia magna and Selenastrum capricornutum MK-510-002
Guidelines:	OECD 203, OECD 202, OECD 201
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Executive Summary

In a 48-hour static acute toxicity laboratory study, water flea neonates were exposed to metconazole at a nominal concentration range of 0.94 to 15.0 mg metconazole/L. Since this range of concentrations did not include a no observed effect concentration, a second test was performed in which daphnids were exposed to nominal concentrations of 0.061, 0.12, 0.24, 0.49, 0.97, 2.0, 3.9, 7.8 and 15.6 mg metconazole/L. Both tests were conducted with 2 replicates per concentration, containing 10 daphnids each. Daphnids were observed for immobility 24 hours and 48 hours after start of exposure.

The biological results are based on nominal concentrations. As the range of concentrations used in the first test with did not include a no observed effect concentration, analysis of toxicity data has been made based on the results of the second test only. In the second test after 48 hours of exposure, no immobility of daphnids was observed in the control and at test item concentrations of up to and including 0.97 mg a.s./L, whereas 5%, 65% and 95% of the daphnids were immobile at the test item concentrations of 2.0, 3.9, 7.8 and 15.6 mg a.s./L. At the highest tested concentration of 15.6 mg a.s./L all daphnids were immobile after 48 hours of exposure.

In a 48-hour static acute toxicity study with *Daphnia magna* the EC_{50} of metconazole was 3.6 mg a.s./L based on nominal concentrations. The NOEC was determined to be 0.97 mg a.s./L (nominal).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Metconazole (BAS 555 F), batch no. F900250, purity: metconazole cis-isomer: 95.2%, metconazole trans-isomer: 0.1% and 1,3,4-triazole analogue: 4.2%.

B. STUDY DESIGN

Test species: Water flea (*Daphnia magna* STRAUS), neonates obtained from culture held at "ICI Brixham Laboratory", originally obtained from Institute National de Recherché Chimique Appliquée, France), < 24 hours old at test initiation.

Test design: Static system (48 hours), Test 1: Control, concentration range of 0.94 to 15.0 mg metconazole/L (nominal) plus control; Test 2: 9 test concentrations item plus control, 2 replicates with 10 daphnids in each; assessment of immobility after 24 and 48 hours.

Endpoints: NOEC, EC_{50} based on immobility of daphnids.

Test concentrations: Test 1: Control, concentration range of 0.94 to 15.0 mg metconazole/L (nominal).
Test 2: Control, 0.061, 0.12, 0.24, 0.49, 0.97, 2.0, 3.9, 7.8 and 15.6 mg metconazole/L (nominal).

Test conditions: Crystallizing dishes, test volume 100 mL, dilution water: reconstituted fresh water; photoperiod: not reported; light intensity: not reported.
Test 1: temperature: 18 - 20°C; pH 8.2 - 8.5; oxygen content: 9.0 mg/L - 9.6 mg/L; total hardness: 166 - 190 mg $CaCO_3$/L; alkalinity: 105 - 115 mg $CaCO_3$/L.
Test 2: temperature: 18 - 20°C; pH 8.3 - 8.6; oxygen content: 8.8 mg/L - 9.2 mg/L; total hardness: 176 - 180 mg $CaCO_3$/L; alkalinity: 107 - 112 mg $CaCO_3$/L.

Analytics: Analytical verification of test item concentrations was conducted using a GC-method with nitrogen selective detection.

Statistics: Descriptive statistics; probit analysis for EC_{50} (48 h) calculation.

II. RESULTS AND DISCUSSION

Analytical measurements: In the first test, analytical verification of test item concentrations was conducted in the test item concentrations of 0.94, 3.8 and 15.0 mg a.s./L at the beginning and at the end of the test. The analysed contents of metconazole ranged from 95% to 100% of nominal at test initiation and from 101% to 109% of nominal at test termination.
In the second test, analytical verification of test item concentrations was only conducted at the beginning of the test in the test item concentrations of 0.061, 0.24, 2.0 and 15.6 mg a.s./L. The analysed contents in the samples were determined to be 100% of nominal concentrations. No analytical verification was conducted for the other test concentrations or at the end of the test.

Biological results: The range of concentrations used in the first test did not include a no observed effect concentration, therefore, analysis of toxicity data has been made based on the results of the second test only.
In the second test after 48 hours of exposure, no immobility of daphnids was observed in the control and at test item concentrations of up to and including 0.97 mg a.s./L, whereas 5%, 65% and 95% of the daphnids were immobile at the test item concentrations of 2.0, 3.9, 7.8 and 15.6 mg a.s./L. At the highest tested concentration of 15.6 mg a.s./L all daphnids were immobile after 48 hours of exposure. The results of the second test are presented in Table 8.2.4.1-1.

Table 8.2.4.1-1: Effects of metconazole on *Daphnia magna* immobility

Concentration [mg/L] (nominal)	**Control**	**0.061**	**0.12**	**0.24**	**0.49**	**0.97**	**2.0**	**3.9**	**7.8**	**15.6**
Immobility (24 h) [%]	0	0	0	0	0	0	0	0	40	100
Immobility (48 h) [%]	0	0	0	0	0	0	5	65	95	100
Endpoints [mg metconazole/L] (nominal)										
EC_{50} (48 h)	3.6 (95% confidence limits: 3.0 - 4.3)									
NOEC (48 h)	0.97									

III. CONCLUSION

In a 48-hour static acute toxicity study with *Daphnia magna* the EC_{50} of metconazole was 3.6 mg a.s./L based on nominal concentrations. The NOEC was determined to be 0.97 mg a.s./L (nominal). The endpoint is not used in the risk assessment due to the reasons outlined above.

CA 8.2.4.2 Acute toxicity to an additional aquatic invertebrate species

This point is not triggered and not addressed via (new) toxicity studies.

CA 8.2.5 Long-term and chronic toxicity to aquatic invertebrates

CA 8.2.5.1 Reproductive and development toxicity to Daphnia magna

The following chronic toxicity test on the water flea *Daphnia magna* performed with the active substance metconazole has not been evaluated previously on EU level. The study was conducted with test material consisting of a different isomeric ratio (cis:trans - 95:5). It confirms the toxicity endpoint of the standard study used for the risk assessment and is submitted here only for completeness and as supplemental data.

Report:	CA 8.2.5.1/1 Toy R., 1991c WL136184 (KNF-S-474c): Chronic toxicity to Daphnia magna MK-523-001
Guidelines:	OECD 202
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Executive Summary

In a 21-day semi-static toxicity test, effects of metconazole to water fleas (*Daphnia magna*) were examined. Neonates less than 24 hours old were exposed to nominal concentrations of 0.0083, 0.042, 0.21, 1.0 and 8.1 mg a.s./L and a dilution water control. All treatment groups and the control consisted of 4 replicates with 10 parent daphnids in each. Adult survival, reproduction and number of moulted exoskeletons were recorded daily over the exposure period of 21 days. Body length was assessed at test termination after 21 days of exposure.

The biological results are based on nominal concentrations. After 21 days of exposure, 10% parent mortality was observed in the control, whereas 5%, 10%, 13% and 57% mortality occurred at the test item concentrations of 0.0083, 0.042, 0.21 and 1.0 mg a.s./L, respectively. At the highest test item concentration, all daphnids were dead at test end. The first brood in the test was produced after 7 - 8 days. White eggs and dead young were produced by *D. magna* at the test item concentration of 1.0 mg a.s./L.
In a 21-day semi-static toxicity study with *Daphnia magna* the NOEC of metconazole was determined to be 0.21 mg a.s./L based on nominal concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Metconazole (BAS 555 F), batch no. F900250, purity: metconazole cis-isomer: 95.2%, metconazole trans-isomer: 0.1% and 1,3,4-triazole analogue: 4.2%.

B. STUDY DESIGN

Test species: Water flea (*Daphnia magna* STRAUS); neonates < 24 hours old at test initiation from culture held at "ICI Brixham Laboratory", originally obtained from Institute National de Recherché Chimique Appliquée, France).

Test design: Semi-static system (21 days), 5 test concentrations plus control, 4 replicates per treatment with 10 daphnids per test vessel; daily assessment of mortality, reproductive performance and number of moulted exoskeletons; assessment of body length at test termination after 21 days of exposure.

Endpoints: LC_{50}; NOEC based on survival, reproduction and growth parameters.

Test concentrations: Control, 0.0083, 0.042, 0.21, 1.0 and 8.1 mg a.s./L (nominal).

Test conditions: Test beakers, test volume 1500 mL; dilution water: reconstituted fresh water; 30 mL soil extract per test vessel; temperature: 17°C - 20°C; pH 7.8 - 8.4; oxygen content: 8.4 mg/L - 10.6 mg/L; total hardness: 120 - 178 mg $CaCO_3$/L; alkalinity: 98 - 125 mg $CaCO_3$/L; light intensity: about 200 - 250 lux; photoperiod 16 hours light : 8 hours dark; feeding: 1 x 10^5 cells/mL *Chlorella vulgaris* per test vessel at test initiation and at renewal of test solutions (2 or 3 days interval).

Analytics: Analytical verification of test item concentrations was conducted using a GC-method with nitrogen selective detection.

Statistics: Descriptive statistics; determination of the NOEC values: Fisher's exact test for mortality data, William's test for moulting data, weighted analysis of variance for length data; probit analysis or moving average angle method for calculation of LC_{50}.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in all treatments at days 0, 3, 6, 8, 10, 13, 15, 17 and 20 in fresh solutions and on days 3 and 6 in old solutions. Measured concentrations of metconazole in fresh solutions were in the range of 80% to 124% of nominal concentrations with one exception (67%) and one sample was lost. The concentrations determined in old test media on days 3 and 6 were in the range 86% to 106% of nominal. As analytical measurements in old media demonstrated the stability of the test item under the test conditions, the following biological results are based on nominal concentrations.

Biological results: After 21 days of exposure, 10% parent mortality was observed in the control, whereas 5%, 10%, 13% and 57% mortality occurred at the test item concentrations of 0.0083, 0.042, 0.21 and 1.0 mg a.s./L, respectively. At the highest test item concentration, all daphnids were dead at test end. The first brood in the test was produced after 7 - 8 days. White eggs and dead young were produced by *D. magna* at the test item concentration of 1.0 mg a.s./L. The results are summarized in Table 8.2.5.1-1.

Table 8.2.5.1-1: Effects of metconazole (21 d) on *Daphnia magna* survival, reproduction and growth

Concentration [mg a.s./L] (nominal)	**Control**	**0.0083**	**0.042** [a)]	**0.21**	**1.0**	**8.1**
Mean survival (21 d) [%]	90	95	90	87	43	0
Mean number of offspring produced per cohort of initially 10 daphnids (21 d)	477	472	553	608	0	0
Cumulative number of moulted exoskeletons produced per cohort of initially 10 daphnids (21 d)	81	75	90	74	50	4
Mean length of surviving daphnids (21 d) [mm]	3.6	3.6	3.7	3.8	3.5	n.d.
Mean percent of live daphnids with live eggs or young (21 d)	92	95	96	100	88	0
	Endpoints [mg metconazole/L] (nominal)					
LC_{50} (21 d)	0.94 (95% confidence limits: 0.54 - 1.4)					
$NOEC_{overall}$ (21 d)	0.21					

n.d. = not determined, all daphnids dead

[a)] Effect parameters at 0.042 mg a.s./L were calculated from 3 test vessels only as the effect responses of the fourth vessel was shown to be statistically significantly different (Fisher's exact test).

III. CONCLUSION

In a 21-day semi-static toxicity study with *Daphnia magna* the NOEC of metconazole was determined to be 0.21 mg a.s./L based on nominal concentrations.

CA 8.2.5.2 Reproductive and development toxicity to an additional aquatic invertebrate species

This point is not triggered and not addressed via (new) toxicity studies.

CA 8.2.5.3 Development and emergence in Chironomus riparius

A spiked water toxicity study with *Chironomus riparius* was already evaluated during the previous Annex I inclusion process. No additional studies are required and no (new) study has been conducted.

CA 8.2.5.4 Sediment dwelling organisms

This point is not triggered and not addressed via (new) toxicity studies.

CA 8.2.6 Effects on algal growth

CA 8.2.6.1 Effects on growth of green algae

The following study on the freshwater green alga *Pseudokirchneriella subcapitata* performed with the active substance metconazole and has not been evaluated previously on EU level. The study was conducted with test material consisting of a different isomeric ratio (cis:trans - 95:5). It confirms the toxicity endpoint of the standard study used for the risk assessment (MK-510-001) and is submitted only for completeness and as supplemental data.

Report:	CA 8.2.6.1/1 [redacted] 1991a WL136184 (KNF-S-474c): Acute toxicity to Oncorhynchus mykiss, Daphnia magna and Selenastrum capricornutum MK-510-002
Guidelines:	OECD 203, OECD 202, OECD 201
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Executive Summary

In a 72-hour static toxicity laboratory study, the effect of metconazole on growth of the green alga *Pseudokirchneriella subcapitata* was investigated. The following nominal concentrations were applied: 0.14, 0.3, 0.7, 1.5, 3.3 and 7.3 mg metconazole/L. Additionally, a dilution water control was set up. Assessment of growth was conducted 24 h, 48 h and 72 h after test initiation.

The biological results are based on nominal concentrations of the test item. After 72 h of exposure the highest no observed effect concentration in this study was 0.3 mg a.s./L.

In a 72-hour algae toxicity test with *Pseudokirchneriella subcapitata*, the E_rC_{50} (72 h) for metconazole was determined to be 2.6 mg a.s./L, the E_bC_{50} was 1.0 mg a.s./L based on nominal concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Metconazole (BAS 555 F), batch no. F900250, purity: metconazole cis-isomer: 95.2%, metconazole trans-isomer: 0.1% and 1,3,4-triazole analogue: 4.2%.

B. STUDY DESIGN

Test species: Unicellular fresh water green alga, *Selenastrum capricornutum* (syn. *Pseudokirchneriella subcapitata* (Reinsch) Korshikov); ATCC 22662; stock obtained from the "American Type Culture Collection", Maryland, U.S.A.

Test design: Static system (72 hours); 6 test concentrations plus a dilution water control; 3 replicates per test item concentration and 6 replicates for the control; daily assessment of cell density.

Endpoints: EC_{50} with respect to growth rate and area under the growth curve after exposure over 72 hours.

Test concentrations: Control (dilution water), 0.14, 0.3, 0.7, 1.5, 3.3 and 7.3 mg metconazole/L (nominal).

Test conditions: Erlenmeyer flasks; test volume: 100 mL; sterile nutrient medium; pH 7.3 - 9.4; oxygen content: 8.4 - 8.7 mg/L; temperature: 21°C - 26°C; initial cell densities: 1 x 10^4 cells/mL; continuous light at about 3000 lux, incubation in orbital incubator (100 cycles/min).

Analytics: Analytical verification of test item concentrations was conducted using a GC-method with nitrogen selective detection.

Statistics: Descriptive statistics, probit analysis for determination of E_bC_{50}; graphical determination of E_rC_{50}; Williams' test for determination of the NOEC value.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each test item concentration at the beginning and at the end of the test. Measured concentrations of metconazole ranged from 79% to 100% of nominal concentrations at test initiation and from 57% to 87% of nominal at test termination. The following biological results are based on nominal concentrations.

Biological results: After 72 h of exposure the highest no observed effect concentration in this study was 0.3 mg a.s./L (Williams' test). The effects on algal growth are summarized in Table 8.2.6.1-1.

Table 8.2.6.1-1: Effect of metconazole on growth of the green alga *Pseudokirchneriella subcapitata*

Concentration [mg a.s./L] (nominal)	**Control**	**0.14**	**0.3**	**0.7**	**1.5**	**3.3**	**7.3**
Inhibition in 72 h (growth rate) [%] #	--	-5.4	4.6	18	25	65	93
Inhibition in 72 h (area under the growth curve) [%]	--	9.9	12	30	52	88	>100
Endpoints [mg metconazole/L] (nominal)							
E_rC_{50} (72 h)	2.6 (95% confidence limits: n.d.)						
E_bC_{50} (72 h)	1.0 (95% confidence limits: 0.72 - 1.4)						
$NOEC_{overall}$ (72 h)	0.3						

n.d. = not determined
# Negative values indicate stimulated growth compared to the control.

III. CONCLUSION

In a 72-hour algae toxicity test with *Pseudokirchneriella subcapitata*, the E_rC_{50} (72 h) for metconazole was determined to be 2.6 mg a.s./L, the E_bC_{50} was 1.0 mg a.s./L based on nominal concentrations. The endpoint is not used in the risk assessment.

CA 8.2.6.2 Effects on growth of an additional algal species

This point is not triggered and not addressed via (new) toxicity studies.

CA 8.2.7 Effects on aquatic macrophytes

The following toxicity study on the aquatic plant *Lemna gibba* performed with the active substance metconazole was conducted due to data requirements for plant growth regulators and has not been evaluated previously on EU level.

Report:	CA 8.2.7/1 Brzozowska K., 2014a BAS 555 F (Reg.No. 4056343) - Lemna gibba CPCC 310 growth inhibition test 2014/1093918
Guidelines:	OECD 221 (2006), EPA 850.4400
GLP:	yes (certified by Bureau for Chemical Substances and Preparations, Lodz, Poland)

Executive Summary

I. MATERIAL AND METHODS

In a 7-day static toxicity laboratory study, the effect of metconazole on the growth of the duckweed *Lemna gibba* was investigated. A filtrate of the loading of 30 mg metconazole/L was used as the highest test item concentration and diluted to derive further test concentrations. This approach resulted in initial mean measured test concentrations of 13.08, 3.287, 0.881, 0.216, 0.049, 0.012 and 0.003 mg a.s./L. Additionally, a water control was set up. Assessment of plant growth and other effects was conducted on days 2, 4 and 7 after test initiation. The percentage growth inhibition, relative to the control, was calculated for each test concentration based on growth rates and final yield for the parameters frond number and plant dry weight.

The biological results are based on initial measured concentrations of the test item. At exposure termination in 0.003 and 0.012 mg a.s./L no changes distinctive from the normal development of plants in the control were observed. In 0.049 and 0.216 mg a.s./L bending down of colonies and shorter roots were observed. In 0.881, 3.287 and 13.08 mg a.s./L shorter roots were observed. Moreover, in 0.216, 0.881 and 3.287 mg a.s./L dark spots on fronds were observed. Statistically significant effects on yield based on dry weight were observed at all test item concentrations, whereas statistically significant effects on growth rate based on frond number and dry weight and yield based on frond number were determined at the six highest test item concentrations.

In a 7-day aquatic-plant test with *Lemna gibba*, the E_rC_{50} of metconazole was determined to be 0.527 mg a.s./L based on frond numbers and >13.08 mg a.s./L based on dry weight (initial measured). The E_yC_{50} was 0.077 mg a.s./L based on frond numbers and 0.555 mg a.s./L based on dry weight (initial measured).

A. MATERIALS

Test item:	Metconazole (BAS 555 F; Reg. no.:4 056 343), batch no. COD-001502; purity: 98.2%.

B. STUDY DESIGN

Test species:	Duckweed (*Lemna gibba* G3); specification CPCC 310, cultures maintained in-house; stock obtained from "Canadian Phycological Culture Centre (CPCC), Department of Biology, University of Waterloo, Ontario, Canada.
Test design:	Static system; test duration 7 days; 7 test item concentrations plus a control, 4 replicates for each test item concentration, 8 replicates for the control; 4 plants with 3 fronds, 12 fronds per replicate at test initiation; assessment of growth and other effects on days 2, 4 and 7.
Endpoints:	EC_{10}, EC_{50} and NOEC with respect to growth rate and yield after exposure over 7 days.
Test concentrations:	Control, 0.003, 0.012, 0.049, 0.216, 0.881, 3.287, 13.08 mg a.s./L (initial mean measured concentrations)
Test conditions:	Glass beakers (600 mL), test volume: 400 mL, 20 x AAP nutrient solution, pH 7.46 - 7.81 at test initiation and pH 8.52 - 9.39 at test termination; temperature: 23.5°C - 24.8°C, continuous light, light intensity: 6510 - 6700 lux.
Analytics:	Analytical verification of the test item was conducted using a LC-method with UV-VIS-detection.
Statistics:	Descriptive statistics, probit method for determination of EC_x values based on frond no. and dry weight, Williams Multiple Sequential t-test procedure and Welch-t test for inhomogeneous variances with Bonferroni-Holm Adjustment for determination of the NOEC value ($\alpha = 0.05$).

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each test item concentration at the beginning and at the end of the test. Mean measured concentrations of metconazole at test initiation were 0.003, 0.012, 0.049, 0.216, 0.881, 3.287 and 13.08 mg/L. Analysed contents of metconazole at test termination ranged from 95.1% to 112.7% of the initially measured concentrations. As analytically determined concentrations at test end were within ± 20% of the initial measured concentrations, the following biological results are based on initial mean measured concentrations.

Biological results: At exposure termination in 0.003 and 0.012 mg a.s./L no changes distinctive from the normal development of plants in the control were observed. In 0.049 and 0.216 mg a.s./L bending down of colonies and shorter roots were observed. In 0.881, 3.287 and 13.08 mg a.s./L shorter roots were observed. Moreover, in 0.216, 0.881 and 3.287 mg a.s./L dark spots on fronds were observed. Statistically significant effects on yield based on dry weight were observed at all test item concentrations, whereas statistically significant effects on growth rate based on frond number and dry weight and yield based on frond number were determined at the six highest test item concentrations (Williams Multiple Sequential t-test Procedure for growth rate data based on frond number and yield data based on both frond number and dry weight, Welch-t test for Inhomogeneous Variances with Bonferroni-Holm Adjustment for growth rate data based on dry weight; $\alpha = 0.05$). Effects on growth rate and yield are summarized below (see Table 8.2.7-1).

Table 8.2.7-1: Effects of metconazole on the growth of duckweed *Lemna gibba*

Concentration [mg a.s./L] (initial measured)	**--**	**0.003**	**0.012**	**0.049**	**0.216**	**0.881**	**3.287**	**13.08**
Inhibition after 7 d [%] (growth rate based on frond no.)	--	0.7	10.5 *	29.4 *	45.5 *	59.6 *	70.9 *	70.0 *
Inhibition after 7 d [%] (growth rate based on dry weight)	--	3.6	12.6 #	21.4 #	29.2 #	34.0 #	37.9 #	42.3 #
Inhibition after 7 d [%] (yield based on frond no.)	--	1.5	21.9 *	50.9 *	68.9 *	80.3 *	87.3 *	87.0 *
Inhibition after 7 d [%] (yield based on dry weight)	--	7.5 *	25.0 *	39.3 *	50.1 *	55.9 *	60.1 *	64.8 *

	Endpoints [mg metconazole/L] (initial measured)
E_rC_{50} (7 d) based on frond no	0.527 (95% confidence limits: 0.226 - 1.334)
E_rC_{10} (7 d) based on frond no	0.004 (95% confidence limits: < 0.003 - 0.018)
E_yC_{50} (7 d) based on frond no	0.077 (95% confidence limits: 0.034 - 0.169)
E_yC_{10} (7 d) based on frond no	0.003 (95% confidence limits: < 0.003 - 0.009)
E_rC_{50} (7 d) based on dry weight	> 13.08
E_rC_{10} (7 d) based on dry weight	0.003 (95% confidence limits: < 0.003 - 0.016)
E_yC_{50} (7 d) based on dry weight	0.555 (95% confidence limits: 0.184 - 2.184)
E_yC_{10} (7 d) based on dry weight	n.d.
$NOEC_{overall}$ (7 d)	< 0.003

n.d. = not determined
* Statistically significant different compared to the control (Williams Multiple Sequential t-test Procedure, α = 0.05).
# Statistically significant different compared to the control (Welch-t test for Inhomogeneous Variances with Bonferroni-Holm Adjustment, α = 0.05).

III. CONCLUSION

In a 7-day aquatic-plant test with *Lemna gibba,* the E_rC_{50} of metconazole was determined to be 0.527 mg a.s./L based on frond numbers and > 13.08 mg a.s./L based on dry weight (initial measured). The E_yC_{50} was 0.077 mg a.s./L based on frond numbers and 0.555 mg a.s./L based on dry weight (initial measured).

CA 8.2.8 Further testing on aquatic organisms

From the literature search the following peer-reviewed scientific study on bioaccumulation and biotransformation of chiral triazole fungicides in rainbow trout (*Oncorhynchus mykiss*) was considered relevant and reliable (with restrictions; RI 2).

Report:	CA 8.2.8/1 [redacted]., 2006a Bioaccumulation and biotransformation of chiral triazole fungicides in rainbow trout (Oncorhynchus mykiss) 2006/1051433
Guidelines:	none
GLP:	no

Executive Summary

In a dietary bioaccumulation study, juvenile rainbow trout (*Oncorhynchus mykiss*) were exposed to dietary concentrations of a mixture of chiral triazole fungicides including metconazole and α-hexachlorocyclohexane (α-HCH). The bioaccumulation and biotransformation of metconazole was investigated in this study during an 8 day uptake phase followed by a 16 day depuration period. Fish were fed a spiked diet containing 28.14 µg metconazole/g wet wt (mean measured) at a feeding rate of 1.5% of the fish body weight. In parallel, a control group which received a diet of untreated food was kept under the same conditions. Fish were randomly sampled from both treatments on days 1, 2, 4 and 8 of the uptake phase and 6 h, 12 h, 18 h, 24 h, 36 h, 2 days, 4 days, 8 days and 16 days of the depuration phase.

Steady state was reached within 1 day. Exposure to the test item did not appear to influence the health of the rainbow trout. There was no significant difference in lipid percentage or liver somatic index between treatment and control fish on any sampling day and there were no signs of stress (*e.g.* coloration change, behavior) or mortality in either treatment. Body weights and whole fish and liver growth rates of the exposed fish were not significant over the course of the study. The biotransformation rate of metconazole accounted for the major proportion (89.5%) of the test item elimination.

In a dietary bioaccumulation study, rainbow trout were fed with a mixture of triazole fungicides and α-HCH containing 28.14 µg metconazole/g wet wt (mean measured) at a feeding rate of 1.5% of the fish body weight. The obtained steady-state biomagnification factor (BMF_{ss}) was 0.019 meaning that metconazole does not accumulate in fish.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Metconazole (BAS 555 F, Reg. no.: 4 056 343); obtained from „EPA Repository (EPA National Pesticide Standard Repository, Ft. Meade, MD, USA); purity: ≥ 97%.

B. STUDY DESIGN

Test species: Rainbow trout (*Oncorhynchus mykiss*), juveniles; initial weight: 17.6 g ± 1.2 g (S.E.); final weight: 25.5 g ± 6.0 g (S.E.); fish obtained from Lake Burton Fish Hatchery, GA, USA.

Test design: One test item treatment plus a control treatment; fish were exposed to food spiked with a mixture of chiral triazole fungicides (bromuconazole, cyproconazole, metconazole, myclobutanil, penconazole, propiconazole, tebuconazole, tetraconazole and triadimefon) and α-HCH for 8 days (uptake) followed by 16 days of clean food (depuration) at 1.5% of the mean weight of the fish (corrected for weight gain after each sampling time); 3 test aquaria per treatment with 15 fish in each (45 fish per treatment); 3 fish were randomly sampled from both treatments (one fish per aquaria) on days 1, 2, 4 and 8 of the uptake phase and 6 h, 12 h, 18 h, 24 h, 36 h, 2 days, 4 days, 8 days and 16 days of the depuration phase; only carcass samples (whole fish minus liver and GI tract to avoid analytes in the undigested food) were used for chemical analysis to obtain bioaccumulation parameters.

Endpoints: Elimination rate (k_d); biotransformation rate; fraction of elimination due to biotransformation; elimination ($t_{1/2}$); time to 95% elimination (t_{95}), BMF_{ss} (Biomagnification factor at steady state).

Test concentrations: Control treatment: diet without the test item; test item treatment: 28.14 ± 1.57 µg metconazole/g wet weight (mean measured ± S.E. in treated food).

Test conditions: Fiberglass aquaria (50 L); re-circulating of dilution water; dilution water: carbon-filtered, dechlorinated tap water; temperature: dilution water was chilled to 12°C; physical-chemical parameters (pH, oxygen content, total hardness, conductivity) not reported in the study; photoperiod: 12 hours light : 12 hours dark; fish diet : commercial fish food (Zeigler finfish starter, Gardner, PA; 50% protein, 15% lipid, 2% fiber); spiked food was prepared by adding the test item dissolved in dichloromethan to commercial fish food and evaporating the solvent to dryness.

Analytics: Determination of test item concentration in the diet and in fish tissue was conducted using a GC method with MS-detection.

Data analysis: Growth rate significance: fitting of all fish weight data to an exponential model (ln fish weight = $a + bt$; where a is a constant, b the growth rate, and t is time in days from the start of the experiment).
Biotransformation rate: = measured depuration rate - minimum depuration rate (Minimum depuration rate = 0.693/half-life (day), half-live was determined from the equation log half-life = $-1.07 + (0.76 \times \log K_{ow}) - (0.05 - \log K_{ow}^2)$($r^2$ = 0.85), which assumes no biotransformation). Log K_{ow} of 3.9 for metconazole was used for calculations.
Elimination rate constant (k_d): fitting of the mean concentration data obtained during depuration sampling to a first order decay curve (ln concentration = $a + k_d t$; a is a constant and t is time in days).
Half-life value ($t_{1/2}$): calculated using $\ln 2/k_d$.
Time to 95% elimination (t_{95}): calculated from $\ln 0.05/k_d$.
Steady state biomagnification factor (BMF_{ss}): calculated from BMF= C_{fish}/C_{food} (C_{fish} is the average concentration assuming steady state in the fish and C_{food} is the average concentration in the food; both concentrations based on lipid content).
Differences between whole body and liver growth rate constants among treatments: Tukey's honestly significant difference (HSD) test ($p < 0.05$) was used to compare percent lipid and liver somatic indices (LSI) of the exposed fish to the controls.

II. RESULTS AND DISCUSSION

Test item concentration in the diet
The concentration of the test item in the diet was determined in spiked and control food. No test item was detected in control food, while the concentration of the test item in treated food was 28.14 ± 1.57 µg metconazole/g wet wt (mean measured ± S.E.).

Test item concentrations in fish
Fish samples were taken on days 1, 2, 4 and 8 of the uptake phase and 6 h, 12 h, 18 h, 24 h, 36 h, 2 days, 4 days, 8 days and 16 days of the depuration phase. The test item was detected in the treated fish on the first collection day, one day after exposure to the spiked food. Steady state was reached within 1 day, consistent with the low $t_{1/2}$. No test item was detected in control fish on any collection day.

Effects of test item exposure on fish health

Exposure to the test item did not appear to influence the health of the rainbow trout. There was no significant difference in lipid percentage or liver somatic index ($p > 0.05$) between treatment and control fish on any sampling day and there were no signs of stress (*e.g.* coloration change, behavior) or mortality in either treatment.

Body weights (two sample t-test, $p = 0.14$), and whole fish and liver growth rates ($p > 0.05$) of the exposed fish were not significant over the course of the study, likely due to the variability in fish size and the short duration of the experiment (24 days total) that did not allow for much growth.

Because of the low coefficient of determinations ($r^2 \leq 0.23$) found for growth rates in both treatments (exposed and control), concentrations were not corrected for growth dilution.

Bioaccumulation parameters

Bioaccumulation parameters for metconazole obtained in the present study are summarized in Table 8.2.8-1. The biotransformation rate of metconazole accounted for the major proportion (89.5%) of the test item elimination.

Table 8.2.8-1: Bioaccumulation parameters (mean value ± S.E.) for metconazole via dietary exposure using juvenile rainbow trout carcass data

Parameter	Results
Elimination rate (k_d) [day^{-1}] [a)]	0.648 ± 0.122 (0.88)
Biotransformation rate [day^{-1}] [b)]	0.580
Fraction of elimination due to biotransformation [%] [c)]	89.5
Elimination ($t_{1/2}$) [day] [d)]	1.1 ± 0.2
Time to 95% elimination (t_{95}) [day] [e)]	4.6 ± 0.9
BMF_{ss} [f)]	0.019

a) Elimination rate constant was calculated using the model ln concentration = $a + b \times$ time for the 16 day elimination period. Coefficient of determination (r^2) for the model is shown in parentheses.

b) Biotransformation rate = measured depuration rate - minimum depuration rate. Minimum depuration rate = 0.693/half-life (day), where half-lives were determined from the equation log half-life = $-1.07 + (0.76 \times \log K_{ow}) - (0.05 - \log K_{ow}^2)$($r^2$ = 0.85), which assumes no biotransformation. Log K_{ow} of 3.9 for metconazole was used for calculations.

c) Percentage of depuration that is estimated to be biotransformed.

d) Half-life ($t_{1/2}$) was calculated from the equation $t_{1/2} = 0.693/k_d$

e) Time to 95% elimination (t_{95}) was calculated from the equation $t_{95} = 2.99/k_d$

f) Biomagnification factor at steady state = C_{fish} (lipid corrected) / C_{food} (lipid corrected).

III. CONCLUSION

In a dietary bioaccumulation study, rainbow trout were fed with a mixture of triazole fungicides and α-HCH containing 28.14 µg metconazole/g wet wt (mean measured) at a feeding rate of 1.5% of the fish body weight. The obtained biomagnification factor (BMF_{ss}) was 0.019 meaning that metconazole does not accumulate in fish.

CA 8.3 Effects on arthropods

CA 8.3.1 Effects on bees

Since Annex I inclusion of the active substance metconazole (BAS 555 F), new studies on honeybees and bumblebees have been performed with the active substance. As a result there are new endpoints, which are considered in the bee risk assessment. Summaries of these new studies are provided below and an overview on studies and endpoints is given in Table 8.3.1-1.

Table 8.3.1-1: List of studies and endpoints with honeybees and the active substance metconazole (BAS 555 F)

Substance	Test species	Endpoint	Value	Reference (BASF DocID)	EU agreed
metconazole	honeybee	72 h acute oral LD_{50}	85 µg a.s./bee	MK-541-001	yes
		96 h acute contact LD_{50}	> 100.0 µg a.s./bee		
	bumblebee	96 h acute oral LD_{50}	111.1 µg a.s./bee	2015/1000441 2015/1137196	no, new study
		96 h acute contact LD_{50}	> 100.0 µg a.s./bee		
	honeybee	10 d chronic LD_{50}	50.0 µg a.s./bee/day	2012/1193216 2015/1137379	no, new study
		10 d chronic NOED	5.480 µg a.s./bee/day		
		10 d chronic LC_{50}	2.938 g a.s./kg food		
		10 d chronic NOEC	0.257 g a.s./kg food		
	honeybee larva	72 h LD_{50}	> 99.2 µg a.s./larva	2012/1193217	no, new study
		72 h LC_{50}	> 2.926 g a.s./kg food		
		72 h NOED	49.6 µg a.s./larva		
		72 h NOEC	1.463 g a.s./kg food		

CA 8.3.1.1 Acute toxicity to bees

CA 8.3.1.1.1 Acute oral toxicity

Report:	CA 8.3.1.1.1/1 Haupt S., 2015a Effects of BAS 555 F (acute contact and oral) on bumblebees (Bombus terrestris L.) in the laboratory 2015/1000441
Guidelines:	Van der Steen (2001), OECD 213 (1998), OECD 214 (1998)
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

Report:	CA 8.3.1.1.1/2 Haupt S., 2015b First final report amendment: Effects of BAS 555 F (acute contact and oral) on bumblebees (Bombus terrestris L.) in the laboratory 2015/1137196
Guidelines:	Van der Steen (2001), OECD 213 (1998), OECD 214 (1998)
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

Executive Summary

In an acute oral toxicity test, bumblebees (adult female worker bumblebees of *Bombus terrestris* L.) were exposed to BAS 555 F. The toxicity of the test item was determined at nominal doses of 12.5, 25.0, 50.0, 100.0 and 200.0 µg BAS 555 F/bumblebee, resulting in an actual uptake of 10.0, 16.4, 32.3, 75.8 and 139.8 µg a.s./bumblebee.

Additionally, bumblebees were treated with dimethoate as a reference item. Further, two control groups were established, one untreated control and one solvent control containing 5% acetone and 1% Tween 20.

After 96 hours of oral exposure, 3.3% mortality was observed in the solvent control, whereas no mortality occurred in the untreated control. After 96 hours, mortality in the treatment groups was 6.7, 3.3, 6.7, 43.3 and 60.0% after consumption of 10.0, 16.4, 32.3, 75.8 and 139.8 µg a.s./bumblebee.

Behavioral abnormalities occurred in a dose dependent manner and decreased over time. During the 24 hour assessment, 2 (of 29 living bees), 6 (of 26 living bees) and 12 (of 26 living bees) of the bees that consumed 10.0, 16.4, 32.3, 75.8 and 139.8 µg a.s./bumblebee were affected or moribund. During the last assessment (96 hours after application), only one bee (of 28 living bees) of the 50.0 µg BAS 555 F/bumblebee treatment group was affected. Bees consuming 10.0 and 16.4 µg BAS 555 F/bumblebee showed no behavioral impairments at any time during the test. In the solvent control group, one bee of 30 living bees was affected during the first assessment 4 hours after application. No further behavioral impairments occurred in the oral test.

In an oral toxicity study with BAS 555 F on bumblebees, the LD_{50} value (96 h) was calculated to be 111.1 µg BAS 555 F/bumblebee.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 555 F (metconazole, Reg. No. 4 056 343); batch no. COD-001502; purity 98.2% (w/w).

B. STUDY DESIGN

Test species: *Bombus terrestris* L. (bumblebee), adult female worker bumblebees derived from a healthy and queen-right colony; source: Biobest Belgium N.V., Ilse Velden 18, 2260 Westerlo, Belgium)

Test design: In a 96 hour test, adults of worker bumblebees of *Bombus terrestris* L. were exposed orally to BAS 550 F (diluted in a feeding solution composed of a 50% (w/v) aqueous sucrose solution containing 5% acetone and 1% Tween 20). In total, 8 treatment groups were set up (5 dose rates of the test item, 1 untreated water control group, 1 solvent control group and 1 dose rate of the reference item) with 30 replicates per treatment and 1 bumblebee per replicate. Assessment of bumblebee mortality and behavioral effects was done after 4, 24, 48, 72 and 96 hours.

Endpoint: Mortality, behavioral impairments.

Reference item: BAS 152 11 I (dimethoate, nominal 400.0 g/L).

Test concentrations: Control (50% (w/v) aqueous sucrose solution)
Solvent control (50% (w/v) aqueous sucrose solution containing 5% acetone and 1% Tween 20)
Test item:

Nominal a.s. dose rate (µg BAS 555 F/bumblebee)	Consumed a.s. (µg BAS 555 F/bumblebee)
12.5	10.0
25.0	16.4
50.0	32.3
100.0	75.8
200.0	139.8

Reference item: 3.26 µg dimethoate/bumblebee nominal (1.83 µg consumed dimethoate/bumblebee)

Test conditions: Temperature: 25.0 °C, relative humidity: 30.0% – 61.0%, photoperiod: 24 h darkness, food: 50% (w/v) aqueous sucrose solution, *ad libitum.*

Statistics: Descriptive statistics; Probit analysis with Abbott correction for oral LD_{50} calculation.

II. RESULTS AND DISCUSSION

After 96 hours of oral exposure, 3.3% mortality was observed in the solvent control, whereas no mortality occurred in the untreated control. After 96 hours, mortality in the treatment groups was 6.7, 3.3, 6.7, 43.3 and 60.0% after consumption of 10.0, 16.4, 32.3, 75.8 and 139.8 µg a.s./bumblebee.
Behavioral abnormalities occurred in a dose dependent manner and decreased over time. During the 24 hour assessment, 2 (of 29 living bees), 6 (of 26 living bees) and 12 (of 26 living bees) of the bees that consumed 10.0, 16.4, 32.3, 75.8 and 139.8 µg a.s./bumblebee were affected or moribund. During the last assessment (96 hours after application), only one bee (of 28 living bees) of the 50.0 µg BAS 555 F/bumblebee treatment group was affected. Bees consuming 10.0 and 16.4 µg BAS 555 F/bumblebee showed no behavioral impairments at any time during the test. In the solvent control group, one bee of 30 living bees was affected during the first assessment 4 hours after application. No further behavioral impairments occurred in the oral test.

The LD_{50} (96 h) was estimated to be 111.1 µg BAS 555 F/bumblebee. The results are summarized in Table 8.3.1.1.1-1.

Table 8.3.1.1.1-1: Toxicity of BAS 555 F to *Bombus terrestris* (bumblebee) in an oral toxicity test

Treatment	Consumed test item	Mean mortality [%] [1)]				
[µg a.s./bumblebee]	[µg a.s./bumblebee]	4 h	24 h	48 h	72 h	96 h
Water control	--	0.0	0.0	0.0	0.0	0.0
Solvent control		0.0	3.3	3.3	3.3	3.3
12.5	10.0	0.0	3.3	3.3	6.7	6.7
25.0	16.4	0.0	3.3	3.3	3.3	3.3
50.0	32.3	0.0	3.3	6.7	6.7	6.7
100.0	75.8	0.0	13.3	40.0	43.3	43.3
200.0	139.8	0.0	13.3	50.0	60.0	60.0
		Endpoint (based on actual uptake)				
LD_{50} (96 h)		111.1 [µg a.s./bumblebee]				

1) Mean of 30 individuals per treatment group

The reference item caused 56.7% mortality after 96 hours.

III. CONCLUSION

In an oral toxicity study with BAS 555 F on bumblebees, the LD_{50} value (96 h) was calculated to be 111.1 µg BAS 555 F/bumblebee.

CA 8.3.1.1.2 Acute contact toxicity

Report:	CA 8.3.1.1.2/1 Haupt S., 2015a Effects of BAS 555 F (acute contact and oral) on bumblebees (Bombus terrestris L.) in the laboratory 2015/1000441
Guidelines:	Van der Steen (2001), OECD 213 (1998), OECD 214 (1998)
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

Report:	CA 8.3.1.1.2/2 Haupt S., 2015b First final report amendment: Effects of BAS 555 F (acute contact and oral) on bumblebees (Bombus terrestris L.) in the laboratory 2015/1137196
Guidelines:	Van der Steen (2001), OECD 213 (1998), OECD 214 (1998)
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

Executive Summary

In an acute contact toxicity limit test with BAS 555 F, young adult worker bumblebees (*Bombus terrestris L.*) were exposed over a period of 96 hours to a dose rate of 100.0 µg BAS 555 F/bumblebee. Additionally, bumblebees were treated with dimethoate as a reference item at a dose of 12.0 µg dimethoate/bumblebee and with either a control (tap water containing 0.1% Triton x-100) or a solvent control (containing pure acetone).

After 96 hours of contact exposure, mortality was determined to be 4.0% in the control and 2.0% in the solvent control. After 96 hours of exposure, no mortality was observed in the BAS 555 F treatment group. No abnormal behavior was observed at any time during the entire 96 hour test period.

In an acute contact toxicity study with BAS 555 F on bumblebees, the LD_{50} value (96 h) was calculated to be > 100.0 µg BAS 555 F/bumblebee.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 555 F (metconazole, Reg. No. 4 056 343); batch no. COD-001502; purity 98.2% (w/w).

B. STUDY DESIGN

Test species: *Bombus terrestris* L. (bumblebee), adult female worker bumblebees derived from a healthy and queen-right colony; source: Biobest Belgium N.V., Ilse Velden 18, 2260 Westerlo, Belgium)

Test design: In a 96 hour limit test, adults of worker bumblebees of *Bombus terrestris* were exposed to a dose of BAS 555 F. In total, 4 treatment groups were set up (1 dose rate of the test item, 1 control group, 1 solvent control group and 1 dose rate of the reference item) with 50 replicates per treatment and 1 bumblebee per replicate. Assessment of bumblebee mortality and behavioral effects was done after 4, 24, 48, 72 and 96 hours.

Endpoint: Mortality, behavioral impairments.

Reference item: BAS 152 11 I (dimethoate, nominal 400.0 g/L).

Test concentrations: Control (tap water containing 0.1% Triton X-100)
Solvent control (pure acetone)
Test item dose rate: 100.0 µg BAS 555 F/bumblebee.
Reference item dose rate: 12.0 µg dimethoate/bumblebee

Test conditions: Temperature: 25.0 °C, relative humidity: 30.0% – 61.0%, photoperiod: 24 h darkness, food: 50% (w/v) aqueous sucrose solution, *ad libitum.*

Statistics: Descriptive statistics.

II. RESULTS AND DISCUSSION

After 96 hours of contact exposure, mortality was determined to be 4.0% in the control and 2.0% in the solvent control. After 96 hours of exposure no mortality was observed in the BAS 555 F treatment group. No abnormal behavior was observed at any time during the entire 96 hour test period.
The LD_{50} (96 h) was estimated to be > 100.0 µg BAS 555 F/bumblebee. The results are summarized in Table 8.3.1.1.2-1.

Table 8.3.1.1.2-1: Toxicity of BAS 555 F to *Bombus terrestris* (bumblebee) in a contact toxicity limit test

Treatment	Mean mortality [%][1)]				
[µg BAS 555 F/bumblebee]	4 h	24 h	48 h	72 h	96 h
Control	0.0	4.0	4.0	4.0	4.0
Solvent control	0.0	2.0	2.0	2.0	2.0
100.0	0.0	0.0	0.0	0.0	0.0
Endpoints					
	[µg BAS 555 F/bumblebee]				
LD_{50} (96 h)	> 100.0				

1) Mean of 50 individuals per treatment group

Mortality of the reference item was determined to be 96.0% after 96 hours.

III. CONCLUSION

In an acute contact toxicity study with BAS 555 F on bumblebees, the LD_{50} value (96 h) was calculated to be > 100.0 µg BAS 555 F/bumblebee.

CA 8.3.1.2 Chronic toxicity to bees

Report:	CA 8.3.1.2/1 Kleebaum K., 2014a Chronic toxicity of BAS 555 F to the honeybee Apis mellifera L. under laboratory conditions 2012/1193216
Guidelines:	Decourty et al. (2005), Suchail et al. (2001), CEB No. 230 (2010)
GLP:	yes (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

Report:	CA 8.3.1.2/2 Kleebaum K., 2015b Amendment no. 1 to final report - Chronic toxicity of BAS 555 F to the honeybee Apis mellifera L. under laboratory conditions 2015/1137379
Guidelines:	Decourty et al. (2005), Suchail et al. (2001), CEB No. 230 (2010)
GLP:	yes (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

Executive Summary

In a 10-day chronic oral toxicity test, 2 - 3 day old worker honeybees (*Apis mellifera* L.) were exposed to a daily application of BAS 555 F diluted in the bee food (50% w/v aqueous sucrose solution). The chronic toxicity of the test item was determined at nominal doses of 10.0, 20.0, 40.0, 80.0 and 160.0 µg a.s./bee/day (effective doses were 5.5, 11.2, 17.3, 36.6 and 71.5 µg a.s./bee/day), corresponding to concentrations of 0.257, 0.514, 1.027, 2.054 and 4.108 g a.s./kg food, respectively. Additionally, honeybees were treated with Dimethoate EC 400 as a reference item at nominal doses ranging from 4.3 to 20.1 ng a.s./bee/day. Untreated diet serving as a control to the reference item and a solvent control containing 1% v/v Tween 20 serving as control to the test item were also included.

The control group showed a mean mortality of 3.3% after 10 days of testing, whereas the solvent control group showed a mean mortality of 11.7%. In the test item group, bees consuming doses of 11.2, 17.3, 36.6, and 71.5 µg a.s./bee/day revealed corrected mortalities of 32.1, 18.9, 45.3 and 58.5%, respectively. These mortalities are statistically significantly increased compared to the control group.
On the last day of the test, 9.1% of the remaining bees of the highest test item dose and 2.2% of the remaining bees of the lowest test item dose were described as moving uncoordinatedly. In the second highest test item dose, 6.9% of the remaining bees were described as being immobile.
The NOED was determined to be 5.480 µg consumed a.s./bee/day, and the NOEC 0.257g a.s./kg food, respectively.

In a 10-day chronic toxicity feeding study with BAS 555 F, the LD_{50} was determined to be 50.0 µg a.s./bee/day, corresponding to an LC_{50} of 2.938 g a.s./kg food.
The NOED was determined to be 5.480 µg consumed a.s./bee/day, and the NOEC 0.257 g a.s./kg food, respectively.

I. MATERIALS AND METHODS

A. MATERIALS

Test item: BAS 555 F, (metconazole, Reg. No. 4 056 343); batch no. COD-001163; analyzed purity: 98.7% w/w (tolerance ± 1.0%).

B. STUDY DESIGN

Test species: *Apis mellifera* L. (honeybee); 2 – 3 day old bees; derived from a healthy and queen-right colony; source: Bienenfarm Kern GmbH, Leipzig, Germany.

Test design: In a 10-day chronic test, young adults of *Apis mellifera* L. were exposed daily to 5 doses of BAS 555 F in treated food (50% w/v aqueous sucrose solution with 1% Tween 20). In total, 11 treatment groups were set up: 5 doses of the test item, 1 untreated control for the reference item, 1 solvent control for the test item, and 4 doses of the reference item, with 3 replicates per dose and 20 bees per replicate. Assessments of bee mortality and behavioral effects were done daily during the study.

Endpoints: Mortality, behavioral impairments.

Reference item: Dimethoate 400 EC (analyzed content of a.s.: 411.7 g/L).

Test doses: Control (for reference item): untreated diet (50% w/v aqueous sucrose solution).
Solvent control (for test item): untreated diet with 1% v/v Tween 20

Test item treatments	
Doses [µg a.s./bee/day]	**Concentrations [g a.s./kg food]**
10.0	0.257
20.0	0.514
40.0	1.027
80.0	2.054
160.0	4.108
Reference item	
Doses [ng dimethoate/bee/day]	**Concentrations [mg dimethoate/kg food]**
4.3	0.1
7.2	0.2
12.0	0.3
20.1	0.5

Test conditions: Temperature: 34°C – 35°C; relative humidity: 57% - 63%, photoperiod: 24 h darkness; food: 50% w/v aqueous sucrose solution.

Statistics: Descriptive statistics; for mortality data Fisher's Exact Binomial Test with Bonferroni Correction (one-sided greater, $\alpha = 0.05$). The median lethal dose/concentration of test and reference item were calculated with Probit and Weibull analysis using linear maximum likelihood regression.

II. RESULTS AND DISCUSSION

The control group showed a mean mortality of 3.3% after 10 days of testing, whereas the solvent control group showed a mean mortality of 11.7%. In the test item group, bees consuming doses of 11.2, 17.3, 36.6, and 71.5 µg a.s./bee/day revealed corrected mortalities of 32.1, 18.9, 45.3 and 58.5%, respectively. These mortalities are statistically significantly increased compared to the control group (Fisher's Exact Binomial Test with Bonferroni Correction, one-sided greater, $\alpha = 0.05$).
On the last day of the test, 9.1% of the remaining bees of the highest test item dose and 2.2% of the remaining bees of the lowest test item dose were described as moving uncoordinatedly. In the second highest test item dose, 6.9% of the remaining bees were described as being immobile.
The NOED was determined to be 5.480 µg consumed a.s./bee/day, and the NOEC 0.257g a.s./kg food, respectively. The results are summarized in Table 8.3.1.2-1.

Table 8.3.1.2-1: Cumulative mortality and toxicity endpoints of honeybees (*Apis mellifera* L.) exposed to BAS 555 F in a chronic oral toxicity test

Treatment [BAS 555 F]			Mortality after 10 days	
Actual daily mean doses [µg a.s./bee/day]	**Overall doses [µg a.s./bee/day]**	**Concentration [g a.s./kg food]**	**Cumulative mortality [%]**	**Corrected cumulative mortality [%]**
Control	Control	Control	3.3	--
Solvent control	Solvent control	Solvent control	11.7	--
5.5	10.0	0.257	23.3	13.2
11.2	20.0	0.514	40.0 *	32.1
17.3	40.0	1.027	28.3 *	18.9
36.6	80.0	2.054	51.7 *	45.3
71.5	160.0	4.108	63.3 *	58.5

Endpoints [1)]		**10 days**
Test item doses [µg a.s./bee/day]	**LD_{50} [2)]**	50.0 (95% CL: 18.2 – 137.8)
	NOED [4)]	5.480
Test item concentrations [g a.s./kg food]	**LC_{50} [3)]**	2.938 (95% CL: 0.983 – 8.782)
	NOEC [4)]	0.257

* Statistically significant difference between treatment group and solvent control group (Fisher's Exact Binomial Test with Bonferroni Correction, one-sided greater, $\alpha = 0.05$).
1) Based on effective doses and analyzed content of BAS 555 F.
2) Calculated by using Probit analysis (linear maximum likelihood regression).
3) Calculated by using Weibull analysis (linear maximum likelihood regression).
4) Fisher's Exact Binominal Test with Bonferroni Correction, (one-sided greater, $\alpha = 0.05$).

The highest dose rate in the reference item (21.0 ng dimethoate/bee/day, corresponding to an actual average daily consumption of 9.7 ng dimethoate/bee) caused a mean mortality of 51.7%.

III. CONCLUSION

In a 10-day chronic toxicity feeding study with BAS 555 F, the LD_{50} was determined to be 50.0 µg a.s./bee/day, corresponding to an LC_{50} of 2.938 g a.s./kg food.
The NOED was determined to be 5.480 µg consumed a.s./bee/day, and the NOEC 0.257 g a.s./kg food, respectively.

CA 8.3.1.3 Effects on honeybee development and other honeybee life stages

Report:	CA 8.3.1.3/1 Kleebaum K., 2015a Acute toxicity of BAS 555 F to honeybee larvae (Apis mellifer L.) under laboratory conditions (in vitro) 2012/1193217
Guidelines:	OECD 237 (2013) Honey bee (Apis mellifera) larval toxicity test single exposure
GLP:	yes (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

Executive Summary

In an acute oral larval toxicity test, four day old honeybee larvae (*Apis mellifera* L.) were exposed to one application of BAS 555 F diluted in the larvae food. The toxicity of the test item was determined with concentrations of 6.2, 12.4, 24.8, 49.6 and 99.2 µg a.s./larva, corresponding to 0.183, 0.366, 0.732, 1.463 and 2.926 g a.s./kg food). Additionally, honeybee larvae were treated with dimethoate as the reference item. Untreated diet served as a control in addition to a solvent control with Tween 20 (1% v/v).

After 72 hours of exposure, a mortality of 5.6% was observed in the control. The solvent control showed a mortality of 8.3% after 72 hours. In the test item groups, after 72 hours, larvae fed with 99.2 µg a.s./larva revealed a mortality of 36.1%, which was statistically significant in comparison to the solvent control group.

After 72 hours of exposure, deviations to the normal food consumption behavior and, correspondingly, in developing an average sized larva occurred in 20 larvae (from the control and all test item groups) when compared to the solvent control. However, no dose-response relationship could be observed in the treatment group, and a test item related effect can therefore be excluded.

In an acute oral larval toxicity study with BAS 555 F on honeybee larvae, the LD_{50} value (72 h) was calculated to be > 99.2 µg a.s./larva (equivalent to an LC_{50} (72 h) > 2.926 g a.s./kg food). The NOED was determined to be 49.6 µg a.s./larva (equivalent to a NOEC = 1.463 g a.s./kg food).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 555 F, (metconazole, Reg. No. 4 056 343); batch no. COD-001163; analyzed purity: 98.7% (tolerance ± 1.0%).

B. STUDY DESIGN

Test species: *Apis mellifera* L. (honeybee); synchronized first instar larvae (one day old); derived from three healthy and queen-right colonies; source: Bienenfarm Kern GmbH, Leipzig, Germany.

Test design: One day old honeybee larvae of *Apis mellifera* were transferred from brood combs to polystyrene grafting cells in 48-well cell culture plates 3 days before start of the treatment. After this, in a 72 hour acute test, the 4 day old larvae were exposed to a single application of BAS 555 F diluted in the larvae food (aqueous sugar solution mixed with royal jelly). In total, 11 treatment groups were set up: 5 doses of the test item, 1 untreated control, 1 solvent control and 4 doses of the reference item with 3 replicates per dose and 12 larvae per replicate. After the day of application, additional feeding of the larvae took place 24 and 48 hours later. Assessments of larval mortality were done after 24, 48 and 72 hours. Additionally, other observations such as small body size or large quantities of remaining food after 72 hours were noted. In an analytical phase of the study, the concentration of the active substance in the test item stock solution was determined.

Endpoints: Mortality (LD_{50}), quantitative observations: body size, remaining food.

Reference item: Dimethoate technical (analyzed purity: 99.8%).

Test doses: Control 1: untreated diet (50% aqueous sugar solution with 50% royal jelly)
Control 2: untreated diet with Tween 20 (1% v/v)
Test item treatments:

Nominal dose/concentration of BAS 555 F	
Doses [µg a.s./larva]	**Concentrations [g a.s./kg food]**
6.2	0.183
12.4	0.366
24.8	0.732
49.6	1.463
99.2	2.926

Reference item treatments: 1.1, 2.2, 4.4 and 8.8 µg dimethoate/larva.

Test conditions: Temperature: 34.0° C – 34.5° C; relative humidity: 93% - 96%, photoperiod: 24 h darkness; food: 50% aqueous sugar solution with 50% royal jelly.

Statistics: Descriptive statistics; Fisher's Exact Binomial Test with Bonferroni Correction for mortality data (one-sided greater, $\alpha = 0.05$) and No Observed Effect Level. The median lethal doses/concentrations of test and reference item were calculated with Probit analysis.

II. RESULTS AND DISCUSSION

After 72 hours of exposure, a mortality of 5.6% was observed in the control. The solvent control showed a mortality of 8.3% after 72 hours. In the test item groups, after 72 hours, only larvae fed with 99.2 µg a.s./larva revealed a mortality of 36.1%, which was statistically significant in comparison to the solvent control group (Fisher's Exact Binomial Test with Bonferroni Correction, one-sided greater, $\alpha = 0.05$).

After 72 hours of exposure, deviations to the normal food consumption behavior and, correspondingly, in developing an average sized larva occurred in 20 larvae (from the control and all test item groups) when compared to the solvent control. However, no dose-response relationship could be observed in the treatment group, and a test item related effect can therefore be excluded.
The results are summarized in Table 8.3.1.3-1.

Table 8.3.1.3-1: Toxicity of BAS 555 F to *Apis mellifera* (honeybee) in an acute oral larval toxicity test after 72 hours

Dosage [µg a.s./larva]	**Concentration [g a.s./kg food]**	**72 h mortality [%]**	
		Absolute	**corrected [1)]**
Control	Control	5.6	--
Solvent control	Solvent control	8.3	--
6.2	0.183	2.8	0.0
12.4	0.366	11.1	3.0
24.8	0.732	8.3	0.0
49.6	1.463	30.6	24.2
99.2	2.926	36.1 *	30.3

	Endpoints [µg BAS 555 F/bee] 72 h
LD_{50} [µg a.s./larva] [2)] (95% confidence limits)	> 99.2
NOED [µg a.s./larva] [3)]	49.6
LC_{50} [g a.s./kg food] [2)] (95% confidence limits)	> 2.926
NOEC [g a.s./kg food] [3)]	1.463

* Statistically significantly different compared to the control (Fisher's Exact Binomial Test with Bonferroni Correction, one-sided greater; $\alpha = 0.05$).
1) According to Schneider-Orelli (1947).
2) Fisher's Exact Binomial Test with Bonferroni Correction, one-sided greater, $\alpha = 0.05$.
3) Median lethal dose after 72 hours of exposure was calculated by using Probit analysis.

In the reference item treatment group, larvae fed with 8.8 µg a.s./larva resulted in a corrected mortality of 64.7% 72 hours after application.

III. CONCLUSION

In an acute oral larval toxicity study with BAS 555 F on honeybee larvae, the LD_{50} value (72 h) was calculated to be > 99.2 µg a.s./larva (equivalent to an LC_{50} (72 h) > 2.926 g a.s./kg food). The NOED was determined to be 49.6 µg a.s./larva (equivalent to a NOEC = 1.463 g a.s./kg food).

CA 8.3.1.4 Sub-lethal effects

No new studies are available.

CA 8.3.2 Effects on non-target arthropods other than bees

No new studies are available.

CA 8.3.2.1 Effects on Aphidius rhopalosiphi

No new studies are available.

CA 8.3.2.2 Effects on Typhlodromus pyri

No new studies are available.

CA 8.4 Effects on non-target soil meso- and macrofauna

Since Annex I inclusion of the active substance metconazole (BAS 555 F), new studies on soil macro organisms have been performed with the active substance and metabolite M555F020 (1,2,4-triazole) to fulfill current data requirements according to Commission Regulation (EU) No. 283/2013. As a result, there are new endpoints which are considered in the respective risk assessment. Summaries of the new studies are provided below and an overview on studies and endpoints is given in Table 8.4-1.

Results of ECx calculations for chronic toxicity studies are provided in a separate document for new and old studies *[see KCA 8.4/1 2016/1002152]*

For studies following guidelines/protocols that were designed to derive a NOEC/NOAEL and have significant limitations for deriving an ECx like limited number of dose groups and/or large dose spacing (e.g. reproductive studies on birds and mammals), no ECx values were calculated.

Table 8.4-1 Toxicity to non-target soil meso- and macrofauna of metconazole

Substance	**Species**	**Endpoint**	**Value [mg/kg dry soil]**	**Reference (BASF Doc ID)**	**EU agreed** (metconazole: DAR, Vol.3, Annex B.9, 2004; EFSA Scientific Report 64, 2006)
metconazole	*Eisenia fetida*	$LC_{50, CORR}$ *	> 500	MK-531-001	yes
		$NOEC_{CORR}$ *	≥ 0.9	MK-534-001	yes
		$NOEC_{CORR}$ *	2.5 **	2014/1028658	no, new study
	Folsomia candida	NOEC	40	2013/1003177	no, new study
	Hypoaspis aculeifer	NOEC	62.5	2014/1028659	no, new study
1,2,4-triazole (= M555F020)	*Eisenia fetida*	NOEC	≥ 1.0	2004/1041154	Yes[1)]
	Folsomia candida	NOEC	1.8	2002/1007851	Yes[2)]
	Hypoaspis aculeifer	NOEC	171	2014/1326895	no, new study

1) EFSA Journal 2014;12(1):3485, 98 pp. doi:10.2903/j.efsa.2014.3485.

2) Study reviewed in EFSA Scientific Report 138, 2008; DAR, Vol. 3, B.9, 2006

* Toxicity endpoint is adjusted using a soil factor of 2 to address the organic content of the soil (peat 10%), and the log P_{ow} of the substance is > 2.

** A new study was conducted, to derive a definitive endpoint for BAS 555 F.

CA 8.4.1 Earthworms – sub-lethal effects

Report: CA 8.4.1/1
Friedrich S., 2014a
Sublethal toxicity of BAS 555 F (Metconazole) to the earthworm Eisenia fetida in artificial soil
2014/1028658
Guidelines: OECD 222 (2004)
GLP: yes
(certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

Executive Summary

In a chronic toxicity study, adults of *Eisenia fetida* (Annelida: Oligochaeta), were exposed to BAS 555 F (metconazole, Reg. No. 4 056 343). The test item was mixed into artificial soil (10% peat) at concentrations of 2.5, 5, 10, 20 and 40 mg BAS 555 F/kg dry soil. For the control treatment, the soil was left untreated. Assessment of mortality, body weight and feeding activity was carried out after 28 days and reproduction (number of juveniles) was assessed after 56 days.

BAS 555 F did not show any statistically significant effects on mortality and body weight. The mortality of adult worms was between 0.0% and 2.5% in the test item treatments and 0.0% in the control group. The weight change of adult worms was between 29.3% and 32.1% in the test item treatments and 30.8% in the control group. In the control, a mean of 143.0 juveniles was counted. In the test item treatment groups, mean numbers of juveniles between 92.0 and 150.5 were counted. The reproduction rate was significantly different compared to the control at 10, 20 and 40 mg BAS 555 F/kg dry soil. The feeding activity in all test item treated groups was comparable to the control.

In a 56-day reproduction study with BAS 555 F (Reg. No. 4 056 343), no unacceptable adverse effects on survival and body weight could be determined at concentrations up to and including 40 mg BAS 555 F/kg dry soil. Statistically significant effects on the number of juveniles of *Eisenia fetida* were determined at 10, 20 and 40 mg BAS 555 F/kg dry soil. Therefore, the NOEC for mortality and body weight was ≥ 40 mg BAS 555 F/kg dry soil, whereas the NOEC for reproduction was 5 mg BAS 555 F/kg dry soil. The EC_{50} for reproduction was estimated to be higher than 40 mg BAS 555 F/kg dry soil, the highest concentration tested.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 555 F (metconazole, Reg. No. 4 056 343), batch no. COD-001502, analyzed purity: 98.2% (± 1.0%).

B. STUDY DESIGN

Test species: *Eisenia fetida*; adult worms with clitellum and weight of 280 – 480 mg, approximately 3 months old; source: W. Neudorff GmbH KG followed by in-house culture.

Test design: In a 56-day test, adults of *Eisenia fetida* were exposed to 5 concentrations of BAS 555 F in treated artificial soil according to OECD 222 (10% peat). In total, 6 treatment groups were set up (5 concentrations of the test item and 1 untreated control group) with 4 replicates for the test item treatments and 8 replicates for the control, 10 adult worms per replicate. The artificial soil was treated and filled into vessels, before the earthworms were introduced on the top of the soil.
Assessment of worm mortality, behavioral effects and weight change was done 28 days after application. After an additional 28 days (56 days after application), reproduction (number of juveniles) was assessed.

Endpoints: Mortality, body weight change, feeding activity, reproduction rate.

Reference item: Nutdazim 50 Flow (carbendazim SC 500). The effects of the reference item were investigated in a separate study.

Test concentrations: Control, 2.5, 5, 10, 20 and 40 mg BAS 555 F/kg dry soil.

Test conditions: Artificial soil according to OECD 222 with 10% peat; pH 6.09 - 6.12 at test initiation, pH 5.70 – 5.79 at test termination; water content 54.9% - 55.2% of its maximum water holding capacity (WHC) at test initiation and 53.8% - 54.9% of WHC at test termination; temperature: 18.0°C – 21.7°C; photoperiod: 16 hours light : 8 hours dark, light intensity: 520 lux, feeding with horse manure.

Statistics: Descriptive statistics Fisher's Exact Binomial Test with Bonferroni Correction for mortality data ($\alpha = 0.05$, one-sided greater).Williams-t-test for weight change and reproduction data ($\alpha = 0.05$, one-sided smaller), Probit analysis (Finney 1971) for calculation of EC_{50}.

II. RESULTS AND DISCUSSION

BAS 555 F did not show any statistically significant effects on mortality (Fisher's Exact Binomial Test with Bonferroni correction, $\alpha = 0.05$, one-sided greater) and body weight (Williams-t-test, $\alpha = 0.05$, one-sided greater). The mortality of adult worms was between 0.0% and 2.5% in the test item treatments and 0.0% in the control group. The weight change of adult worms was between 29.3% and 32.1% in the test item treatments and 30.8% in the control group. In the control, a mean of 143.0 juveniles was counted. In the test item treatment groups, mean numbers of juveniles between 92.0 and 150.5 were counted. The reproduction rate was significantly different compared to the control at 10, 20 and 40 mg BAS 555 F/kg dry soil (Williams-t-test, $\alpha = 0.05$, one-sided greater). The feeding activity in all test item treated groups was comparable to the control.

The results are summarized in Table 8.4.1-1.

Table 8.4.1-1: Effects of BAS 555 F on *Eisenia fetida* in a 56-day reproduction study

BAS 555 F [mg a.s./kg dry soil]	**Control**	**2.5**	**5**	**10**	**20**	**40**
Mortality (day 28) [%]	0.0	2.5	0.0	0.0	2.5	0.0
Weight change (day 28) [%]	30.8	29.3	32.1	31.7	30.4	30.1
Number of juveniles (day 56)	143.0	141.0	150.5	119.0 *	105.3 *	92.0 *
Reproduction (day 56) [% of control]	100	98.6	105.2	83.2	73.6	64.3
Endpoints [mg BAS 555 F/kg dry soil]						
$NOEC_{mortality, body\ weight}$ (day 28)	≥ 40					
$NOEC_{reproduction}$ (day 56)	5					
EC_{50} (day 56)	> 40					

* Statistically significantly different compared to the control (Williams-t-test, $\alpha = 0.05$, one-sided smaller).

In a separate study the reference item Nutdazim 50 Flow (carbendazim SC 500) had a significant effect on biomass increase and reproduction of *Eisenia fetida*. The reproduction rate was clearly inhibited by 39.0% and 100% compared to the control at the tested concentrations of 5 and 10 mg product/kg dry soil dry.

III. CONCLUSION

In a 56-day reproduction study with BAS 555 F (Reg. No. 4 056 343) no unacceptable adverse effects on survival and body weight could be determined at concentrations up to and including 40 mg BAS 555 F/kg dry soil. Statistically significant effects on the number of juveniles of *Eisenia fetida* were determined at 10, 20 and 40 mg BAS 555 F/kg dry soil. Therefore, the NOEC for mortality and body weight was ≥ 40 mg BAS 555 F/kg dry soil, whereas the NOEC for reproduction was 5 mg BAS 555 F/kg dry soil. The EC_{50} for reproduction was estimated to be higher than 40 mg BAS 555 F/kg dry soil, the highest concentration tested.

CA 8.4.2 Effects on non-target soil meso- and macrofauna (other than earthworms)

CA 8.4.2.1 Species level testing

Report:	CA 8.4.2.1/1 Ganssmann M., 2013b Effects of BAS 555 F (Metconazole) on reproduction of the Collembola Folsomia candida in artificial soil with 5% peat 2013/1003177
Guidelines:	OECD 232 (2009)
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

Executive Summary

The effects of BAS 555 F (metconazole, Reg. No. 4 056 343) on mortality and reproduction of Collembola (*Folsomia candida*) were investigated in a chronic laboratory study over 28 days. Six concentrations (10, 20, 40, 80, 160 and 320 mg BAS 555 F/kg dry soil) were incorporated into the soil (5% peat) with 4 replicates per treatment (each containing 10 collembolans). An untreated control with 8 replicates was included. Assessment of adult mortality and reproduction rate (number of juveniles) was carried out after 28 days.

After 28 days of exposure, the mortality was not statistically significantly increased compared to the control at any test item concentration except the concentration of 320 mg BAS 555 F/kg dry soil, where 33.0% of the exposed collembolans were dead.

The mean reproduction values in the test item groups were not statistically significantly different up to and including the concentration of 40 mg BAS 555 F/kg dry soil. At 80, 160 and 320 mg BAS 555 F/kg dry soil, the reproduction was statistically significantly reduced compared to the control. No behavioral abnormalities were observed in any of the treatment groups.

In a 28-day reproduction study with BAS 555 F (Reg. No. 4 056 343) on collembolans (*Folsomia candida*), the NOEC for reproduction was 40 mg BAS 555 F/kg dry soil, whereas the NOEC for mortality was 160 mg BAS 555 F/kg dry soil. The LC_{50} was > 320 mg BAS 555 F/kg dry soil.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 555 F (metconazole, Reg. No. 4 056 343), batch no. COD-001163, analyzed purity: 98.7% (± 1.0%).

B. STUDY DESIGN

Test species: Collembola (*Folsomia candida*), juveniles (10-12 days old); source: in-house culture.

Test design: 28-day test in treated artificial soil according to OECD 232 (5% peat); artificial soil was filled in glass vessels after treatment with different concentrations of the test item before collembolans were introduced on top of the soil. 7 treatment groups (6 test item concentrations, 1 control) were set up with 8 replicates for control and 4 replicates for test item, each with 10 collembolans. Assessment of adult mortality, behavioral effects and reproduction rate was carried out after 28 days.

Endpoints: Mortality, reproduction rate, behavioral effects.

Reference item: Boric acid. The effects of the toxic reference item were investigated in a separate study.

Test concentrations: Control, 10, 20, 40, 80, 160 and 320 mg BAS 555 F/kg dry soil.

Test conditions: Artificial soil according to OECD 232 (5% peat); pH 6.2 – 6.5 at test initiation, pH 5.7 - 5.9 at test termination; water content at study initiation 50.8% - 51.3% of maximum water holding capacity (WHC) and 48.3% - 50.5% of maximum WHC at test termination; temperature: 18.0°C – 22.0°C; photoperiod: 16 h light : 8 h dark; light intensity: 400 - 800 lux; food: approximately 2 mg granulated dry yeast at the start of the test and after 14 days.

Statistics: Descriptive statistics. Fisher's Exact Test for mortality ($\alpha = 0.05$, one-sided greater), Williams t-test for reproduction ($\alpha = 0.05$, one-sided smaller).

II. RESULTS AND DISCUSSION

After 28 days of exposure, the mortality was not statistically significantly increased compared to the control at any test item concentration except the concentration of 320 mg BAS 555 F/kg dry soil, where 33.0% of the exposed collembolans were dead (Fisher's Exact Test, $\alpha = 0.05$, one-sided greater).

The mean reproduction values in the test item groups were not statistically significantly different up to and including the concentration of 40 mg BAS 555 F/kg dry soil. At 80, 160 and 320 mg BAS 555 F/kg dry soil, the reproduction was statistically significantly reduced compared to the control (Williams-t-test, $\alpha = 0.05$, one-sided smaller). No behavioral abnormalities were observed in any of the treatment groups.

The results are summarized in Table 8.4.2.1-1

Table 8.4.2.1-1: Effects of BAS 555 F on Collembola (*Folsomia candida)* in a 28-day reproduction study

BAS 555 F [mg/kg dry soil]	**Control**	**10**	**20**	**40**	**80**	**160**	**320**
Mortality (day 28) [%]	8	5	10	3	13	15	33 *
No. of juveniles (day 28)	729	842	711	707	561 *	538 *	527 *
Reproduction (day 28) [% of control]	--	115	98	97	77	74	72
Endpoints [mg BAS 555 F/kg dry soil]							
$NOEC_{reproduction}$	40						
$NOEC_{mortality}$	160						
EC_{50}	> 320						
LC_{50}	> 320						

* Statistically significant compared to the control (Williams-t-test, $\alpha = 0.05$, one-sided smaller).

In a separate study, the reference item boric acid showed statistically significant effects on mortality and reproduction at concentrations of ≥ 53.7 mg/kg soil. The EC_{50} for reproduction was calculated to be 59.9 mg/kg soil.

III. CONCLUSION

In a 28-day reproduction study with BAS 555 F (Reg. No. 4 056 343) on collembolans (*Folsomia candida*), the NOEC for reproduction was 40 mg BAS 555 F/kg dry soil, whereas the NOEC for mortality was 160 mg BAS 555 F/kg dry soil. The LC_{50} was > 320 mg BAS 555 F/kg dry soil.

Report: CA 8.4.2.1/2
Schulz L., 2014b
Effects of BAS 555 F (Metconazole) on the reproduction of the predatory mite Hypoaspis aculeifer
2014/1028659
Guidelines: OECD 226 (2008)
GLP: yes
(certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

Executive Summary

The effects of BAS 555 F (Reg. No. 4 056 343) on mortality and reproduction of the predatory mite *Hypoaspis aculeifer* were investigated in a chronic laboratory study over 14 days. The test item was mixed into artificial soil at concentrations of 15.63, 31.25, 62.5, 125 and 250 mg BAS 555 F/kg dry soil. Test item treatments were replicated four times each. As a control treatment, the soil was prepared with acetone (solvent). Reproduction and mortality assessments of the mites were carried out after 14 days of exposure.

After 14 days of exposure, mortality rates of 0.0% - 7.5% were recorded in the test item treatment groups. In the solvent control, the mortality rate was 1.3%. The observed mortality rates for adult mortality in the test item treatment groups compared to the solvent control were not statistically significant. Reproduction rates in the 15.63, 31.25, 62.5, 125 and 250 mg BAS 555 F/kg dry soil were 231.5, 223.0, 202.3, 116.8 and 28.8 juveniles, respectively. The mean reproduction in the solvent control reached 208.1 juveniles. The test item showed no statistically significantly adverse effects on reproduction up to and including 62.5 mg BAS 555 F/kg dry soil. However, BAS 555 F caused statistically significant effects on reproduction at 125 and 250 mg BAS 555 F/kg dry soil.

In a 14-day reproduction study with BAS 555 F (Reg. No. 4 056 343) on predatory soil mites (*Hypoaspis aculeifer*), the NOEC for mortality was determined to be ≥ 250 mg BAS 555 F/kg dry soil. The NOEC for reproduction was determined to be 62.5 mg BAS 555 F/kg dry soil. The LC_{50} was determined to be > 250 mg BAS 555 F/kg dry soil.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 555 F (metconazole, Reg. No. 4 056 343), batch no. COD-001163, analyzed purity: 98.7% (± 1.0%).

B. STUDY DESIGN

Test species: *Hypoaspis aculeifer*, adult females with an age difference of 2 days; source: in-house culture.

Test design: 14-day laboratory test on effects of BAS 555 F on mortality and reproduction of soil mites. Artificial soil (5% peat) was treated with different concentrations of the test item and filled in glass vessels before predatory mites were introduced on top of the soil; 6 treatment groups (1 solvent control, 5 test item concentrations); 8 replicates for solvent control and 4 replicates for test item treatments, each with 10 soil mites; assessment of adult mortality and reproduction performed after 14 days.

Endpoints: Mortality and reproduction rate after 14 days.

Reference item: Dimethoate (analyzed purity: 99.8%, tolerance ± 1.0%). The effects of the reference item were investigated in a separate study.

Test concentrations: Untreated control (prepared with acetone only), 15.63, 31.25, 62.5, 125 and 250 mg BAS 555 F/kg dry soil.

Test conditions: Artificial soil according to OECD 226 (5% peat); pH 5.7 – 5.8 at test initiation, pH 5.5 – 5.6 at test termination; water content at test initiation 47.69% - 50.48% of maximum water holding capacity (WHC) and 46.65% - 51.79% of maximum WHC at test termination; temperature: 19.7°C – 21.2°C; photoperiod: 16 h light : 8 h dark; light intensity: 510 lux; food: cheese mites (*Tyrophagus putrescentiae*).

Statistics: Descriptive statistics; Fisher's Exact Binomial Test with Bonferroni Correction for mortality ($\alpha = 0.05$, one-sided greater), Williams t-test for reproduction ($\alpha = 0.05$, one-sided smaller).

II. RESULTS AND DISCUSSION

After 14 days of exposure, mortality rates of 0.0% - 7.5% were recorded in the test item treatment groups. In the solvent control, the mortality rate was 1.3%. The observed mortality rates for adult mortality in the test item treatment groups compared to the solvent control were not statistically significant (Fisher's Exact Binomial Test with Bonferroni Correction, $\alpha = 0.05$, one-sided greater). Reproduction rates in the 15.63, 31.25, 62.5, 125 and 250 mg BAS 555 F/kg dry soil were 231.5, 223.0, 202.3, 116.8 and 28.8 juveniles, respectively. The mean reproduction in the solvent control reached 208.1 juveniles. The test item showed no statistically significantly adverse effects on reproduction up to and including 62.5 mg BAS 555 F/kg dry soil (Williams-t-test, $\alpha = 0.05$, one-sided smaller). However, BAS 555 F caused statistically significant effects on reproduction at 125 and 250 mg BAS 555 F/kg dry soil (Williams-t-test, $\alpha = 0.05$, one-sided smaller).

The results are summarized in Table 8.4.2.1-2

Table 8.4.2.1-2: Effects of BAS 555 F on predatory mites (*Hypoaspis aculeifer*) in a 14-day reproduction study

BAS 555 F [mg/kg dry soil]	**Control**	**15.63**	**31.25**	**62.5**	**125**	**250**
Mortality (day 14) [%]	1.3	7.5	2.5	0.0	0.0	0.0
No. of juveniles (day 14)	208.1	231.5	223.0	202.3	116.8 *	28.8 *
Reproduction (day 14) [% of solvent control]	100	111	107	97	56	14
Endpoints [mg BAS 555 F/kg dry soil]						
$NOEC_{mortality}$	≥ 250					
$NOEC_{reproduction}$	62.5					
LC_{50}	> 250					
EC_{50} (95% confidence limit)	138.2 (127.6 – 149.7)					

* Statistically significant compared to the control (Williams-t-test, $\alpha = 0.05$, one-sided smaller).

III. CONCLUSION

In a 14-day reproduction study with BAS 555 F (Reg. No. 4 056 343) on predatory soil mites (*Hypoaspis aculeifer*), the NOEC for mortality was determined to be ≥ 250 mg BAS 555 F/kg dry soil. The NOEC for reproduction was determined to be 62.5 mg BAS 555 F/kg dry soil. The LC_{50} was determined to be > 250 mg BAS 555 F/kg dry soil.

Report:	CA 8.4.2.1/3 Schulz L., 2014c 1,2,4-triazole - CGA71019 - Effects on the reproduction of the predatory mite Hypoaspis aculeifer 2014/1326895
Guidelines:	OECD 226 (2008)
GLP:	yes (certified by Pflanzenschutzamt der Landwirtschaftskammer Hannover, Hannover, Germany)

Executive Summary

The effects of the test item CGA71019 (=1,2,4-triazole, =M555F020, referred to as "test item" below) on survival and reproduction of the soil mite *Hypoaspis aculeifer* were investigated in a laboratory study over 14 days. The test item was mixed into artificial soil according to OECD 226 (5% peat) at concentrations of 9.07, 16.3, 29.4, 52.9, 95.3, 171, 309, 556 and 1000 mg test item/kg dry soil. For the control treatment, the soil was left untreated. 8 replicates for the control and 4 replicates for the test item treatments were prepared, respectively; each containing 10 adult soil mites. Assessment of mortality and reproduction was carried out after 14 days.

Adult soil mite mortality rates of 0.0% to 10.0% were recorded in the test item treatment groups, compared to 3.8% mortality in the control group. This resulted in corrected mortality rates ranging from -1.3% to 6.5% in the treatment groups. The observed mortality rates in the test item treatment groups compared to control were not statistically significant.

The mean number of juveniles was 187.0 in the control and 212.8, 200.3, 203.5, 239.0, 244.8, 184.0, 116.3, 41.8 and 20.0 at concentrations of 9.07, 16.3, 29.4, 52.9, 95.3, 171, 309, 556 and 1000 mg test item/kg dry soil, respectively. The test item caused no statistically significantly adverse effects on reproduction up to and including a test concentration of 171 mg test item/kg dry soil. Statistically significant effects on reproduction could be observed at 309, 556 and 1000 mg test item/kg dry soil.

In a 14-day reproduction study with the test item CGA71019 (=1,2,4-triazole, =M555F020) on soil mites (*Hypoaspis aculeifer*), the LC_{50} was estimated to be > 1000 mg test item/kg dry soil. The NOEC for mortality was determined to be ≥ 1000 mg test item/kg dry soil, while the NOEC for reproduction was determined to be 171 mg test item/kg dry soil.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: CGA71019 (=1,2,4-triazole, =M555F020, metabolite of BAS 555 F, metconazole); batch no. R 200; analyzed purity: 99.0% (tolerance ± 2.0%).

B. STUDY DESIGN

Test species: Soil mites: *Hypoaspis aculeifer* (CANESTRINI); age: adults with an age difference of 2 days.

Test design: The effects of the test item on mortality and reproduction of the soil mite *Hypoaspis aculeifer* were investigated in a chronic laboratory experiment over a time period of 14 days according to OECD 226. Different concentrations of the test item were homogeneously mixed into the artificial soil (5% peat) which was then filled into glass vessels after which the soil mites were introduced on top of the soil; 10 treatment groups (9 test item concentrations, control); 8 replicates/control group and 4 replicates/test item treatment group each with 10 soil mites. Assessment of adult mortality and reproduction effects was carried out after 14 days.

Endpoints: Mortality and reproduction rate (no. juveniles) after 14 days.

Reference item: Dimethoate EC 400 (content of a.s. dimethoate: 400 g/L nominal). The effects of the reference item were investigated in a separate study.

Test concentrations: Control, 9.07, 16.3, 29.4, 52.9, 95.3, 171, 309, 556 and 1000 mg test item/kg dry soil.

Test conditions: Artificial soil according to OECD 226 (5% peat), pH 6.0 at test initiation, pH 5.7 - pH 6.0 at test termination; water content at test initiation 49.47% - 51.83% of maximum water holding capacity (WHC) and 48.5% - 51.52% of maximum WHC at test termination; temperature 19.5 °C – 21.2 °C; photoperiod: 16 h light : 8 h dark; light intensity: 511 lux. Feeding of mites with *Tyrophagus putrescentiae*.

Statistics: Descriptive statistics; Fisher’s Exact Binomial Test with Bonferroni Correction for mortality ($\alpha = 0.05$) and Williams-t-test for reproduction ($\alpha = 0.05$), Probit Analysis for EC-values.

II. RESULTS AND DISCUSSION

Adult soil mite mortality rates of 0.0% to 10.0% were recorded in the test item treatment groups, compared to 3.8% mortality in the control group. This resulted in corrected mortality rates ranging from -1.3% to 6.5% in the treatment groups. The observed mortality rates in the test item treatment groups compared to control were not statistically significant (Fisher's Exact Binomial Test with Bonferroni Correction, $\alpha = 0.05$).

The mean number of juveniles was 187.0 in the control and 212.8, 200.3, 203.5, 239.0, 244.8, 184.0, 116.3, 41.8 and 20.0 at concentrations of 9.07, 16.3, 29.4, 52.9, 95.3, 171, 309, 556 and 1000 mg test item/kg dry soil, respectively. The test item caused no statistically significantly adverse effects on reproduction up to and including a test concentration of 171 mg test item/kg dry soil. Statistically significant effects on reproduction could be observed at 309, 556 and 1000 mg test item/kg dry soil (Williams-t-test, $\alpha = 0.05$). The results are summarized in Table 8.4.2.1-3.

Table 8.4.2.1-3: Effects of the test item CGA71019 (=1,2,4-triazole, =M555F020) on *Hypoaspis aculeifer* in a 14-day reproduction study

CGA71019 [mg/kg dry soil]	**Control**	**9.07**	**16.3**	**29.4**	**52.9**	**95.3**	**171**	**309**	**556**	**1000**
Mortality (day 14) [%]	3.8	10.0	2.5	0.0	2.5	2.5	0.0	0.0	2.5	5.0
Mean no. of juveniles (day 14)	187.0	212.8	200.3	203.5	239.0	244.8	184.0	116.3 *	41.8 *	20.0 *
Reproduction (day 14) [% of control]	--	-13.8	-7.1	-8.8	-27.8	-30.9	1.6	37.8	77.7	89.3
Endpoints [mg CGA71019/kg dry soil]										
$NOEC_{mortality}$	≥ 1000									
$NOEC_{reproduction}$	171.0									
LC_{50}	> 1000									

* Statistically significantly different compared to the control (William's t-test, $\alpha = 0.05$).

III. CONCLUSION

In a 14-day reproduction study with the test item CGA71019 (=1,2,4-triazole, =M555F020) on soil mites (*Hypoaspis aculeifer),* the LC_{50} was estimated to be > 1000 mg test item/kg dry soil. The NOEC for mortality was determined to be ≥ 1000 mg test item/kg dry soil, while the NOEC for reproduction was determined to be 171 mg test item/kg dry soil.

CA 8.5 Effects on nitrogen transformation

Since Annex I inclusion of the active substance metconazole (BAS 555 F), no new studies on nitrogen transformation have been performed with the active substance. In contrast to the last Annex I inclusion, a risk assessment for soil microorganisms will be presented for the metabolite 1,2,4-triazole (= M555F020, see chapter M-CP 10.5.). A study on the effects of 1,2,4-triazole on nitrogen transformation is available and was already EU reviewed (EFSA Scientific Report, 138, 2008 and DAR, Vol. 3, B.9, 2006), see Table 8.5-1 below. A study on the effects of the representative formulation, BAS 555 01 F on nitrogen transformation was performed. Reference to formulation studies is made in chapter M-CP 10.5.

Table 8.5-1: EU Endpoint: Effects on Soil Microbial Activity - Ecotoxicological endpoints for soil micro-organisms

Test substance	Test design	EU agreed endpoints	Reference (BASF DocID)
1,2,4-triazole (= M555F020)	N	No unacceptable effects up to and including 0.333 mg/kg dry soil, equivalent to 0.25 kg/ha [1)]	2000/1021861

1) Study reviewed in EFSA Scientific Report, 138, 2008, DAR, Vol. 3, B.9, 2006.

CA 8.6 Effects on terrestrial non-target higher plants

Since Annex I inclusion of the active substance metconazole (BAS 555 F) no new studies on non-target plants have been performed with the active substance. However, for the active substance metconazole, EU agreed endpoints on non-target plants are available. For details, reference is made to the relevant EU documents. Studies on the effects of the representative formulation, BAS 555 01 F on non-target plants have been performed. Reference is made to chapter M-CP 10.6.

CA 8.6.1 Summary of screening data

No new studies are available.

CA 8.6.2 Testing on non-target plants

No new studies are available with the active substance. Studies were performed with the formulated product and reference is made to chapter M-CP 10.6.

CA 8.7 Effects on other terrestrial organisms (flora and fauna)

No new studies are available.

CA 8.8 Effects on biological methods for sewage treatment

The results of the already peer-reviewed and accepted study are still valid and are summarized in Table 8.8-1. No new study has been performed.

Table 8.8-1: Effects on biological methods for sewage treatment

Test item	Study type	Endpoint [mg a.s./L]	Reference (BASF DocID)	EU agreed
BAS 555 F (metconazole)	Respiration inhibition test (inhibition of oxygen consumption activated sludge from wastewater plant)	EC_{50} >1000	MK-690-005	yes

CA 8.9 Monitoring data

No monitoring studies assessing ecotoxicological effects of metconazole are available.

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Metconazole

Document M-CA, Section 9

LITERATURE DATA

Compiled by:

[redacted]

Telephone:
Telefax:
E-mail:

Version history[1]

Date	Data points containing amendments or additions and brief description	Document identifier and version number
27/Jan/2016	Additional TDM Literature data for Metabolism & residue data	Document MCA Section 9 Version 2 (BASF DocID 2016/1036511)

[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 How to revise an Assessment Report

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CA 9 LITERATURE DATA..... Fehler! Textmarke nicht definiert.

Metconazole

A literature search on Metconazole was performed by the BASF Group Information Center. The Literature Search Report on Metconazole describes the general search and evaluation process as well as details on search profiles, search histories and summary tables.

The complete search report is provided in K-CA 9 (BASF DocID 2015/1176049).

The first step of the search result processing based on summary records was done by the Information Center and involved the separation into "hits" and "ballast" (obviously irrelevant records). The “ballast” was not further processed.

The “hits” were further evaluated by the scientific experts and categorized into “not relevant”, “not reliable”, and “used for dossier”. This is documented in EXCEL files which are attached to the search report in K-CA 9 with the file names as listed below (alphabetical order):

Analytics:	Metconazole Literature Analytics
Consumer Safety:	Metconazole Literature Metabolism and Residues in Animals
Ecotoxicology:	Metconazole Literature Ecotox Aquatic
Environmental Fate:	Metconazole Literature Environmental Fate
	Metconazole Literature Ecotox General
	Metconazole Literature Ecotox Terrestric
	Metconazole Literature Ecotox Wildlife
	Metconazole Literature Metabolism and Residues in Plants
Product Chemistry:	Metconazole Literature Product Chemistry
Toxicology:	Metconazole Literature Toxicology

The hits in Analytics, Ecotox Terrestric and Ecotox Wildlife, Environmental Fate, Metabolism and Residues in Animals and Plants as well as Product Chemistry did not contribute to the risk assessment and were therefore not further discussed in the dossier. Hits considered as relevant and reliable for Toxicology are discussed in M-CA 5.8.2.

In addition, the most recent evaluations from US EPA and CA PMRA are provided in K-CA 9:

US EPA (2006): Pesticide Fact Sheet. Metconazole. New Chemical Tolerances Established.
(BASF DocID 2006/7017233)

US EPA (2008): Metconazole: FQPA Human Health Risk Assessment for Proposed New Uses on Small Grains, Stone Fruits, Tree Nuts, Peanuts, Soybeans, and Sugar Beets.
(BASF DocID 2008/7023558)

US EPA (2014): Metconazole – Human Health Risk Assessment for a Section 3 Registration of New Uses on Dry Shelled Pea and Beans (Except Soybean) Crop Subgroup 6C and Sunflower Crop Subgroup 20B; Crop Group Expansion to Rapeseed Subgroup 20A; and Crop Group Conversions to Fruit, Stone, Group 12-12; and Nut, Tree, Group 14-12.
(BASF DocID 2014/7004822)

CA PMRA (2011): Metconazole. Evaluation Report ERC2011-02.
(BASF DocID 2011/7009735)

CA PMRA (2013): Metconazole. Proposed Registration Decision. PRD2013-11.
(BASF DocID 2013/7005957)

CA PMRA (2015): Metconazole. Registration Decision. RD2015-01.
(BASF DocID 2015/7001835)

Triazole Derivative Metabolites (TDMs)

A literature search on TDMs was performed for the TDMG. The detailed methodology employed and the results obtained for Toxicology, Ecotoxicology, Environmental Fate and Metabolism & residues data are provided in the ~~three~~ four other documents of M-CA 9. (BASF DocID 2015/1228510; BASF DocID 2015/1228511, BASF DocID 2015/1228512 and BASF DocID 2015/1276731)

The hits in Toxicology, Environmental Fate, Ecotoxicology and Metabolism & residues data did not contribute to the risk assessment and were therefore not further discussed in the dossier.

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Document M-CA, Section 10

CLASSIFICATION AND LABELLING OF THE ACTIVE SUBSTANCE

Compiled by:

[redacted]

Telephone: [redacted]
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Version history[1]

Date	Data points containing amendments or additions and brief description	Document identifier and version number

[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 How to revise an Assessment Report

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CA 10 CLASSIFICATION AND LABELLING OF THE ACTIVE SUBSTANCE..... 4

CA 10 CLASSIFICATION AND LABELLING OF THE ACTIVE SUBSTANCE

Table 10-1: The following harmonized classification and labelling was adopted for metconazole:

Legislation	Classification	Labelling	Concentration limits
Regulation (EC) No 1272/2008	Hazard class and category code: Acute Tox. 4 (oral) Repr. 2 (unborn child) Aquatic Chronic 2 Hazard statement code: H302, H411, H361d	Pictogram signal word code: GHS07 GHS08 GHS09 Warning Hazard statement code: H302, H411, H361d	

New evaluation leads to a change of classification for aquatic acute and chronic hazard, since the lowest acute and chronic endpoints for metconazole are below 1.0 mg and 0.1 mg/L, respectively (see Table 10-3, 7 d E_rC_{50} with swollen duckweed and 95-d ELS fish NOEC). Additionally, M-factors are indicated for highly toxic substances (i.e. H400 or H410 classified). The resulting acute and chronic M-factors for metconazole are 1 and 10, respectively. Therefore, BASF proposed the following classification and labelling for metconazole:

Table 10-2: Proposed Classification and Labelling for Metconazole according to (EC) No 1272/2008

Legislation	Classification	Labelling	Concentration limits
Regulation (EC) No 1272/2008	Hazard class and category code: Acute Tox. 4 (oral) Repr. 2 (unborn child) Aquatic Acute 1 Aquatic Chronic 1 Hazard statement code: H302, H400, H410, H361d	Pictogram signal word code: GHS07 GHS08 GHS09 Warning Hazard statement code: H302, H410, H361d	M-factor$_{(acute)}$ = 1 M-factor$_{(chronic)}$ = 10

Table 10-3: Ecotoxicology/Environment data relevant for Classification of metconazole [1)]

Study Type (duration)	Results	Reference (BASF DocID / Name)
Oncorhynchus mykiss (96 h)	96 h LC_{50} = 2.1 mg/L [2)]	1990/7000167 / MK-510-001
***Oncorhynchus mykiss* (95 d)**	**95 d NOEC = 0.00291 mg/L**	**2001/1015080 / --**
Daphnia magna (48 h)	48 h EC_{50} = 4.2 mg/L	1990/7000167 / MK-510-001
Daphnia magna (21 d)	21 d NOEC = 0.160 mg/L	2002/1004678 / --
Selenastrum capricornutum (72 h)	72 h E_rC_{50} = 2.2 mg/L 72 h NOEC = 0.38 mg/L	1990/7000167 / MK-510-001
***Lemna gibba* (7 d) [$]**	**7 d E_rC_{50} = 0.527 mg/L** 7 d E_rC_{10} = 0.004 mg/L	**2014/1093918 / --**
Biodegradation	Metconazole is not readily biodegradable	-- / MK-690-001, MK-690-002

[$] Study was not submitted during Annex I inclusion process of the active substance (for details see chapter CA 8.2).
1) The lowest acute and chronic endpoint (basis for classification) is marked in **bold**.
2) Lowest EC_{50} value after 72 h in the 96 h test.