Triticonazole

Document M-CA, Section 1

IDENTITY OF THE ACTIVE SUBSTANCE

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CA 1 IDENTITY OF THE ACTIVE SUBSTANCE

CA 1.1 Applicant

BASF Agro B.V., Arnhem (NL) - Zuerich (Wädenswil) Branch
Im Tiergarten 7
8055 Zuerich
Switzerland

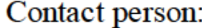
Contact person:

Alternative contact:

CA 1.2 Producer

Manufacturer of triticonazole (legal entity):

BASF Agro B.V., Arnhem (NL) - Zuerich (Wädenswil) Branch
Im Tiergarten 7
8055 Zuerich
Switzerland



Contact person:

Location of the manufacturing site of triticonazole:

Confidential information - data provided separately (Document J)

CA 1.3 Common Name Proposed or ISO-accepted and synonyms

Triticonazole

CA 1.4 Chemical Name (IUPAC and CA nomenclature)

IUPAC: rac-(5*E*)-5-(4-chlorobenzylidene)-2,2-dimethyl-1-(1*H*-1,2,4-triazol-1-ylmethyl)cyclopentanol

CA: (5*E*)-5-[(4-chlorophenyl)methylene]-2,2-dimethyl-1-(1*H*-1,2,4-triazol-1-ylmethyl)cyclopentanol

CA 1.5 Producer's Development Code Numbers

BAS 595 F,
Reg. No. 4378513
former Reg. No. RPA400727

CA 1.6 CAS, EC and CIPAC Numbers

CAS No: 131983-72-7
EC No: Not allocated
CIPAC No: 652

CA 1.7 Molecular and Structural Formula, Molar Mass

Molecular formula: $C_{17}H_{20}ClN_3O$

Structural formula:

Molar mass: 317.82

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

Minimum purity of the technical active substance: 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

Triticonazole TGAI does not contain impurities of toxicological, eco-toxicological or environmental concern.

CA 1.11 Analytical Profile of Batches

Confidential information - data provided separately (Document J)

Triticonazole

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	EC Method A.1	998.5 g/kg	Information previously reported and peer-reviewed: First crystalline form: 137°C Second crystalline form: 141°C	Y	EFSA Scientific Report (2005) 33, 1-69
	EC Method A.1	998.5 g/kg	Information previously reported and peer-reviewed: Starts slightly decomposing after melting. Significant decomposition above 180°C		
CA 2.2 Vapour pressure, volatility	EC Method A4 OECD 104	986 g/kg	9×10^{-8} Pa at 25°C	Y	[see KCA 2.2/1 2014/1001862]
	Calculation	N/A	Henry's Law Constant (K_H) = 3.07×10^{-6} Pa m^3/mole	Not relevant	[see KCA 2.2/2 2015/1137136]
			Henry's Law Constant (K_H) = 1.2×10^{-6} Pa m^3/mole (Vapour pressure and water solubility measured or extrapolated at 20°C were used for the calculation)	Not relevant	[see KCA 2.2/3 2015/1256390]
CA 2.3 Appearance (Physical state, colour)	Visual assessment	998.5 g/kg	Powder	Y	DAR, Volume 3, B2.1, 2003
		998.5 g/kg	White		
		957 & 959 g/kg	Information previously reported and peer-reviewed: Powder with or without agglutinated mass		EFSA Scientific Report (2005) 33,

Test or Study & Data Point	Guideline and method	Test material purity and specification	Findings	GLP Y/N	Reference
		957 & 959 g/kg	Information previously reported and peer-reviewed: White		1-69
CA 2.4 Spectra (UV/VIS, IR, NMR, MS), molar extinction at relevant wavelengths, optical purity	OECD 101	993 g/kg	Information previously reported and peer-reviewed: Neutral medium (MeOH/Water 100:10) Wavelength (nm) / Molar absorbance coefficient ($L \times mol^{-1} \times cm^{-1}$) 212 / 23879 263 / 25731 No significant modifications were observed between the spectrum obtained in neutral, acidic or basic media.	Y	EFSA Scientific Report (2005) 33, 1-69
		L85-136, 994 g/kg	UV absorption at 290 nm: in methanol: 1555 $L \times mol^{-1} \times cm^{-1}$ in water: 1884 $L \times mol^{-1} \times cm^{-1}$ in water (acidic conditions): 1734 $L \times mol^{-1} \times cm^{-1}$ in water (basic conditions): 1869 $L \times mol^{-1} \times cm^{-1}$ The absorption at 320 nm is negligible (<< 10 $L \times mol^{-1} \times cm^{-1}$) in all tested solutions.		[see KCA 2.4/1 2015/1256391]
		993 g/kg	Information previously reported and peer-reviewed: Wave number (cm^{-1}) / Group assignment 3140 / OH stretching (associated)		DAR, Volume 3, B2.1, 2003
		993 g/kg	Information previously reported and peer-reviewed: 1H NMR Spectrum δ (ppm) / Multiplicity, J (Hz) 8.11 / singlet 7.85 / singlet 7.23 / doublet, 8.5 7.05 / doublet, 8.5		

Test or Study & Data Point	Guideline and method	Test material purity and specification	Findings	GLP Y/N	Reference
			5.59 triplet, 2.4 4.36/4.21 doublet, 13.7 3.14 singlet (broad) 2.64 multiplet 1.75 multiplet 1.13 singlet 0.90 singlet		
		993 g/kg	Information previously reported and peer-reviewed: m/z Assignment 317 molecular ion M^+ 235 base peak $(M\text{-}CH_2Tz)^+$ 83 $(CH_3Tz)^+$ Tz = Triazole		
CA 2.5 Solubility in water	EC Method A.6, OECD 105	993 g/kg	Information previously reported and peer-reviewed: Distilled water (pH 7.3 – 8.7; 20°C): 9.3 mg/L Buffer solution pH 5 (20°C): 7.7 mg/L Buffer solution pH 9 (20°C): 8.3 mg/L	Y	EFSA Scientific Report (2005) 33, 1-69
CA 2.6 Solubility in organic solvents	EC Method A.6 Flask method	959 g/kg	Information previously reported and peer-reviewed: Solvent Solubility at 20°C (g/L) Hexane: 0.12 Toluene: 12.6 Methanol: 18.2 2-Propanol: 7.6 1-Octanol: 6.2 Dichloromethane: 191.0 Acetone: 74.5 Ethyl acetate: 48.6	Y	EFSA Scientific Report (2005) 33, 1-69

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	EC Method A8 OECD 117	993 g/kg	Information previously reported and peer-reviewed: Log P_{ow} = 3.29 ± 0.04 at 20°C Independent of the pH value	Y	EFSA Scientific Report (2005) 33, 1-69
	EC Method A8 OECD 117	RPA 404766 993 g/kg	pH 4: Log P_{OW} = 1.6 at 25°C pH 7: Log P_{OW} = 1.6 at 25°C pH 10: Log P_{OW} = 1.5 at 25°C	Y	[see KCA 2.7/1 2014/1001865]
	EC Method A8 OECD 117	RPA 407922 995 g/kg	pH 4: Log P_{OW} = 1.9 at 25°C pH 7: Log P_{OW} = 1.6 at 25°C pH 10: Log P_{OW} = 0.04 at 25°C	Y	[see KCA 2.7/2 2014/1001866]
	EC Method A8 OECD 117	RPA 406341 918 g/kg	pH 4: Log P_{OW} = 2.2 at 25°C pH 7: Log P_{OW} = 2.2 at 25°C pH 10: Log P_{OW} = 2.1 at 25°C	Y	[see KCA 2.7/3 2014/1001864]
	EC Method A8 OECD 117	RPA 406203 999 g/kg	pH 4: Log P_{OW} = 3.5 at 25°C pH 7: Log P_{OW} = 3.5 at 25°C pH 10: Log P_{OW} = 3.5 at 25°C	Y	[see KCA 2.7/4 2014/1001863]
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		Information previously reported and peer-reviewed: No dissociation is expected in aqueous solution. The examination of the chemical structure shows that there is no substituent which could be easily ionised and make the substance ionisable over the range of pH 3-9.	Not relevant	EFSA Scientific Report (2005) 33, 1-69

Test or Study & Data Point	Guideline and method	Test material purity and specification	Findings	GLP Y/N	Reference
CA 2.9 Flammability and self-heating	EC Method A.10	968 g/kg	Information previously reported and peer-reviewed: Not highly flammable	Y	EFSA Scientific Report (2005) 33, 1-69
	Method 14.3.4 of the UN RTDG	971 g/kg	Information previously reported and peer-reviewed: No self-ignition or exothermic behaviour was observed when the test substance was maintained at 140°C for 25 hours.	N	DAR, Volume 3, B2.1, 2003
CA 2.10 Flash point			Not applicable as the melting point is > 40°C		
CA 2.11 Explosive properties	EC Method A.10	911 g/kg	Information previously reported and peer-reviewed: Not explosive	Y	EFSA Scientific Report (2005) 33, 1-69
CA 2.12 Surface Tension	EC Method A5 OECD 105	986 g/kg	90% saturated solution: 72.0 mN/m at 20°C	Y	[see KCA 2.12/1 2014/1001862]
CA 2.13 Oxidising properties	EC Method A.17	968 g/kg	Information previously reported and peer-reviewed: The test material has been determined to be non-oxidising.	Y	EFSA Scientific Report (2005) 33, 1-69
CA 2.14 Other studies			None		

Summary of Data Points CA 2.1 to CA 2.14

Triticonazole is a white powder with a melting point of 137 to 141°C. It has a very low vapour pressure and is very slightly volatile. It has low solubility in water (8-9 mg/L) but is soluble in organic solvents. It does not dissociate in water and has a partition coefficient (log Pow) of 3.3, indicating a potential for bioaccumulation. Triticonazole does not possess explosive or oxidising properties and is neither flammable nor auto-flammable, indicating that it does not present problems during transport or storage. There are no implications for classification.

Triticonazole

Document M-CA, Section 3

FURTHER INFORMATION ON THE ACTIVE SUBSTANCE

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CA 3 FURTHER INFORMATION ON THE ACTIVE SUBSTANCE

CA 3.1 Use of the Active Substance

Triticonazole is a contact and systemic fungicide, used as seed treatment against seed and soil borne diseases such as *Tilletia* spp, *Ustilago* spp, *Fusarium* spp in cereals and *Fusarium* spp, *Ustilago* and *Sphacelotheca* of maize.

The representative formulation is a seed treatment product (Premis, BAS 595 01 F) containing 25 g a.i./L and is used at the following rate: 5 g a.i. (0.2 L product)/100 kg seeds, i.e. 12.5 g a.i./ha at a max. seeding rate of 250 kg/ha.

CA 3.2 Function

Fungicide

CA 3.3 Effects on Harmful Organisms

Triticonazole is active as a contact and systemic fungicide seed treatment; target fungal pathogens are killed or suppressed. It shows an apoplastic (upwards) distribution inside the plant after penetration.

CA 3.4 Field of Use Envisaged

Agriculture

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

Triticonazole is used to control a range of seed and soil borne diseases such as *Tilletia* spp, *Ustilago* spp, *Fusarium* spp in cereals and *Fusarium* spp, *Ustilago* and *Sphacelotheca* of maize.

CA 3.6 Mode of Action

Triticonazole 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 14α-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 sterol biosynthesis inhibitors (SBI).

It is active as a contact and systemic fungicide. It shows an apoplastic (upward) distribution inside the plant after penetration. When applied as a seed treatment, the product is slowly absorbed by the seedlings through the seed, teguments and the root.

The biological activity of the Triticonazole enantiomers on cereal diseases after seed treatment application was investigated (Strathmann and Dombo, 2005, BASF DocID 2005/1044280): Both the R-enantiomer and the S-enantiomer showed biological activity, and both enantiomers contribute to the broad-spectrum activity of the racemic Triticonazole.

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

Triticonazole should present the same cross-resistance patterns as other sterol demethylation inhibitor (DMI) fungicides. A resistance risk analysis was conducted in 2013. No reports on a reduced sensitivity to demethylation inhibitors (DMIs) for the target pathogens exist at the current time. There is no cross-resistance within the SBI-group, i.e. between morpholines and DMI fungicides. Likewise there is no cross resistance or a correlation in the sensitivity to SBI fungicides and other modes of action. Baseline data are not available. No monitoring data and no reports on field failure are available for the target pathogens. The FRAC (Fungicide resistance action committee) working group described the DMI-fungicides in general as medium-risk compounds. The pathogen risk is assessed as follows:

- Low risk pathogens: *Tilletia caries, Ustilago nuda, Pyrenophora graminea*
- Medium risk pathogens: *Microdochium spp., Fusarium spp.*.

The combined resistance risk of *Pyrenophora graminea, Tilletia caries, Ustilago nuda* and DMIs is concluded to be low and that of *Microdochium spp.* and *Fusarium spp.* and DMIs to be medium. For common bunt (*Tilletia caries*) and loose smut (*Ustilago nuda*) resistance development would only have consequences if it developed in a crop destined for seed production. Development of resistant isolates in a food crop would be inconsequential because the resistant propagules would be removed from the population at harvest. This further lowers the chances of resistance becoming a problem.

The objective of anti-resistance management strategies is the reduction of selection pressure to avoid or delay the occurrence of resistance. This can be achieved by good agricultural practice, which leads to less infection pressure (e.g. phytosanitary measurements, cultivation of less susceptible varieties, appropriate crop cultivation unfavourable for the target pathogens). Another important resistance management strategy is the restriction of use. By their very nature, cereal seed treatments are only applied once per season.

Since population size of pathogens is lower at disease onset than when already established in the field, selection pressure is less when using preventive applications rather than curative or eradicative spray schemes. A seed treatment is the most preventive application that can be made. This is from a resistance management point of view an optimal timing that is also an effective resistance management (van den Berg et al. 2013).

BASF is a member of the FRAC SBI Working Group and will promote effective anti-resistance management strategies.

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

Precautions for safe handling:
Handle in accordance with good industrial hygiene and safe practice. Avoid contact with skin, eyes and clothing. The wearing of closed work clothes is recommended. Remove contaminated clothing immediately and dispose of safely. Store work clothing separately. Keep away from food, drink and animal feeding stuffs. No eating, drinking, smoking or tobacco use at the place of work. Hands and/or face should be washed down before breaks and at the end of the shift.

Where required, the following PPE should be used:
Respiratory protection: Breathing protection if dusts or breathable aerosols/dust are formed. Wear respiratory protection if ventilation is inadequate. 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 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 goggle) (e.g. EN 166).

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

Conditions for safe storage:
Segregate from food and animal feeds. Protect against moisture, heat (temperatures > 40°C) and direct sunlight.

<u>Transport classification</u>:
UN No: UN3077
Proper Shipping Name: ENVIRONMENTALLY HAZARDOUS SUBSTANCE, SOLID, N.O.S. (contains TRITICONAZOLE)
Hazard Class: 9
Packing Group: III
Environmental hazard: Marine Pollutant

<u>Fire-fighting measures:</u>
Products of combustion include carbon monoxide, hydrogen chloride, carbon dioxide, halogenated hydrocarbons, hydrocarbons, and nitrogen oxides. Wear self-contained breathing apparatus and chemical-protective clothing. Use water spray, dry powder, foam, carbon dioxide extinguishers. Keep containers cool by spraying with water if exposed to fire.

<u>Environmental precautions</u>:
Do not discharge into the subsoil/soil. Do not discharge into drains/surface waters/groundwater.

CA 3.9 Procedures for Destruction or Decontamination

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

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

Personal precautions, protective equipment, and emergency procedures:
Use personal protective clothing. Avoid contact with the skin, eyes, and clothing.
In the event of an accident, the following first aid measures should be observed:

General advice:	Avoid contact with the skin, eyes, and clothing. Take off immediately all contaminated clothing. First aid personnel should pay attention to their own safety. If the patient is likely to become unconscious, place and transport in stable sideways position (recovery position). If difficulties occur: Obtain medical attention. Show container, label, and/or safety data sheet to physician.
If inhaled:	Keep patient calm, remove to fresh air, seek medical attention.
On skin contact:	After contact with skin, wash immediately with plenty of water and soap. If irritation develops, seek medical attention.
On contact with eyes:	Immediately wash affected eyes for at least 15 minutes under running water with eyelids held open, consult an eye specialist.
On ingestion:	Rinse mouth immediately and then drink plenty of water, seek medical attention. Do not induce vomiting unless told to by a poison control center or doctor. Never induce vomiting or give anything by mouth if the victim is unconscious or having convulsions.

Methods and materials for containment and cleaning up:
Sweep/shovel up spillages. Dispose of absorbed material in accordance with regulations. Collect waste in suitable containers, which can be labeled and sealed. Clean contaminated floors and objects thoroughly with water and detergents, observing environmental regulations. Incinerate or take to a special waste disposal site in accordance with local authority regulations.

BASF
We create chemistry

Triticonazole

Document M-CA, Section 4

ANALYTICAL METHODS

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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

The method R-566-12-93 (determination of the active substance content by reversed phase HPLC with U.V. detection) was submitted and peer-reviewed in the Annex I Inclusion process and is still valid. Please refer to the Draft Assessment Report (DAR), Volume 3, Annex B.5.1.1, 2003.

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

Triticonazole technical does not contain impurities of toxicological, eco-toxicological and environmental concern.

Significant impurities and additives:

CONFIDENTIAL INFORMATION please refer to document JCA.

CA 4.1.2 Methods for risk assessment

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

Report: CA 4.1.2/1
Bruns G., Tauber R., 1998a
Validation of the method of analysis for Triticonazole (RPA 400727) and its metabolites in soil and turf
R012011
Guidelines: EPA 164-1
GLP: yes

Principle of the method

Method MS 90.01 (modified) was developed for the determination of triticonazole and its metabolites M595F002 (RPA 406341) and M595F014 (RPA 406203) in soil and turf by LC-MS with a limit of quantification (LOQ) of 0.005 mg kg^{-1}.
Residues of triticonazole and its metabolites are extracted from soil or turf using a double extraction with an acetone/NH_4OH solution. An aliquot of the combined extracts are concentrated by rotary evaporation and the concentrated extract is cleaned-up on a C18 SPE cartridge. The eluent is concentrated and analyzed by gradient LC-MS.

Recovery findings The method proved to be suitable to determine triticonazole and its metabolites M595F002 (RPA 406341) and M595F014 (RPA 406203) in soil and turf. Samples were spiked with the analytes at the limit of quantification of 0.005 mg kg^{-1} (LOQ) to 10x LOQ. All average recovery values were between 70% and 120%. The detailed results are given in the table below (Table 4.1.2-1).

Table 4.1.2-1: Results of the method validation for the determination of triticonazole and its metabolites M595F002 (RPA 406341) and M595F014 (RPA 406203) in soils/turfs

Soil / Turf	Analyte	m/z	Fortification level [mg kg^{-1}]	Number of Replicates	Mean Recovery [%]	RSD [%]
LUCAMA, NC soil	Triticonazole	318.1 → 70	0.005	5	77	7.0
			0.025	5	88	11
	M595F002	334.1 → 70	0.005	5	91	9.9
			0.025	5	109	10
	M595F014	318.1 → 70	0.005	5	85	13
			0.025	5	81	12
WATSONVILLE, CA soil	Triticonazole	318.1 → 70	0.005	3	80	6.6
			0.050	3	77	14
	M595F002	334.1 → 70	0.005	3	105	7.7
			0.050	3	93	5.4
	M595F014	318.1 → 70	0.005	3	91	8.4
			0.050	3	80	15
EPHARATA, WA soil	Triticonazole	318.1 → 70	0.005	3	96	10
			0.025	3	84	17
	M595F002	334.1 → 70	0.005	3	120	8.8
			0.025	3	91	11
	M595F014	318.1 → 70	0.005	3	106	3.8
			0.025	3	76	14
LUCAMA, NC turf	Triticonazole	318.1 → 70	0.005	5	84	17
			0.025	5	83	6.3
	M595F002	334.1 → 70	0.005	5	85	11
			0.025	5	90	5.1
	M595F014	318.1 → 70	0.005	5	78	6.7
			0.025	5	76	3.7
WATSONVILLE, CA turf	Triticonazole	318.1 → 70	0.005	3	102	18
			0.050	3	77	13
	M595F002	334.1 → 70	0.005	3	110	12
			0.050	3	92	11
	M595F014	318.1 → 70	0.005	3	85	16
			0.050	3	78	13
EPHARATA, WA turf	Triticonazole	318.1 → 70	0.005	3	85	5.4
			0.025	3	76	9.1
	M595F002	334.1 → 70	0.005	3	91	8.9
			0.025	3	85	9.8
	M595F014	318.1 → 70	0.005	3	75	17
			0.025	3	72	5.6

Linearity Good linearity (r2 > 0.98) was observed in the range of 5 ng mL^{-1} to 100 ng mL^{-1} for the reported mass transitions of triticonazole and its two metabolites.

Specificity Residues of triticonazole and its metabolites M595F002 (RPA 406341) and M595F014 (RPA 406203) in soil and turf were confirmed by LC-MS/MS analysis. The analysis gave similar results (see Table 4.1.2-2) for selected extracts. Under the described conditions, the method is specific for the determination of triticonazole and its metabolites M595F002 (RPA 406341) and M595F014 (RPA 406203) in soil and turf.

Table 4.1.2-2: Comparison of soil and turf results for LC-MS analysis and confirmation analysis by LC-MS/MS

Sample	Fortification level [mg kg^{-1}]	Recovery [%]					
		Triticonazole		M595F002		M595F014	
		LC-MS	LC-MS/MS	**LC-MS**	LC-MS/MS	**LC-MS**	LC-MS/MS
LUCAMA, NC soil	**0.005**	**72**	94	**86**	120	**78**	88
	0.025	**76**	96	**92**	116	**76**	92
WATSONVILLE, CA soil	**0.005**	**76**	90	**96**	104	**86**	86
	0.025	**80**	104	**94**	124	**86**	96
EPHARATA, WA soil	**0.005**	**106**	88	**124**	116	**110**	88
	0.025	**76**	100	**80**	112	**68**	88
LUCAMA, NC turf	**0.005**	**68**	68	**84**	72	**72**	72
	0.025	**76**	72	**96**	84	**76**	68
WATSONVILLE, CA turf	**0.005**	**84**	96	**98**	100	**68**	94
	0.025	**68**	54	**92**	88	**68**	46
EPHARATA, WA turf	**0.005**	**82**	76	**84**	82	**60**	80
	0.025	**84**	76	**92**	96	**76**	68

Matrix Effects In the context of the study matrix effects were not reported.

Limit of Quantification The method has a limit of quantification (LOQ) of 0.005 mg kg^{-1} for soil and turf, resulting from the lowest concentration level successfully tested within recovery experiments and calculated LOQ values.

Limit of Determination The method has a limit of determination (LOD) of 0.002 mg kg^{-1}, corresponding to the lowest calibration level used.

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

Stability Working Solutions The standard solutions were made using acetonitrile. Each product prepared in acetonitrile and stored at 4 ± 3°C was stable for up to 5.5 months.

Extract Stability In context of the study report it was stated, that extracts of triticonazole, M595F002 (RPA 406341) and M595F014 (RPA 406203) were stored in acetonitrile at -20°C until analyzed. Further details are not given.

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

Conclusion The method MS 90.01 for analysis of triticonazole, M595F002 (RPA 406341) and M595F014 (RPA 406203) in soil and turf used LC-MS for final determination, results were checked by applying a confirmatory LC/MS/MS method. It could be demonstrated that the method fulfils the requirements with regard to linearity, specificity, repeatability, limit of quantification, and recoveries and is therefore applicable to correctly determine residues of triticonazole, M595F002 (RPA 406341) and M595F014 (RPA 406203) in soils and turfs.

Report: CA 4.1.2/2
Doran A.M. et al., 2001a
Triticonazole - Method validation for Triticonazole, RPA 406341 and RPA 404766 in soil
B003339

Guidelines: none

GLP: yes
(certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Principle of the method

The analytical method (CLE 198/120-02R) has been validated for the determination of triticonazole, M595F002 (RPA 406341) and M595F001 (RPA 404766) in soil. Soil is extracted with acetone/ammonium hydroxide using sonication. Extracts are purified using soil solid phase extraction (SPE) applying a C18 phase. Samples are evaporated under a stream of nitrogen and are further analysed by reverse phase LC-MS/MS with a limit of quantification (LOQ) of 0.002 mg kg^{-1}.

Recovery findings The method proved to be suitable to determine triticonazole and its metabolites M595F002 (RPA 406341) and M595F001 (RPA 404766) in soil. Samples were spiked with the analytes at the limit of quantification (LOQ; 0.002 mg kg^{-1}) to 10x LOQ (0.02 mg kg^{-1}). All average recovery values were within the acceptable range 70% and 110%. The detailed results are given in the table below (Table 4.1.2-3).

Table 4.1.2-3: Results of the method validation for the determination of triticonazole and its metabolites M595F002 (RPA 406341) and M595F001 (RPA 404766) in soils

Soil	Analyte	m/z	Fortification level [mg kg^{-1}]	Number of Replicates	Mean Recovery [%]	RSD [%]
Soil, Type 1	**Triticonazole**	318.1 → 70	0.002	5	82.4	4.2
			0.020	5	86.6	2.4
	M595F002	334.1 → 70	0.002	5	83.7	4.7
			0.020	5	89.3	3.6
	M595F001	318.1 → 70	0.002	5	82.9	4.3
			0.020	5	87.3	7.2
Soil, Type 2	**Triticonazole**	318.1 → 70	0.002	5	81.8	2.8
			0.020	5	89.8	1.8
	M595F002	334.1 → 70	0.002	5	86.7	4.7
			0.020	5	92.1	3.6
	M595F001	318.1 → 70	0.002	5	75.8	6.7
			0.020	5	88.3	3.3

Linearity	Good linearity (r > 0.996) was observed in the range of 0.50 ng mL^{-1} to 500 ng mL^{-1} for the two mass transitions of triticonazole and its two metabolites.
Specificity	No significant interferences due to reagents or matrices. Residues of triticonazole and its metabolites M595F002 (RPA 406341) and M595F001 (RPA 404766) in soil were confirmed by LC-MS/MS analysis. The analysis gave similar results (see Table 4.1.2-3) for selected extracts. Under the described conditions, the method is specific for the determination of triticonazole and its metabolites M595F002 (RPA 406341) and M595F001 (RPA 404766) in soil.
Matrix Effects	In context of the study matrix effects were not reported.
Limit of Quantification	The method has a limit of quantification (LOQ) of 0.002 mg kg^{-1} for each analyte.
Limit of Determination	The limit of determination (LOD) of method was not reported. According to the lowest calibration level of about 0.0005 mg kg^{-1} a limit of determination (LOD) of about 0.0005 mg kg^{-1} or even less can be anticipated.
Repeatability	The relative standard deviations (RSD, %) for all fortification levels were below 20%.
Stability Working Solutions	Each stock calibration standard solution indicated sufficient stability for 5.5 months, when stored in acetonitrile at + 4°C.
Extract Stability	In context of the study report it was stated, that extracts of triticonazole, M595F002 (RPA 406341) and M595F001 (RPA 404766) were stored in 5 ml acetonitrile +4°C until analyzed. Further details are not given.
Reproducibility	Reproducibility of the method was not determined within the validation study.
Conclusion	The method no. 0051 for analysis of triticonazole, M595F002 (RPA 406341) and M595F001 (RPA 404766) in soil uses LC-MS/MS for final determination, which is a highly specific technique. It could be demonstrated that the method fulfils the requirements with regard to linearity, specificity, repeatability,

Report: CA 4.1.2/3
Beck I.-C., 2007a
Development and validation of an analytical method for the determination of Triticonazole and its dihydroxy metabolites in drinking and in surface water, using LC/MS/MS
2007/1035039

Guidelines: EEC 91/414 Annex II (Part A Section 4.2), SANCO/825/00 rev. 7 (17 March 2004), SANCO/3029/99 rev. 4 (11 July 2000)

GLP: yes
(certified by Ministerium fuer Umwelt und Verkehr Baden-Wuerttemberg, Stuttgart)

Principle of the method

A LC-MS/MS method for the determination of triticonazole and its three dihydroxy metabolites M595F013 (RPA 407922), M595F002 (RPA 406341), and M595F001 (RPA 404766) in drinking and in surface water with a limit of quantification (LOQ) of 0.05 µg/L (per analyte) was developed and validated. Solid-phase extraction (SPE) using ENV+ cartridges retains all analytes from acidified 50-mL aliquots of water, followed by elution with 10 mL of acetone. LC-MS/MS monitors for each of the four analytes two parent-daughter ion transitions (MRMs) for quantitation and confirmation. Similar MS/MS fragmentation pattern of the three dihydroxy metabolites M595F013 (RPA 407922), M595F002 (RPA 406341) and M595F001 (RPA 404766) require chromatographic separation.

Recovery findings Method validation was accomplished by analyzing for each water type 2 blank control specimens, 5 replicate specimens fortified at LOQ, and 5 replicate specimens fortified at 10x LOQ. Method validation acceptance criteria were fully met with average recovery rates ranging from 84 % to 101 % and relative standard deviations (RSD) of ≤ 14 %. Recovery results for all analytes, each MRM and both water types are summarized below (Table 4.1.2-4).

Table 4.1.2-4: Results of the method validation for the determination of triticonazole and its metabolites M595F013 (RPA 407922), M595F002 (RPA 406341) and M595F001 (RPA 404766) in drinking and surface water

Fortification level [µg/L]	Analyte	Triticonazole		M595F013		M595F002		M595F001	
	Transition [m/z]	318.4 → 70	318.4 → 125	334.1 → 70	334.1 → 141	334.1 → 70	334.1 → 229.1	334.1 → 70	334.1 → 125
Drinking water by SPE and LC-MS/MS									
0.05	Average [%]	97	99	95	90	91	92	97	94
	RSD [%]	7	8	9	7	10	9	11	9
0.5	Average [%]	94	94	92	91	94	92	95	91
	RSD [%]	2	2	1	3	1	3	1	2
Surface water by SPE and LC-MS/MS									
0.05	Average [%]	93	98	86	84	84	91	101	100
	RSD [%]	9	7	11	9	12	10	5	6
0.5	Average [%]	98	90	94	91	91	89	90	91
	RSD [%]	1	3	9	14	8	11	2	3

Linearity	Good linearity (r > 0.999) was observed in the range of 0.25 ng mL^{-1} to 100 ng mL^{-1} for both mass transitions of triticonazole and its three metabolites.
Specificity	LC-MS/MS using a Thermo Aquasil HPLC column (C18 phase, 150 mm length, 3 mm i.d., 3 µm particle size) result in chromatographic separation of the three dihydroxy metabolites, as the three dihydroxy metabolites showed similar fragmentation patterns. Only the phenolic metabolite M595F013 (RPA 407922) give a selective ion transition at 334 m/z → 141 m/z, which was used for confirmation. Thus LC-MS/MS provides sufficient selectivity by monitoring for each of the analytes two daughter ions.
Matrix Effects	Blank control water specimens gave for all analytes and for all MRMs no signals interfering with detection of the analytes, supporting a limit of detection of 0.01 µg/L or 20 % of the LOQ.
Limit of Quantification	The method has a limit of quantification (LOQ) of 0.05 µg/L for triticonazole and its three dihydroxy metabolites M595F013 (RPA 407922), M595F002 (RPA 406341), and M595F001 (RPA 404766) in drinking and in surface water.
Limit of Determination	The method has a limit of determination (LOD) of 0.01 µg/L.
Repeatability	The relative standard deviations (RSD, %) for all fortification levels were below 20%.
Stability Working Solutions	All standard solutions were stored refrigerated when not in use. Stability of standard solutions was demonstrated by consistent LC-MS/MS results throughout the duration of the experimental phase of the study.
Extract Stability	In context of the study report it was stated, that sample extracts were subsequently analysed after SPE-extraction. Further details are not given.
Reproducibility	Reproducibility of the method was not determined within the validation study.
Conclusion	The method P1252G for analysis of triticonazole and its three dihydroxy metabolites M595F013 (RPA 407922), M595F002 (RPA 406341) and M595F001 (RPA 404766) in drinking and in surface water uses LC-MS/MS for final determination, which is a highly specific technique. It could be demonstrated that the method fulfilled the requirements during the time of conduction (SANCO/825/00 rev.7).

Report: CA 4.1.2/4
Chambers J. et al., 2014c
Method validation for the determination of Triticonazole in soil
2014/1036830

Guidelines: SANCO/825/00 rev. 8.1 (16 November 2010), EU Regulation 544/2011 (10 June 2011) implementing Regulation No 1107/2009, EU Regulation 1107/2009 with Regulation 545/2011 (former Annex III)

GLP: yes
(certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Principle of the method

Samples of soil (10g) are weighed into plastic extraction bottles (125 mL) and acetone/water, 4/1, v/v (100 mL) is added. The samples are shaken on a mechanical wrist-action shaker for 1 hour then centrifuged at 4000 rpm for 2 minutes. The supernatant is decanted into a volumetric flask (200 mL). The soil sample is extracted again using acetone/water, 4/1, v/v (100 mL). After the sample has been shaken on a mechanical wrist-action shaker for 1 hour and centrifuged at 4000 rpm for 2 minutes, the supernatant is combined with the first extract in the volumetric flask and the final volume adjusted to 200 mL with acetone/water, 4:1, v/v. An aliquot (0.1 mL) is diluted with acetone/water, 1:1, v/v (0.9 mL). The samples are analysed by high performance liquid chromatography with tandem mass specific detection (LC-MS/MS), in positive ion mode, using a Phenomenex Luna C18(2) column (50 mm x 2.0 mm, 5 µm particle size) and gradient elution with mobile phases of water containing 0.1% formic acid and acetonitrile containing 0.1% formic acid. Quantification is performed using external standards. The ion transition m/z 318 → 70 is used for quantitation and the ion transition m/z 318 → 125 is used for confirmation.

Specificity

LC-MS/MS monitoring two mass transitions is considered to be a highly specific technique.

No interferences at >30% of the LOQ were present at the retention time of interest in control matrix samples.

Linearity

Linearity of detector response was demonstrated using six matrix matched external standard solutions across the working range of 0.0510 ng/mL to 3.67 ng/mL. The results are presented in Table 4.1.2-5 below.

Table 4.1.2-5: Linearity Data

Analyte	Ion Transition (m/z)	Concentration Range (ng/mL)	Correlation Coefficient (r)	Slope	Intercept
Soil	318 → 70	0.0510 – 3.67	0.9990	5.09×10^5	1.21×10^4
	318 → 125	0.0510 – 3.67	0.9980	2.99×10^4	386

Accuracy (Recovery)

Recovery data was generated from six samples fortified at the LOQ and six samples fortified at 10 x LOQ. The mean percentage recoveries obtained were within the guideline requirements and are presented in Table 4.1.2-6 below.

Table 4.1.2-6: Precision and Accuracy Data

Matrix	Fortification Level (mg/kg)	Recoveries (%)	Mean Recovery (%)	Acceptable Recovery (%)	RSD (%)	Acceptable RSD (%)
Soil	**Ion transition m/z 318 → 70 (quantification)**					
	0.0541	92, 111, 104, 104, 101, 101	102	70 – 120	6.1	20
	0.541	104, 104, 101, 97, 100, 105	102	70 – 120	3.0	20
	Ion transition m/z 318 → 125 (confirmation)					
	0.0541	91, 104, 97, 97, 102, 98	98	70 – 120	4.5	20
	0.541	95, 102, 96, 101, 101, 98	99	70 – 120	3.0	20

Precision (Repeatability)

Repeatability data was generated from six samples fortified at the LOQ and six samples fortified at 10 x LOQ. The relative standard deviations (RSD) obtained were within the guideline requirements and are presented in Table 4.1.2-6.

Limit of Quantification (LOQ)

The limit of quantification (LOQ), defined as the lowest fortification level where acceptable precision and accuracy data were obtained, was demonstrated to be 0.05 mg/kg for Triticonazole in soil.

Conclusion

The analytical procedure has been successfully validated in terms of specificity, linearity, precision, accuracy and LOQ, in accordance with all of the requirements of SANCO/825/00 rev. 8.1.

Report: CA 4.1.2/5
Class T., 2014d
Determination of Triticonazole in drinking and in surface water
2014/1036831
Guidelines: SANCO/825/00 rev. 8.1 (16 November 2010)
GLP: yes
(certified by Landesamt fuer Umwelt, Messungen und Naturschutz Baden Wuerttemberg, Karlsruhe, Germany)

Report: CA 4.1.2/6
Class T., 2015b
Report amendment No. 1 - Determination of Triticonazole in drinking and in surface water
2015/1173435
Guidelines: SANCO/825/00 rev. 8.1 (16 November 2010)
GLP: yes
(certified by Landesamt fuer Umwelt, Messungen und Naturschutz Baden Wuerttemberg, Karlsruhe, Germany)

Principle of the method

Samples of acidified drinking and surface water (0.1% formic acid) are transferred to an autosampler vial and analysed by direct injection high performance liquid chromatography with tandem mass specific detection ((DI-) LC-MS/MS) in positive polarity mode, using a Phenomenex Aqua C18 column (50 x 2 mm, 5µm particle size) and gradient elution with mobile phases of water containing 0.1% formic acid and methanol containing 0.1% formic acid. Quantification is performed using external standards. The ion transition m/z 318 → 70 is used for quantification and the ion transition for 318 → 125 is used for confirmation.

Specificity

LC-MS/MS monitoring two mass transitions is considered to be a highly specific technique.

No interferences at >30% of the LOQ were present at the retention time of interest in control matrix samples.

Linearity

Linearity of detector response was demonstrated using six matrix matched external standard solutions across the working range of 0.010 to 1.5 ng/mL. The results are presented in Table 4.1.2-7 below.

Table 4.1.2-7: Linearity Data

Matrix	Ion Transition (m/z)	Concentration Range (ng/mL)	Correlation Coefficient (r)	Slope	Intercept
Drinking Water	318 → 70	0.010 – 1.5	0.9999	2.15×10^6	573
	318 → 125	0.010 – 1.5	0.9998	1.62×10^5	292
Surface Water	318 → 70	0.010 – 1.5	0.9999	2.18×10^6	3450
	318 → 125	0.010 – 1.5	0.9999	1.61×10^5	-115

Accuracy (Recovery)

Table 4.1.2-8: Precision and Accuracy Data

Matrix	Fortification Level (µg/L)	Recoveries (%)	Mean Recovery (%)	Acceptable Recovery (%)	RSD (%)	Acceptable RSD (%)
Drinking water	**Ion transition m/z 318 → 70 (quantification)**					
	0.05	115, 114, 112, 113, 115	114	70-120	1.0	20
	0.5	112, 112, 111, 111, 111	111	70-120	0.4	20
	Ion transition m/z 318 → 125 (confirmation)					
	0.05	112, 117, 114, 112, 111	113	70-120	2.1	20
	0.5	108, 111, 111, 111, 111	110	70-120	1.3	20
Surface water	**Ion transition m/z 318 → 70 (quantification)**					
	0.05	112, 111, 114, 116, 114	113	70-120	1.7	20
	0.5	107, 108, 110, 109, 114	112	70-120	2.2	20
	Ion transition m/z 318 → 125 (confirmation)					
	0.05	112, 118, 112, 117, 111	114	70-120	2.8	20
	0.5	108, 107, 108, 112, 110	109	70-120	1.7	20

Precision (Repeatability)

Repeatability data was generated from five samples of each matrix fortified at the LOQ and five samples fortified at 10 x LOQ. The relative standard deviations (RSD) obtained for each fortification level were within the guideline requirements of less than 20% and are presented in Table 4.1.2-8 above.

Limit of Quantification (LOQ)

The limit of quantitation (LOQ), defined as the lowest fortification level where acceptable precision and accuracy data were obtained, was determined to be 0.050 µg/L for Triticonazole in surface and drinking water.

Conclusion

The analytical procedure has been successfully validated in terms of specificity, linearity, precision and LOQ, in accordance with all of the requirements of SANCO/825/00 rev. 8.1.

Report: CA 4.1.2/7
Stanislowski T., 2014b
Development and validation of an analytical method for the determination of Triticonazole in air
2014/1036833
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

ORBO™-43 absorbent (XAD) tubes fortified with triticonazole are flushed with air (36°C, 94% relative humidity) at 1 mL/min for 6 hours. The analyte is extracted with ethyl acetate (3 mL) by ultra-sonicating the samples for approximately 3 minutes then transferring the extract to a graduated centrifuge vial. The extraction process is repeated a further two times and the extracts are combined in the graduated centrifuge vial and diluted to 10 mL with ethyl acetate. The samples are analysed by gas chromatography with tandem mass specific detection (GC-MS/MS) in positive ion mode, using an Agilent VF-5ms column (30 m x 0.25 mm x 0.25 µm) and an oven temperature program. Quantification is performed using external standards. The ion transition m/z 235 → 182 is used for quantitation and the ion transition for 235 → 217 is used for confirmation.

Specificity

GC-MS/MS monitoring two mass transitions is considered to be a highly specific technique.

No interferences at >30% of the LOQ were present at the retention time of interest in control matrix samples.

Breakthrough

No significant breakthrough of triticonazole was observed on the back sections of the absorbent tubes.

Linearity

Linearity of detector response was demonstrated using seven external standard solutions across the working range of 50 to 3500 ng/mL. The results are presented in Table 4.1.2-9 below.

Table 4.1.2-9: Linearity Data

Analyte	Ion Transition (m/z)	Concentration Range (ng/mL)	Coefficient of Determination (R^2)	Slope	Intercept
Triticonazole	235 → 182	50 – 3500	0.9949	5638.51	-271641
	235 → 217	50 – 3500	0.9968	4754.57	-209332

Accuracy (Recovery)
Recovery data was generated from five samples fortified at the LOQ and five samples fortified at 10 x LOQ. The mean percentage recoveries obtained at each fortification level were within the guideline requirements and are presented in Table 4.1.2-10 below.

Table 4.1.2-10: Precision Data – Air

Matrix	Fortification Level (µg/adsorption tube)	Recovery %	Mean Recovery (%)	Acceptable Recovery (%)	RSD (%)	Acceptable RSD (%)
Air	**Ion transition m/z 235 → 182 (quantification)**					
	2.7	84, 72, 75, 89, 82	80	70 – 120	9	20
	27	76, 94, 99, 97, 81	89	70 – 120	11	20
	Ion transition m/z 235 → 217 (confirmation)					
	2.7	83, 74, 75, 91, 78	80	70 – 120	9	20
	27	77, 93, 100, 96, 82	90	70 – 120	11	20

Precision (Repeatability)
Repeatability data was generated from five samples fortified at the LOQ and five samples fortified at 10 x LOQ. The relative standard deviations (RSD) obtained for each fortification level were within the guideline requirements and are presented in Table 4.1.2-10.

Limit of Quantification (LOQ)
The limit of quantitation (LOQ), defined as the lowest fortification level where acceptable precision and accuracy data were obtained, was determined to be 2.7 µg per adsorption tube for Triticonazole in air (corresponding to about 7.5 µg/m^3).

Conclusion
The analytical procedure has been successfully validated in terms of specificity, linearity, precision, accuracy and LOQ, in accordance with all of the requirements of SANCO/825/00 rev. 8.1.

(b) Methods in soil, water and any additional matrices used in support of efficacy studies
Not relevant.

(c) Methods in feed, body fluids and tissues, air and any additional matrices used in support of toxicological studies
As Triticonazole is not classified as toxic, no method of analysis is required.

(d) Methods in body fluids, air and any additional matrices used in support of operator, worker, resident and bystander exposure studies
No exposure studies were conducted with triticonazole. Consequently, such methods of analysis are not required.

(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

Report: CA 4.1.2/8
Stanislowski T., 2014c
Development and validation of an analytical method for the determination of the Triticonazole in various crop types
2014/1031578
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

Samples of tomato and orange (10 g) and samples of wheat grain and rape seed (5 g) are weighed into 50 mL screw-capped centrifuge vials. Acetonitrile (10 mL) is added to the samples and water (10 mL) is added to the wheat grain and rape seed samples only and the samples are shaken vigorously for 1 minute. Magnesium sulfate (4 g), sodium chloride (1 g), trisodium citrate dehydrate (1 g) and disodium hydrogencitrate sesquihydrate (0.5 g) are added to the samples and the samples are shaken vigorously for 1 minute then centrifuged at 3000 rpm for 5 minutes. The rape seed samples are transferred to a freezer overnight and then centrifuged for 1 minute at 4000 rpm. The raw extracts (6 mL) are then transferred to Dispersive SPE (dSPE) PSA Clean Up Tube (Supelco 55228-U). A spatula of C18 material is added to the rape seed samples only, and the samples are shaken for 30 seconds and centrifuged for 5 minutes at 3000 rpm. An aliquot of the extract (0.50 mL) is transferred to an autosampler vial and acidified with 5% formic acid in acetonitrile (5 µL) and vortex-mixed. Water containing 0.1% formic acid (0.50 mL) is added to the samples and the samples vortex-mixed. The orange, wheat grain and rape seed samples are filtered through a syringe filter before analysis. The samples are analysed by high performance liquid chromatography with tandem mass specific detection (LC-MS/MS) in positive ion mode, using a Phenomenex Luna C18 column (50 x 2.0 mm, 5 µm particle size) and gradient elution with mobile phases of methanol + 0.1% formic acid and water + 0.1% formic acid. Quantification is performed using external standards. The ion transition m/z 318 → 70 is used for quantitation and the ion transition for m/z 318 → 125 is used for confirmation.

Specificity

LC-MS/MS monitoring two mass transitions is considered to be a highly specific technique.

No interferences at >30% of the LOQ were present at the retention time of interest in control matrix samples.

Linearity

Linearity of detector response was demonstrated using six matrix matched external standard solutions across the working range of 1.0 to 60 ng/mL for tomato and orange and across the working range of 0.5 to 30 ng/mL for wheat grain and rape seed. The results are presented in Table 4.1.2-11 below.

Table 4.1.2-11: Linearity Data

Analyte	Ion Transition (m/z)	Concentration Range (ng/mL)	Correlation Coefficient (r)	Slope	Intercept
Tomato	318 → 70	1.0 – 60	0.9996	4.8×10^4	-6.06×10^3
	318 → 125	1.0 – 60	0.9995	3.06×10^3	-525
Orange	318 → 70	1.0 – 60	0.9998	1.32×10^4	-1.29×10^3
	318 → 125	1.0 – 60	0.9994	809	-86.1
Wheat Grain	318 → 70	0.5 – 30	0.9997	3.66×10^4	787
	318 → 125	0.5 – 30	0.9999	2.27×10^3	60.1
Rape Seed	318 → 70	0.5 – 30	0.9999	2.26×10^4	3.09×10^3
	318 → 125	0.5 – 30	0.9998	1.41×10^3	240

Accuracy (Recovery)

Recovery data was generated from five samples fortified at the LOQ and five samples fortified at 10 x LOQ for each matrix. The mean percentage recoveries obtained were within the guideline requirements and are presented in Table 4.1.2-12 to Table 4.1.2-15 below.

Table 4.1.2-12: Accuracy and Precision Data – Tomato

Matrix	Fortification Level (mg/kg)	Recoveries (%)	Mean Recovery (%)	Acceptable Recovery (%)	RSD (%)	Acceptable RSD (%)
Tomato	**Ion transition m/z 318 → 70 (quantification)**					
	0.01	105, 102, 101, 99, 103	102	60 – 120	2	30
	0.1	99, 95, 94, 94, 95	95	70 – 120	2	20
	Ion transition m/z 318 → 125 (confirmation)					
	0.01	102, 97, 98, 96, 101	99	60 – 120	3	30
	0.1	97, 93, 93, 95, 94	94	70 – 120	2	20

Table 4.1.2-13: Accuracy and Precision Data – Orange

Matrix	Fortification Level (mg/kg)	Recoveries (%)	Mean Recovery (%)	Acceptable Recovery (%)	RSD (%)	Acceptable RSD (%)
Orange	**Ion transition m/z 318 → 70 (quantification)**					
	0.01	101, 100, 102, 103, 101	101	60 – 120	1	30
	0.1	102, 102, 98, 102, 101	101	70 – 120	2	20
	Ion transition m/z 318 → 125 (confirmation)					
	0.01	101, 98, 107, 100, 102	102	60 – 120	3	30
	0.1	101, 101, 98, 101, 100	100	70 – 120	2	20

Table 4.1.2-14: Accuracy and Precision Data – Wheat Grain

Matrix	Fortification Level (mg/kg)	Recoveries (%)	Mean Recovery (%)	Acceptable Recovery (%)	RSD (%)	Acceptable RSD (%)
Wheat Grain	**Ion transition m/z 318 → 70 (quantification)**					
	0.01	103, 100, 98, 100, 98	100	60 – 120	2	30
	0.1	99, 96, 97, 98, 96	97	70 – 120	1	20
	Ion transition m/z 318 → 125 (confirmation)					
	0.01	97, 98, 95, 96, 93	96	60 – 120	2	30
	0.1	100, 96, 98, 98, 96	98	70 – 120	2	20

Table 4.1.2-15: Accuracy and Precision Data – Rape Seed

Matrix	Fortification Level (mg/kg)	Recoveries (%)	Mean Recovery (%)	Acceptable Recovery (%)	RSD (%)	Acceptable RSD (%)
Rape Seed	**Ion transition m/z 318 → 70 (quantification)**					
	0.01	99, 98, 98, 92, 88	95	60 – 120	5	30
	0.1	93, 91, 90, 89, 86	90	70 – 120	3	20
	Ion transition m/z 318 → 125 (confirmation)					
	0.01	98, 95, 96, 88, 92	94	60 – 120	4	30
	0.1	93, 90, 89, 88, 84	89	70 – 120	4	20

Precision (Repeatability)

Repeatability data was generated from five samples fortified at the LOQ and five samples fortified at 10 x LOQ for each matrix. The relative standard deviations (RSD) obtained were within the guideline requirements and are presented in Table 4.1.2-12 to Table 4.1.2-15.

Limit of Quantification (LOQ)

The limit of quantitation (LOQ), defined as the lowest fortification level where acceptable precision and accuracy data were obtained, was determined to be 0.01 mg/kg for Triticonazole in various crop types.

Conclusion

The analytical procedure has been successfully validated in terms of specificity, linearity, precision, accuracy and LOQ, in accordance with all of the requirements of SANCO/825/00 rev. 8.1.

Report: CA 4.1.2/9
Stanislowski T., 2014d
Development and validation of an analytical method for the determination of the Triticonazole in foodstuffs of animal origin
2014/1031579
Guidelines: SANCO/825/00 rev. 8.1 (16 November 2010)
GLP: yes
(certified by Landesamt fuer Umwelt, Messungen und Naturschutz Baden Wuerttemberg, Karlsruhe, Germany)

Principle of the method

Samples of whole milk, eggs, bovine meat and liver (5.0 g) and samples of fat (2.5 g) are weighed into 50 mL screw-capped centrifuge vials. The fat sample is warmed/ melted in a water bath at 40°C. Water (milk: 5 mL, eggs, meat and liver: 6 mL) is added along with acetonitrile (10 mL) and the samples are shaken vigorously for 1 minute. Water (10 mL) is added to the fat sample along with acetonitrile (10 mL) and the samples are warmed in a water bath set at 40°C before being shaken vigorously for 1 minute. Magnesium sulfate (4 g), sodium chloride (1 g), trisodium citrate dehydrate (1 g) and disodium hydrogencitrate sesquihydrate (0.5 g) are added to the samples and the samples are shaken vigorously for 1 minute then centrifuged at 3000 rpm for 5 minutes. The egg and fat samples are transferred to a freezer for 4 hours and then centrifuged for 1 minute at 4000 rpm. The raw extract (6 mL) is transferred to Dispersive SPE (dSPE) PSA Clean Up Tube (Supelco 55228-U). A spatula of C18 material is added to the egg and fat samples only, and the samples are shaken for 30 seconds and centrifuged for 5 minutes at 3000 rpm. An aliquot of the extract (1.0 mL) is transferred to an autosampler vial and acidified with 5% formic acid in acetonitrile (10 µL). The milk, egg, meat and liver samples are diluted by a factor of 5 using acetonitrile/water (2/6, v/v), containing 0.1% formic acid. The fat samples are diluted by a factor of 2.5 using water containing 0.1% formic acid. The samples are analysed by high performance liquid chromatography with tandem mass specific detection (LC-MS/MS) in positive ion mode, using a Phenomenex Luna C18 column (50 x 2.0 mm, 5 µm particle size) and gradient elution with mobile phases of methanol + 0.1% formic acid and water + 0.1% formic acid. Quantification is performed using external standards. The ion transition m/z 318 → 70 is used for quantitation and the ion transition for m/z 318 → 125 is used for confirmation.

Specificity

LC-MS/MS monitoring two mass transitions is considered to be a highly specific technique.

No interferences at >30% of the LOQ were present at the retention time of interest in control matrix samples.

Linearity

Linearity of detector response was demonstrated using six external standard solutions across the working range of 0.20 to 20.0 ng/mL. The results are presented in Table 4.1.2-16 below.

Table 4.1.2-16: Linearity Data

Analyte	Ion Transition (m/z)	Concentration Range (ng/mL)	Correlation Coefficient (r)	Slope	Intercept
Triticonazole	318 → 70	0.20 – 20.0	1.0000	3.4×10^4	532
	318 → 125	0.20 – 20.0	1.0000	2.06×10^3	-5.34

Accuracy (Recovery)

Recovery data was generated from five samples of each matrix fortified at the LOQ and five samples fortified at 10 x LOQ. The mean percentage recoveries obtained were within the guideline requirements and are presented in Table 4.1.2-17 to Table 4.1.2-21 below.

Table 4.1.2-17: Precision and Accuracy Data – Bovine Meat

Matrix	Fortification Level (mg/kg)	Recoveries (%)	Mean Recovery (%)	Acceptable Recovery (%)	RSD (%)	Acceptable RSD (%)
Bovine Meat	**Ion transition m/z 318 → 70 (quantification)**					
	0.01	114, 101, 99, 103, 102	104	60 – 120	6	30
	0.1	101, 90, 87, 85, 87	90	70 – 120	7	20
	Ion transition m/z 318 → 125 (confirmation)					
	0.01	107, 107, 101, 102, 102	104	60 – 120	3	30
	0.1	104, 89, 88, 84, 86	90	70 – 120	8	20

Table 4.1.2-18: Precision and Accuracy Data – Bovine Liver

Matrix	Fortification Level (mg/kg)	Recoveries (%)	Mean Recovery (%)	Acceptable Recovery (%)	RSD (%)	Acceptable RSD (%)
Bovine Liver	**Ion transition m/z 318 → 70 (quantification)**					
	0.01	100, 96, 99, 95, 98	98	60 – 120	2	30
	0.1	97, 95, 86, 82, 80	88	70 – 120	9	20
	Ion transition m/z 318 → 125 (confirmation)					
	0.01	100, 94, 103, 98, 96	98	60 – 120	4	30
	0.1	97, 96, 85, 83, 80	88	70 – 120	9	20

Table 4.1.2-19: Precision and Accuracy Data – Whole Milk

Matrix	Fortification Level (mg/kg)	Recoveries (%)	Mean Recovery (%)	Acceptable Recovery (%)	RSD (%)	Acceptable RSD (%)
Whole Milk	**Ion transition m/z 318 → 70 (quantification)**					
	0.01	102, 102, 99, 82, 76	92	60 – 120	13	30
	0.1	79, 109, 108, 74, 80	90	70 – 120	19	20
	Ion transition m/z 318 → 125 (confirmation)					
	0.01	98, 102, 93, 79, 71	89	60 – 120	15	30
	0.1	79, 110, 107, 73, 79	90	70 – 120	19	20

Table 4.1.2-20: Precision and Accuracy Data – Eggs

Matrix	Fortification Level (mg/kg)	Recoveries (%)	Mean Recovery (%)	Acceptable Recovery (%)	RSD (%)	Acceptable RSD (%)
Eggs	**Ion transition m/z 318 → 70 (quantification)**					
	0.01	94, 94, 94, 102, 97	96	60 – 120	4	30
	0.1	92, 85, 85, 80, 90	86	70 – 120	5	20
	Ion transition m/z 318 → 125 (confirmation)					
	0.01	95, 94, 96, 102, 98	97	60 – 120	3	30
	0.1	91, 85, 83, 81, 90	86	70 – 120	5	20

Table 4.1.2-21: Precision and Accuracy Data – Fat

Matrix	Fortification Level (mg/kg)	Recoveries (%)	Mean Recovery (%)	Acceptable Recovery (%)	RSD (%)	Acceptable RSD (%)
Fat	**Ion transition m/z 318 → 70 (quantification)**					
	0.01	83, 90, 96, 92, 93	91	60 – 120	6	30
	0.1	92, 87, 89, 89, 91	90	70 – 120	2	20
	Ion transition m/z 318 → 125 (confirmation)					
	0.01	81, 87, 95, 94, 91	89	60 – 120	6	30
	0.1	92, 90, 90, 90, 89	90	70 – 120	1	20

Precision (Repeatability)

Repeatability data was generated from five samples of each matrix fortified at the LOQ and five samples fortified at 10 x LOQ. The relative standard deviations (RSD) obtained were within the guideline requirements and are presented in Table 4.1.2-17 to Table 4.1.2-21.

Limit of Quantification (LOQ)

The limit of quantitation (LOQ), defined as the lowest fortification level where acceptable precision and accuracy data were obtained, was determined to be 0.01 mg/kg for Triticonazole in foodstuffs of animal origin.

Conclusion

The analytical procedure has been successfully validated in terms of specificity, linearity, precision, accuracy and LOQ, in accordance with all of the requirements of SANCO/825/00 rev. 8.1.

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

Report:	CA 4.1.2/10 Weber S., 2006a Validation of the analytical method 562/0: Determination of BAS 595 F (Reg.No. 4378513) in different plant matrices 2006/1009635
Guidelines:	EEC 96/46, SANCO/825/00 rev. 6 (20 June 2000), 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)
GLP:	yes (certified by Landesamt fuer Umweltschutz und Gewerbeaufsicht, Mainz, Germany)

Principle of the methods:
BAS 595F is extracted from different plant matrices with a mixture of methanol and water. An aliquot of the extract is centrifuged and partitioned against dichloromethane. The final determination of BAS 595 F is performed by HPLC-MS/MS.

Recovery findings:

The method proved to be suitable for analysis of Triticonazole in lettuce, bean, lemon, rape and corn (grain, straw and forage). In all matrices tested, the mean recovery values were between 70% and 110%. The detailed results are given in Table 4.1.2-22.

Table 4.1.2-22: Validation data for analytical methods for the determination of Triticonazole

Reference	Sample Matrix	Test Substance	Fortification level [mg/kg]	Average recovery [%]	RSD [%]	No. of analyses
CA 4.1.2/10	Lettuce	Triticonazole (m/z 318 →70)	0.01	80	4.7	5
			0.1	85.9	2.2	5
		Triticonazole (m/z 318 →125)	0.01	88.9	17.1	5
			0.1	87.1	3.1	5
	Bean	Triticonazole (m/z 318 →70)	0.01	89.7	2.8	5
			0.1	91	1.9	5
		Triticonazole (m/z 318 →125)	0.01	95	9.4	5
			0.1	92.9	1.6	5
	Lemon	Triticonazole (m/z 318 →70)	0.01	91.6	4.1	5
			0.1	88.7	3.7	5
		Triticonazole (m/z 318 →125)	0.01	92.5	18.9	5
			0.1	89.3	3.9	5
	Rape	Triticonazole (m/z 318 →70)	0.01	91.6	7.2	5
			0.1	80.6	10.4	5
		Triticonazole (m/z 318 →125)	0.01	85.6	5.9	5
			0.1	80.1	10.4	5
	Corn grain	Triticonazole (m/z 318 →70)	0.01	95.7	13.4	5
			0.1	86.3	2.7	5
		Triticonazole (m/z 318 →125)	0.01	96.5	11.1	5
			0.1	87.8	3.3	5
	Corn straw	Triticonazole (m/z 318 →70)	0.01	89.5	2.0	5
			0.1	83.6	1.0	5
		Triticonazole (m/z 318 →125)	0.01	91.6	8.7	5
			0.1	86.2	3.0	5
	Corn forage	Triticonazole (m/z 318 →70)	0.01	90.3	2.0	5
			0.1	82.1	3.1	5
		Triticonazole (m/z 318 →125)	0.01	91.5	8.9	5
			0.1	83.1	5.5	5

Linearity: Good linearity was observed in the range of 0.025 to 2.5 ng/mL for triticonazole (external reference standard).

Specificity: No significant interferences due to reagents or matrices.

Limit of Quantification: The limit of quantitation for BAS 595 F is 0.01 mg/kg in all plant matrices.

Repeatability The relative standard deviations (RSD, %) for all commodities were below 20%. The detailed values are shown in Table 4.1.2-22.

Conclusion The method has been successfully validated and fulfills the EU requirements with regard to specificity, repeatability and limit of quantitation. The mean recovery was between 70 and 110% and the relative standard deviation (RSD, %) was lower than 20%.

Report: CA 4.1.2/11
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 318. Analysis was accomplished using an YMC ProC18 column and an acetonitrile-pure water gradient with formic acid as modifier.
The limit of quantification (LOQ) of the method is 0.001 mg L^{-1}.
The multi-method was further developed for additional analytes and could therefore be found cited under the BASF method no. APL0500/03 too.

Recovery findings

The method proved to be suitable to determine BAS 595 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 100% and 101%. The detailed results are given in the table below.

Table 4.1.2-23: Results of the method validation for the determination of BAS 555 F in water

<table>
<tr><th>Matrix</th><th>Analyte</th><th>Fortification level [mg L⁻¹]</th><th>Number of Replicates</th><th>Mean Recovery [%]</th><th>RSD [%]</th><th>Overall Recovery [%]</th><th>RSD [%]</th></tr>
<tr><td rowspan="2">Ft-Mix Water (Tap-Water)</td><td rowspan="5">triticonazole Reg.No.4378513</td><td>0.001</td><td>5</td><td>100</td><td>0.7</td><td rowspan="2">100</td><td rowspan="2">1.1</td></tr>
<tr><td>0.1</td><td>5</td><td>101</td><td>1.0</td></tr>
<tr><td>AAP-Water</td><td>0.001</td><td>1</td><td>102</td><td>--</td><td>--</td><td>--</td></tr>
<tr><td>M4-Water</td><td>0.001</td><td>1</td><td>101</td><td>--</td><td>--</td><td>--</td></tr>
<tr><td>OECD-Water</td><td>0.001</td><td>1</td><td>101</td><td>--</td><td>--</td><td>--</td></tr>
</table>

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 595 F. The identification and quantification is based on the selected ion monitoring of ESI MS molecular ion signal M318 characteristic for the analyte. Under the described conditions the method is specific for the determination of BAS 595 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 595 F in water. It could be demonstrated that the method fulfils the requirements with regard to linearity, specificity, accuracy, repeatability, and limit of quantification.

Further methods for concentration control are reported, where necessary, along with the respective ecotoxicological 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.

CA 4.2 Methods for post-approval control and monitoring purposes

(a) Methods for the determination of all components included in the monitoring residue definition as submitted in accordance with the provision of point 6.7.1 in order to enable Member States to determine compliance with established maximum residue levels (MRLs); they shall cover residues in or on food and feed of plant and animal origin

Report:	CA 4.2/1 Jarrett H., 2014a Independent laboratory validation for the determination of Triticonazole in various crop types 2014/1031576
Guidelines:	EU Regulation 544/2011 (10 June 2011) implementing Regulation No 1107/2009, EU Regulation 1107/2009 with Regulation 545/2011 (former Annex III), SANCO/825/00 rev. 8.1 (16 November 2010)
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Principle of the method

Samples of tomato and orange (10 g) and samples of wheat grain and rape seed (5 g) are weighed into 50 mL screw-capped centrifuge vials. Acetonitrile (10 mL) is added to the samples and water (10 mL) is added to the wheat grain and rape seed samples only and the samples are shaken vigorously for 1 minute. Magnesium sulfate (4 g), sodium chloride (1 g), trisodium citrate dehydrate (1 g) and disodium hydrogen citrate sesquihydrate (0.5 g) are added to the samples and the samples are shaken vigorously for 1 minute then centrifuged at 3000 rpm for 5 minutes. The rape seed samples are transferred to a freezer overnight and then centrifuged for 1 minute at 4000 rpm. The raw extracts (6 mL) are then transferred to Dispersive SPE (dSPE) Clean Up Tube containing PSA (150 mg) and magnesium sulphate (900 mg). A spatula of C18 material (150 mg) is added to the rape seed samples only, and the samples are shaken for 30 seconds and centrifuged for 5 minutes at 3000 rpm. An aliquot of the extract (1 mL) is transferred to an autosampler vial and acidified with 5% formic acid in acetonitrile (10 µL) and vortex-mixed. Water containing 0.1% formic acid (1 mL) is added to the samples and the samples vortex-mixed. The orange, wheat grain and rape seed samples are filtered through regenerated cellulose syringe filters (0.45 µm) before analysis. The samples are analysed by high performance liquid chromatography with tandem mass specific detection (LC-MS/MS) in positive ion mode, using a Phenomenex Luna C18 (2) column (50 x 2.0 mm, 5 µm particle size) and gradient elution with mobile phases of methanol + 0.1% formic acid and water + 0.1% formic acid. Quantification is performed using external standards. The ion transition m/z 318 → 70 is used for quantitation and the ion transition for m/z 318 → 125 is used for confirmation.

Specificity

LC-MS/MS monitoring two mass transitions is considered to be a highly specific technique.

No interferences at >30% of the LOQ were present at the retention time of interest in control matrix samples.

Linearity

Linearity of detector response was demonstrated using seven matrix matched external standard solutions across the working range of 0.5 to 53 ng/mL for wheat grain and rape seed and across the working range of 0.8 to 69 ng/mL for tomato and orange. The results are presented in Table 4.2-1 below.

Table 4.2-1: Linearity Data

Analyte	Ion Transition (m/z)	Concentration Range (ng/mL)	Correlation Coefficient (r)	Slope	Intercept
Tomato	318 > 70	0.8 – 69	0.9999	2.19 x 10^5	2.37 x 10^4
	318 > 125	0.8 – 69	0.9999	2.2 x 10^4	348
Orange	318 > 70	0.8 – 69	0.9999	1.36 x 10^5	1.31 x 10^4
	318 > 125	0.8 – 69	1.0000	1.33 x 10^4	1.85 x 10^3
Wheat Grain	318 → 70	0.5 – 53	0.9997	2.22 x 10^5	2.95 x 10^4
	318 → 125	0.5 – 53	0.9997	2.2 x 10^4	1.15 x 10^3
Rape Seed	318 → 70	0.5 – 53	1.0000	2.24 x 10^5	7.97 x 10^3
	318 → 125	0.5 – 53	0.9999	2.23 x 10^4	-1.26 x 10^3

Accuracy (Recovery)

Recovery data was generated from at least five samples fortified at the LOQ and at least five samples fortified at 10 x LOQ for each matrix. The mean percentage recoveries obtained were within the guideline requirements and are presented in Table 4.2-2 to Table 4.2-5 below.

Table 4.2-2: Accuracy and Precision Data – Tomato

Matrix	Fortification Level (mg/kg)	Recoveries (%)	Mean Recovery (%)	Acceptable Recovery (%)	RSD (%)	Acceptable RSD (%)
Tomato	**Ion transition m/z 318 → 70 (quantification)**					
	0.01	110, 108, 108, 110, 106, 106	108	60 – 120	1.6	30
	0.1	110, 111, 110, 109, 108, 107	109	70 – 120	1.2	20
	Ion transition m/z 318 → 125 (confirmation)					
	0.01	105, 106, 106, 106, 104, 102	105	60 – 120	1.4	30
	0.1	110, 110, 111, 109, 107, 106	109	70 – 120	1.8	20

Table 4.2-3: Accuracy and Precision Data – Orange

Matrix	Fortification Level (mg/kg)	Recoveries (%)	Mean Recovery (%)	Acceptable Recovery (%)	RSD (%)	Acceptable RSD (%)
Orange	Ion transition m/z 318 → 70 (quantification)					
	0.01	111, 109, 109, 106, 108, 103	108	60 – 120	2.7	30
	0.1	109, 110, 112, 113, 114, 113	112	70 – 120	2.0	20
	Ion transition m/z 318 → 125 (confirmation)					
	0.01	112, 105, 108, 107, 106, 99	106	60 – 120	4.1	30
	0.1	109, 110, 113, 114, 115, 113	113	70 – 120	2.0	20

Table 4.2-4: Accuracy and Precision Data – Wheat Grain

Matrix	Fortification Level (mg/kg)	Recoveries (%)	Mean Recovery (%)	Acceptable Recovery (%)	RSD (%)	Acceptable RSD (%)
Wheat Grain	Ion transition m/z 318 → 70 (quantification)					
	0.01	98, 104, 101, 99, 105	101	60 – 120	2.9	30
	0.1	106, 106, 117, 116, 115, 110	112	70 – 120	4.4	20
	Ion transition m/z 318 → 125 (confirmation)					
	0.01	98, 99, 98, 98, 101	99	60 – 120	1.4	30
	0.1	105, 107, 119, 119, 116, 110	113	70 – 120	5.6	20

Table 4.2-5: Accuracy and Precision Data – Rape Seed

Matrix	Fortification Level (mg/kg)	Recoveries (%)	Mean Recovery (%)	Acceptable Recovery (%)	RSD (%)	Acceptable RSD (%)
Rape Seed	Ion transition m/z 318 → 70 (quantification)					
	0.01	100, 99, 100, 98, 103, 101	100	60 – 120	1.6	30
	0.1	101, 105, 101, 100, 103, 101	102	70 – 120	2.0	20
	Ion transition m/z 318 → 125 (confirmation)					
	0.01	97, 100, 101, 93, 100, 101	99	60 – 120	3.0	30
	0.1	101, 106, 100, 100, 105, 99	102	70 – 120	2.8	20

Precision (Repeatability)

Repeatability data was generated from at least five samples fortified at the LOQ and at least five samples fortified at 10 x LOQ for each matrix. The relative standard deviations (RSD) obtained were within the guideline requirements and are presented in Table 4.2-2 to Table 4.2-5.

Limit of Quantification (LOQ)

The limit of quantitation (LOQ), defined as the lowest fortification level where acceptable precision and accuracy data were obtained, was determined to be 0.01 mg/kg for Triticonazole in various crop types.

Conclusion

The analytical procedure has been successfully validated in terms of specificity, linearity, precision, accuracy and LOQ, in accordance with all of the requirements of SANCO/825/00 rev. 8.1.

Report:	CA 4.2/2 Chambers J.G. et al., 2014a Independent laboratory validation for the determination of Triticonazole in foodstuffs of animal origin 2014/1031577
Guidelines:	EU Regulation 544/2011 (10 June 2011) implementing Regulation No 1107/2009, EU Regulation 1107/2009 with Regulation 545/2011 (former Annex III), SANCO/825/00 rev. 8.1 (16 November 2010)
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Principle of the method

Samples of whole milk, eggs, bovine meat and liver (5.0 g) and samples of fat (2.5 g) are weighed into 50 mL screw-capped centrifuge vials. The fat sample is warmed/ melted in a water bath at 40°C. Water (milk: 5 mL, eggs, meat and liver: 6 mL) is added along with acetonitrile (10 mL) and the samples are shaken vigorously for 1 minute. Water (10 mL) is added to the fat sample along with acetonitrile (10 mL) and the samples are warmed in a water bath set at 40°C before being shaken vigorously for 1 minute. Magnesium sulfate (4 g), sodium chloride (1 g), trisodium citrate dehydrate (1 g) and disodium hydrogen citrate sesquihydrate (0.5 g) are added to the samples and the samples are shaken vigorously for 1 minute then centrifuged at 3000 rpm for 5 minutes. The egg and fat samples are transferred to a freezer for 4 hours and then centrifuged for 1 minute at 4000 rpm. The raw extract (6 mL) is transferred to Dispersive SPE (dSPE) Clean Up Tube containing PSA (150 mg) and magnesium sulphate (900 mg). A spatula of C18 material (150 mg) is added to the egg and fat samples only, and the samples are shaken for 30 seconds and centrifuged for 5 minutes at 3000 rpm. An aliquot of the extract (1.0 mL) is transferred to an autosampler vial and acidified with 5% formic acid in acetonitrile (10 µL). The milk, egg, meat and liver samples are diluted by a factor of 5 using acetonitrile/water (2/6, v/v), containing 0.1% formic acid. The fat samples are diluted by a factor of 2.5 using water containing 0.1% formic acid. The samples are analysed by high performance liquid chromatography with tandem mass specific detection (LC-MS/MS) in positive ion mode, using a Phenomenex Luna C18 (2) column (50 x 2.0 mm, 5 µm particle size) and gradient elution with mobile phases of methanol + 0.1% formic acid and water + 0.1% formic acid. Quantification is performed using external standards. The ion transition m/z 318 → 70 is used for quantitation and the ion transition for m/z 318 → 125 is used for confirmation.

Specificity

LC-MS/MS monitoring two mass transitions is considered to be a highly specific technique.

No interferences at >30% of the LOQ were present at the retention time of interest in control matrix samples.

Linearity

Linearity of detector response was demonstrated using five external standard solutions across the working range of 0.20 to 20.0 ng/mL. The results are presented in Table 4.2-6 below.

Table 4.2-6: Linearity Data

Analyte	Ion Transition (m/z)	Concentration Range (ng/mL)	Correlation Coefficient (r)	Slope	Intercept
Triticonazole	318 → 70	0.20 – 20.0	0.9975	4.99×10^5	1.01×10^4
	318 → 125	0.20 – 20.0	0.9977	5.6×10^4	3.26×10^3

Accuracy (Recovery)

Recovery data was generated from at least five samples of each matrix fortified at the LOQ and at least five samples fortified at 10 x LOQ. The mean percentage recoveries obtained were within the guideline requirements and are presented in Table 4.2-7 to Table 4.2-11 below.

Table 4.2-7: Precision and Accuracy Data – Bovine Meat

Matrix	Fortification Level (mg/kg)	Recoveries (%)	Mean Recovery (%)	Acceptable Recovery (%)	RSD (%)	Acceptable RSD (%)
Bovine Meat	**Ion transition m/z 318 → 70 (quantification)**					
	0.01	97, 102, 107, 110, 107, 108, 106	105	60 – 120	4.0	30
	0.1	93, 108, 94, 94, 91, 89	95	70 – 120	7.1	20
	Ion transition m/z 318 → 125 (confirmation)					
	0.01	97, 102, 106, 87, 110, 86, 90	97	60 – 120	9.8	30
	0.1	92, 110, 94, 95, 90, 89	95	70 – 120	7.9	20

Table 4.2-8: Precision and Accuracy Data – Bovine Liver

Matrix	Fortification Level (mg/kg)	Recoveries (%)	Mean Recovery (%)	Acceptable Recovery (%)	RSD (%)	Acceptable RSD (%)
Bovine Liver	**Ion transition m/z 318 → 70 (quantification)**					
	0.01	87, 88, 87, 97, 97, 90	91	60 – 120	5.2	30
	0.1	82, 84, 84, 85, 83, 84	83	70 – 120	1.1	20
	Ion transition m/z 318 → 125 (confirmation)					
	0.01	92, 90, 86, 95, 97, 98	93	60 – 120	5.1	30
	0.1	82, 84, 84, 84, 82, 83	83	70 – 120	1.3	20

Table 4.2-9: Precision and Accuracy Data – Whole Milk

Matrix	Fortification Level (mg/kg)	Recoveries (%)	Mean Recovery (%)	Acceptable Recovery (%)	RSD (%)	Acceptable RSD (%)
Whole Milk	**Ion transition m/z 318 → 70 (quantification)**					
	0.01	114, 124, 111, 117, 120, 113	116	60 – 120	4.1	30
	0.1	98, 96, 95, 95, 88, 98	95	70 – 120	3.8	20
	Ion transition m/z 318 → 125 (confirmation)					
	0.01	115, 123, 110, 117, 120, 111	116	60 – 120	4.3	30
	0.1	99, 97, 96, 96, 89, 98	96	70 – 120	3.6	20

Table 4.2-10: Precision and Accuracy Data – Eggs

Matrix	Fortification Level (mg/kg)	Recoveries (%)	Mean Recovery (%)	Acceptable Recovery (%)	RSD (%)	Acceptable RSD (%)
Eggs	**Ion transition m/z 318 → 70 (quantification)**					
	0.01	110, 109, 110, 113, 112, 124	113	60 – 120	4.9	30
	0.1	98, 108, 98, 98, 95	99	70 – 120	4.9	20
	Ion transition m/z 318 → 125 (confirmation)					
	0.01	110, 108, 109, 114, 110, 124	112	60 – 120	5.2	30
	0.1	98, 107, 97, 98, 96	99	70 – 120	4.4	20

Table 4.2-11: Precision and Accuracy Data – Fat

Matrix	Fortification Level (mg/kg)	Recoveries (%)	Mean Recovery (%)	Acceptable Recovery (%)	RSD (%)	Acceptable RSD (%)
Fat	**Ion transition m/z 318 → 70 (quantification)**					
	0.01	95, 95, 95, 98, 97, 100	97	60 – 120	2.1	30
	0.1	70, 75, 71, 73, 74, 73	73	70 – 120	2.5	20
	Ion transition m/z 318 → 125 (confirmation)					
	0.01	95, 95, 97, 98, 100, 100	98	60 – 120	2.2	30
	0.1	71, 75, 70, 74, 74, 72	73	70 – 120	2.3	20

Precision (Repeatability)

Repeatability data was generated from at least five samples of each matrix fortified at the LOQ and at least five samples fortified at 10 x LOQ. The relative standard deviations (RSD) obtained were within the guideline requirements and are presented in Table 4.2-7 to Table 4.2-11.

Limit of Quantification (LOQ)

The limit of quantification (LOQ), defined as the lowest fortification level where acceptable precision and accuracy data were obtained, was determined to be 0.01 mg/kg for Triticonazole in foodstuffs of animal origin.

Conclusion

The analytical procedure has been successfully validated in terms of specificity, linearity, precision, accuracy and LOQ, in accordance with all of the requirements of SANCO/825/00 rev. 8.1.

(b) Methods for the determination of all components included for monitoring purposes in the residue definitions for soil and water as submitted in accordance with the provisions of point 7.4.2

Report: CA 4.2/3
Chambers J. et al., 2014b
Independent laboratory validation for the determination of Triticonazole in drinking water
2014/1036832

Guidelines: SANCO/825/00 rev. 8.1 (16 November 2010), EU Regulation 1107/2009 with Regulation 544/2011 (former Annex II), EU Regulation 1107/2009 with Regulation 545/2011 (former Annex III)

GLP: yes
(certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Report: CA 4.2/4
Chambers J., 2015a
Battelle UK report amendment certification - Independent laboratory validation for the determination of Triticonazole in drinking water
2015/1177041

Guidelines: SANCO/825/00 rev. 8.1 (16 November 2010), EU Regulation 1107/2009 with Regulation 544/2011 (former Annex II), EU Regulation 1107/2009 with Regulation 545/2011 (former Annex III)

GLP: yes
(certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Principle of the method

Samples of acidified drinking water (0.1% formic acid) are transferred to an autosampler vial and analysed by direct injection high performance liquid chromatography with tandem mass specific detection (LC-MS/MS) in positive polarity mode, using a Phenomenex Luna C18 (2) column (50 x 2 mm, 5 µm particle size) and gradient elution with mobile phases of water containing 0.1% formic acid and methanol containing 0.1 % formic acid. Quantification is performed using external standards. The ion transition m/z 318 → 70 is used for quantification and the ion transition for m/z 318 → 125 is used for confirmation.

Specificity

LC-MS/MS monitoring two mass transitions is considered to be a highly specific technique.

No interferences at >30% of the LOQ were present at the retention time of interest in control matrix samples.

Linearity

Linearity of detector response was demonstrated using six matrix matched external standard solutions across the working range of 0.00984 to 1.51 ng/mL. The results are presented in Table 4.2-12 below.

Table 4.2-12: Linearity Data

Matrix	Ion Transition (m/z)	Concentration Range (ng/mL)	Correlation Coefficient (r)	Slope	Intercept
Drinking Water	318 → 70	0.00984 – 1.51	0.9995	6.34×10^5	5.47×10^3
	318 → 125	0.00984 – 1.51	0.9997	7.51×10^4	900

Accuracy (Recovery)

Table 4.2-13: Precision and Accuracy Data

Matrix	Fortification Level (µg/L)	Recoveries (%)	Mean Recovery (%)	Acceptable Recovery (%)	RSD (%)	Acceptable RSD (%)
Drinking water	**Ion transition m/z 318 → 70 (quantification)**					
	0.0493	77, 81, 81, 77, 86, 82	81	70-120	4.2	20
	0.493	92, 91, 94, 94, 98, 92	94	70-120	2.5	20
	Ion transition m/z 318 → 125 (confirmation)					
	0.0493	73, 80, 99, 73, 84, 78	81	70-120	12.0	20
	0.493	92, 89, 92, 93, 97, 89	92	70-120	3.2	20

Precision (Repeatability)

Repeatability data was generated from six samples of each matrix fortified at the LOQ and six samples fortified at 10 x LOQ. The relative standard deviations (RSD) obtained for each fortification level were within the guideline requirements of less than 20% and are presented in Table 4.2-13 above.

Limit of Quantification (LOQ)

The limit of quantitation (LOQ), defined as the lowest fortification level where acceptable precision and accuracy data were obtained, was determined to be 0.050 µg/L for Triticonazole in drinking water.

Conclusion

The analytical procedure has been successfully validated in terms of specificity, linearity, precision and LOQ, in accordance with all of the requirements of SANCO/825/00 rev. 8.1.

(c) Methods for the analysis in air of the active substance and relevant breakdown products formed during or after application, unless the applicant shows that exposure of operators, workers, residents or bystanders is negligible

Methods for concentration control in air are reported in chapter 4.1.2.

(d) Methods for the analysis in body fluids and tissues for active substances and relevant metabolites

There is a discrepancy between the regulation 283/2013 and the guidance document SANCO/825/00 rev 8.1. The regulation 283/2013 gives a link to the Official Journal of the European Union for a listing of valid guidance documents. For the monitoring methods only the SANCO 825/00 rev. 8.1 is named.

Based on the guidance document, an analytical method for the determination of active substance in body fluids is only required if the active substance is classified as toxic or very toxic (T, T+) or classified according GHS as follows: Acute toxicity (cat. 1-3), CMR (cat. 1) or STOT (cat. 1). As Triticonazole does not belong to these classes of compounds no method in body fluids is necessary for monitoring purposes.

Triticonazole

Document M-CA, Section 5

TOXICOLOGICAL AND METABOLISM STUDIES ON THE ACTIVE SUBSTANCE

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[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

The metabolism and toxicological studies were conducted with certified triticonazole technical grade active ingredient (TGAI). The TGAI is a stable racemic mixture, as shown via storage testing (see Doc J).

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

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

Three studies were conducted using the Sprague-Dawley rat orally administered with ^{14}C-Triticonazole. In a preliminary study the animals received one single dose of 500 mg/kg [redacted]. An A.D.M.E study [redacted] was also performed in male and female rats using single oral dosing at the nominal levels of 500 mg/kg (high dose) and 5 mg/kg (low dose) and a repeated oral dosing at a dose rate of 5 mg/kg for a period of 14 days with the non-radiolabelled Triticonazole followed by a radioactive dose on the fifteenth day. A bile excretion study [redacted] was carried out in order to provide supplementary information concerning the biliary excretion of ^{14}C-Triticonazole.
The studies have been part of the previous evaluation and are therefore not submitted again in this dossier. For reasons of convenience, a short summary of the main conclusions is given below.

Category of test	Dose ranges	Results	Reference (BASF DocID)
RPA 400727 A.D.M.E study in the rat	Low dose 5 and high dose 500 mg/kg bw/d (single oral ^{14}C-exposure) male and female Sprague Dawley rats	BAS 595 F showed a rapid elimination mainly via faeces and to a much lower percentage via urine in both sexes. The radioactivity in the tissues was very low. Parent and a number of hydroxylated metabolites were found in faeces. No parent was found in urine. No major differences were observed between male and female. The metabolisation was more intense in the lower dose level.	R013078
Triticonazole rat bile excretion study	5 and 500 mg/kg bw	The elimination of radiolabel via bile differs between the two dose rates. Only low levels of radioactivity in the tissues	R012111

Summary of toxicokinetic studies:
The absorption, distribution, metabolism and excretion of Triticonazole by Sprague-Dawley rats has been investigated using ^{14}C-test material.

* = position of radiolabel

Absorption
The degree of absorption of ^{14}C-Triticonazole, as calculated by the sum of the radioactivity found in the urine, expired air, cage wash and tissues (single oral dosing from [redacted]), was found to be very low at high dose rate: below 5%dose for both sexes. For the low dose rates the degree of absorption of ^{14}C-Triticonazole was higher and a sex difference was also observed. The mean percentages were found to be around 14%dose for male rats and around 33%dose for female rats. Results obtained from the biliary excretion study [redacted] led to the same qualitative results, the degree of absorption being greater for the Low dose (99.5% absorbed) than the High dose (33.8% absorbed). This difference was probably due to the non-dissolution of the majority of the test compound during its intestinal transit for the high dose group. Therefore an important part of the test compound was not available for the absorption. This hypothesis was supported in the ADME study [redacted] by the fact that a greater percentage of parent material was found in faeces from the high dose (67%dose) compared to the %dose found in the low dose group (1%dose).

Distribution
The distribution of radioactivity was found to be low following both single and repeated administration of ^{14}C-Triticonazole. Percentages of radioactivity found in the Single oral high dose group were 0.09%dose and 0.08%dose for males and females respectively. The levels of radioactivity in the tissues of the single oral low dose group animals accounted for a mean of 0.41% of the dose for the males and 0.11% for the females which was similar to the levels observed for the repeated low dose group where a mean of 0.57% of the dose was found in the tissues for the males and 0.22% for the females. The preliminary study [redacted] led to the same range of concentrations. In all cases the mean %dose found for male rats were slightly higher than those found for female rats. These very low levels of radioactivity found in tissues appear to be a reflection of the slightly higher elimination rates observed at the two dose rates.
In terms of tissue concentrations, the increase in concentrations observed for the high dose group was not proportional to the dose.

The single oral low dose group females were found to possess tissue concentrations that ranged between non-detected (heart, brain, spleen, muscle, bone & marrow and residual carcass) and 0.04 µg.equiv./g (adrenals). The males were found to have mean tissue concentrations between non-detected (brain) and 0.07 µg.equiv./g (plasma). The repeated low dose group possessed tissue concentrations that ranged from non-detected (brain, muscle and bone & marrow) to 0.07 µg.equiv./g (adrenals) for females and between 0.01 µg.equiv./g to 0.18 µg.equiv./g for males [redacted].

For the high dose group [redacted], the highest tissue concentrations were observed in the skin & fur and liver for both sexes.

Metabolism

Metabolism of Triticonazole was found to be rapid with only low levels (< 1.7% dose) of parent material found in the faecal extract samples (males and females) collected over the 24 hours following dosing for the two low dose experiments (single & repeated oral dosing - [redacted] [redacted]. The levels of parent material also decreased rapidly in the high dose experiment.

The following tables present a summary of the metabolite structures found for the ADME study [redacted]:

RPA N°		M595F006 RPA 406972		M595F005 RPA 405826/404886		M595F000 Triticonazole	
Structure							
Group	Sex	Males	Females	Males	Females	Males	Females
SOHD	Urine	0.78	2.51	n.d.	n.d.	n.d.	n.d.
	Faeces	6.58	2.58	5.66	5.21	79.74	85.19
	Total	**7.36**	**5.09**	**5.66**	**5.21**	**79.74**	**85.19**
SOLD	Urine	4.64	24.84	n.d.	n.d.	n.d.	n.d.
	Faeces	33.80	17.61	19.61	26.21	1.34	1.85
	Total	**38.44**	**42.45**	**19.61**	**26.21**	**1.34**	**1.85**
ROLD	Urine	2.18	11.23	n.d.	n.d.	n.d.	n.d.
	Faeces	34.16	21.59	15.14	24.12	0.23	0.18
	Total	**36.34**	**32.82**	**15.14**	**24.12**	**0.23**	**0.18**
RPA N°		n.a.		n.a.		n.a.	
Structure							
Sample	Sex	Males	Females	Males	Females	Males	Females
SOHD	Urine	0.62	0.13	n.d.	n.d.	0.07	0.05
	Faeces	1.53	0.25	1.37	0.37	0.54	1.61
	Total	**2.15**	**0.38**	**1.37**	**0.37**	**0.61**	**1.66**
SOLD	Urine	3.65	0.48	n.d.	n.d.	0.40	0.26
	Faeces	10.64	2.00	10.37	6.89	1.54	8.37
	Total	**14.29**	**2.48**	**10.37**	**6.89**	**1.94**	**8.63**
ROLD	Urine	4.02	0.49	n.d.	n.d.	0.52	0.23
	Faeces	13.88	2.06	7.58	8.02	1.58	6.65
	Total	**17.90**	**2.55**	**7.58**	**8.02**	**2.10**	**6.88**
RPA N°		M595F007 RPA 406780		M595F001 RPA 404766		M595F002 RPA 406341	
Structure							

n.d. = not detected
n.a. = analytical standards not currently available
SOHD = Single Oral High Dose group (500 mg/kg)
SOLD = Single Oral Low Dose group (5 mg/kg)
ROLD = Repeat Oral Low Dose group (5 mg/kg)

The major component in the faecal extracts from the high dose group was identified as parent material. M595F005 (RPA 405826/404886) was also identified as being one of the major metabolites. The major components in the faecal extracts from the low dose experiments were found to be M595F005 (RPA 405826/404886) and M595F006 (RPA 406972). Three other, more polar components were present in quantities up to 12.01% of the administered radioactivity of which two were found to be M595F005 (RPA 405826/404886) with an additional hydroxyl group and M595F006 (RPA 406972) with an additional hydroxyl group respectively.

No parent material or M595F005 (RPA 405826/404886) was observed in urine samples. A metabolite fraction was identified as being M595F006 (RPA 406972). The LC/MS analyses also indicated the presence of low levels of M595F001 (RPA 404766), M595F007 (RPA 406780) and M595F002 (RPA 406341) in the urine samples.

Following oral dosing, Triticonazole was extensively metabolised. As can be seen from the above data the proportion of the administered dose identified was high. This is especially true for the high dose group where ca. 98% of the administered radioactivity was assigned a structure. For the two low dose groups structures assigned to ca. 88% and ca. 82% of the administered dose for the single and the repeat low dose groups respectively. All metabolites representing ≥5% of the administered dose have been identified.

Elimination

The major route of elimination following oral administration of Triticonazole (for the single & repeated oral dosing experiments -) was the faeces followed by the urine. The rate of elimination was rapid with the majority being completed within 48 hours of dose administration.

The majority of the administered ^{14}C-Triticonazole observed in the blood was both rapidly absorbed and eliminated at both dose levels showing no significant difference between the sexes or whole blood and plasma.

Biliary Excretion study

The recoveries obtained from that study were different.

Male and female Sprague Dawley rats were administered [Phenyl U-14C]-Triticonazole at the nominal dose rates of 5 and 500 mg/kg. The recovery of radioactivity at 48 hours post administration were found to be quantitative with means of 100.46% and 99.68% for the low and the high dose groups respectively.

The low dose group results revealed that the mean proportions of the administered dose eliminated for the males and females respectively were: 95.24% and 87.50% in bile, 3.14% and 11.95% in urine, and 1.02% and 0.54% in faeces. No sex difference could be discerned. In contrast, the high dose group elimination results indicated that the mean proportions of the administered dose eliminated for the males and the females were: 29.90% and 22.38% in bile, 58.99% and 46.34% in faeces, 1.28% and 8.22% in urine. In this group, the elimination of the radiolabel occurred principally *via* the faeces in both sexes, the rate being similar between males and females and with a large inter-individual variation.

The mean measurable levels of radioactivity found in the low dose group tissues were found to range between 0.0002 µg equivalents/g (stomach contents, males) to 0.0993 µg equivVg (plasma, males). Higher concentrations were observed in the blood and plasma from the male rats compared with the female rats whereas levels in the carcass samples were the same. These results indicate that the levels of Triticonazole and/or its metabolites were very low at 48 hours after a single oral dose at the rate of 5 mg/kg. The mean measurable levels of radioactivity found in the high dose group tissues were found to range between 1.9 µg equivalents/g (blood, females) to 895.8 µg equiv./g (intestinal contents, females). No differences in concentrations were observed in the blood and plasma between the sexes although there was a reasonably high inter-individual variability in the results.
The absorption level of Triticonazole can be evaluated from the recoveries obtained in urine (plus cage wash), bile and tissues. The intestinal and stomach contents were excluded because it was not possible to say whether the radioactivity present was due to passive diffusion across the gut wall or had simply not been absorbed. Consequently, the absorption level of Triticonazole at low and high doses, can be summarised as follows:

Estimated Level of Oral Absorption for Triticonazole (in % of the administered dose)		
	5 mg/kg	500 mg/kg
Male Rats	98.8	31.8
Female Rats	100.2	35.8
Mean	99.5	33.8

The differences between the two dose levels are most likely to be due to dissolution effects. At the much lower dose level of 5 mg/kg the majority, if not all, the Triticonazole had time to be dissolved in the gut/intestinal fluids and thereafter absorbed. At the higher dose level of 500 mg/kg it is probable that a large proportion of the administered dose did not have time to dissolve in the gut or intestinal fluids and was therefore proportionately less available for absorption. The results from the A.D.M.E. study support this hypothesis in that the faeces from the high dose group were found to contain much higher levels of parent material (ca. 67% dose) than the low dose group (ca. 1% dose).

The discrepancy suggested that some of the metabolites and/or parent material excreted *via* the bile could be re-absorbed (enterohepatic circulation) and subsequently re-excreted *via* the urine.

Conclusion
In conclusion, when administered to rats, Triticonazole is well absorbed (especially at low levels), widely distributed but at low concentrations, extensively metabolised and rapidly excreted following after oral ingestion. The metabolic steps involved hydroxylation. Small differences were observed between the sexes in elimination patterns and metabolite profiles.

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

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.). In the absence of validated test methods or guidance documents, and in agreement with the RMS Austria, this data requirement is waived in accordance to SANCO Guidance Document SANCO/10181/2013-rev 2.1 (13 May 2013). Nevertheless, a study for the comparative in vitro metabolism is ongoing and will be submitted in the first quarter of 2016. The respective Dossier section will be amended accordingly.

Additional literature data

Report:	CA 5.1.2/1 Mazur C.S.,Kenneke J.F., 2007 a Cross-species comparison of Conazole fungicide metabolites using rat and rainbow trout (Onchorhynchus mykiss) hepatic microsomes and purified human CYP 3A4 2008/1103236
Guidelines:	none
GLP:	no

In the present study, in vitro metabolic profiles were determined for thirteen conazole fungicides (among them triticonazole) using rat and rainbow trout (Oncorhynchus mykiss) liver microsomes and purified human Cyp 3A4. In the following, only the results obtained with rat microsomes and the human protein are briefly summarized. Frozen hepatic microsomes from male Sprague-Dawley rats at a protein concentration of 20 mg microsome protein/ml were purchased from In Vitro Technologies (Baltimore, MD). Human recombinant Cyp 3A4 was purchased from In Vitro Technologies (Baltimore, MD). The screening assays (20 – 40 µM) were conducted using 10 pmol of recombinant Cyp3A4.

Analysis of all conazole samples was performed using LC/MS/MS analytical methods (with the retention times and the mass peak given).

For triticonazole, parent was identified in rat microsomes and human Cyp 3A4 at a retention time of 20.72 and 20.15 min (mass peak of 318 m/z). One metabolite Triti-M1 at a retention time of 11.89 in rat microsomes and 11.60 in human Cyp 3A4. The molecular mass of this metabolite is 334 m/z.

Conclusion: From this limited information, there is no indication for a difference in rat and human metabolism of triticonazole.

CA 5.2 Acute Toxicity

Studies evaluated in the draft monograph of triticonazole by the Rapporteur Member State Austria (2003):
Triticonazole (BAS 595 F) has been tested in various species and via different routes of administration. All studies are scientifically valid; however, some of the studies have been conducted before the release of study guidelines and are without GLP according to the usual practice in those days. These studies have been evaluated by European authorities and Austria as Rapporteur Member State (European Commission Peer Review Program) and are, for the convenience of the reviewer, listed in Table 5.2-1. Brief summaries of the respective studies were extracted from the monograph of triticonazole and are provided under the respective chapters.

Table 5.2-1: Summary of already peer-reviewed acute toxicity studies with triticonazole as available in the DAR (2003)

Route	Purity; Species/Sex	Dose range (vehicle)	Result Classification	Reference (BASF Doc ID)
Oral	95% - 99.7% Rat, CD, m/f	800 and 2000 mg/kg bw (water/MC)	LD_{50} (m/f) > 2000 mg/kg bw	R012999
Dermal	97.1% Rat, CD, f	2000 mg/kg bw (undiluted)	LD_{50} (m/f) >2000 mg/kg bw	R013017
Inhalation	97.1% Rat, CD, m/f	1.4 mg/L	LC_{50} (m/f): > 1.4 mg/L (4 h, nose only)	R013028
	90.76% Rat, Sprague-Dawley, m/f	2.63 mg/L	LC_{50} (m/f): > 2.63 mg/L (4 h, nose only)	C014044
	90.76% Rat, Sprague-Dawley, m/f	5.61 mg/L	LC_{50} (m/f): > 5.61 mg/L (4 h, nose only)	C014043
Skin irritation	97.1% Rabbit, NZW	0.5 g/animal (moistened with water)	Not irritating	R013022
Eye irritation	97.1% Rabbit, NZW, m	0.1 mL/animal	Not irritating	R013018
	97.2% Rabbit, NZW, f	0.1 mL/animal	Not irritating	R012105
Skin sensitization M & K test	96.4% Guinea Pig, Dunkin Hartley, m/f	Intradermal induction: 5% Epidermal induction: 50% Challenge: 50%	Not sensitizing	R013081
Skin sensitization Buehler-test	97.0% Guinea pig, Dunkin Hartley, m/f	Epidermal induction: 50% Challenge: 10% and 50%	Not sensitizing	R013063

NZW: New Zealand White

Based on the data available at that time, the following EU agreed endpoints were given in the EFSA Scientific Report (33, 1-69) from 2005:

Acute toxicity	
Rat LD_{50} oral:	> 2000 mg/kg bw
Rat LD_{50} dermal:	> 2000 mg/kg bw
Rat LC_{50} inhalation:	> 5.61 mg/L (4-h, nose/mouth)
Skin irritation:	Non-irritant
Eye irritation:	Non-irritant
Skin sensitization (M&K/Buehler):	Not skin sensitizing

Submission of not yet peer-reviewed studies in this AIR3-Dossier:

No new oral, dermal and inhalation acute toxicity, and skin and eye irritation studies according to current criteria have been performed with triticonazole. In the year 2005 a new Buehler test has been conducted with triticonazole including data conducted with a positive control substance (BASF DocID 2006/1001981). A robust study summary of this study is provided below. Further in accordance with the data requirements of Commission Regulation SANCO/11802/2010 an in vitro NRU-Phototoxicity study in Balb/c 3T3 cells has been performed and the study is summarized in detail in chapter CA 5.2.7. All new studies are submitted within the AIR3 process and are presented in Table 5.2-2.

Table 5.2-2: Summary of newly available acute toxicitiy studies with triticonazole

Type of study	**Species/Sex**	**purity / vehicle**	**Result**	**Reference (BASF DocID)**
Skin sensitization Buehler test*	Guinea pig, Dunkin Hartley, f	90.3% / propylene glycol	Epidermal induction: 50% Challenge: 25% and 50% Not sensitizing	2006/1001981
in vitro NRU-Phototoxicity study	Balb/c 3T3 cells	91.3% / DMSO 1% in PBS	Not phototoxic	KCA 5.2.7/1 2013/1089154

Considering all available studies triticonazole is not acutely toxic by the oral, dermal, and inhalation route. Furthermore triticonazole is no skin or eye irritant, not a skin sensitizer and not phototoxic.

The proposed endpoints based on all available studies are shown below in Table 5.2-3.

Table 5.2-3: Proposed acute toxicity endpoints of triticonazole

Study type/species	Results	Classification
		Reg. EC 1272/2008 (CLP)
Acute oral toxicity, rat	LD_{50} > 2000 mg/kg bw	-
Acute dermal toxicity, rat	LD_{50} > 2000 mg/kg bw	-
Acute inhalation toxicity, rat	LC_{50} > 5.61 mg/L	-
Dermal irritation, rabbit	Not irritating	-
Eye irritation, rabbit	Not irritating	-
M & K test / Buehler-test, guinea pig	Not sensitizing (M & K test) Not sensitizing (Buehler-tests)	-
***In vitro* NRU Phototoxicity Test, Balb/c 3T3 cells**	**Not phototoxic**	-

* new endpoints or values differing from the current agreed EU endpoints are marked in bold

Under consideration of all available data the classification of triticonazole will not differ under Reg. EC 1272/2008 (CLP) compared to the EU agreed endpoints given in the EFSA Scientific Report (33, 1-69) from 2005. The List of endpoints will be adapted accordingly as shown below:

Acute toxicity (SANCO/11802 data point 5.2)

Rat LD_{50} oral	LD_{50} > 2000 mg/kg bw No classification required
Rat LD_{50} dermal	LD_{50} > 2000 mg/kg bw No classification required
Rat LC_{50} inhalation	LC_{50} > 5.61 mg/L No classification required
Skin irritation	Not irritating No classification required
Eye irritation	Not irritating No classification required
Skin sensitization (test method used and result)	Not sensitizing (M & K / Buehler) No classification required
Phototoxicity	Not phototoxic No classification required

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

CA 5.2.1 Oral

Guidelines: In compliance with OECD guideline 401 (1987) and US EPA Pesticide Assessment Guidelines, Subdivision F, No 81-1
GLP: Yes
Acceptance: The study was considered acceptable in the EU registration process 2003.

Material and method:
Groups of 5 rats/sex (strain: CD Sprague-Dawley; source:) weighing between 99 and 118 g (5-week old) received a single dose of 0 (vehicle control) and 2000 mg/kg bw triticonazole (batch no. BD 1074; purity 99.3 %, suspended in 0.5 % w/v methylcellulose in distilled water) by oral gavage. After administration all animals were kept under observation for 14 days. Clinical observations were made daily. Body weights were recorded on the day before dosing and on days 1, 8 and 15. At termination all surviving rats were examined at necropsy for macroscopic abnormalities.

Findings:
Clinical signs and mortality: There were no treatment-related deaths. Signs of reaction to treatment were confined to decreased motor activity and ataxia in one male and all 5 females on day 1 occurring within half an hour after dosing. There was complete recovery in all rats by day 2. No treatment related effects on body weights/weight gain were observed.
Pathology: No gross pathological findings were noted in the rats at the final sacrifice on day 15.

Conclusion:
Triticonazole (suspended in 0.5 % w/v methylcellulose in distilled water) is of low acute toxicity in rats after oral administration. The LD_{50} is higher than 2000 mg/kg bw in males and females.

CA 5.2.2 Dermal

Guidelines: In compliance with OECD guideline 402 (1987)
GLP: Yes
Acceptance: The study was considered acceptable in the EU registration process 2003.

Material and method:
Groups of five male and five female rats (strain: CD, remote Sprague Dawley origin; source:) weighing between 216 and 244 g received a topical application of triticonazole technical (batch no. DA646; purity 97.1 %; moistened with 0.2 ml distilled water) at a dose level of 2000 mg/kg bw, which was placed on a gauze patch (5 x 5 cm), applied on the clipped dorsum and occluded with aluminum foil and a waterproof adhesive bandage. After the 24-hour exposure period, the bandage and foil were removed and the treated skin was washed with water. Within the 14 days observation period, clinical signs were recorded twice per day, body weights of the animals were recorded on the day before treatment and on days 1, 8 and 15. At termination, all animals were subjected to a macroscopic post mortem examination.

Findings:
There were no mortalities or signs of systemic toxicity observed during the 14-day study period. Local signs of irritation (very slight to well defined erythema and eschar formation) at the site of administration were observed in 2 females from day 3 to 10 after treatment. One of these two rats also showed loss of skin flexibility (days 3 – 6) and sloughing (days 7 – 10). The other rat affected demonstrated slight exfoliation. Body weight gains were normal for all animals and there were no abnormal necropsy findings at termination of the study.

Conclusion:
Triticonazole is of low acute dermal toxicity in rats. The LD_{50} was higher than 2000 mg/kg bw for males and females.

CA 5.2.3 Inhalation

Guidelines: In compliance with OECD guideline 403 and US EPA guideline 81-3
GLP: Yes
Acceptance: The study was considered acceptable in the EU registration process 2003.

Material and method:
A group of five male and five female rats (strain: CD-Sprague Dawley; source:) weighing between 180 and 220 g were exposed for four hours (nose-only) to an atmosphere containing triticonazole (batch no. DA646; purity 954 g/kg) at a concentration of 1.4 mg/L air (measured gravimetrically and stated to be the maximum practicable concentration). The nominal chamber concentration was 10.68 mg/L. The MMAD was not indicated in the report. However, the mean proportion of particles of inhalable size derived from cascade impactor samples and measured once during each hour of the exposure period was 47.4 % < 6 µm and 29.8 % of particles < 3.5 µm. After exposure, the rats were kept under observation for 15 days. Body weights were recorded before treatment and then daily until the end of the observation period. At termination, all animals were subjected to a detailed macroscopic examination and the organ weights of lungs, liver and kidneys were recorded.

Findings:
Clinical signs and mortality:
There were no deaths as a result of exposure. During exposure, wet fur and excessive salivation was observed in two males and one female. These findings were not present at observations performed 30 minutes after completion of the exposure. No further clinical signs were noted throughout the remainder of the observation period.
Body weight:
Slight body weight loss/reduced body weight gain was recorded for 3 males and 3 females on the day following exposure only.
Pathology:
At termination of the study, there were no abnormal findings at necropsy. The weights of lungs, liver and kidneys were unaffected by exposure.

Conclusion:
The acute inhalation LC_{50} (4 hours exposure) for triticonazole in male and female rats is > 1.4 mg/L air.

Guidelines: In compliance with OECD guideline 403 and US EPA guideline 81-3
GLP: Yes
Acceptance: The study was considered acceptable in the EU registration process 2003.

Material and method:
Groups of 5 males and 5 females rats (strain: HSD:Sprague-Dawley; source:) weighing between 189 and 347 g were exposed nose-only for 4 hours to an atmosphere containing triticonazole (batch no. OP9750215; purity: 90.76%). The concentration of test material (fine powder) in the test atmosphere was measured gravimetrically. The nominal chamber concentration was 6.96 mg/L and the achieved concentration was 2.63 mg/L air with an average MMAD of 2.4 µm. The particle size, taken from the breathing zone of the animals, was derived from cascade impactor samples and reported as 50 % of particles < 3.2 µm. Observations (including individual body weight measurements on days 0 before treatment, 7 and 14) were made for 14 days following exposure, after which the rats were sacrificed and examined at necropsy for abnormal tissues or organs.

Findings:
Clinical signs and mortality:
No treatment-related deaths were observed throughout the study. Clinical signs were confined to wet fur, piloerection and decreased activity in both sexes. All animals had recovered by day 3. Body weight gain was unaffected by the administration of the test article.
Pathology:
At termination of the study, no significant gross necropsy findings were noted.

Conclusion:
The acute inhalation LC_{50} (4 hours exposure) for triticonazole in male and female rats is > 2.63 mg/L air.

Guidelines: In compliance with OECD guideline 403 and US EPA guideline 81-3
GLP: Yes
Acceptance: The study was considered acceptable in the EU registration process 2003.

Material and method:
Five male and five female Sprague-Dawley rats (strain HSD; source: weighing between 183 and 289 g were exposed for 4 hours via nose only inhalation to a dust aerosol (dynamic conditions) of triticonazole (batch no. OP9750215; purity: 90.76 %) at an exposure (gravimetric) concentration of 5.61 mg/L air (nominal concentration 19.7 mg/L). The mass median aerodynamic diameter (MMAD) was reported to be 7 µm with a geometric standard deviation of 9.4 µm after 2¼ hours of exposure and 4.7 µm with a geometric standard deviation of 8.7 after 3¼ hours of exposure. Analyses taken from the breathing zone of the animals after 3¼ hours of exposure showed that 50 % of the particles were 4.7 µm or less. After exposure, the rats were kept under observation for 14 days. Body weights were recorded before treatment and on days 7 and 14. At termination, all animals were subjected to a detailed macroscopic examination.

Findings:
Clinical signs and mortality:
There were no mortalities observed during the 14-day study period. Body weight gain was unaffected by treatment. Clinical signs, already occurring during exposure comprised of the fur coated with urine and/or faeces, decreased activity and piloerection and were seen in all animals. In one female, sensitivity to touch on day 1 after exposure was noted. All animals had completely recovered by day 6.
Pathology: At termination of the study, no macroscopic necropsy findings were noted.

Conclusion:
The acute inhalation LC_{50} (4 hours exposure) for triticonazole in male and female rats is > 5.61 mg/L air.

CA 5.2.4 Skin irritation

Guidelines: In compliance with OECD guideline 404 (1981)
GLP: Yes
Acceptance: The study was considered acceptable in the EU registration process 2003.

Material and method:
Triticonazole (batch no. DA646; purity 97.1 %; 0.5 g a.i. moistened with 0.2 ml of distilled water) was applied to a 6 cm x 6 cm area of the clipped dorsal region of 3 male New Zealand White rabbits (source:) under a semi-occlusive dressing for a 4-hour exposure period. Following the exposure period, the test sites were washed with warm water to remove any remaining test material and dried. The skin sites were examined 1, 24, 48 and 72 hours after treatment and reactions were assessed according to the criteria of Draize (1959).

Findings:
Clinical signs: Throughout the study period, all treated sites appeared normal; no oedema or erythema were noted. In addition, no signs of systemic toxicity were observed. Triticonazole showed a primary irritation score of 0.00 when applied for 4 hours (semi-occluded) to intact rabbit skin.

Conclusion:
According to the results of the study, triticonazole is not irritant to the intact shaved rabbit skin.

CA 5.2.5 Eye irritation

█████████████████

Guidelines: In compliance with OECD guideline 405 (1987)
GLP: Yes
Acceptance: The study was considered acceptable in the EU registration process 2003.

Material and method:
Groups of 6 adult male New Zealand white rabbits (source: ████████████████) received a single application of 100 mg of triticonazole (batch no. DA646, purity 97.1 %) into the conjunctival sac of the right eye. The eyelids were held together for one second following instillation. The left eyes remained untreated and served as a control. The eyes were examined for ocular reactions 1 hour after the instillation, and then 1, 2, and 3 days thereafter. In addition to standard assessments, pain response, area of cornea affected and conjunctival discharge were also investigated. As a result of seeing a severe response in one of the 3 animals initially used, a further 3 rabbits were tested and ocular reactions were examined and scored. Grading and scoring of the ocular lesions were performed using the numerical scoring system according to the relevant guidelines.

Findings:
After instillation, a slight initial pain response was observed in three animals, the other three rabbits being unaffected. During the first 24 hours following treatment, slight injection of the conjunctival vessels was observed in all rabbits. In addition, a very slight discharge was evident in four rabbits at the 1 hour examination. No corneal effects or conjunctival chemosis was noted in any rabbit. Very slight iritis was also observed in two rabbits at the one and 24 hour examinations. The eyes of five rabbits were apparently normal within 48 hours after treatment. In one rabbit, iritis was evident from the 24 hour observation through to 14 days with a crescent shaped lesion in the pupil at 7 and 14 days, which was considered to be iris tissue adhered to the lens as a result of the iridial congestion. The same animal showed moderate conjunctival redness from 1 hour through day 14 and also pannus formation at the day 14 examination.
Mean scores (24 – 72 hours) in the 5 animals not showing a severe response were 0.0 (conjunctival chemosis), 0.1 (conjunctival redness), 0.0 (corneal opacity), and 0.1 (iridial lesions), respectively. Mean scores for the severely affected rabbit were 0.0 for corneal opacity and conjunctival chemosis, 1.0 for iridial lesions and 1.7 for redness of the conjunctiva. Since the findings were considered to be an irreversible lesion of the eye, the animal was subsequently killed. This unexpected reaction in one animal only was considered likely to be idiosyncratic in nature by the study author.

Conclusion:
The results of five animals showed no evidence of distinct eye irritation induced by triticonazole, but the presence of irreversible lesions in one animal is of serious concern. Normally, the results of the study would lead to consideration of classification, however it has to be noted that a second eye irritation study is available and evaluated hereinafter.

Guidelines: In compliance with OECD guideline 405 (1987) and Directive EEC 92/69 Method B5 (1992)
GLP: Yes
Acceptance: The study was considered acceptable in the EU registration process 2003.

Material and method:
Approximately 100 mg of triticonazole (batch no. 013951; purity 97.2 %) were placed into the conjunctival sac of the left eye of 6 female New Zealand White rabbits (source:). The eyelids were gently held together for one second following application. The collateral eyes remained untreated and served as a control. The animals were observed twice daily for clinical signs and mortality during a 72-hour period. The local eye irritation was assessed 1 hour after the instillation, and then 24, 48 and 72 hours thereafter. Grading and scoring of the ocular lesions were performed using the numerical scoring system given in the mentioned OECD guidelines.

Findings:
All six animals showed a slight redness of the conjunctiva one hour after application which was accompanied by slight discharge in one case. However, the redness had disappeared on day 1. According to the classification system, the overall mean scores from the 24, 48, and 72 hour observations for redness, chemosis, corneal opacity and iritis were 0.0, each.

Conclusion:
According to the results of this study, triticonazole is non-irritating to the rabbit eye.

CA 5.2.6 Skin sensitisation

Guidelines: In compliance with OECD guideline 406 (1981) (Maximisation test)
GLP: Yes
Deviation: No positive control was run concurrently in the study.
Acceptance: The study was considered acceptable in the EU registration process 2003.

Material and method:
20 guinea pigs (10 males, 10 females; strain: Dunkin Hartley; source:) were given triticonazole (batch no. DA 646; purity 96.4 %) intradermally and topically. Additionally 10 male and 10 female guinea pigs were used as negative control group. The concentrations used for the treatment in this study were based on the results of a preliminary screening study. In the main study, intradermal induction (two sites per dose, 0.1 ml/injection) was performed with (i) Freunds Complete Adjuvant (FCA), (ii) 5 % w/v triticonazole in propyleneglycol and (iii) 5 % w/v triticonazole in a mixture of propylene glycol and FCA by intradermal injections into the dermis on either side of the dorsal median line parallel to the spinal column at the scapular region. Control animals received similar injections except triticonazole was replaced with vehicle. The day of intradermal induction was designated day 1. On day 7, 10 % (w/v) sodium lauryl sulphate in petrolatum was applied to the clipped dorsal skin of all animals. Topical induction (for 48 hours under occlusive dressing) was carried out on day 8 using a concentration of 50 % triticonazole in propylene glycol (0.6 ml/animal) for test animals and propylene glycol only for control animals. On day 22, the challenge phase was performed on all guinea pigs (controls and test animals) by applying 50% triticonazole in propylene glycol dermally for 24 hours under occlusive dressing on the right flank (5 x 5 cm area) while the left flank received the vehicle only. A separate site on the right flank was challenged with 10 % triticonazole in propylene glycol. The dressings were removed 24 hours later and skin reactions were quantified 24 and 48 hours thereafter.

Findings:
In the preliminary study, signs of skin irritation (slight to moderate erythema) were observed 24 und 48 hours after intradermal application of either 0.1, 0.3, 0.5, 1, 3, or 5 % w/v triticonazole in propylene glycol. It was stated in the report that 5 % was the maximum concentration that would pass through a hypodermic needle. Topical application of triticonazole in propylene glycol at either 5, 10, 30 or 50 % w/v induced no dermal response 24 and 48 hours after removal of the dressing.

In the main study, mild to moderate skin reactions were observed in most animals (test and controls) following intradermal injection. Also after topical induction, mild skin irritation and exfoliation was evident in almost all animals (test and control animals). Following topical challenge with 50 % test material in propylene glycol, erythema was noted in 3 males and 3 females in the control groups (barely perceptible or slight) and in 2 males and 2 females in the test groups (barely perceptible). In addition, exfoliation was evident in 3 males and 5 females in the controls compared with 5 males and 8 females in the test group. Following topical challenge with 10 % triticonazole in propylene glycol, a barely perceptible erythema was noted in one female in the test group only. In addition, exfoliation was noted in 3 control females and one male and one female from the test groups. Topical challenge with propylene glycol alone produced no skin reactions.

Conclusion:
It was concluded that under the conditions of the Maximization test, triticonazole did not cause delayed contact hypersensitivity in guinea pigs. The results do not indicate classification of the test material for skin sensitization.

Guidelines: In compliance with OECD guideline 406 (1981) (modified Buehler-test)
GLP: Yes
Deviation: No positive control was run concurrently in the study.
Acceptance: The study was considered acceptable in the EU registration process 2003.

Material and method:
10 male and 10 female Dunkin/Hartley guinea pigs (source: [redacted]) received topical applications of triticonazole (batch no. DA 646, purity 97.0 %) in propylene glycol (50 % w/v, 0.25 ml/patch) on their shaven left flanks (5 x 5 cm) for 6 hours under occlusive dressing. The induction phase consisted of nine applications each, on days 1, 3, 5, 8, 10, 12, 15, 17, and 19 of the study period. The concentration tested was based on a preliminary test with the concentration of 50 % being considered to be "sub-irritant". 5 male and 5 females serving as controls were treated in the same way, except that the vehicle only was used in the induction phase. On day 29, all test and control animals were challenged by a 6 hours occluded topical application of 50 % w/v triticonazole in propylene glycol (0.25 ml) to one site, 10 % w/v to the second site and propylene glycol alone to the third on their shaven right flanks. Dermal reactions were assessed on the morning following each induction application, and at 24 and 48 hours after removal of the occlusive dressings.

Findings:
In the preliminary test, very faint erythema was evident in one (of four) animals 24 and 48 hours after patch removal following testing of 50 % and 10 % w/v triticonazole in propylene glycol. During the induction phase of the main study, very faint erythema at the application site was observed in one male during the first week of induction and at the majority of animals during the second and third week of induction with 50 % w/v triticonazole in propylene glycol. No reaction was observed amongst the control animals. After challenge application with 50 % w/v, no dermal reactions were observed in treated or control animals.
Challenge application of 10 % w/v produced very faint erythema in one female of the test group animals and no reactions amongst controls. Challenge with propylene glycol alone did not cause any dermal reactions.

Conclusion:
It was concluded that under the conditions of this study, repeated occluded dermal application of triticonazole did not cause delayed contact hypersensitivity in guinea pigs. The results do not indicate classification of the test material for skin sensitization.

Report: CA 5.2.6/1
[redacted]
BAS 595 F - BUEHLER test in guinea pigs
2006/1001981
Guidelines: OECD 406, EPA 870.2600, JMAFF No 12 Nosan No 8147, EEC 96/54 B 6
GLP: yes
(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

For the determination of potential sensitizing properties of the test substance BAS 595 F (Batch: COD-000601, Purity: 90.3%), a BUEHLER test based on the method of Buehler, E.V. (1965) was conducted with one control (10 animals) and one treated (20 animals) test groups using female Dunkin Hartley Guinea pigs (HsdPoc: DH).

The test-substance concentrations for the main test were selected based on the results of the pretests. The inductions were performed on days 0, 7 and 14 using 50% test substance preparation in the test group or using the vehicle propylene glycol in the control group. A challenge was carried out 14 days after the last induction using 25% and 50% test substance preparation in propylene glycol.

After the first induction no skin irritation was observed in all animals of the control and test group. The second and third induction caused discrete or patchy to moderate and confluent erythema in several animals of the control and test group, each.

After the challenge discrete or patchy or moderate and confluent erythema was noticed in the application sites of the 50% test substance preparation of 1/10 animal of the control group and 2/20 animals of the test group. No skin reactions were noticed in all application sites of the 25% test substance preparation and the vehicle propylene glycol.

Based on the results of this study, it was concluded that BAS 595 F does not have a sensitising effect on the skin of the guinea pig in the BUEHLER Test under the test conditions chosen. Thus, no classification is required according to Regulation (EC) No. 1272/2008.

(DocID 2006/1001981)

I. MATERIAL AND METHODS

1. Test Material: BAS 595 F

Description:	not specified
Lot/Batch #:	COD-000601
Purity/content:	90.3%
Stability of test compound:	The stability of the formulation under storage conditions over the study period was guaranteed.

2. Vehicle and/or positive control: Propylene glycol

3. Test animals:

Species:	Guinea pigs
Strain:	Dunkin Hartley, HsdPoc: DH
Sex:	female
Age:	6 - 7 weeks
Weight on day 0:	318 - 394 g
Source:	[redacted]
Acclimation period:	7 days
Diet:	Kliba-Labordiät (Kanninchen & Meerschweinchen-haltung "GLP"), Provimi Kliba SA, Kaiseraugst, Basel, 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 (floor area: min. 2000 cm^2)

4. Environmental conditions:

Temperature:	20 - 24°C
Humidity:	30 - 70%
Air changes:	Central air-conditioning system
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 experimental work: 08-Nov-2005 - 27-May-2006 (In-life phase: 15-Nov-2005 (1st induction) to 15-Dec-2005 (last observation))

2. Animal assignment and treatment:

The skin sensitizing potential of BAS 595 F was assessed using the Guinea Pig BUEHLER Test. This test consists of a pre-test for the determination of suitable induction and challenge concentrations and the BUEHLER test itself.
For this, female guinea pigs were randomly distributed to groups according to the instructions of "Salfi, R.: A Long-Period Random Number Generator with Application to permutation, Compstat 1974, pp. 28 - 35".

At least 15 hours before each test substance application at the appropriate application sites. If necessary, additionally at least 2 hours before evaluation of the skin reactions. A check for any dead or moribund animal was made twice each workday and once on weekends or public holidays.
Evaluations of the skin reactions were performed according to the grading scale of Magnusson and Kligman (The Identification of Contact Allergens by Animal Assay. The Guinea Pig Maximization Test. J. Invest. Dermatol. 52, 268 - 276 (1969)).

3. Preliminary tests

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 were applied to the skin of the left and right flanks under an occlusive dressing for 6 hours. The dressing consisted of rubberized linen patches (4 x 4 cm, Russka), patches of Idealbinde (5 x 5 cm from Pfälzische Verbandstoff-Fabrik) and Fixomul®Stretch adhesive fleece (Beiersdorf AG). The test was performed on 3 animals per test concentration.
The evaluation of the skin reactions was performed 1, 24 and 48 h after removal of the patch. In accordance with the test guideline a slightly irritating concentration should be used in the main test for induction, whereas the maximum non-irritant concentration should be applied for challenge.
In the pre-test, concentrations of 25 and 50% test substance preparations in propylene glycol were analysed. Since no skin irritation was noticed 24 and 48 hours after removal of the patch, both test item concentrations were assessed as suitable for the main test.

4. Main study - Induction

Inductions were performed on days 0, 7 and 14 on the same application area using 50% test item suspension in propylene glycol.
2 x 2 cm gauze patches (6 layers surgical gauze Ph. Eur. Lohmann GmbH & Co. KG) containing the 0.5 mL of the test substance were applied for 6 hours to the skin of the flank under an occlusive dressing. The dressing consisted of rubberized linen patches (4x4 cm, Ruska), patches of Idealbinde (5 x 5 cm from Pfälzische Verbandstoff-Fabrik) and Fixomul®stretch adhesive fleece (Beiersdorf AG).
The evaluation of the skin reactions was performed 24 hours after removal of the patch.
The control group animals were treated with the vehicle propylene glycol in the same way as the test group animals.

5. Challenge

The challenge was carried out 14 days after the last induction using 0, 25 and 50% test item preparations in propylene glycol.
For this 0.5 mL of the test substance preparation was applied under occlusive conditions to the intact flank (right flank anterior and posterior: test substance formulations and left flank posterior: vehicle propylene glycol) of the animals for 6 hours. The patches were prepared as described above for the epidermal pre-test. Skin reactions were determined 24 and 48 hours after removal of the patches.

6. Positive controls

A positive control (reliability check) with a known sensitiser was not performed in this study. However, separate positive control studies were performed twice a year in the laboratory. The positive control with α-hexylcinnamaldehyde (HCA) techn. 85% showed that the test system was able to detect sensitizing compounds under the laboratory conditions chosen.

7. Evaluation of results:

The number of animals with skin findings at 24 and/or 48 hours after removal of the patch was taken into account for the determination of the sensitisation rate.
The evaluation "sensitising" results if at least 15% of the test animals exhibit skin reactions (grade $\geq$ 1) in this test.
The evaluation is based on the criteria of the CLP Regulation (EC) No. 1272/2008).

8. Analysis of treatment solutions:

A concentration control analysis of both test substance preparations (50% and 25%) used for the challenge application was performed. The stability and homogeneity of the test substance in the vehicle was determined indirectly by the concentration control analysis. The homogeneity of the test substance preparation during application was provided by stirring with a spatula (50%) or with a magnetic stirrer (25%).

9. Statistics:

Not performed in this study

II. RESULTS AND DISCUSSION

A. PRE-TEST

After application of 50% and 25% test substance preparations in propylene glycol no skin irritation was noticed 24 and 48 hours after removal of the patch [see Table 5.2.6-1]

Table 5.2.6-1: Skin irritation scores – preliminary test

Animal No.	Weight [g]	Findings 1 hour after removal of the patch		Findings 24 hours after removal of the patch		Findings 24 hours after removal of the patch	
Application site		right flank	left flank	right flank	left flank	right flank	left flank
Concentration in doubly distilled water		50%	25%	50%	25%	50%	25%
4	748	1	2	0	0	0	0
5	711	1	1	0	0	0	0
6	788	1	2	0	0	0	0

Based on the data from the pre-test, a test concentrations of 50% BAS 595 F was used for the epidermal inductions and both concentrations of 25 and 50% BAS 595 F were used for the challenge.

B. INDUCTION

After the first induction no skin irritation was observed in all animals of the control and test group. The second and third induction caused discrete or patchy to moderate and confluent erythema in several animals of the control and test group, each. The data is summarized in Table 5.2.6-2

Table 5.2.6-2: Skin reactions in animals treated with 50% BAS 595 F or vehicle

Description	Findings 24 hours after beginning of application					
	Propylene glycol			50% BAS 595 F		
	1st	2nd	3rd	1st	2nd	3rd
Grade 0	10/10	8/10	7/10	20/20	14/20	15/20
Grade 1	-	2/10	2/10	-	5/20	5/20
Grade 2	-	-	1/10	-	1/20	-

x/y: number of animals with findings / number of animals tested

C. CHALLENGE

After the challenge discrete or patchy or moderate and confluent erythema was noticed in the application sites of the 50% test substance preparation of one animal of the control group and two animals of the test group. No skin reactions were noticed in all application sites of the 25% test substance preparation and the vehicle propylene glycol [see Table 5.2.6-3 and Table 5.2.6-4]. Thus, 10% of guinea pigs applied for this test revealed skin reaction after challenge that is below the value of 15% that would trigger a classification.

Table 5.2.6-3: Challenge skin reaction scores 24 and 48 hours in the control group

Skin findings	Propylene glycol		25% BAS 595 F		50% BAS 595 F	
	24 h	48 h	24 h	48 h	24 h	48 h
Grade 0	10/10	10/10	10/10	10/10	9/10	9/10
Grade 1	-	-	-	-	-	-
Grade 2	-	-	-	-	1/10	1/10

x/y: number of animals with findings / number of animals tested

Table 5.2.6-4: Challenge skin reaction scores 24 and 48 hours in the test group

Skin findings	Propylene glycol		25% BAS 595 F		50% BAS 595 F	
	24 h	48 h	24 h	48 h	24 h	48 h
Grade 0	20/20	20/20	20/20	20/20	18/20	18/20
Grade 1	-	-	-	-	1/20	2/20
Grade 2	-	-	-	-	1/20	-

x/y: number of animals with findings / number of animals tested

D. OBSERVATIONS

No clinical signs of systemic toxicity or mortality were observed.

E. BODY WEIGHTS

The expected body weight gain was generally observed in the course of the study.

F. POSITIVE CONTROL

Results with HCA are summarized in Table 5.2.6-5. The positive control HCA, techn. 85% showed that the guinea pig strain used was sufficiently sensitive for detection of skin sensitizing compounds in the Buehler test.

Table 5.2.6-5: Results of positive control substance test (HCA) in the Buehler test

	Challenge					
	Positive control 15% HCA in Lutrol® E 400			Vehicle control: Lutrol® E 400		
	24 h	48 h	Total	24 h	48 h	Total
Control group*	0/10	0/10	0/10	0/10	0/10	0/10
Test group	19/20	16/20	19/20	0/20	0/20	0/20

* Induction (epicutaneous): HCA 20% in Lutrol® E 400
x/y: number of positive reactions/number of animals tested (reading at 24 h and/or 48 h after removal of the patch)

III. CONCLUSION

Based on the results of this study and applying the evaluation criteria, it was concluded that BAS 595 F does not have skin sensitising properties. Thus, classification of BAS 595 F as a skin sensitizer is not required according to CLP Regulation (EC) No. 1272/2008.

CA 5.2.7 Phototoxicity

Report: CA 5.2.7/1
Cetto V.,Landsiedel R., 2013a
BAS 595 F (Triticonazole) - In vitro 3T3 NRU phototoxicity test
2013/1089154

Guidelines: OECD 432 (2004) In vitro 3T3 NRU Phototoxicity test, (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.41 No. L 142

GLP: yes
(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Report: CA 5.2.7/2
Cetto V.,Landsiedel R., 2015 a
Amendment No. 1 to the report: BAS 595 F (Triticonazole) - In vitro 3T3 NRU phototoxicity test
2015/1257100

Guidelines: OECD 432 (2004) In vitro 3T3 NRU Phototoxicity test, (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.41 No. L 142

GLP: yes
(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Deviations: None

Executive Summary

Triticonazole (Batch COD-001440; purity 91.3%) was tested for its ability to induce phototoxic effects in Balb/c 3T3 cells *in vitro*, as the absorption spectrum showed a borderline absorption at 290 nm (see Chapter MCA 2.4). There was no absorption seen at 320 nm. The criteria to conduct a phototoxicity experiment were thus only hardly fulfilled (see Official Journal of the European Union L 93, Volume 56). Nevertheless a photo-cytotoxicity was estimated by the means of the Neutral Red Uptake (NRU) method and using a solar simulator designed to emit light mimicking the absorption spectrum of natural sunlight. A single experiment was carried out with and without UV/VIS irradiation. Vehicle and positive controls were included into the study. Based on the range-finding phototoxicity test the same concentrations were used in the main experiment with and without irradiation: 4.6 - 10 - 21.5 - 46.4 - 100 - 215.4 - 464.2 - 1100 µg/mL.

Test substance precipitation in culture medium at the end of treatment was observed at 100 µg/mL and above in the absence and presence of UV/VIS irradiation. In addition, no changes in cell morphology were observed at the end of exposure period without and with irradiation. After treatment with the test substance, no cytotoxic effects indicated by Neutral Red absorbance values of below 50% of control were observed in the Main Experiment in the absence and the presence of UV/VIS irradiation. Therefore, no EC_{50} values could be calculated. Based on these observations a formal PIF = *1 has to be used to characterize the result. The positive control chlorpromazine led to the expected cytotoxicity both with and without UV/VIS irradiation (PIF: 36.7).
Thus, under the experimental conditions of this study, triticonazole (BAS 595 F) is considered **not to be a phototoxic** substance in the *in vitro* 3T3 NRU Phototoxicity Test using Balb/c 3T3 cells.
(DocID 2013/1089154)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material Triticonazole (BAS 595 F)

Description:	Solid; white
Lot/Batch #:	COD-001440
Purity:	91.3% (tolerance ± 1%)
Stability of test compound:	The stability of the test substance under storage conditions over the test period was guaranteed until 11 Feb 2014 by the sponsor, and the sponsor holds this responsibility. The homogeneity of the test substance was ensured by mixing prior to preparation of test substance solutions.
Solvent used:	DMSO

2. Control Materials:

Vehicle control:	The vehicle control cultures with and without irradiation only contained the vehicle used for the test substance/positive control at the same concentration and volume as used for the test substance and the positive control.
Solvent/final concentration:	DMSO 1% (v/v)
Positive control compounds:	Chlorpromazine (CPU) was dissolved in DMSO

A complete 96-well plate containing 8 concentrations was performed in parallel to demonstrate sensitivity of the test method.

Without irradiation	1.9-3.8-7.5-15-30-60-90-180 µg/mL
With irradiation	0.03-0.05-0.1-0.2-0.4-0.8-1.6-3.2 µg/mL

3. Test organisms: The BaIb/c 3T3, clone A31, cell line was isolated from the muscle tissue of mouse embryo. This fibroblast cell line has a high proliferation rate (doubling time 16 - 20 hours) and a high plating efficiency (>70%)) of untreated cells both necessary for the appropriate performance of the study. The Balb/c 3T3 cell line which was used in this experiment was obtained from the “European Collection of Cell Cultures” Salisbury, Wiltshire SP4 OJG, UK (date 09 Aug 2006) and is stored at -196°C (liquid nitrogen).

4. Culture media and reagents:

Culture medium: Dulbecco's Modified Eagle's Medium (DMEM) supplemented with
- 10% (v/v) newborn calf serum (NCBS)
- 4 mM L-glutamine
- 100 IU penicillin
- 100 µg/mL streptomycin

Neutral Red solution:
- 0.4 g Neutral Red powder (NR; Sigma N4638)
- 100 mL deionized water

Neutral Red medium:
- 1 mL Neutral Red solution
- 79 mL culture medium (DMEM incl. supplements)

Incubated overnight at 37° C with 5% CO_2 and filtered with a 0.22 µm filter prior to use.

Other solutions and reagents:
- phosphate buffered saline (PBS) without Ca/Mg
- trypsin/EDTA solution (0.05%; 0.02%)
- Neutral Red desorb solution (1 mL acetic acid, 50 mL ethanol, 49 mL deionized water)

5. Irradiation source: The Sol 500 solar simulator (Dr. Hönle AG, 82166 Gräfelfing, Germany) used with filter H1. This irradiation source is recommended in the Annex 3 of OECD TG 432.

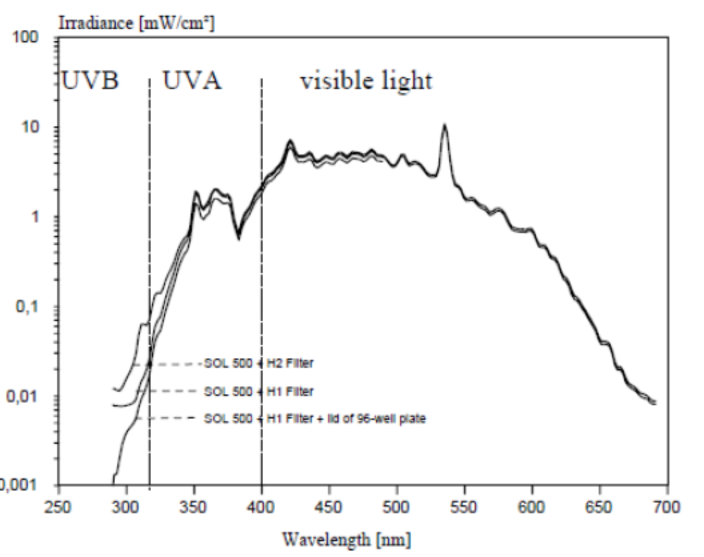


Figure 5.2.7-1: Spectral power distribution of a filtered solar simulator

(Source: OECD TG 432, 2004, Annex 3)
As shown in Figure 5.2.7-1 it mainly emits light in the UVA and visible range, (which is usually associated with high direct cytotoxicity) and to a lesser extent in the UVB range (which is associated with high cytotoxicity and regarded to be of less relevance in the context of substance induced phototoxicity (OECD TG 432). However, the experimental setting was shown to be sufficient to detect phototoxic effects also fo chemicals typically absorbing in the UVB range, e.g. the concurrent positive control chlorpromazine (absorption peak at 309 nm) or Amiodarone (absorption peak 242 nm and 300 nm) (shoulder). The exposure rates were determined with UV-meter RM-21 (Dr. Gröbel GmbH, 76275 Ettlingen, Germany).

6. Test concentrations:

Pretest: Up to 1100 µg/mL with and without irradiation.

NRU test conditions: An appropriate amount of test article substance was taken up in the vehicle, shaken thoroughly and diluted in accordance with the planned doses under light protection conditions immediately before administration.

The experiment was performed in 96 well plates in one experiments (6 replicates per concentration with and without irradiation; two plates per substance (test substance or positive control) were prepared.) The test substance concentrations were:

Without: 4.6; 10.00; 21.5; 46.4; 100.0, 215.4, 464.2, 1100.0 µg/mL

With: 4.6; 10.00; 21.5; 46.4; 100.0, 215.4, 464.2, 1100.0 µg/mL

B. TEST PERFORMANCE:

1. Dates of experimental work: 02/19/2013 00:00:00 - 03/13/2013 00:00:00

2. Treatment and NRU Phototoxicity test:

Two 96 well-plates per substance (test substance or positive control) were used for cultivation of cells (1.5 x 10^5 cells/well). After an attachment period of about 24 hours the cells were washed once with 100 µL PBS and subsequently treated with the respective substance (8 concentrations each with 6 replicates of the test substance or the positive control) and the vehicle control in parallel for 1 hour in the dark (5% (v/v) CO_2, ≥90% humidity; 37° C). Then, one microplate per substance was irradiated for 50 minutes with UV/VIS (UV intensity underneath the lid 1.5 - 2.1 mW/cm² = 5 J/cm²) whereas the respective reference plate was kept in the dark for the same period. After test substance removal and washing step (100 µL PBS) the cells were incubated in culture medium overnight. The medium was removed after 24 hours, the cells washed again, 100 µL medium containing 50 µg/mL Neutral red was added and the plates were incubated for another 3 hours. Each step was performed under light protected conditions in the lab to prevent uncontrolled photo activation. Afterwards, the cells were washed and the dye was extracted by Neutral Red desorb solution. Cytotoxicity was determined by measuring the Neutral Red Uptake using a microplate reader (Perkin Elmer, Waltham, Massachusetts, US; Wallac 1420 multilabel counter) equipped with a 550 nm filter to read the absorption of the extracted dye. The absorption shows a linear relationship with the number of surviving cells.

3. Evaluation/Assessment

For the assessment of the phototoxic potential of a compound two prediction models are currently available:

- The Photo-Irritancy-Factor Prediction model for substances which allow the comparison of two equi-effective concentrations (EC_{50}) in the concurrently performed experiments in the presence and absence of light. This model includes the special case of absence of cytotoxicity in the presence and absence of light for substances obviously showing no phototoxic potential (see below).
- The Mean Photo Effect prediction model which is used if no equi-effective concentrations (EC_{50}) are obtained in the absence and presence of UV light. This special case does not apply to this study. Even though described in the report this case is not described in this summary.

3.1 Cytotoxicity

The mean absorbance values obtained for each test group of every plate were used to calculate the percentage of cell viability relative to the respective vehicle control, which is arbitrarily set at 100 %.

$$Cytotoxicity\,[\%] = \frac{\text{Absorbance}_{\text{mean}} \text{ of the test group}}{\text{Absorbance}_{\text{mean}} \text{ of the vehicle control}} \times 100$$

In case of cytotoxicity, an EC_{50} value (Inhibition concentration 50% relative to the respective vehicle control) was calculated by a linear interpolation method (linear dose-response curve).

3.2 Photo-Irritancy-Factor

If no cytotoxicity occurs to determine an EC_{50} value in the concurrently performed experiments in the absence and presence of UV light up to the highest applied test concentration it has to be considered that the test substance has no phototoxic potential.

In this case, a formal PIF = *1 is used to characterize the result:

$$PIF = *1 = \frac{C\max(-UVA)}{C\max(+UVA)}$$

resulting in the following classification rule:

PIF = *1	no phototoxic potential predicted

3.3 Other parameters

pH:
The pH was measured at least for the two top doses and for the vehicle controls with and without irradiation.

Osmolarity:
Osmolarity was measured at least for the two top doses and for the vehicle controls with and without irradiation.

Solubility:
Test substance precipitation was checked immediately after treatment and at the end of treatment.

Cell morphology:
Test cultures of all test groups were examined microscopically before staining with NRU, which allows conclusions to be drawn about attachment of the cells.

4. Statistics:

No special statistical tests were performed.
Mean absorbance values and standard deviations were calculated from the single values using calculation software (e.g. MS Excel). The calculations were made using the unedited values. For the report the values were rounded, therefore there may be deviations in the given relative values. If technical errors occurred in single wells (outlier) at least 4 single values per test group were sufficient for calculating reliable mean values. Outliers are defined as values that have half or double the value of the respective mean.

5. Acceptance criteria:

The assay has to be considered valid if the following criteria are met:

- The vehicle control needs to fulfill the following criteria:
  - The mean OD550 value (with and without UV/VIS irradiation) should be ≥ 0.3.
  - Cell viability after irradiation should be at least 80% of the concurrent non-irradiated vehicle control.
  - The standard deviation of the mean values of both vehicle control rows should not exceed ± 15%.
- The positive control chlorpromazine needs to fulfill the following criteria:
  - the EC_{50} value should be in the ranges:
    With irradiation (+UV/VIS): 0.1 - 2.0 µg/mL
    Without irradiation (-UV/VIS): 7.0 - 90.0 µg/mL
  - and the PIF ≥ 6.

II. RESULTS AND DISCUSSION

A. CELL MORPHOLOGY AND TREATMENT CONDITIONS

Osmolarity and pH values were not influenced by test substance treatment. In this study, in the absence and the presence of UV/VIS irradiation precipitation in culture medium was observed at test substance concentrations of 100 µg/mL and above. In addition, no changes in cell morphology were observed at the end of exposure period with and without irradiation.

B. CYTOTOXICITY OF THE TEST SUBSTANCE

After treatment with the test substance, no cytotoxic effects indicated by Neutral Red absorbance values of below 50% of control were observed in the main experiment in the absence and the presence of UV/VIS irradiation. Therefore, no EC_{50} values could be calculated.
Based on these observations a formal PIF = *1 has to be used to characterize the result.

Table 5.2.7-1: Mean relative cytotoxicity of BAS 595 F with and without UV/VIS irradiation in Balb 3T3 cells

Test group	UV/VIS irradiation*	Precipitation**	Mean $OD_{corr.}$ ***	Cytotoxicity [% of control]
Vehicle control (1% DMSO)	-	-	0.405	100.0
BAS 595 F				
4.6 µg/mL	-	-	0.345	85.1
10.0 µg/mL	-	-	0.350	86.3
21.5 µg/mL	-	-	0.361	89.0
46.4 µg/mL	-	-	0.369	91.1
100.0 µg/mL	-	+	0.412	101.7
215.4 µg/mL	-	+	0.394	97.2
464.2 µg/mL	-	+	0.428	105.7
1100.0 µg/mL	-	+	0.324	79.9
Vehicle control (1% DMSO)	+	-	0.399	100.0
BAS 595 F				
4.6 µg/mL	+	-	0.375	94.0
10.0 µg/mL	+	-	0.385	96.5
21.5 µg/mL	+	-	0.388	97.3
46.4 µg/mL	+	-	0.388	97.2
100.0 µg/mL	+	+	0.395	99.0
215.4 µg/mL	+	+	0.428	107.3
464.2 µg/mL	+	+	0.436	109.2
1100.0 µg/mL	+	+	0.436	109.3

*: Irradiation with Sol500 solar simulator for 50 minutes (approx. 5 J/cm²)
**: Precipitation in PBS at the end of exposure period
***: Mean OD corrected: mean absorbance (test group) minus mean absorbance (blank)

C. CYTOTOXICITY OF THE POSITIVE CONTROL

After treatment with the positive control chlorpromazine clear cytotoxic effects indicated by Neutral Red absorbance values of below 50% of control were observed in the absence and the presence of UV/VIS irradiation at least in the highest applied concentrations.
Without UV/VIS irradiation, there was a decrease in the cell number from 30.0 µg/mL (EC_{50}: 24.3 µg/mL) onward. The cell densities were distinctly reduced. With UV/VIS irradiation, there was a decrease in the cell number at 0.8 µg/mL (EC_{50}: 0.7 µg/mL) and above. Based on the EC_{50} values a PIF of 36.7 (phototoxic potential) was obtained (see Table 5.2.7-2).

Table 5.2.7-2: Mean relative cytotoxicity of Chlorpromazine with and without UV/VIS irradiation in Balb/c 3T3 cells

Test group	UV/VIS irradiation	Mean OD *	Mean $OD_{corr.}$ **	Relative Cytotoxicity [% of control]	Standard deviation [%]
Blank	-	0.035	-	-	-
Vehicle control 1	-	0.411	0.375	-	5.8
Vehicle control 2	-	0.407	0.372	-	4.1
Vehicle control mean (1% DMSO)	-	0.409	0.374	100.0	4.9
Chlorpromazine					
1.9 µg/mL	-	0.446	0.411	109.8	5.4
3.8 µg/mL	-	0.457	0.422	112.8	3.4
7.5 µg/mL	-	0.468	0.433	115.9	5.7
15.0 µg/mL	-	0.413	0.378	101.2	4.2
30.0 µg/mL	-	0.103	0.068	18.3	4.0
60.0 µg/mL	-	0.035	0.000	-0.1	0.1
90.0 µg/mL	-	0.036	0.001	0.4	0.8
180.0 µg/mL	-	0.035	0.000	0.1	0.1
Blank	+	0.036	-	-	-
Vehicle control 1	+	0.343	0.307	-	4.5
Vehicle control 2	+	0.392	0.356	-	3.4
Vehicle control mean (1% DMSO)	+	0.367	0.332	100.0	8.6
Chlorpromazine					
0.03 µg/mL	+	0.348	0.313	94.2	4.2
0.05 µg/mL	+	0.348	0.313	94.2	2.9
0.10 µg/mL	+	0.347	0.312	94.0	3.0
0.20 µg/mL	+	0.339	0.304	91.6	4.0
0.40 µg/mL	+	0.308	0.273	82.2	2.6
0.80 µg/mL	+	0.144	0.109	32.7	6.8
1.60 µg/mL	+	0.037	0.001	0.3	0.6
3.20 µg/mL	+	0.039	0.004	1.2	0.3

*: Mean absorbance at 550 nm of 6 wells, in general
**: Mean absorbance (test group) minus mean absorbance (blank)

III.CONCLUSIONS

According to the results of the present study, triticonazole (BAS 595 F) is considered **not to be a phototoxic** substance in the *in vitro* 3T3 NRU Phototoxicity Test. No phototoxic potential of triticonazole is expected at realistic outdoor conditions.

CA 5.3 Short-Term Toxicity

Studies evaluated in the draft monograph of triticonazole by the Rapporteur Member State Austria (2003):

Triticonazole (BAS 595 F) has been tested in various species and via different routes of administration. All studies are scientifically valid; however, some of the studies have been conducted before the release of study guidelines and are without GLP according to the usual practice in those days. These studies have been evaluated by European authorities and Austria as Rapporteur Member State (European Commission Peer Review Program) and are, for the convenience of the reviewer, listed in Table 5.3-1. Brief summaries of the respective studies were extracted from the monograph of triticonazole and are provided under the respective chapters.

Table 5.3-1: Summary of already peer-reviewed short term toxicity studies with triticonazole as available in the DAR (2003)

Study	Dosages (mg/kg bw/ day)	NOAEL (mg/kg bw/day)	Main adverse effect	Reference
4 weeks oral F-344 rat 0, 500, 1500, 5000, 15000 and 50000 ppm/diet	Males: 0, 50.1, 152.3, 513.2, 1494, 4802 mg/kg bw/d Females: 0, 52.4, 151.3, 489.4, 1476, 4945 mg/kg bw/d	cannot be determined	– clinical signs – decreased body weight and retarded body weight gain – haematological findings – hypoglycaemia – organ weight changes (liver ↑, uterus ↓, prostate ↓) – histological alterations in liver and uterus *(at ≥ 1476 mg/kg bw in females)**	(R013012)
13 weeks oral CD rat 0, 25, 250, 12500, and 25000 ppm/diet	Males: 0, 2.0, 19.8, 1117.0, 2309.3 mg/kg bw/d Females: 0, 2.2, 22.3, 1183.5, 2368.8 mg/kg bw/d	19.8 (♂)/22.3 (♀) mg/kg bw/d	– clinical signs (hair loss) – decreased body weight gain – haematological and clinical chemistry changes – organ weight changes (liver ↑, Ovaries** ↑) – histological alterations in liver and adrenals *(at ≥ 12500 ppm in m & f)*	(R013029)
6 weeks oral CD-1 mouse 0, 25, 250, 12500, and 25000 ppm/diet	Males: 0, 77, 233, 851, 3270 mg/kg bw/d Females: 0, 98.8, 286, 982, 4091 mg/kg bw/d	cannot be determined	– mortalities and clinical signs – decreased body weight and retarded body weight gain – organ weight changes (liver ↑) – histological alterations in the liver	(C044272)
13 weeks oral CD-1 mouse 0, 2500, 5000 and 8000 ppm/diet	Males: 0, 382.8, 807.6 and 1426.2 mg/kg bw/d Females: 0, 503.8, 969.2 and 1657.6 mg/kg bw/d	cannot be determined	– decreased body weight and retarded body weight gain – organ weight changes (liver ↑) – histological alterations in the liver	(R013027)
4 weeks oral Beagle dog	0, 10, 30, 100, and 300 mg/kg bw/d via capsule	30 mg/kg bw/d	– clinical signs – decreased body weight gain – haematological and clinical chemistry findings – organ weight changes (liver ↑, thymus ↓) – histological alterations in the liver	(R013014)

Study	Dosages (mg/kg bw/ day)	NOAEL (mg/kg bw/day)	Main adverse effect	Reference
52 weeks oral Beagle dog	0, 2.5, 25, and 150 mg/kg bw/d via capsule	2.5 mg/kg bw/d	– clinical (neurological) signs – decreased body weight gain – cataractogenic effects (150 mg/kg - pathology) – haematological and clinical chemistry findings – organ weight changes (liver ↑, adrenals ↑ at 150 mg/kg bw) – histological alterations in adrenals (at 150 mg/kg bw)	(R013082)
3 weeks dermal CD rat	0, 100, 300, and 1000 mg/kg bw/d	1000 mg/kg bw/d	– no effects observed at any dose level	(R012966)

**comments in italics were included by BASF and not included in the respective table of the DAR, but are in line with the assessment of the RMS*
**no histopathological correlate was seen for the ovary weight changes

The short term toxicity of triticonazole has been investigated in rats and mice following a 90 day exposure period and in dogs following a 1-year treatment period. The dosages of these studies were selected based on the results of 4 to 6-week preliminary studies. In addition, a 21-day dermal study was conducted in rats.
In a 90 day dietary study in the rat, there was clear evidence of systemic toxicity at high dose level (12500 and 25000 ppm). Treatment-related findings included reductions in body weight gain and food consumption, haematological and clinical chemistry changes, organ weight effects and histopathological findings. The liver and the adrenals were identified as the major target organs. Vacuolation of the adrenal cortex was noted in all groups but, based on severity grade and microscopic appearance, the findings at the dose levels of 25 and 250 ppm were considered to be typical of spontaneous changes commonly found in untreated animals. The short term NOAEL for triticonazole in the rat can be set at 250 ppm (equivalent to 19.8 (males) and 22.3 (females) mg/kg bw/d).
Also in mice, continuous dietary treatment during 90 days produced severe systemic toxicity at all dose levels with the liver being identified as the major target organ showing organ weight changes associated with histopathological alterations (hypertrophy, fatty vacuolation, necrosis, and increased mitotic activity). However, being a preliminary range-finding study, no short-term NOAEL could be determined in mice.
In the 1-yr dog-study, clear systemic toxicity was evident at the high dose (150 mg/kg) including cataractogenic effects, decreased body weight gains, haematological and clinical chemistry findings and increased liver and adrenal weights. Increased adrenal weights were associated with histopathological changes. Clinical chemistry findings together with organ weight effects suggest toxicological effects on the liver, but there were no histopathological alterations seen in the liver. Based on a moderate reduction in body weight gain and also clinical chemistry findings in females at 25 mg/kg bw/d, the NOAEL for this study is considered to be 2.5 mg/kg bw/d. This conclusion was also agreed at the Expert Meeting in October 2004 (EPCO 14).

Dermal application of triticonazole at dose levels up to 1000 mg/kg bw/d to rats for 21 days did neither produce local effects nor systemic toxicity. The NOAEL for this study was 1000 mg/kg bw/d (the highest dose tested).

Submission of not yet peer-reviewed studies in this AIR3-Dossier:

No new short-term toxicity studies have been performed with triticonazole.

Under consideration of all available data the classification of triticonazole will not differ under Reg. EC 1272/2008 (CLP) compared to the EU agreed endpoints given in the EFSA Scientific Report (33, 1-69) from 2005. The List of endpoints will be adapted accordingly as shown below:

Short term toxicity

Target / critical effect:	Biochemical changes indicative of liver effects in females. Other target organs affected at doses >LOAEL were the adrenals (rat; dog: cortical fatty vacuolation and degeneration of *zona reticularis*) and eye (dog: cataracts at high dose level).
Lowest relevant oral NOAEL/NOEL	1-yr dog: 2.5 mg/kg bw/d
Lowest relevant dermal NOAEL/NOEL	21-d rat: > 1000 mg/kg bw/d
Lowest relevant inhalation NOAEL/NOEL	no data –not required

The entry into the list of endpoints (Doc N1) is provided here:

Short-term toxicity

Target organ / critical effect	Rat: liver (hypertrophy, biochemical changes), adrenals (cortical fatty vacuolation and degeneration of zona reticularis at high dose levels > 1000 mg/kg bw) Dog: adrenals (cortical fatty vacuolation) and eye (cataracts at high dose levels)	
Relevant oral NOAEL	1-yr dog: 2.5 mg/kg bw 90-day rat: 250 ppm (equivalent to 19.8 and 22.3 mg/kg bw in males and females)	
Relevant dermal NOAEL	21-day rat: > 1000 mg/kg bw	
Relevant inhalation NOAEL	No data - not required	

For convenience of the reviewer brief summaries of the respective studies as extracted from the monograph of triticonazole are provided below.

CA 5.3.1 Oral 28-day study

Rat, 4 week feeding study, 0, 500, 1500, 5000, 15000, 50000 ppm (R013012)

Guidelines: This study was performed as a range finding study for a subsequent 90-day study. No specific test guideline was mentioned.
GLP: Yes
Acceptance: The study was considered acceptable in the EU registration process 2003.

Material and methods:
Groups of 5 male and 5 female F-344 rats per dose group received triticonazole (batch: YG 2156/1; purity 98.9%) at dietary doses of 0, 500, 1500, 5000, 15000, or 50000 ppm for 4 weeks. The dose levels were equivalent to 0, 50.1, 152.3, 513.2, 1494 and 4802 mg/kg bw/d in males and 0, 52.4, 151.3, 489.4, 1476 and 4945 mg/kg bw/d in females.

Findings:
General observations:
There were no deaths during the study period related to treatment. Clinical signs (marked hair loss on the head and dorsal surface, piloerection, hunched posture) were noted throughout the entire treatment period for all animals of both sexes receiving 50000 ppm.
Body weight:
Body weight gains of males receiving 5000 ppm and above and females receiving 50000 ppm were significantly lower than those of the respective control values throughout the study period. For females receiving 5000 and 15000 ppm, body weight gains were also lower during the first four days of treatment, but unaffected thereafter. Animals of both sexes receiving 1500 ppm showed only slightly lower weight gain. Mean body weights and weight gain of animals receiving 500 ppm were similar to that of the controls over the treatment period. There was also a dose-related decrease in food consumption in animals of both sexes treated with 5000 (90 - 93%), 15000 (84 – 88%) and 50000 ppm (72 – 76%) compared with the respective control values.
Ophthalmoscopy:
Ophthalmoscopic examinations revealed no evidence of treatment-related changes at any dose group.
Clinical chemistry and haematology:
Evaluation of haematological parameters after three weeks of treatment revealed significant dose-related effects in animals receiving 5000 ppm and higher, consisting of lower haematocrit values, lower haemoglobin concentration, lower total leucocyte counts (both neutrophils and lymphocytes) and lower platelet counts.

Concerning clinical chemistry, treatment-related findings were limited to a dose-related hypoglycaemia among rats receiving >5000 ppm. Also noted, relative to control values, were higher ALT activities in both sexes at 50000 ppm; higher AST activities in males at 5000 and 50000 ppm; higher AP activities in females at 15000 and 50000 ppm, low creatinine levels among males at 5000 ppm or more; higher cholesterol concentrations in males at 5000, 15000 and 50000 ppm and in females receiving 1500, 5000 and 15000 ppm. However, it was stated that the majority of the individual values in all of these respects were within the range of expectancy for young F-344 rats at this laboratory. In view of the minimal nature of the intergroup differences, which were often not strictly dosage-related in degree, none of these changes could unequivocally be ascribed to treatment with triticonazole.
Urinalysis:
When compared with control animals (pH 6.2), significant lower urinary pH values were noted in males treated at 15000 (pH 6.0; $p<0.05$) and 50000 (pH 5.8; $p<0.001$) ppm. In addition, slightly lower urinary protein content was noted in males treated with 5000 ppm and more than in controls. A marked ketonuria was evident in animals of both sexes of the top dose.
Organ weight:

Organ weight analysis after 4 weeks of treatment revealed dose-related increased absolute and relative liver weights among animals receiving 5000 ppm and above. The prostate weights of males receiving 5000 ppm or more were lower than those of their respective controls. Lower uterus weights were also noted among all treated females when compared with controls. In all these instances the changes showed a clear dose-relationship. In addition, absolute testes weights were significantly lower in males which received 50000 ppm.
Necropsy:
Macroscopic examination of animals killed after 4 weeks of treatment revealed abnormal (pale) caecal contents among animals which received 50000 ppm.
Histopathology:
Histopathological examinations identified the liver and the uterus as the main target organs. In the liver, atypical fatty centriacinar and microvascular panacinar vacuolation was seen in males treated with 50000 ppm and in females which received 15000 and 50000 ppm. Necrosis of individual hepatocytes was noted in in males at 15000 and 50000 ppm and in one female treated with 15000 ppm. Also hepatocyte hypertrophy was evident in four females and in one male at the top dose level. In the uterus, reduction of the endometrial stroma was observed among females receiving 15000 and 50000 ppm giving the organ a rather atrophic appearance. No treatment-related changes were observed at 5000 ppm and below.

Conclusion
Clear evidence of toxicity was noted at concentrations of 5000 ppm triticonazole when administered to F-344 rats for four weeks via diet (corresponding to 513.2 and 489.4 mg/kg bw in males and females); the liver and uterus were identified as the major target organs. Since effects on the uterus weight were recorded at all dose levels, a NOAEL could not be determined. It was concluded in the report that the highest concentration of triticonazole which would be suitable for prolonged dietary administration to rats is between 5000 and 15000 ppm.

Mice, 6 weeks feeding study, 0, 500, 1500, 5000, 15000, 50000 ppm (C019001)

Guidelines: This study was performed as a range finding study for a subsequent 90-day study. No specific test guideline was mentioned.

GLP: Yes

Acceptance: The study was considered acceptable in the EU registration process 2003 but due to major limitations of parameters the study was regarded only as supplementary information.

Material and methods:

Groups of 12 male and 12 female mice (strain: CD-1; source: [redacted]) were fed diet containing 0, 500, 1500, 5000, 15000 or 50000 ppm triticonazole (batch no. YG 2156/1; purity 98.9%), equivalent to 0, 77, 233, 851 and 3270 mg/kg bw/d in males and 0, 98.8, 286, 982 and 4091 mg/kg bw/d in females, respectively (data for mice treated at 50000 ppm are not given) for 6 weeks.

Findings:

General observations:

All animals treated with 50000 ppm and also one male and ten females receiving 15000 ppm died or were killed in extremis within the first week of treatment. Before death, the mice displayed signs of ill-health including hunched posture, piloerection, flaccid muscle tone, hypoactivity, hypothermia and marked body weight loss and emaciation.

Body weight and food consumption:

During the first three days of dosing, marked dose-related decreases in body weights were reported in animals receiving 5000 ppm and above. Thereafter the body weight gain of males treated with 5000 ppm and of females treated with 5000 and 15000 ppm was superior to that of the respective control values. Body weights of males receiving 15000 ppm remained more or less static during the study period. Bodyweight gains of animals receiving 500 and 1500 ppm were unaffected by treatment.

Food consumption of mice receiving 50000 ppm was negligible and of mice treated with 15000 ppm was marked lower than that of controls during the first week of treatment. Thereafter, food intake in this dose group (15000 ppm) was only slightly lower than that of controls. Food consumption of animals receiving <5000 ppm was unaffected by treatment. However, food conversion ratio was also lower than that of controls for males receiving 5000 ppm.

Organ weights:

Organ weight analysis from animals killed after 6 weeks of treatment revealed clear dose-related increased absolute and relative liver weights in all treated groups when compared with controls. Higher liver weights were also noted for some of the mice killed or dying during the treatment period. All other inter-group differences occasionally attaining statistical significance were considered not to be of toxicological relevance.

Pathology:

Macroscopic examination after six weeks of treatment revealed liver enlargement in animals receiving 5000 ppm and more. There was also a range of histopathological findings related to treatment. The major finding was hypertrophy of periacinar hepatocytes in males and females at > 1500 ppm. In males which received 15000 ppm and in females which received 5000 ppm, this finding was frequently associated with fatty vacuolation. In addition, increased ploïdy of hepatocytes was noted at these dose levels. Proliferation of bile duct cells, increased incidences of inflammatory cells in the portal area and focal mineralization were also evident in males receiving 5000 and 15000 ppm.

Conclusion
Taking into account the findings of this study, a NOAEL cannot be determined as treatment-related findings were observed at all dose levels with the liver identified as the target organ. However, it was concluded in the report, that the maximum-tolerated dose for repeated dietary administration of triticonazole in mice lies between 1500 and 5000 ppm.

Dog, 4 weeks feeding study, 0, 10, 30, 100, and 300 mg/kg bw/d (R013014)

Guidelines: This study was performed as a range finding study for a subsequent repeated dose study. No specific test guideline was mentioned.

GLP: Yes

Acceptance: The study was considered acceptable in the EU registration process 2003 but due to major limitations of parameters the study was regarded only as supplementary information.

Material and methods:

Triticonazole (batch no. YG 2156/1; purity 95.4%) was given to groups of 2 male and 2 female beagle dogs (source [redacted]) at dose levels of 0 (empty capsule), 10, 30, 100 and 300 mg/kg bw/d in gelatin capsules for 28 consecutive days.

Findings:

General observations:

No treatment-related mortalities occurred at any dose level. Signs of reaction to treatment were confined to only one male dog receiving 300 mg/kg bw and consisted of ataxia, abnormal gait, hypoactivity, circling and head shaking. These signs were observed approx. 5 – 6 hours after dosing on days 3 to 5 of treatment and there was a complete recovery by the following morning. Subsequently, no signs of reaction to treatment were seen.

Body weight:

Decreases in overall body weight gains were observed in animals of both sexes receiving 300 mg/kg when compared with control animals. This effect was less evident in males (63% of controls) than in females (40% of controls) and was considered to arise from initial body weight loss during the first 3 days of treatment. The body weight gains of all other treated animals were unaffected by treatment. There was also a decrease in food consumption in females receiving 300 mg/kg (92% of controls). Neurological and veterinary examinations and ophthalmoscopy revealed no treatment-related changes in any dose group.

Clinical chemistry and haematology:

Haematological investigations exhibited higher haematocrit value, haemoglobin concentration and erythrocyte count in one female receiving 300 mg/kg after 25 days of treatment. Clinical chemistry analysis revealed significant treatment-related elevated activities of alkaline phosphatase in male dogs receiving 100 and 300 mg/kg, and in female dogs receiving 300 mg/kg compared to control values. There were no other haematological or biochemical changes in the plasma that could be attributed to treatment. In addition, no treatment-related changes were seen in animals of the other dose-groups.

Urinalysis:

The composition of the urine was unaffected by treatment with triticonazole at any dose level.

Organ weight analysis:

When compared with control animals, treatment-related findings were limited to slight increase in relative liver weights in males receiving 300 mg/kg and in females receiving 100 and 300 mg/kg bw/d. Absolute liver weight was only higher in males of the top dose. In addition, absolute and relative thymus weights of males receiving 100 and 300 mg/kg were low in comparison with those of control males.

Pathology:
There were no macroscopic changes after four weeks which could be attributed to treatment with triticonazole. Upon histopathological examination, treatment-related findings were limited to periacinar hypertrophy of hepatocytes with associated fatty vacuolation in both males receiving 300 mg/kg. There were no other findings considered to be related to treatment.

Conclusion:
Following continuous oral administration of triticonazole to Beagle dogs for four weeks, mild systemic toxicity (reduced body weight gain, biochemical and haematological changes, organ weight effects and histopathological findings) was observed at the dose level of 300 mg/kg bw/d. The liver was identified as the target organ. Based on increased activity of the alkaline phosphatase and higher liver weights, evident at 100 mg/kg bw/d, the NOAEL for this study was established at 30 mg/kg bw/d.

CA 5.3.2 Oral 90-day study

Rat, 13 week feeding study, 0, 500, 1500, 5000, 15000, 50000 ppm (R013029)

Guidelines: This study was performed in compliance with the US EPA guideline 82-1.
GLP: Yes
Acceptance: The study was considered acceptable in the EU registration process 2003.

Material and methods:
Groups of 10 male and 10 female CD rat (source: [redacted]) received triticonazole (batch no. YG 2160/1, purity 98.2% used for the first nine weeks; batch no. YG 2156/1, purity 98.9% used subsequently) with their diet at dose levels of 0, 25, 250, 12500 and 25000 ppm for 13 weeks (equivalent to 0, 2.0, 19.8, 1117.0 and 2309.3 mg/kg bw/d in males and 0, 2.2, 22.3, 1183.5 and 2368.8 mg/kg bw/d in females).

Findings:
General observations:
No treatment-related mortalities occurred at any dose level. One female receiving 250 ppm was killed in extremis during week 13 of the study following a renal failure (not attributed to treatment). Clinical signs were limited to a generalized hair loss (predominantly affected on head, ventral and dorsal body surface) that persisted until week 9 in animals treated at 12500 ppm and throughout the study in all animals treated with 25000 ppm.
Body weight and food consumption:
During the first 5 weeks of dosing, marked significant decreases in body weight gains were reported in rats treated at 12500 and 25000 ppm. Thereafter, overall body weight gains were comparable with controls. The overall body weight gains of males receiving 12500 and 25000 ppm were 80% and 70% of control values, resp., whereas terminal body weight gains in females receiving 12500 and 25000 ppm were 75% and 70% of control values, respectively. Food consumption was depressed in animals at 25000 ppm, especially during weeks 1 – 9. Food consumption of males and females receiving 12500 ppm was also low until weeks 5 and 7 respectively. The overall efficiency of food conversion at both high dose levels was also reduced compared with controls. Body weight gains and food consumption in animals treated at 25 and 250 ppm were comparable with control animals.
Ophthalmoscopy:
Ophthalmoscopic examinations revealed no treatment-related effects at any dose level. Urinalysis: When compared with control animals, treatment-related findings consisted of lower protein content of the urine in males at 12500 and 25000 ppm and slightly lower urinary pH in males at 25000 ppm and in females at all dose levels. However, the slight effect noted in females at 25 ppm was considered incidental.
Clinical chemistry and haematology:
Haematological investigations indicated a number of treatment-related effects in the two higher dose groups (for details see Table 5.3.2-1).

Table 5.3.2-1: Haematological findings (after 12 weeks of treatment) (group mean values)

	Dose group level [ppm]									
	Males					Females				
	0	25	250	12500	25000	0	25	250	12500	25000
Packed cell volume (PCV) [%] (SD)	45 (1)	46 (1)	46 (2)	45 (1)	43** (2)	45 (1)	46 (2)	46 (2)	44 (2)	43** (2)
Haemoglobin [g%] (SD)	15.8 (0.5)	16.2 (0.3)	16.1 (0.8)	15.8 (0.3)	15.2** (0.3)	15.9 (0.6)	15.9 (0.6)	16.0 (0.7)	15.1* (0.8)	15.0** (0.7)
Erythrocytes [10^{-12}/l] (SD)	8.95 (0.32)	8.85 (0.26)	8.94 (0.43)	8.55* (0.34)	8.47** (0.18)	8.40 (0.38)	8.59 (0.43)	8.59 (0.37)	8.40 (0.36)	8.28 (0.55)
MCV [cμ] (SD)	51 (1)	52 (2)	52 (2)	53** (2)	51 (2)	54 (2)	53 (2)	54 (2)	52** (1)	51*** (2)
MCH [pg] (SD)	18 (1)	18 (1)	18 (1)	19** (1)	18 (1)	19 (1)	19* (1)	19 (1)	18*** (1)	18** (1)
Total white blood cells [10^{-9}/l] (SD)	13.7 (1.3)	17.4** (2.9)	14.6 (3.9)	13.9 (2.3)	13.3 (2.2)	8.8 (1.7)	7.4 (2.0)	7.2 (1.1)	10.2 (2.4)	12.7*** (3.1)

*p<0.05, **p<0.01, ***p<0.001

When compared with control values, lower packed cell volumes (PCV) and haemoglobin concentrations (males and females at 25000 ppm), lower erythrocyte counts (males at 12500 and 25000 ppm), lower MCV and MCH (females at 12500 and 25000 ppm) and higher total leucocyte and lymphocyte counts (females at 25000 ppm) were observed. In addition, examination of blood smears revealed more spherocytes and a greater prevalence of anisocytosis in males treated at 25000 ppm and in females at > 12500 ppm. There were no haematological changes that were considered treatment-related at 25 and 250 ppm.
Clinical chemistry analysis in plasma revealed also changes relative to controls in animals treated at 12500 and 25000 ppm which were considered treatment-related (see Table 5.3.2-2)

Table 5.3.2-2: Selected clinical chemistry findings (after 12 weeks of treatment) (group mean values)

	Dose group level [ppm]									
	Males					Females				
	0	25	250	12500	25000	0	25	250	12500	25000
Alkaline phosphatase (AP) [iU/l] (SD)	90 (6)	93 (14)	104* (17)	97 (16)	120*** (18)	51 (13)	61 (15)	55 (14)	59 (13)	57 (9)
Cholesterol [mg%] (SD)	54 (9)	59 (12)	52 (14)	77*** (10)	72** (15)	77 (11)	86 (15)	80 (7)	142*** (20)	157*** (21)
Glucose [mg%] (SD)	138 (18)	155* (21)	150 (12)	133 (15)	121* (19)	115 (13)	122 (6)	123 (15)	115 (15)	98** (10)
Total protein [g%] (SD)	6.8 (0.2)	7.0* (0.2)	6.7 (0.2)	6.9 (0.3)	6.8 (0.2)	7.3 (0.2)	7.2 (0.2)	7.3 (0.3)	7.6** (0.2)	7.7*** (0.3)

*p<0.05, **p<0.01, ***p<0.001

Higher plasma alkaline phosphatase (males at 25000 ppm), higher cholesterol concentrations (males and females at 12500 and 25000 ppm), reduced glucose levels (males and females at 25000 ppm), higher plasma protein concentrations (females at 12500 and 25000 ppm) were associated with some changes in the different protein fractions (lower albumin concentration and higher α1-globulin concentration with a concomitant reduction in the albumin to globulin ratio at 12500 ppm; increased β-globulin concentration at 12500 and 25000 ppm). Other intergroup differences attaining statistical significance at some dose levels when compared with controls were considered as biological variability with no toxicological relevance because they were not dose-related or were within the normal range of controls.

Pathology:
Macroscopic examination of animals at the terminal sacrifice exhibited enlarged livers in females at 25000 ppm and each one female showed enlarged ovaries in each group receiving 250, 12500 and 25000 ppm (Animal no. 71, 85, 93). Analysis of organ weights revealed a number of differences between groups which were considered to be secondary to the reduction of bodyweight. The critical effects which were considered significant and treatment-related were increased absolute and relative liver weights in animals treated at > 12500 ppm, and increased absolute and relative ovary weights at the same dose levels (see Table 5.3.2-3).

Table 5.3.2-3: Relevant organ weight and necropsy findings

	Dose group level [ppm]									
	Males					Females				
	0	25	250	12500	25000	0	25	250	12500	25000
Organ weight changes (mean group values)										
Adrenals abs. weight [g] (SD)	0.058 (0.011)	0.056 (0.011)	0.064 (0.011)	0.054 (0.007)	0.053 (0.010)	0.071 (0.008)	0.073 (0.007	0.088** (0.009)	0.053 (0.006)	0.056**
Adrenals rel. weight [%] (SD)	0.0107 (0.0023)	0.0100 (0.0014)	0.0120 (0.0024)	0.0120 (0.0015)	0.0126 (0.0011)	0.0210 (0.0020)	0.0225 (0.0027)	0.0261** (0.0027)	0.0188 (0.0019)	0.0205 (0.0043)
Liver abs. weight [g] (SD)	17.4 (1.1)	17.7 (3.0)	17.3 (1.7)	19.1 (1.8)	18.6 (2.4)	11.2 (1.7)	12.3 (1.2)	12.0 (1.2)	15.4** (2.7)	17.3** (1.5)
Liver rel. weight [%] (SD)	3.19 (0.21)	3.16 (0.24)	3.22 (0.37)	4.23** (0.30)	4.50** (0.30)	3.29 (0.37)	3.79** (0.27)	3.57 (0.17)	5.51** (0.83)	6.36** (0.46)
Ovaries abs. weight [g] (SD)						0.090 (0.018	0.098 (0.025)	0.105 (0.045)	0.120** (0.014)	0.114* (0.027)
Ovaries rel. weight [%] (SD)						0.0267 (0.0055)	0.0303 (0.0081)	0.0313 (0.0124)	0.0432** (0.0060)	0.0416** (0.0087)
Group incidences of histopathological changes in the liver and adrenals										
Liver										
Periacinar hepatocyte hypertrophy	0/10	0/10	2/10	6/10*	10/10***	0/10	0/10	0/9	6/10*	9/10***
Centriacinar fatty vacuolation	0/10	3/10	1/10	1/10	2/10	3/10	1/10	4/9	7/10	10/10***
Adrenals										
Degeneration of zona reticularis	0/10	1/10	0/10	0/10	0/10	0/10	0/10	0/9	9/10***	10/10***
Cortical fatty vacuolation										
slight	2/10	2/10	3/10	3/10	2/10			1/9	3/10	8/10
mild				3/10	1/10					
moderate				2/10	2/10				1/10	2/10
marked				2/10	4/10					
severe					1/10					

*p<0.05, **p<0.01, ***p<0.001

Animal no. 71 from the 250 ppm dose group had the highest individual ovary weight (0.217 g, which is more than 2-fold above the mean values of all dose groups) compared to all treated animals of all groups. Together with the absence of a histopathological change, this suggests a single incidental effect unrelated to treatment. As there were no histopathological changes observed in none of the individual female animals, the weight changes of the ovaries in the two top doses were considered to be of doubtful toxicological significance.

Microscopic examination revealed treatment-related changes in the liver and the adrenals. In the liver significant increased incidences of minimal to slight periacinar hypertrophy and minimal to slight centriacinar hepatocytic fatty vacuolation were evident in animals at 12500 and 25000 ppm. Marginal hepatocyte hypertrophy was also recorded in two males that had received 250 ppm. In the adrenal cortex, degeneration of the *zona reticularis* in females at 12500 and 25000 ppm, and a significantly higher incidence of fatty vacuolation of the *zona fasciculata* (and also of the *zona reticularis* in more severe cases) was evident in males and in females at > 12500 ppm. This was conclusively confirmed by a peer-review (Isaac K., 2000, C014049 and Gopinath C., 2000, C014047). There were no other histopathological findings considered to be related to treatment with triticonazole.

Conclusion:
Continuous dietary administration of triticonazole to CD rats during 90 days produced clear evidence of toxicity at 12500 and 25000 ppm (corresponding to dose levels of >1000 and >2000 mg/kg bw, exceeding the limit doses), with the liver and the adrenals being identified as major target organs. Hypertrophy and fatty vacuolation was seen in liver and degeneration of zona reticularis and fatty vacuolation was observed in adrenals of females and males at doses of ≥ 12500 ppm.
The NOAEL of this study is 250 ppm (equivalent to 19.8 and 22.3 mg/kg bw/d in males and females, respectively).

Mice, 13 weeks feeding study, 0, 2500, 5000, 8000 ppm (R013027)

Guidelines: This study was performed as a range finding study for the subsequent carcinogenicity study. It was performed in accordance with the US EPA Guideline 82-1.
GLP: Yes
Acceptance: The study was considered acceptable in the EU registration process 2003 but due to major limitations of parameters the study was regarded only as supplementary information.

Materials and methods:
Groups of 12 male and 12 female CD-1 mice (source: [redacted]) received triticonazole (batch no. BD 1074; purity 99.3%) in their diet at concentrations of 0, 2500, 5000 or 8000 ppm (equivalent to 0, 382.8, 807.6 and 1426.2 mg/kg bw in males and 0, 503.8, 969.2 and 1657.6 mg/kg bw in females) for 90 days. Animals were observed at least twice daily for clinical signs and mortality. Body weights were recorded prior to dosing, on days 3 and 7 and then weekly. Food consumption was recorded weekly. Neither haematology nor clinical chemistry parameters were investigated. At necropsy, all animals were subjected to a detailed macroscopic pathological examination and the weights of selected organs (brain, heart, kidneys, liver, lungs, spleen, testes, and uterus) were recorded. Histopathological examinations were performed on the liver only.

Findings:
General observations: neither treatment-related clinical signs nor mortalities which could be related to treatment with triticonazole were noted throughout the study period. Concerning body weight, there was a significant depression of weight gain in all treated groups. This effect was particularly marked during the first week with initially body weight loss during the first 3 days of dosing. Nevertheless, food consumption was unaffected by treatment at any dose group when compared with controls.

Organ weight analysis revealed dose-related increases in absolute and relative liver weights compared with controls. In addition, dose-related lower organ weights of uterus plus cervix were evident among all treated females, but statistical significance was achieved only at the highest dose group. Macroscopic examination revealed high incidences of enlarged livers in all treated groups. Additionally, the livers of several animals were described as “swollen”. There were no other findings which were attributed to treatment. Histologically, periacinar and/or panacinar hepatocytic fatty vacuolation was evident in all treated mice. These vacuoles were confirmed as fat. Further findings which occurred in all treated animals comprised hepatocyte hypertrophy, coagulative necrosis of hepatocytes, hepatocyte necrosis and bile plug formation. In addition, increased mitotic activity of hepatocytes was seen in males and females receiving 8000 ppm and in females receiving 2500 ppm test compound.

Conclusion:
Based on treatment-related findings, particularly of significant hepatic toxicity, which were seen at all dose groups, a NOAEL could not be established for this study. However, it was concluded, that a dose level of 1500 ppm is suitable as the highest dose for the subsequent mouse carcinogenicity study.

Dog, 52 weeks feeding study, 0, 2.5, 25, and 150 mg/kg bw/d (R000173)

Guidelines: This study was conducted according to US EPA/FIFRA guideline 83-1 (1984).
GLP: Yes
Acceptance: The study was considered acceptable in the EU registration process 2003.

Material and methods:
Groups of 4 male and 4 female beagle dogs (source: [redacted]) received triticonazole (batch no. DA 646; purity 96.6 – 97.1%) in gelatine capsules at daily dose levels of 0 (empty capsule), 2.5, 25 and 150 mg/kg bw/d for 52 weeks. Dosing was performed after feeding.

Findings:
General observations:
There were no mortalities which could be related to treatment at any dose level.
Remark: One female dog receiving 25 mg/kg was killed for animal welfare reasons during week 5 following a six days period of deterioration in condition including severe pain of the joints. Examination of blood samples indicated high neutrophil and monocyte count, markedly high plasma AP and high plasma cholesterol and albumin concentrations. Significant findings at histopathology revealed myofibre degeneration, inflammation of skeletal muscles, multifocal perivascular changes in the brain and slight erythrocytosis in lymph nodes. Since the symptoms and severe findings were not repeated in any other dog, this death was considered incidental and not related to treatment.
Clinical signs as a reaction to treatment were confined to animals receiving 150 mg/kg and comprised neurological changes, higher incidences of red and thickened pinnae and skin, red gums, externally-visible lenticular opacities, ocular discharge and partially ”closed eyes”. Clinical signs of neurological origins were seen between weeks 6 and 11 of treatment, generally taking the form of tremor of the head and neck (noted in 2 males and all 4 females between 30 minutes and 4 hours after dosing), short-duration convulsions (noted in one male and female during week 11, both before and after dosing), ataxia and reluctance to walk (observed in one female during week 9). There was complete recovery for all these signs.

Body weight gain:
Overall body weight gains of males and females receiving 150 mg/kg were significantly lower when compared with those of the controls. In addition, although the difference did not reach statistical significance, the overall body weight gains of females receiving 25 mg/kg were also marked lower (- 25%) in comparison with controls. Weight gains of males receiving 2.5 mg/kg were lower than the controls, but in the absence of effects in males at 25 mg/kg, this was not considered related to treatment.
Food consumption was considered to have been unaffected to treatment.

Ophthalmoscopic examinations:
Early cataract formation was noted in 4 males and 2 females receiving 150 mg/kg at week 13 with a further female of the top dose affected at week 19. These changes originally presented as anterior and posterior capsular opacities of varying severity progressed to total cataract formation with opacification of the lens nucleus, equatorial vacuolation and a clear cortical zone between the two. During cataractogenesis concomitant uveitis including conjunctivitis, blepharospasms, miosis and photophobia were observed. From week 32 onwards, no examination of the retina was possible in animals displaying a cataract. Lens degeneration was also seen during histopathology (see below). The rate of tear secretion was unaffected at any dose level. There were no ophthalmoscopic changes that were considered treatment-related at 2.5 and 25 mg/kg bw/d.
Remark: It was stated by the study author that the cause of this lenticular degeneration is not clearly known but such effects were observed with some hypocholesterolemic agents, and the administration of triticonazole was clearly associated with lowered plasma cholesterol concentrations in dogs.

Clinical chemistry and haematology
Haematological treatment-related changes were limited to higher platelet counts at week 24 and 50 in female dogs receiving 150 mg/kg and also higher haematocrit values and haemoglobin concentrations evident in one male and 2 females of the top dose at week 12 of the study, when compared with control values. There were no other changes at 2.5 and 25 mg/kg considered to be related to treatment.

Clinical chemistry analysis revealed a number of treatment-related changes in animals receiving 150 mg/kg. A summary of the relevant haematology and clinical chemistry values is given in Table 5.3.2-4.

Table 5.3.2-4: Relevant haematological and clinical chemistry findings (group mean values)

	Dose group level [ppm]							
	Males				Females			
	0	2.5	25	150	0	2.5	25	150
Platelet count [10-9/l] (SD)								
Before treatment	400 (137)	367 (23)	394 (58)	363 (67)	440 (83)	403 (62)	362 (37)	413 (44)
Week 12	328 (89)	325 (47)	324 (42)	351 (74)	361 (26)	326 (53	311 (6)	421 (67)
Week 24	280 (75)	296 (28)	302 (28)	336 (59)	327 (35)	302 (70)	272 (24)	418* (45)
Week 50	345 (112)	338 (35)	376 (29)	431 (32)	380 (62)	383 (104)	406 (34)	517* (32)
Alkaline phosphatase (AP) [iU/l] (SD)								
Before treatment	111 (27)	110 (23)	109 (2)	127 (15)	118 (34)	100 (30)	106 (15)	93 (9)
Week 12	68 (15)	74 (6)	88 (22)	437*** (197)	83 (14)	71 (16)	111*[1]	326*** (90)
Week 24	51 (10)	73 (46)	86 (16)	705*** (342)	63 (6)	64 (9)	107***[1] (18)	385*** (141)
Week 50	68 (29)	66 (38)	85 (11)	1029** (717)	72 (5)	72 (16)	113**[1] (17)	600*** (161)
Alanine aminotransferase (ALT) [iU/l] (SD)								
Before treatment	17 (4)	21 (3)	19 (3)	17 (2)	20 (6)	17 (3)	21 (6)	15 (2)
Week 12	24 (5)	32 (7)	33 (6)	44** (13)	28 (2)	25 (5)	34 (4)	43 (27)
Week 24	27 (4)	29 (8)	32 (7)	52* (26)	27 (5)	24 (2)	32 (8)	47* (21)
Week 50	37 (5)	36 (7)	37 (4)	60** (17)	27 (4)	32 (5)	36 (10)	58** (22)
Aspartate aminotransferase (AST) [iU/l] (SD)								
Before treatment	30 (4)	29 (5)	31 (7)	27 (1)	26 (3)	27 (7)	31 (6)	26 (7)
Week 12	30 (5)	41** (7)	36 (5)	31 (1)	28 (3)	34 (3)	30 (4)	31 (8)
Week 24	28 (3)	35 (12)	28 (3)	22 (1)	24 (4)	24 (2)	24 (2)	26 (2)
Week 50	39 (6)	42 (9)	39 (6)	29* (2)	29 (3)	30 (5)	27 (3)	31 (2)
Cholesterol [mg%] (SD)								
Before treatment	184 (19)	153 (28)	163 (27)	174 (21)	148 (17)	153 (14)	152 (24)	170 (6)
Week 12	163 (19)	140 (42)	144 (33)	95** (26)	145 (17)	143 (15)	137 (32)	121 (27)
Week 24	161 (24)	144 (40)	128 (27)	78** (15)	146 (16)	138 (14)	103* (22)	105* (33)
Week 50	163 /24)	143 (41)	124 (33)	73** (17)	159 (21)	163 (17)	157 (59)	112 (37)
Protein [g%] (SD)								
Before treatment	5.3 (0.2)	5.1 (0.2)	5.3 (0.3)	5.4 (0.3)	5.4 (0.3)	5.2 (0.3)	5.5 (0.2)	5.3 (0.3)
Week 12	5.6 (0.2)	5.2 (0.4)	5.3 (0.4)	5.1 (0.4)	5.1 (0.2)	5.4 (0.1)	5.2 (0.3)	5.1 (0.2)
Week 24	5.9 (0.2)	5.6 (0.7)	5.4 (0.3)	5.0** (0.1)	5.5 (0.2)	5.5 (0.1)	5.1** (0.1)	5.1** (0.2)
Week 50	6.0 (0.3)	5.8 (0.3)	5.7 (0.4)	5.4** (0.1)	6.0 (0.1)	5.8 (0.3)	5.8 (0.3)	5.3** (0.4)

*p<0.05, **p<0.01, ***p<0.001, [1]Result of statistical evaluation after exclusion of high dose values

In comparison with controls, alkaline phosphatase (AP) activity was markedly high after 12 weeks, and became higher as the study progressed. In addition, lower cholesterol concentrations, total plasma protein and albumin concentrations were noted at this dose level when compared with controls. Plasma alanine amino-transferase (ALT) activities were consistently higher whereas creatinine concentration was consistently lower than in controls at this dose level. Among animals receiving 25 mg/kg, similar treatment-related findings on AP activities, cholesterol and protein concentrations were observed although these changes tended to be less marked, and in some cases were only transient. However, statistical significant intergroup difference from control values (after exclusion of high dose values) were reached for AP activities in females at weeks 12, 24 and 50, and for cholesterol and protein concentrations in females at week 24, when compared with controls. One female receiving 25 mg/kg also had a consistently higher plasma ALT activity than the controls. There were no clinical chemistry changes in animals receiving 2.5 mg/kg bw/d.

Urinalysis:
Treatment-related changes were limited to animals of the top dose level consisting of significantly increased specific gravity in males at weeks 24 and 50 and in females at all sampling times and a lower urinary volume in females at weeks 24 and 50.
Organ weight:
Organ weight analysis revealed increased values of absolute and relative adrenal weights and liver weights, resp., in males and females receiving 150 mg/kg bw, when compared with control values. In addition, a slight increase in relative kidney weights in females and marked decreases in absolute and relative prostate weights in males were noted at this dose level (see Table below).

Table 5.3.2-5: Organ weight changes of selected organs (group mean values)

	Dose group level [ppm]							
	Males				Females			
	0	2.5	25	150	0	2.5	25	150
Adrenals								
Absolute weights [g] (SD)	1.41 (0.07)	1.45 (0.26)	1.30 (0.09)	1.88 (0.62)	1.78 (0.13)	1.60 (0.36)	1.58 (0.33)	1.69 (0.41)
Relative weights [%] (SD)	0.0098 (0.0012)	0.0108 (0.0020)	0.0092 (0.0010)	0.0139 (0.0038)	0.0130 (0.0007)	0.0125 (0.0021)	0.0132 (0.0019)	0.0174* (0.0033)
Kidneys								
Absolute weights [g] (SD)	61 (4)	62 (7)	66 (6)	64 (5)	58 (5)	55 (3)	53 (5)	54 (7)
Relative weights [%](SD)	0.42 (0.04)	0.46 (0.01)	0.46 (0.03)	0.47 (0.02)	0.42 (0.01)	0.44 (0.05)	0.45 (0.04)	0.56** (0.06)
Liver								
Absolute weights [g] (SD)	496 (38)	417 (30)	504 (77)	539 (62)	472 (64)	424 (61)	415 (55)	475 (88)
Relative weights [%](SD)	3.45 (0.54)	3.12 (0.36)	3.53 (0.41)	4.03 (0.57)	3.46 (0.57)	3.38 (0.57)	3.48 (0.30)	4.93* (0.75)
Prostate								
Absolute weights [g] (SD)	8.18 (2.02)	8.93 (3.88)	8.92 (1.56)	2.86* (0.74)				
Relative weights [%](SD)	0.0570 (0.0169)	0.0651 (0.0228)	0.0626 (0.0113)	0.0211* (0.0048				

*p<0.05, **p<0.01

The lower absolute and relative prostate weights are an isolated finding in dogs. There is no respective findings on organ weights or histopathology on male reproductive organs (prostate, seminal vesicles, epididymides, testes) in rats or mice in any of the relevant studies in Sprague-Dawley rat or CD-1 mice. The high dose results (> 500 mg/kg bw) of the F-344 rat, seen in the 28-day study are not considered further. Further, there was no histopathological correlate seen in the prostate of the high dose dogs.

Macroscopic pathology:
An apparent enlargement of the liver was observed in two males treated at 25 mg/kg bw and in two males and two females treated at 150 mg/kg bw, although a similar finding was also recorded for one control male. Another observation at the macroscopic examination was thickening of the skin (predominantly pinnae and hocks) at a higher incidence in dogs treated with 150 mg/kg bw, compared to controls.

Histopathology:
Microscopic examinations of animals at the terminal sacrifice revealed histopathological changes in the eyes and adrenals of dogs treated with 150 mg/kg bw. In the eyes, marked degeneration of the lens was observed in all males and 3 females at this dose level. In addition, one of these females showed a slight retinal oedema. In the adrenals, a higher incidence of slight to minimal vacuolation of the *zona fasciculata* was evident in animals receiving 150 mg/kg bw than compared to the controls (see Table 5.3.2-6).

Table 5.3.2-6: Group incidences of relevant histopathological findings

	Dose group level [ppm]							
	Males				**Females**			
	0	**2.5**	**25**	**150**	**0**	**2.5**	**25**	**150**
Adrenals								
Vacuolation of zona fasciculate - minimal	1/4	0/4	1/4	1/4	0/4	0/4	0/3	0/4
Vacuolation of zona fasciculate - slight	0/4	1/4	1/4	3/4	0/4	0/4	1/3	3/4
Eyes								
Lenticular degeneration	0/4	0/4	0/4	4/4	0/4	0/4	0/3	3/4

A pathological re-evaluation has been conducted by an external pathologist ([redacted] BASF DocID 2015/1197310). According to this re-evaluation a slightly different grading for the findings is suggested (for details see Chapter MCA 5.8.3).

It has to be noted that in the light of the apparent neurological clinical signs, microscopic examination of brain, spinal cord and sciatic nerves did not reveal any abnormal treatment-related finding.

Conclusion:
The continuous administration of triticonazole to Beagle dogs for 52 weeks produced clear evidence of toxicity at 150 mg/kg bw/d. Although the difference did not reach statistical significance, the overall body weight gain of females receiving 25 mg/kg was also markedly lower (-25%) and considered to be of toxicological significance. The liver, the eyes and the adrenals were identified as target organs. The clinical chemistry findings (at ≥ 25 mg/kg bw) along with higher liver weight and macroscopic liver enlargement (at 150 mg/kg bw) suggest effects on this organ, but there were no histopathological findings seen at any dose level in the liver. The increased adrenal weight at 150 mg/kg bw was associated with histopathological changes in the adrenal cortex (increased cortical fatty vacuolation). In the eyes, cataractogenic effects were observed after high dose administration only.
The NOAEL of this study was set at 2.5 mg/kg bw/d during the European Peer Review based on body weight gain and clinical chemistry changes in females seen at 25 mg/kg bw.

CA 5.3.3 Other routes

Rat, 3 weeks dermal study, 0, 100, 300, and 1000 mg/kg bw/d (R012966)

Guidelines: This study was conducted according to US EPA guideline 82-2 and in compliance with OECD 410 (1981).
GLP: Yes
Deviations: Adrenals were not weighed; only a limited number of organs/tissues was examined microscopically.
Acceptance: The study was considered acceptable in the EU registration process 2003.

Material and methods:
4 Groups of 5 male and 5 female rats (strain: Crl:CD(SD)BR VAF/Plus; source: ██████ ██████) were exposed dermally to triticonazole (batch no.: OP9550347; purity 97.2%; dissolved in water) at dose levels of 0 (vehicle control), 100, 300 and 1000 mg/kg bw/d. The test article was applied 6 hours per day, on seven consecutive days per week for 3 weeks (23 days). The fur on the dorsal area of the animals was clipped to reveal an area of skin, equivalent to approximately 10% of the body surface (approx. 25 cm2). Triticonazole was applied to a gauze patch which was moistened with the appropriate amount of reverse osmosis water (approx. 2 µL/mg test material). The gauze patch was placed on the skin and secured with non-irritating tape. The application site was covered with an elastic latex bandage. At the end of each exposure period, the treated skin area was cleaned with water to remove any residual test substance.

Findings:
General observations: No treatment-related mortalities occurred at any dose level. One female given 1000 mg/kg was found dead on day 17. There was no obvious cause of death apparent at necropsy; therefore the death of this animal was not considered to be related to treatment. There were no differences in mean body weights, body weight gains and food consumption. The cutaneous application of triticonazole produced neither apparent clinical signs of systemic toxicity nor local effects on the treated skin sites. Haematology and clinical chemistry data showed no treatment-related effects at any dose level. Small but statistically significant differences for several parameters were considered incidental and unrelated to administration because they were inconsistent between the sexes and did not exhibit a relationship to dose. There were no organ weight findings, and no gross necropsy or microscopic findings indicative of an adverse effect of treatment after repeated dermal application of up to 1000 mg/kg triticonazole.

Conclusion:
Neither signs of systemic toxicity nor local treatment effects of the skin were observed in treated animals under the study conditions. The NOAEL was found to be 1000 mg/kg bw/d (the highest dose tested).

Comparison with CLH criteria

A comparison with CLH criteria is conducted using the results of the 13-week studies in rats, and mice and the two dog studies (28-day and 1 year). In 13-week study in rats, toxicological effects occur only at doses of > 1000 mg/kg bw, which is beyond a dose to be considered for STOT RE classification. The lowest dose administered in the 13-week mouse study was 2500 ppm (corresponding to 383 and 504 mg/kg bw), which is also well above the trigger values used for classification with STOT RE according to the CLP Guidance.
The effects seen in the 28-day study in Beagle dogs were reduced body weight gain, biochemical and haematological changes, lower thymus and higher liver and weights at ≥ 100 mg/kg bw, accompanied by hypertrophies and fatty vacuolations in liver at 300 mg/kg bw and histopathological findings). The continuous administration of triticonazole to Beagle dogs for 52 weeks produced clear evidence of toxicity at 150 mg/kg bw/d. At 150 mg/kg bw increased adrenal weights and increased incidences of cortical fatty vacuolations were observed. At the same dose cataractogenic effects in the eyes and increased liver weights associated with macroscopic liver enlargement were observed. At the next lower dose of 25 mg/kg bw only marginal effects on lower body weights in females and clinical chemistry findings indicative for liver toxicity. These changes are considered to be adaptative and reversible and not relevant for classification.
As potentially classification-relevant effects occur only at the dose of 150 mg/kg bw in dogs, no classification with STOT RE is warranted.

CA 5.4 Genotoxicity Testing

Studies evaluated in the draft monograph of the Rapporteur Member State Austria (2003): A sufficient data-package of *in vitro* genotoxicity studies in bacterial and mammalian cell systems and of *in vivo* genotoxicity studies conducted with triticonazole is available. These studies were performed guideline-compliant at that time and have been evaluated by European authorities and Austria as Rapporteur Member State (European Commission Peer Review Program) and were considered to be acceptable. For the convenience of the reviewer, these studies are summarized below in table form as extracted from the monograph in Table 5.4-1.

Submission of not yet peer-reviewed studies in this AIR3-Dossier: A new Ames test has been conducted (BASF DocID 2014/1192479; summarized below), as well as an Ames test with additionally spiked impurities (summarized in Doc J of this dossier).

Table 5.4-1: *In vitro* and *in vivo* mutagenicity data with triticonazole

Study type	Exposure	Result	Reference
In vitro studies			
Reverse mutation assay (S. typhimurium TA 1535, TA 1537, TA 98, TA 100)	0, 25, 79, 250, 790 and 2500 µg/plate (dissolved in DMSO)	negative (±S9)	May K. (1991) (R013016)
*Reverse mutation assay (S. typhimurium TA 98, TA 100, TA 1535 and TA 1537, and a strain of E. coli WP2 uvrA)**	*0, 33, 100, 333, 1000, 2750, 5500 µg/plate (dissolved in DMSO)*	*negative (±S9)*	*2014/1192479*
Chinese Hamster V79 cell/HGPRT locus gene mutation assay	0, 62.5, 125, 250, 500 and 1000 µg/mL (dissolved in DMSO)	negative (±S9)	Lloyd J.M. (1991) (R013019)
Chromosomal aberration assay in cultured human lymphocytes	0, 10, 20, 40, 50 and 60 µg/mL (- S9) 0, 125, 250 and 500 µg/mL (+ S9) (dissolved in DMSO)	no structural aberrations (±S9) increased polyploidy at 250 and 500 µg/mL (+S9) [questionable signif.]**	Dance C.A. (1992) (R013062)
Chromosomal aberration assay in cultured human lymphocytes	0-800 µg/L (dissolved in DMSO) evaluation performed at: (I) 274.4, 392, 560 µg/mL (±S9) (II) 337.5, 450, 600 µg/mL (+S9) 253.1, 337.5, 450 µg/mL (-S9)	negative (±S9)	Marshall R. (1997) (R012107)
Unscheduled DNA synthesis assay in rat hepatocytes	0, 7.81, 15.6, 31.3, and 62.5 µg/mL (dissolved in DMSO)	negative	Foster B. (1992) (R013061)

Study type	Exposure	Result	Reference
Literature data			
High Throughput Screen genotoxicity assays (GreenScreen, CellCiphr, CellSensor)	Up to 200 µM	negative	2009/1130462
***In vivo* studies**			
Micronucleus test in CD-1 mice	0, 25, 125, and 625 mg/kg bw (single oral dose [gavage]; dissolved in 0.5% aqueous methyl cellulose)	negative	Edwards C. (1992) (R012061)

**Study in italics is a new Ames test, which has been conducted for the Japanese registration, a robust study summary is included below*
***the polyploidies occur without a clear dose-response relationship and were also seen in one solvent control of an experiment without S-9 mix*

The summary of genotoxicity, as given in the monograph of triticonazole is still valid:
"Triticonazole was tested in *in vitro* and *in vivo* mutagenicity assays measuring different end points of potential mutagenicity such as gene mutation in bacteria and in mammalian cells, and chromosomal aberration and UDS in somatic cells. Results from these studies showed that triticonazole did not induce gene mutation in any of the bacterial tester strains of *S. typhimurium*, or gene mutation in mammalian cells in culture (CH-V79 assay). No potential for clastogenicity was observed in the *in vitro* chromosome aberration assay in human lymphocytes or in the *in vitro* UDS assay in rat hepatocytes as well.
In the *in vivo* mouse micronucleus assay, a clear negative result was obtained. Therefore, it can be concluded that triticonazole has no genotoxic potential of relevance to human risk assessment."

Thus, the conclusion for relevant endpoints for the current re-registration remains as follows:

Genotoxicity

In vitro studies	No evidence of genotoxic potential
In vivo studies in somatic cells	No evidence of genotoxic potential
In vivo studies in germ cells	No evidence of genotoxic potential

And is summarized in the following way in DocN2 of this Dossier:

Genotoxicity

In vitro studies	Two Ames tests are available in TA 1535, 1537, 98, 100 and a strain of E. coli WP2 uvrA): Negative (±S9) HGPRT assay in V79 cells: Negative (±S9) Two Chromosome aberration in cultured human lymphocytes available: Negative (±S9) Unscheduled DNA synthesis assay in rat hepatocytes: Negative	
In vivo studies	Micronucleus test in CD-1 mice: Negative	
Photomutagenicity	Not conducted, as no validated test method or guidance document is available.	
Potential for genotoxicity	Triticonazole is unlikely to be genotoxic	

CA 5.4.1 *In vitro* studies

In vitro mutagenicity in bacteria (1991, May, R013016)

Guidelines: According to OECD 471 (1983) and US EPA guideline 798.5265 (1985)
Deviations: The stability of the test compound in the solvent was not determined. No statistical analysis of the results was performed. Neither S. typhimurium TA 102 nor E. coli strains were used.
GLP: Yes
Acceptance: The study was considered acceptable in the EU registration process 2003.

Material and method:
Triticonazole (batch no. DA 646; purity 97.1%) was tested in the Ames test using histidine dependent auxotrophic mutants of Salmonella typhimurium (strains TA 1535, TA 1537, TA 98 and TA 100). Triticonazole (dissolved in DMSO) was added at concentrations of 0 (solvent control), 25, 79, 250, 790 and 2500 µg/plate (0.1 mL of each concentration/plate) in the presence and absence of S-9 mix (liver preparations from Aroclor 1254-induced male Sprague-Dawley rats).

Findings:
In both trials, cytotoxicity (thinning of the background lawn of non-revertant cells and reduction of revertant colony numbers) was observed in all strains at 2500 µg/plate with and without S-9 mix. However, no increases in revertant colony numbers over control counts were obtained with any of the strains tested following treatment with triticonazole at any concentration, either in the presence and absence of metabolic activation. The positive control materials elicited the expected positive responses.

Conclusion:
It can be concluded that triticonazole was not mutagenic when tested at dose levels up to 2500 µg/plate in this *in vitro* test system.

In vitro mutagenicity in mammalian cells (1991, Lloyd, R013019)

Guidelines: According to OECD 471 (1983) and US EPA guideline 798.5265 (1985)
Deviations: The stability of the test compound in the solvent was not determined. No statistical analysis of the results was performed. Neither S. typhimurium TA 102 nor E. coli strains were used.
GLP: Yes
Acceptance: The study was considered acceptable in the EU registration process 2003.

Material and method:
Triticonazole (batch no. DA 646; purity 97.1 %; dissolved in DMSO) was tested for its ability to induce point mutations at the *HGPRT*-locus in Chinese Hamster V79 cells (V79-4/I clone 9 3/12) obtained from Shell Research Ltd. England). The V79 cells (7.5 x 105 cells per flask) were exposed to triticonazole for a period of 3 hours at concentrations of 0, 62.5, 125, 250, 500 and 1000 µg/mL without and with metabolic activation (1.5 mL liver S-9 homogenate fraction from Aroclor 1254 induced male CD rats) in two replicate cultures.

Findings:
In the preliminary cytotoxicity test, there was no evidence of dose-related cytotoxicity observed at any concentration of triticonazole tested in either activated or non-activated cultures. However, precipitation of the test material was observed at 700 µg/mL, and there was extensive precipitation at 3500 µg/mL, forming aggregates of the test material and so dose levels of 62.5 – 1000 µg/mL were selected for the mutagenicity experiment.
Also in the main mutation assay, precipitation of the test material was noted at concentrations of 500 and 1000 µg triticonazole/mL, both with and without S-9 mix. There was no indication of dose-related cytotoxicity (assessed by plating efficiency) immediately following treatment with triticonazole, and no obvious effect following the expression period at any dose level, neither in the absence nor in the presence of S-9 mix in either of the two experiments.
Exposure of V79 cells to triticonazole up to 1000 µg/mL produced no real increase in 6-TGr colony numbers or in mutant frequencies in either of the two mutation assays, compared to the solvent control. Isolated small increases in mutation frequencies in some cultures were noted: In the first assay without S-9 mix, an increased mutation frequency was observed in cultures at 250 µg/mL compared to solvent control but this effect was neither reproduced in the replicate of this experiment nor in the second experiment. In the mutation assays with S-9 mix, very small increases in mutation frequencies were observed in cultures exposed to concentrations of 62.5 and 125 µg/mL (first test) and in cultures treated with 250 and 500 µg/mL (second test), but no increases were seen in the respective replicate cultures. A marked positive response was seen with the positive controls EMS and DMBA.

Conclusion:
It was concluded that, under the conditions of this test, triticonazole showed no evidence of mutagenic activity at the HGPRT gene locus, when V79 cells were treated in the absence or presence of S-9 mix.

In vitro clastogenicity in human lymphocytes (1992, Dance, R013062)

Guidelines: According to OECD 473 (1983)
Deviations: Only one sampling time was included; the number of metaphases scored is in excess of that required by the mentioned guideline.
GLP: Yes
Acceptance: The study was considered acceptable in the EU registration process 2003.

Material and method:
Cultures of human lymphocytes from a single male donor were exposed to triticonazole (batch no DA646; purity 97.1 %, dissolved in DMSO) without and with metabolic activation (liver preparations from Aroclor 1254-induced male CD rats) and the lymphocytes examined for chromosomal damage. The concentrations used in the main cytogenetic study (0, 10, 20, 40, 50 and 60 µg/mL without S-9, and 0, 125, 250 and 500 µg/mL with S-9) were based on a preliminary toxicity test with concentrations of 62.5, 125, 250, 500 and 1000 µg/mL tested.
In the cytogenetic study, triplicate cultures per concentrate were incubated either for 24 hours (without S-9) or 3 hours (with S-9) followed by a recovery period of 21 hours. In each case, colcemid was added (0.5 µg/mL) 3 hours before the end of the incubation. As positive control substances, chlorambucil (2 µg/mL) and cyclophosphamide (6 µg/mL) were used for the non-activation test and for metabolic activation test, respectively.

Findings:
In the preliminary cytotoxicity test, precipitation of the test material was apparent in cultures treated at 500 and 1000 µg/mL. In the presence of S-9 mix, no significant toxicity was seen in all cultures treated with triticonazole at concentrations < 250 µg/mL; reductions in mitotic activity of approx. 25 % and 37 % were evident at 500 and 1000 µg/mL, resp., compared to solvent control. In the absence of S-9 mix, marked cytotoxicity was apparent at all test concentrations with reductions in mitotic activity of approx. 81 %, 96 %, 72 %, 82 % and 80 % at 62.5, 125, 250, 500 and 1000 µg/mL, respectively.
In the cytogenetic study, precipitation of test material was apparent in cultures treated with triticonazole at 500 µg/mL. In the absence of S-9 mix, a dose-related cytotoxicity was apparent at the higher concentrations tested (40, 50 and 60 µg/mL) with reductions in mitotic activity (compared to solvent control) of 14 %, 27 % and 50 % respectively No toxicity was evident at the lower concentrations; therefore slides from these cultures were not analysed for chromosomal damage. In the presence of S-9 mix, there was no evidence of toxicity at any concentration tested. Metaphase analysis revealed no biologically or statistically significant increases (p>0.05) in the frequency of cells with structural aberrations including or excluding gaps in cultures treated with triticonazole with and without metabolic activation. Clear increases of chromosome aberrations were apparent in both positive controls demonstrating the sensitivity of the test system.

Incidence of polyploid cells: No increase in the incidence of polyploid cells were observed in cultures treated with triticonazole in the absence of S-9 mix. In the presence of S-9 mix, some increase in the number of polyploid cells (outside the historical control range of 0 – 3 per culture) was noted in two cultures treated with 250 µg/mL and, to a lesser extent, in one culture at 500 µg/mL. However, also in one solvent control culture (without S-9 mix), a higher incidence of polyploid cells than expected was noted. It was suggested by the study author that the increased incidence of polyploidy might be indicative of an effect on the spindle formation or function or may be due to a non-specific effect associated with precipitation of the test compound. However, it has to be noted that there was no clear dose-response in the absence of any effect on the mitotic index seen in this study. Also, no such effect on numerical aberrations was evident in the more recent chromosomal aberration study in human lymphocytes (Marshall R.; 1997, BASF DocID R012107) with a comparable concentration range tested (the study described below). Therefore, the biological significance of this finding in a single experiment can be questioned.

Conclusion:
It was concluded that triticonazole, under the conditions of the test, caused no increase in the incidence of structural chromosomal aberrations. The apparent increase in polyploidy cells, without a clear dose-response relationship and also occurring in one solvent control culture cannot be considered of real biological significance.

In vitro clastogenicity in human lymphocytes (1997, Marshall, R012107)

Guidelines: According to OECD 473 (1983) and US EPA guideline 84-2
Deviations: No deviations mentioned.
GLP: Yes
Acceptance: The study was considered acceptable in the EU registration process 2003.

Material and method:
Cultures of human lymphocytes (stimulated to divide by treatment with phytohaemagglutinin) from a male [first experiment] and a female donor [second experiment] were exposed to triticonazole (batch no. OP9550347; purity: 90.9 %; dissolved in DMSO) with and without metabolic activation (liver preparations from Aroclor 1254-induced male Sprague-Dawley rats) in two independent experiments at concentrations of 0 (solvent control), 7.751, 11.07, 15.82, 22.6, 32.28, 46.12, 65.88, 94.12, 134.5, 192.1, 274.4, 392.0, 560 and 800 µg/mL (limit of solubility in the vehicle) [first experiment] and 0 (solvent control), 33.79, 45.05, 60.07, 80.09, 106.8, 142.4, 189.8, 253.1, 337.5, 450, 600 and 800 µg/mL [second experiment]. All samples were run in duplicate (A, B) for each concentration tested. In the first experiment the cells were exposed to triticonazole for 20 h (-S9) or 3 h (+S9). In the second experiment an additional delayed sampling time was performed (44 h; ±S9).

Findings:
Based on the evaluation of the mitotic index, concentrations of 274.4, 392 and 560 µg/mL (mitotic inhibition 51 % [- S-9] and 56 % [+ S-9] at 560 µg/mL) were used in the first experiment for analysis of chromosomal aberrations. In the second experiment, the selected concentrations for analysis were 253.1, 337.5 and 450 µg/mL (mitotic inhibition 50% [- S-9] at 450 µg/mL) and 337.5, 450 and 600 µg/mL (mitotic inhibition 49 % [+ S-9] at 600 µg/mL), respectively. In experiment 1, treatment of cells with triticonazole in the absence of S-9 resulted in some cultures in statistically significant increased frequencies of cells with structural aberrations. However, the effect seen in one replicate of the intermediate (392 µg/mL) and high concentration level (560 µg/mL) was neither reproduced in both replicates of this experiment, nor in the second experiment, and therefore not considered to be of relevance.
In the second experiment, a total of only 137 cells could be analysed from cultures treated with 337.5 µg/mL in the presence of S-9 and sampled at 20 hours. Since the frequency of aberrations in the cells examined was very low, and no evidence of an increase in aberrant cells was apparent at higher concentrations, this deficiency was not considered to have affected the validity at this dose level. The only finding in this experiment was a small but statistically significant increase in cells with structural aberrations (without S-9) at the 44 hour sampling, but it was stated in the report that the frequency of aberrant cells fell within the historical negative control ranges.
Concerning numerical aberrations (including endo-reduplication, hyperploidy and polyploidy), the frequencies of cells with aberrations seen in all test-article-treated cultures fell within the historical negative control ranges.
In all negative controls, chromosome aberration frequencies were within historical normal ranges while they were significantly increased in both positive control cultures.

Conclusion:
Following two independent experiments that were performed in the absence and presence of a rat liver metabolic activation system, triticonazole did not induce structural or numerical chromosomal aberrations in cultured human lymphocytes when tested at dose levels up to toxic concentrations.

In vitro DNA repair in rat hepatocytes (1992, Foster, R013061)

Guidelines: In compliance with EC B.18 (67/548/EC)
Deviations: No deviations mentioned.
GLP: Yes
Acceptance: The study was considered acceptable in the EU registration process 2003.

Material and method:
Hepatocytes were isolated from male Sprague Dawley CD rats (source: Charles River UK Ltd.) and cultured for a preliminary toxicity test using concentrations of 0 (solvent control), 62.5, 125, 250, 500 and 1000µg/mL triticonazole (batch no. DA646; purity 97.1 %; dissolved in DMSO). Cultures were incubated at 37°C for 18 hours and the toxicity of the test material was then evaluated using the Neutral Red Test.
In the main UDS assay, triplicate cultures per concentration were exposed to the test substance at concentrations of 0 (solvent control), 7.81, 15.6, 31.3, 62.5 and 125 µg/mL, and [3H]-labelled thymidine (10 µCi/mL) for 18 hours, washed and then incubated for a further 24 hours before fixing. A second set of cultures were treated identically but without [3H] thymidine to determine viability of the cells. Two slides per concentration from cultures with [3H] thymidine treatment were investigated for UDS by autoradiography, with 50 non-S-phase cells per slide being scored for nuclear and cytoplasmic grain count. As positive control, 2-Acetylaminofluorene (2.2 µg/mL) was used.

Findings:
In the preliminary toxicity test, precipitation was noted at concentrations of > 250 µg/mL. In addition, assessment using the Neutral Red Test revealed that hepatocytes treated with 125 and 250 µg/mL showed a marked reduced viability (36.1 % and 37 %, respectively) compared with the solvent control (100 %). Cultures treated with 62.5 µg/mL showed a slight reduced viability of 90.7 %.
In the main assay, hepatocyte viability was slightly reduced at concentrations < 62.5 µg/mL compared with solvent control as determined by the Neutral Red Test. However, the cells appeared healthy when viewed microscopically. At 125 µg/mL a marked decrease in viability was seen (59.3 %). The nuclear and cytoplasmic grain counts of triticonazole treated cultures did not differ significantly from the solvent controls. The net nuclear grain counts from cultures treated at < 62.5 µg/mL were also similar to those in negative controls. The only evidence for an increase in mean net nuclear grain count was at the toxic concentration of 125 µg/mL, but this was only slight compared with vehicle control, and there was no increase in the number of cells in repair. A clear significant increase in NNG count was seen with the positive control substance 2-AAF.

Conclusion:
It was concluded that under the conditions of this test, there was no evidence of DNA damage leading to UDS in rat hepatocytes treated *in vitro* with triticonazole.

New *in vitro* mutagenicity studies conducted with triticonazole:
A new Ames test has been conducted with triticonazole TGAI and is summarized in more detail below. One further Ames test has been conducted with triticonazole TGAI including some spiked impurities. This test is summarized in Doc J of this dossier.

Report:	CA 5.4.1/1 Woitkowiak C., 2014b BAS 595 F (Triticonazole) - Salmonella typhimurium / Escherichia coli reverse mutation assay 2014/1192479
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 TA 98, TA 100, TA 1535 and TA 1537, and a strain of *E. coli* WP2 uvrA were exposed with Triticonazole (Batch: COD-001440, Purity: 91.3%) in the presence and absence of metabolic activation for 48 hours. Vehicle (DMSO) and positive controls were included in each experiment. In the Ames standard plate test (SPT) and the pre-incubation test (PIT), the test item was tested in six concentrations in a range of 33 to 5500 µg/plate with and without S9 mix (phenobarbital/ß-naphthoflavone-induced rat liver S9 fraction).

Precipitation of the test substance was found from about 1000 µg/plate onward with and without S9 mix. In SPT, a weak bacteriotoxic effect was occasionally observed at 2750 and 5500 µg/plate with and without S9, respectively. In PIT, bacteriotoxicity was observed depending on the strain and test conditions from about 333 µg/plate onward.
An increase in the number of his^+ and try^+ revertants was not observed in SPT and PIT either without S9 mix or after the addition of a metabolizing system. The positive controls induced the appropriate response in the corresponding strains, thus demonstrating the sensitivity of the test system. The vehicle control induced number of revertants that was within the range of historical control data for each strain.

Based on the results of the present study, the test substance BAS 595 F (Triticonazole) is not mutagenic in the Ames standard plate test and pre-incubation test under the experimental conditions chosen.

(BASF DocID 2014/1192479)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material BAS 595 F (Triticonazole)

Description:	solid / white
Lot/Batch #:	COD-001440
Purity:	91.3% (tolerance +/-1.0%)
Stability of test compound:	The stability of the test substance is guaranteed until 01-Jan-2019 as indicated by the sponsor.
Solvent used:	DMSO

2. Control Materials:

Negative control:	sterility controls were performed in accordance with the experimental design, but without addition of bacterial suspension.
Vehicle control:	The vehicle control with and without S9 mix only contained the vehicle used for the test substance at the same concentration and volume for all tester strains.
Solvent/final concentration:	100 µL/plate

Positive control compounds tested without addition of metabolic activation system:

Strain	Mutagen	Solvent	Conc. [µg/plate]
TA 100	N-methyl-N'-nitroso-guanidine (MNNG)	DMSO	5
TA 1535	N-methyl-N'-nitroso-guanidine (MNNG)	DMSO	5
TA 1537	9-Aminoacridine (AAC)	DMSO	100
TA 98	4-nitro-o-phenylene-diamine (NOPD)	DMSO	10
WP2 uvrA	4-Nitroguinoline-N-oxide (4-NQO)	DMSO	5

Positive control compounds tested with addition of rat metabolic activation system:

Strain	Mutagen	Solvent	Conc. [µg/plate]
TA 100	2-Aminoanthracene	DMSO	2.5
TA 1535	2-Aminoanthracene	DMSO	2.5
TA 1537	2-Aminoanthracene	DMSO	2.5
TA 98	2-Aminoanthracene	DMSO	2.5
WP2 uvrA	2-Aminoanthracene	DMSO	60

3. Activation:

S9 was produced from the livers of approximately 5 male Wistar rats (Crl:WI(Han); source: [redacted]), weighting 200 – 300 g, that received 80 mg/kg bw phenobarbital i. p. and 80 mg/kg bw ß-naphthoflavone orally on 3 consecutive days.

The rat liver S9-mix was prepared freshly prior each experiment 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%

4. Test organisms:

S. typhimurium strains: TA 98, TA 100, TA 1535, TA 1537
E. coli strains: WP2 uvrA
The bacterial strains are checked for the following characteristics at regular intervals: deep rough character (rfa), ampicillin resistance (R factor plasmid), and UV-light sensitivity (absence of uvrB and uvrA genes in Salmonella and E. coli strains, respectively).

Histidine and tryptophan auxotrophy was automatically proven in each experiment via the spontaneous rate.

5. Test concentrations:

SPT/PIT: TA 98, TA 100, TA 1535, TA 1537 and WP2 uvrA were exposed to dose levels of 0, 33, 100, 333, 1000, 2750 and 5500 µg/plate with and without metabolic activation.

B. TEST PERFORMANCE:

1. Dates of experimental work: 22-Jul – 01-Aug-2014,
Finalization date: 20-Oct-2014

2. Standard plate test (SPT):

A mixture of 2-mL portions of warm (42 - 45°C) soft agar (0.8% agar and 0.6% NaCl, supplemented with 10% of 0.5 mM L-histidine + 0.5 mM (+)-biotin or 0.5 mM L-tryptophan), 0.1 mL test substance preparation or vehicle, 0.1 mL fresh bacterial culture, 0.5 mL S9 mix (in tests with metabolic activation) or 0.5 mL of 100 mM sodium phosphate buffer (in tests without metabolic activation) was poured onto minimal agar in Petri dishes. Each Petri dish contained about 20 mL minimal agar (1.5% agar supplemented with 2.0% salts of Vogel-Bonner Medium E and 2.0% glucose). Each concentration and the controls were tested in triplicates. After incubation at 37°C for 48 - 72 hours in the dark, the bacterial colonies (his^+ or try^+ revertants) were counted using the Sorcerer Image Analysis System with the software program Ames Study Manager (Perseptive Instruments Ltd., Haverhill, UK). Colonies were counted manually, if precipitation of the test substance hindered the counting using the Image Analysis System.

3. Pre-incubation test (PIT):

0.1 mL test substance preparation or vehicle, 0.1 mL bacterial suspension and 0.5 mL S9 mix (in tests with metabolic activation) or 0.5 mL phosphate buffer (in tests without metabolic activation) were incubated at 37°C for 20 minutes using a shaker. Subsequently, 2 mL soft agar was added and the mixtures were poured onto the agar plates within approximately 30 seconds. Each concentration and the controls were tested in triplicates. After incubation at 37°C for 48 - 72 hours in the dark, the bacterial colonies (his^+ or try^+ revertants) were counted using the Sorcerer Image Analysis System with the software program Ames Study Manager (Perseptive 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:

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 109 cells per mL were used.

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 number 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. ANALYTICAL DETERMINATIONS

Homogeneity of the test substance preparations at room temperature was verified analytically for a period of 4 hours and was ensured by mixing before generation of the test substance preparations and application.

B. TOXICITY

In SPT, a weak bacteriotoxic effect (slight decrease in the number of his^+ revertants) was occasionally observed at 2750 and 5500 µg/plate with and without S9, respectively.
In PIT, bacteriotoxicity (reduced his^- or trp^- background growth, slight decrease in the number of his^+ and trp^+ revertants) was observed depending on the strain and test conditions from about 333 µg/plate onward.

C. SOLUBILITY

Test substance precipitation was observed from about 1000 µg/plate onward with and without S9 mix.

D. MUTATION ASSAYS

In the SPT and PIT experiments with and without metabolic activation no relevant increase in number of revertants was observed in any strain tested [see Table 5.4.1-1]. The positive controls yielded revertant numbers in a range expected for the respective strains and thus demonstrated the sensitivity of the test system. The vehicle control induced number of revertants that was within the historical data range of each strain.

Table 5.4.1-1: Standard plate and pre-incubation tests with BAS 595 F (Triticonazole) - Mean number of revertants

Experiment 1: Standard plate test										
Strain	**TA 100**		**TA 1535**		**TA 1537**		**TA 98**		**WP2 uvrA**	
Metabol. Activation	**-S9**	**+S9**	**-S9**	**+S9**	**-S9**	**+S9**	**-S9**	**+S9**	**-S9**	**+S9**
Vehicle control										
DMSO	58.0	60.7	12.0	11.7	5.0	8.0	20.0	28.7	51.7	64.7
SD	7.9	8.1	1.0	2.5	3.6	3.5	4.4	4.0	5.5	4.2
Test item [µg/plate]										
33	61.3	54.3	9.3	7.7	3.7	5.3	17.3	25.0	54.0	70.7
SD	8.0	9.3	1.2	1.5	1.2	3.1	4.2	6.2	7.2	1.5
100	70.7	62.0	11.3	7.7	6.0	5.0	14.7	29.3	52.3	66.0
SD	4.2	5.6	1.2	2.3	2.6	1.0	2.1	3.5	13.1	3.0
333	62.3	55.7	12.7	7.0	6.7	5.0	15.7	23.7	54.0	74.0
SD	6.0	14.8	3.8	1.7	0.6	3.6	1.5	5.7	6.2	8.5
1000	70.3	55.3	13.0	12.3	5.7	6.7	17.3	23.7	43.0	72.3
SD	14.8	1.5	3.5	3.1	1.5	2.9	2.3	5.5	2.0	8.0
2750	59.0	49.7	10.0	7.3	4.7	6.7	15.7	21.0	49.7	47.0
SD	1.0	13.0	3.0	1.5	0.6	1.2	2.5	3.6	1.5	36.9
5500	52.3	62.3	10.3	10.0	5.0	4.7	14.3	17.3	53.0	56.7
SD	8.1	2.5	1.5	2.0	1.0	0.6	2.5	1.5	4.6	1.5
Pos. control										
§	5178.0	1568.3	5894.0	203.7	2413.7	185.3	393.7	1622.0	925.3	157.0
SD	257.6	92.4	64.2	5.0	296.4	37.8	44.2	41.3	11.5	9.0
Experiment 2: Pre-incubation test										
Strain	**TA 100**		**TA 1535**		**TA 1537**		**TA 98**		**WP2 uvrA**	
Metabol. activation	**-S9**	**+S9**	**-S9**	**+S9**	**-S9**	**+S9**	**-S9**	**+S9**	**-S9**	**+S9**
Vehicle control										
DMSO	63.0	65.3	9.3	8.3	7.3	8.3	18.3	29.7	44.0	47.7
SD	12.5	4.5	1.2	1.5	3.5	0.6	3.1	2.5	6.0	5.5
Test item										
33	57.0	59.7	9.3	7.3	8.3	7.3	19.0	22.0	33.0	50.7
SD	1.7	2.3	3.2	1.2	3.5	2.5	5.3	5.0	5.3	9.5
100	62.0	54.3	10.0	7.0	6.7	9.0	24.3	25.3	34.7	42.3
SD	4.4	7.6	4.4	1.0	3.2	2.6	4.5	7.8	5.0	9.5
333	68.7	55.3	11.3	10.7	3.3	6.7	24.0	24.0	30.0	47.3
SD	6.4	8.1	4.0	0.6	1.2	1.5	4.4	2.6	9.8	4.5
1000	49.0	65.0	7.3	8.7	4.7	5.0	17.0	23.7	28.7	35.3
SD	7.0	3.0	3.1	2.1	1.2	1.0	3.6	6.0	2.5	3.8
2750	35.0	51.3	8.0	7.7	3.7	2.7	13.0	22.7	23.7	41.0
SD	4.4	7.0	2.0	0.6	1.5	1.2	4.4	4.2	4.7	1.7
5500	38.3	35.0	7.3	3.7	3.0	2.3	14.0	18.7	27.7	32.3
SD	7.1	9.0	1.5	1.5	1.0	1.5	3.0	1.5	7.1	5.5
Pos. control										
§	2930.0	2091.7	962.3	138.0	1701.0	151.0	399.3	1387.3	380.3	128.7
SD	88.1	108.5	226.6	25.9	113.1	10.4	9.1	70.8	73.6	21.2

§ = Compound and concentrations see Material and Methods (I.A.2.) above

III. CONCLUSION

Based on the results of the present study, the test substance BAS 595 F (Triticonazole) is not mutagenic in the Ames standard plate test and pre-incubation test under the experimental conditions chosen.

Additional literature data

Report: CA 5.4.1/2
Knight A.W. et al., 2009a
Evaluation of high-throughput genotoxicity assays used in profiling the US EPA ToxCast chemicals
2009/1130462
Guidelines: none
GLP: no

Executive Summary of the Literature

Three high-throughput screening (HTS) genotoxicity assays-GreenScreen HC GADD45a-GFP (Gentronix Ltd.), CellCiphr p53 (Cellumen Inc.) and CellSensor p53RE-bla (Invitrogen Corp.) were used to analyze the collection of 320 predominantly pesticide active compounds being tested in Phase I of US. Environmental Protection Agency's ToxCast research project. Between 9% and 12% of compounds were positive for genotoxicity in the assays. However, results of the varied tests only partially overlapped, suggesting a strategy of combining data from a battery of assays. The HTS results were compared to mutagenicity (Ames) and animal tumorigenicity data. Overall, the HTS assays demonstrated low sensitivity for rodent tumorigens, likely due to: screening at a low concentration, coverage of selected genotoxic mechanisms, lack of metabolic activation and difficulty detecting non-genotoxic carcinogens. Conversely, HTS results demonstrated high specificity, >88%. Overall concordance of the HTS assays with tumorigenicity data was low, around 50% for all tumorigens, but increased to 74-78% (vs. 60% for Ames) for those compounds producing tumors in rodents at multiple sites and, thus, more likely genotoxic carcinogens. The aim of the present study was to evaluate the utility of HTS assays to identify potential genotoxicity hazard in the larger context of the ToxCast program, to aid prioritization of environmentally relevant chemicals for further testing and assessment of carcinogenicity risk to humans.

For triticonazole negative results were observed in all three screening assays, indicating that triticonazole has no genotoxic properties supporting available *in vitro* and *in vivo* data.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: Triticonazole
Description: not specified
Batch/purity #: not specified
Stability of test compound: not specified

2. Control Materials:
Vehicle control: GreenScreen HC GADD45a-GFP: DMSO
CellCiphr p53: DMSO
CellSensor p53 RE-bla: DMSO
Positive control: GreenScreen HC GADD45a-GFP: not specified
CellCiphr p53: mentioned, but not specified
CellSensor p53 RE-bla: Nultilin-3 (12 µM)

3. Test Organisms: GreenScreen HC GADD45a-GFP: human lymphoblastoid TK6 cell line (GADD45a-GFP reporter strain and out-of-frame EGFP gen control strain)
CellCiphr p53: human HepG2 cell line
CellSensor p53 RE-bla: HCT-116 cell line

4. Test Concentrations: GreenScreen HC GADD45a-GFP: 50, 100 and 200 µM
CellCiphr p53: 0.39 – 200 µM
CellSensor p53 RE-bla: 1.2 nM – 92 µM

B. TEST PERFORMANCE:

1. Cytotoxicity assay:

GreenScreen HC GADD45a-GFP:
Inhibition of cell proliferation was detected by reduction of optical absorbance.

CellCiphr p53:
Cell loss was recorded by measuring the cell counts after Hoechst 3342 staining with Arrayscan HCS Reader, and IC_{50} values were calculated.

CellSensor p53 RE-bla:
not specified

2. Genotoxicity assay:

GreenScreen HC GADD45a-GFP:
Human *GADD45a* mediated growth arrest and DNA damage was recorded by p53 regulated induction of GFP protein via fluorescence. A control strain containing an out-of-frame EGFP gene with non-functional GFT protein was used for corrections of auto-fluorescence or non-specific induced cellular fluorescence.

CellCiphr p53:
DNA damage was recorded by measurement of p53 activation via a fluorescent anti-p53 antibody (Alexa Fluor 488). Half-maximal activity (AC_{50}) values were determined by fitting the data to the Hill equation using the Condoseo module of Genedata Screener (Genedata AG, Basel, Switzerland).

CellSensor p53 RE-bla:
Activation of the p53 controlled beta-lactamase were recorded via proprietary "GeneBLAzer" technology based on fluorescence resonance energy transfer (FRET). Data were expressed as the ratio of emissions at 460 nm/530 nm (excitation at 405 nm). For primary data analysis, readings for each titration point were first normalized relative to the Nultilin-3 control (12 µM, 100%) and wells containing the vehicle only (basal, 0%), and then corrected by applying a pattern correction algorithm using control plates containing the DMSO diluent alone. Concentration–response titration points for each compound were fitted to the Hill equation and concentrations of half-maximal activity (AC_{50}) and maximal response (efficacy) values were calculated.

3. Evaluation criteria:

GreenScreen HC GADD45a-GFP:
If the cell density relative to a vehicle-treated control fell below 80% at 1 test concentration the compound was deemed cytotoxic and if extended over 2 or 3 concentrations, strongly cytotoxic. Otherwise the compound was considered negative for cytotoxicity.
If induction of GFP fluorescence relative to a vehicle-treated control exceeded 50% at 1 test concentration the compound was deemed genotoxic and if extended over 2 or 3 concentrations, strongly genotoxic. Otherwise the compound was considered negative for genotoxicity.

CellCiphr p53:
A positive result was concluded if the p53 AC_{50} was calculated to be below 200 µM, provided the AC_{50} was lower than the IC_{50} for cell loss/cytotoxicity at for that time point.

CellSensor p53 RE-bla:
A positive result was concluded if the p53 AC_{50} was calculated to be below 92 µM.

II. RESULTS AND DISCUSSION

Negative result were obtained with triticonazole in the GreenScreen HC GADD45a-GFP, CellCiphr p53 and CellSensor p53RE-bla assays tested up to 200 µM.

III. CONCLUSION

According to the results of the present study, the test substance triticonazole is not mutagenic in the GreenScreen HC GADD45a-GFP, CellCiphr p53 and CellSensor p53RE-bla assays under the experimental conditions chosen here.

CA 5.4.2 *In vivo* studies in somatic cells

In vivo clastogenicity in bone marrow erythrocytes in mice (1992, [redacted]

Guidelines: According to OECD 474 (1982) and EEC B.12 (92/69/EEC)
Deviations: No deviations mentioned.
GLP: Yes
Acceptance: The study was considered acceptable in the EU registration process 2003.

Material and method:
Triticonazole (batch no. DA 646, purity 97.1 %, suspended in 0.5 % methyl cellulose in distilled water) was administered to groups of male and female CD-1 mice (source: [redacted]) by gastric intubation: Single treatments have been performed at doses of 0 (vehicle control), 25, 125 and 625 mg/kg bw. These dose levels were based on a preliminary study using dose levels of 625, 1250, 2500 and 5000 mg/kg bw in order to estimate the maximum tolerated dose (including histological evaluation of bone marrow smear).
In the main study, 5 male and 5 female mice from all dose levels were killed 24 hours after administration of the test substance, and further groups of 10 mice each having received 0 and 625 mg/kg bw, respectively, were killed after 48 and 72 hours, respectively From each mouse, bone marrow smears from the femurs were obtained, and the frequencies of micronucleated cells per 2000 erythrocytes were scored. Calculated values of micronuclei per 1000 polychromatic erythrocytes were analysed statistically (Mann-Whitney U-test). The ratio of polychromatic/mature cells was also calculated for each animal. The positive control group (5 males and 5 females) received a single oral dose of chlorambucil (30 mg/kg bw) and bone marrow smears were taken 24 hours after dosing

Findings:
In the preliminary test, all animals treated at > 1250 mg/kg bw were sacrificed in extremis between 1 and 2 hours after dosing. Mice dosed at 625 mg/kg showed transient signs like hunched posture, hyperactivity and piloerection. In addition, mice treated at this dose level lost weight during the 24 hours post dosing but had regained the weight loss within 48 or 72 hours. Histological evaluation of the bone marrow smear did not show evidence of changes in the ratio of polychromatic/mature erythrocytes.
In the main study, all mice treated at 125 and 625 mg/kg bw showed transient over-activity after dosing. In addition, some mice treated at 625 mg/kg showed transient piloerection and hunched posture. There was also a slight body weight loss in six of 10 mice treated at 625 mg/kg and sacrificed after 24 hours and in nine of the ten mice given chlorambucil. No signs were seen in the vehicle control groups.
Histological investigations did not show any significant differences ($p > 0.05$) in the frequencies of micronucleated polychromatic cell in any dose group treated with triticonazole and at any of the three time points investigated. Group mean values were closely similar to mean control group values at all termination times. There was also no effect on the ratio polychromatic/mature erythrocytes. In mice given the positive control, a clear increase in the incidence of micronucleated polychromatic erythrocytes was seen.

Conclusion:
It can be concluded that, under the conditions of this test, triticonazole did not induce chromosomal damage leading to micronucleus formation in polychromatic erythrocytes of treated mice at doses up to 625 mg/kg bw.

CA 5.4.3 *In vivo* studies in germ cells

No *in vivo* studies in germ cells were performed with triticonazole.

Photomutagenicity

According to the Commission Regulation (EU) No 283/2013 of 1 March 2013 (data requirements for active substances, in accordance with Regulation (EC) No 1107/2009 a “special testing requirement in relation to photomutagenicity may be indicated by the structure of a molecule. If the Ultraviolet/visible molar extinction/absorption coefficient of the active substance and its major metabolites is less than 1000 L x mol^{-1} x cm^{-1}, photomutagenicity testing is not required.”

Although the UV/VIS molar extinction/absorption coefficient of triticonazole is less than 1000 L x mol^{-1} x cm^{-1}, no photomutagenicity has been conducted, as no validated test methods or guidance documents are available for this endpoint. This waiving argumentation is based on SANCO Guidance document (SANCO/10181/2013-rev. 2.1 (13 May 2013).

CA 5.5 Long-Term Toxicity and Carcinogenicity

Studies evaluated in the draft monograph of the Rapporteur Member State Austria of Sept. 2005:

Studies evaluated in the triticonazole (BAS 595 F) draft monograph of Rapporteur Member State Austria of Sept. 2005 consisted of one chronic toxicity/oncogenicity study in rats and one carcinogenicity study in mice. These studies have been evaluated by European Authorities and Austria as the Rapporteur Member State and were considered to be acceptable. For the convenience of the reviewer, these studies are summarized below as extracted from the monograph. The results are summarized in Table 5.5-1.

Study	Dosages (mg/kg bw/day)	NOAEL (mg/kg bw/day)	Main adverse effect	Reference and year
Chronic toxicity/oncogenicity study in CD-rats (dietary administration of 0, 5, 25, 750,5000 ppm for 99/100 weeks)	Males: 0, 0.2, 1.0, 29.4, 203.6 mg/kg bw/d Females: 0, 0.3, 1.3, 38.3, 286.6 mg/kg bw/d	NOAEL (males): 29.4 mg/kg bw/d NOAEL (females): 38.3 mg/kg bw/d	– retarded body weight gain – haematological and clinical chemistry findings – histological changes in adrenals (degeneration)* and liver (fatty vacuolation) <u>no oncogenic potential</u>	(R013100)
Oncogenicity study in CD-1 mice (dietary administration of 0, 15, 150, 1500 ppm for 78 weeks)	Males: 0, 1.8, 17.4, 202.2 mg/kg bw/d Females: 0, 2.1, 20.1, 209.5 mg/kg bw/d	NOAEL (males): 17.4 mg/kg bw/d NOAEL (females): 20.1 mg/kg bw/d	– retarded body weight gain – increased liver and adrenal weights** – histological changes in liver and lymph nodes*** <u>no oncogenic potential</u>	(R013143)

*the degenerative effects (increased incidences of multinucleated cells) decreased in severity with increasing exposure duration, showing hardly any treatment-relationship at the terminal sacrifice time points
**relative adrenal weight increases were only seen at the interim sacrifice not at the terminal sacrifice and were not accompanied by a histopathological correlate
***the findings in the lymph nodes were not statistically significant, when all animals (scheduled and earlier sacrifices) are included in the calculation, thus a treatment-relationship is doubtful

In a 2-year combined chronic toxicity/carcinogenicity study in rats, continuous dietary administration of triticonazole produced clear evidence of toxicity at 5000 ppm. In addition to reduced body weight gain and reduced efficiency of food conversion, there were some changes in haematology and clinical chemistry in both sexes, and also histopathological non-neoplastic findings in the liver and the adrenal cortex. Changes in the eye lens (evident in males at 5000 ppm after 98 weeks of treatment only) were considered to be normal age-related changes and not an effect of treatment. Although the poor survival rate limits the value of the rat carcinogenicity study, there was no convincing evidence of any treatment-related hyperplastic or oncogenic response. Increased incidences of benign pituitary adenoma and keratoacanthoma of the skin were noted but were considered to be coincidental and not indicative of an oncogenic potential.

Further evidence suggesting that triticonazole is not oncogenic is demonstrated by the lack of any oncogenic effect in the valid mouse carcinogenicity study and the negative genotoxicity studies. The NOAEL for this chronic toxicity/carcinogenicity study in the rat is 750 ppm (equivalent to 29.4 in males and 38.3 mg/kg bw/d in females, respectively).

In the mouse carcinogenicity study, continuous dietary administration of triticonazole for 78 weeks produced clear evidence of toxicity at 1500 ppm. Body weight gain was reduced during throughout the dosing period and increased liver weight was associated with histopathological non-neoplastic changes (hepatocytic fatty vacuolation).There was no indication of oncogenic potential at any dose level. On the basis of these results, the dose level of 150 ppm (equivalent to 17.4 in males and 20.1 mg/kg bw/d in females, respectively) was the NOAEL of this study.
Overall there was no evidence of a carcinogenic potential.

Based on the available data, the following endpoint was determined during the last Annex I listing of triticonazole concerning:

Long term toxicity and carcinogenicity

Target/critical effect	liver (increased organ weight, fatty vacuolation and hypertrophy)
Lowest relevant NOAEL / NOEL	18 months mouse: 17.4 mg/kg bw/d
Carcinogenicity	overall no evidence of a carcinogenic potential

Studies submitted in this AIR3 dossier:
There are no new studies available with triticonazole that affect the overall evaluation for long-term toxicity and carcinogenicity, however all relevant effects observed in both species are considered. Thus, the conclusion for relevant endpoints for the current re-registration is as follows:

Long-term toxicity and carcinogenicity

Long-term effects (target organ/critical effect)	Rat: liver (terminal sacrifice: fatty vacuolation) & adrenals (interim sacrifices only: multinucleated cells in the cortex) Mouse: liver (fatty vacuolation)	
Relevant long-term NOAEL	2-year, rat: 750 ppm (29.4 and 38.3 mg/kg bw in males and females) 18-month, mouse: 150 ppm (17.4 and 20.1 mg/kg bw in males and females)	
Carcinogenicity (target organ, tumour type)	No carcinogenic potential	
Relevant NOAEL for carcinogenicity	2-year, rat: ≥ 5000 ppm (203.6 and 286 mg/kg bw in males and females) 18-month, mouse: 1500 ppm (202.2 and 209.5 mg/kg bw in males and females)	

For convenience of the reviewer brief summaries of the studies listed in the DAR of the draft monograph of triticonazole are provided below.

Rat, 99/100 weeks feeding study, 0, 5, 25, 750, 5000 ppm (R013100)

Guidelines: This study was conducted according to the EPA Pesticide Assessment Guidelines, Subdivision F, No. 83-5.
GLP: Yes
Acceptance: The study was considered acceptable in the EU registration process 2003.

Remark:
The intended duration of the study was 104 weeks, but given the survival rates, the terminal sacrifice was initiated after 99 weeks for males and 100 weeks for females to ensure sufficient survival of at least 25 % for terminal investigations. It is noted that the survival rate did not meet the requirements for a 50 % survival rate at 24 months specified in the relevant guidelines in order to establish a negative result for carcinogenicity. It was stated in the report that the early termination reflects the decline in CD rats longevity noted in this laboratory and others over recent years.
Although the value of the study is limited by the poor survival, detailed examination of pathological and histopathological data exhibited no convincing indication of any treatment-related hyperplastic or oncogenic response. Therefore, it can be concluded that the rats survived long enough to allow a valid interpretation of the potential oncogenicity of the test compound. Additionally, minor deviations from the protocol are noted but do not limit the scientific validity of the study.

Material and method:
Groups of 50 male and 50 female CD rats (source: [redacted]) received triticonazole (batch no. DA 646; purity 97 %) with the diet at dose levels of 0, 5, 25, 750 and 5000 ppm (equivalent to mean achieved doses of 0, 0.2, 1.0, 29.4 and 203.6 mg/kg bw/d [males] and 0, 0.3, 1.3, 38.3 and 286.6 mg/kg bw/d [females] for two years (99 weeks [♂], 100 weeks [♀]). AAdditional 15 animals/sex and dose group were sacrificed after 26 and 53 weeks of treatment. Test diets were prepared twice weekly; concentrations, stability and homogenicity of the test diets were confirmed periodically by analysis. All animals were inspected at least daily for morbidity, mortality and clinical signs, and weekly for a more detailed examination including palpation. Body weights were recorded at weekly intervals for the first 14 weeks of treatment and once every two weeks thereafter. Food consumption was recorded weekly. Ophthalmoscopy was performed before commencement of treatment (all animals) and after 24, 50 and 98 weeks (control groups and high dose group animals only). Biochemistry (total protein, albumin, globulin, calcium, phosphate, sodium, potassium, urea, creatinine, glucose, cholesterol, bilirubin, chloride, AST, ALT, AP, CPK), haematology (haematocrit, haemoglobin concentration, RBC, MCHC, MCV, MCH, WBC total, WBC differential, platelets count, reticulocyte count, prothrombin time) and urinalysis (colour, volume, pH, specific gravity, protein, glucose, ketones, bilirubin, urobilinogen, nitrite, blood cells, sediments) were conducted in 10 males and females from each dose group after 24, 52 and 76 weeks and at termination.

At the end of dosing, each animal was subjected to a gross pathology examination and selected organs were weighed (adrenals, brain, heart, kidneys, liver, lungs, ovaries, pituitary, prostate, spleen, testes, thymus, thyroid, uterus). Histopathology was performed in a wide range of tissues (adrenals, brain, caecum, colon, duodenum, epididymides, eyes and optic nerve, femur and marrow, heart, ileum, jejunum, kidneys, liver, lungs, lymph nodes [mandibular, mesenteric], mammary gland, oesophagus, ovaries, pancreas, pituitary, prostate, rectum, salivary glands, sciatic nerve, smooth muscle, spinal cord, spleen, sternum, stomach, testes, thymus, thyroid with parathyroid, trachea, urinary bladder, uterus and vagina) on all interim (26 or 52 weeks) and terminal phase control and high-dosed animals. In addition, the kidneys, liver and lungs were examined for all animals, and the adrenals for all animals assigned to the 26 week interim phase and for all females assigned to the 52 week interim phase. Microscopy was also performed in all animals killed or dying during the study from all groups and from all rats in respect of tissues considered to exhibit a reaction to treatment.

Findings:
General observations: There were no treatment-related clinical signs of toxicity at any dose level. A tabulated summary is given below:

Table 5.5-1: Number of surviving animals among the 50 rats assigned to each group and time taken to reach < 50% survival

	Control		5 ppm		25 ppm		750 ppm		5000 ppm	
	M	F	M	F	M	F	M	F	M	F
Survival rate (week 99 for animals and week 100 for females										
Surviving animals (%)	14/50 (28)	19/50 (38)	23/ (46)	14/50 (28)	14/50 (28)	16(50) (32)	17/50 (34)	18/50 (36)	21/50 (42)	28/50 (56)
Weeks	85	94	96	94	93	94	87	90	94	>100

There were also no treatment-related significantly adverse intergroup differences in mortalities and survival, respectively. However, a trend towards higher survival rates was evident in treated animals. At 5000 ppm, there was a significant reduction in body weight gain of approximately -20 % and -29 % in males and females respectively, during the first week of dosing when compared with the respective control values. Body weight gains of females in this group remained lower than controls throughout the study, being 79 % of controls at week 88 (until the senescent decline of body weights). Males receiving 5000 ppm were similarly but less markedly affected (92 % of controls at week 76). Body weight gains of animals in the other dose groups were similar to controls. Food consumption was not affected by treatment. However, lower efficiency of food conversion was evident in females receiving 5000 ppm during the first 14 weeks of treatment and in males during the first week.
Ophthalmoscopic examination of control and high dosed animals did not reveal any treatment-related findings after 24 or 50 weeks of treatment, but there was an apparent increase in the incidence of lenticular sclerosis in males receiving 5000 ppm after 98 weeks of treatment when compared with control animals and on posterior capsular opacity plaque (not statistically significant, [redacted] C028256). Details on the ophthalmoscopic findings are given in **Table 5.5-1**.

Table 5.5-2: Incidence of lens findings at ophthalmoscopic examination after 98 weeks and of selected lens findings before and 24, 50 and 98 weeks of treatment in the high dose compared to controls

Findings in the lens (98 weeks, unless indicated otherwise)	Males		Females	
	Controls	5000 ppm	Controls	5000 ppm
Number of animals affected/Number of animals examined (% affected)				
Anterior suture line opacity	7/14 (50%)	6/22 (27%)	7/22 (32%)	13/30 (43%)
Anterior subcapsular opacity	0/14	0/22	2/22 (9%)	1/30 (3%)
Posterior capsular opacity (central)	4/14 (29%)	5/22 (23%)	2/22 (9%)	5/30 (17%)
Posterior capsular opacity plaque (before treatment)[1]	0/80 (0%)	0/80 (0%)	0/80 (0%)	0/80 (0%)
Posterior capsular opacity plaque (24 weeks)[1]	1/80 (1%)	0/80 (0%)	0/79 (0%)	2/80 (2.5%)
Posterior capsular opacity plaque (50 weeks)[1]	2/14 (14%)	0/14 (0%)	0/15 (0%)	0/14 (0%)
Posterior capsular opacity plaque (98 weeks)[1] HCD (M: 0-19.0%, F: 0-21.4%)[2]	1/14 (7%)	5/22 (23%)*	1/22 (4.5%)	1/30 (3%)
Anterior polar opacity	1/14 (7%)	2/22 (9%)	1/22 (4.5%)	1/30 (3%)
Anterior polar cataract	0/14	1/22 (4.5%)	0/22	0/30
Arcuate opacity	0/14	0/22	3/22 (14%)	1/30 (3%)
Sclerosis (before treatment)[1]	0/80 (0%)	0/80 (0%)	0/80 (0%)	1/80 (1%)
Sclerosis (24 weeks)[1]	0/80 (0%)	0/80 (0 %)	0/79 (0%)	0/80 (0%)
Sclerosis (50 weeks)[1]	0/14 (0%)	0/14 (0%)	0/15 (0%)	0/14 (0%)
Sclerosis (98 weeks)[1] HCD (M: 0 – 11.1%; F: 0-20.0%)[2]	1/14 (7%)	9/22 (41%)	15/22 (68%)	21/30 (70%)
Nuclear sclerosis	1/14 (7%)	0/22	1/22 (4.5%)	1/30 (3%)
Ventral forward protrosis	0/14	0/22	1/22 (4.5%)	0/30
Obscured (due to keratitis or poorly) dilated pupil	0/14	3/22 (14%)	0/22	0/30

[1]Statistical analysis has only been performed on the findings "posterior capsular opacity plaque" and "lens sclerosis"
*$p<0.05$
[2]only 6 studies had been identified, where these specific finding have been recorded, as opthalmoscopic investigations were not conducted at terminal examination in all studies, because the nomenclature had changed and there are not so many studies with this rat strain available ( )

A treatment relationship of the findings posterior capsular opacity and lens sclerosis in males was considered unlikely based on the fact, that

- The overall number of examined animals is low and only 14 male control animals were examined vs 22 high dose animals.
- The posterior capsular opacity plaque findings in high dose males were not statistically significant.
- The comparison of posterior capsular opacity plaque incidence with historical control data compiled from 6 studied performed in the same performing laboratory showed that the incidence in the male treated group (23%) was close to the upper limit of normal range in the performing laboratory (19%).
- Morphological characteristics were representative of the normal ageing nuclear sclerosis change in rats. Lenticular sclerosis, as recorded during the ophthalmology exams at the late timepoint (week 98) is considered to be a normal aging ocular lesion in senescent laboratory rats

- The longer survival rate of males treated at 5000 ppm (22 male animals in high dose group vs 14 animals in controls) most probably contributed to the late development of this age-related lesion.
- There is a 3-fold difference between the highest historical control value (20%) for lens sclerosis and the concurrent control females (68%) and a similar roughly 3-fold difference between the males incidence in historical controls (11.1%) and the treated males of the triticonazole study (41%), indicating the high variability of this finding

This assessment is supported by the fact that these observations appeared only after 98 weeks but were not evident during the intermediate examinations at 24 and 50 weeks. It is also noteworthy that no treatment-related eye lesions were seen at necropsy on macroscopic or microscopic examination at any dose group. Further it is worth to mention, that there was no indications of lenticular changes of the type observed in the associated study in dogs at any of the ophthalmic examinations (see 1-year dog study in Chapter MCA 5.3).

Haematological analysis of blood samples obtained after 76 and 97 weeks of treatment indicated, in comparison with controls, significantly lower platelet counts (-16 % at week 76 and –19 % at week 97) and a longer prothrombin time in females receiving 5000 ppm at week 76 and 97. Platelet counts in males receiving 5000 ppm were also slightly but significantly lower after 24 (-10 %) and 76 weeks (-17 %) of treatment. Variations of these parameters in other treated groups were seen on isolated occasions, but no clear pattern was observed. Also other inter-group differences occasionally attained statistical significance were not considered to be of toxicological relevance. There were no haematological findings that were considered treatment-related at 5, 25 and 750 ppm.
Clinical chemistry analysis revealed slightly, but consistently and significantly, lower plasma Alanine amino transferase (ALT) activities in animals receiving 5000 ppm. A similar change was apparent among animals receiving 750 ppm after 50 and 76 weeks of treatment, although statistical significance was not attained. The plasma aspartate amino-transferase (AST) activity of animals receiving 5000 ppm was slightly but consistently lower than controls, although statistical significance was not reached on any occasion (see Table 5.5.-3). There were also significantly lower cholesterol concentrations at week 76 in males receiving 5000 ppm and in females at week 76 and 97. No clear pattern was obtained for the alkaline phosphatase (AP) levels in plasma. The isolated statistically significant changed at single time points or dose groups are considered incidental and not treatment related. Similar changes of cholesterol concentrations were reported in females treated at lower dose levels with no evidence of a clear dose-related pattern. Therefore, these changes were considered not to be adverse.

Table 5.5-3: Clinical chemistry findings at different time points (group mean values)

	Dose group level [ppm]									
	Male					**Female**				
	0	**5**	**25**	**750**	**5000**	**0**	**5**	**25**	**750**	**5000**
Alkaline phosphatase (AP) [iU/l] (SD)										
Week 24	82 (17)	82 (14)	72 (15)	67 (21)*	70 (12)	38 (9)	38 (13)	32 (7)	32 (7)	33 (7)
Week 50	68 (39)	76 (33)	66 (11)	61 (12)	60 (9)	32 (11)	25 (6)	28 (14)	21 (6)*	22 (4)*
Week 76	61 (10)	74 (19)	61 (26)	61 (22)	62 (18)	25 (8)	27 (10)	31 (12)	30 (9)	26 (8)
Week 97	56 (17)	73 (48)	46 (18)	93 (135)	58 (24)	23 (10)	29 (13)	27 (14)	39 (22)*	26 (12)
Alanine amino transferase (ALT) [iU/l] (SD)										
Week 24	34 (5)	35 (5)	31 (5)	34 (9)	25(5)**	30 (5)	32 (8)	30 (9)	28 (6)	22 (3)*
Week 50	43 (19)	42 (24)	50 (34)	28 (4)	24 (6)	44 (18)	49 (14)	46 (19)	34 (9)	29 (10)*
Week 76	49 (32)	99 (172)	38 (16)	28 (19)	27 (12)	42 (21)	43 (17)	37 (11)	29 (7)	28 (6)*
Week 97	43 (33)	25 (8)*	28 (14)	31 (20)	24 (11)*	41 (25)	29 (8)	32 (14)	39 (16)	25 (7)*
Aspartate amino-transferase (AST) [iU/l] (SD)										
Week 24	97 (23)	100 (23)	80 (13)	97 (27)	83 (13)	76 (11)	86 (18)	76 (13)	78 (8)	67 (7)
Week 50	88 (26)	91 (39)	95 (28)	78 (16)	76 (8)	97 (36)	117 (39)	111 (43)	95 (21)	84 (37)
Week 76	97 (31)	225 (413)	83 (20)	84 (20)	79 (15)	89 (24)	92 (23)	79 (20)	89 (22)	76 (11)
Week 97	83 (32)	74 (18)	83 (24)	73 (29)	72 (16)	83 (27)	75 (30)	74 (17)	99 (61)	70 (21)
Total cholesterol [mg%]										
Week 24	61 (12)	63 (18)	66 (12)	75 (17) *	68 (14)	94 (34)	78 (14)	106 (41)	79 (22)	118 (22)
Week 50	103 (28)	120 (35)	129 (76)	91 (28)	102 (25)	117 (29)	114 (29)	107 (20)	135 (70)	110 (22)
Week 76	134 (56)	110 (36)	120 (41)	135 (51)	78 (25)**	157 (74)	111 (28)*	103 (24)**	101 (24)**	116 (27)*
Week 97	194 (84)	155 (61)	171 (96)	166 (64)	158 (61)	192 (118)	185 (65)	112 (36)*	130 (56)*	130 (24)*

*p<0.05, **p<0.01

There were no other haematological or clinical chemistry finding considered to be associated with treatment in any dose group. There were no significant results recorded at urinalysis throughout the study. However, generally lower urinary volumes and higher specific gravities among animals receiving 5000 ppm were noted at most occasions, although the differences did not always attain statistical significance.

Pathology:
There were no clearly treatment-related macroscopic abnormalities detected at any of the scheduled necropsies or at the necropsies of premature decedents. Organ weight analysis revealed slightly but significantly increased relative liver (+23 %; p<0.01) and spleen weights (+21 %; p<0.01) in females receiving 5000 ppm sacrificed after 26 weeks of treatment. The absolute weights were also slightly higher than controls in these animals but statistical significance was not attained. Males at this interim sacrifice were not affected. There were no other treatment-related effects on organ weights of either of these organs after 52 weeks or at termination.

Histopathological examination at the interim sacrifices after 26 and 53 weeks of treatment revealed no neoplastic findings considered related to treatment. Non-neoplastic findings were limited to the adrenals of animals treated with 5000 ppm. A statistically higher incidence of multinucleated cells in the *zona fasciculata* were noted in 9/15 females after 26 weeks of treatment (compared to 0/15 in controls) and in 3/14 females after 53 weeks of treatment (compared to 0/15 in controls). In addition after 53 weeks of treatment, an increased incidence of chronic inflammation was observed in the *zona fasciculata* of 4/14 females receiving 5000 ppm (compared to 0/15 in the control group). At the terminal phase only 3/50 females showed multinucleated cells in the *zona fasciculata* of the adrenals and only 1/50 females had a chronic inflammation (all without statistical significance). Based on a re-evaluation of an external pathologist (see Chapter MCA 5.8, BASF DocID 2015/1197310) a treatment-related change in the adrenal findings in females is doubtful. In males very marginal (not statistically significant) increased incidences of cortical fatty vacuolation in adrenals were seen at the 26 and 53 weeks sacrifice timepoint only. A summary of the relevant non-neoplastic findings in adrenals, liver and lungs is given in Table 5.5-4

Table 5.5-4: Group incidences of relevant non-neoplastic findings in adrenals, liver and lungs at the interim and terminal sacrifices (terminals and decedents)

Findings	Dose group level [ppm]									
	Male					Female				
	0	5	25	750	5000	0	5	25	750	5000
Adrenals (26 weeks)										
Multinucleated cells (Z. f.)	0/15	0/15	0/15	0/15	0/15	0/15	0/15	0/15	0/15	9/15***
Cortical fatty vacuolation	4/15	2/15	2/15	3/15	7/15	0/15	0/15	1/15	0/15	1/15
Adrenals (53 weeks)										
Multinucleated cells (Z. f.)	0/14	-	-	-	0/14	0/15	0/15	0/14	0/14	3/14
Chronic inflammation (Z. f.)	0/14	-	-	-	0/14	0/15	0/15	0/14	0/14	4/14*
Cortical fatty vacuolation	1/14	-	-	-	3/14	1/15	0/15	0/14	0/14	0/14
Adrenals (terminal)										
Multinucleated cells (Z. f.)	0/50	0/35	0/42	0/40	0/50	0/50	0/46	0/48	0/46	3/50
Chronic inflammation (Z. f.)	0/50	0/35	0/42	0/40	0/50	0/50	0/46	0/48	0/46	1/50
Cortical fatty vacuolation	11/50	10/35	7/42	10/40	13/50	8/50	4/46	4/48	6/46	11/50
Liver (terminal)										
Centriacinar fatty vacuolation	6/50	3/50	5/50	5/50	9/50	16/50	15/50	11/50	23/50	33/50**
Lungs (terminal)										
Accumulation of alveolar macrophages	2/50	1/50	0/50	1/50	2/50	0/50	3/50	1/50	1/50	7/50*

*p<0.05, **p<0.01, ***p<0.001; Z f.: Zona fasciculata

The mechanism of the degenerative changes observed in the adrenal cortex of females after treatment with 5000 ppm triticonazole is not known, a more comprehensive discussion can be found in Chapter MCA 5.8 of this dossier. It is noteworthy to mention, that neither an evidence for treatment-related cortical adenocarcinoma (incidences in M: 0//50, 0/35, 0/42, 0/40, 0/50; F: 1/50, 1/46, 0/48, 1/46, 0/50 for controls, 5, 25, 750, 5000 ppm), cortical adenoma (incidences in M: 0//50, 1/35, 0/42, 0/40, 1/50; F: 3/50, 1/46, 0/48, 0/46, 3/50 for controls, 5, 25, 750, 5000 ppm), nor for medullary phaeochromocytoma (incidences in M: 5/50, 6/35, 5/42, 3/40, 4/50; F: 1/50, 2/46, 0/48, 0/46, 1/50 for controls, 5, 25, 750, 5000 ppm).

Non-neoplastic findings at the terminal sacrifice considered to be treatment-related were confined to the liver and the lungs: In the liver, a significant increased incidence of centriacinar fatty vacuolation was reported for females receiving 5000 ppm and some evidence (no statistical significance was achieved) of increased fatty vacuolation of hepatocytes in males at 5000 ppm and in females at 750 ppm. (However, no special staining techniques, e.g. to confirm the presence of fat were used.) The lung findings were a significant increase in the incidence of accumulation of alveolar macrophages in females receiving 5000 ppm, but as it was only observed in a few animals of one sex, it was considered of doubtful toxicological significance.

Concerning neoplastic findings, statistically significant increases in the incidences of benign lesions were limited to the pituitary gland and the skin. A tabulated summary can be found below:

Table 5.5-5: Group incidences of neoplastic findings in pituitary and skin at the terminal phase

Findings	Dose group level [ppm]									
	Male					Female				
	0	5	25	750	5000	0	5	25	750	5000
Pituitary (animals killed or dying during the study) (%)										
Number examined	38	30	38	36	30	32	38	32	33	23
Adenoma	13 (34.2 %)	16 (53.3%)	19 (50%)	18 (50%)	17 (56.7%)	21 (65.6%)	25 (65.8%)	23 (71.9%)	29* (87.9%)	16 (69.6%)
Carcinoma	0	1 (3.3%)	0	2 (5.6%)	0	3 (9.4%)	5 (13.2%)	1 (3.1%)	1 (3.0%)	2 (8.7%)
Pituitary (all animals) (%)										
Number examined	50	38	43	43	50	49	45	44	44	50
Adenomas** HCD (104 wk): M: 42.9 – 60%) HCD (90-95 wk): M: 36.7 – 46.2%)	19 (38%)	24 (63.2%)	24 (55.8%)	25 (58.1%)	29* (58%)	32 (65.3%)	31 (68.9%)	34 (77.3%)	40 (90.9%)	32 (64%)
Carcinomas	0	1 (2.6%)	2 (4.7%)	2 (4.7%)	0	3 (6.1%)	5 (11.1%)	1 (2.3%)	1 (2.3%)	2 (4%)
Skin (all animals) (%)										
Number examined	20	22	21	24	27	9	11	10	10	8
Papilloma	4 (20%)	2 (9.1%)	1 (4.8%)	1 (4.2%)	3 (11.1%)	0	1 (9.1%)	1 (10%)	0	0
Keratocanthoma	0	5* (22.7%)	2 (9.5%)	2 (8.3%)	6* (22.2%)	0	1 (9.1%)	0	0	1 (12.5%)

*p<0.05; **HCD provided by Renault, D (C028249)

In the pituitary gland, benign adenomas were observed in males (29/50; p< 0.05) treated at 5000 ppm compared to 19/50 in control males. The statistically significant increase for this tumour is coincidental due to an unusually low incidence in male concurrent control animals. The incidence in the males receiving 5000 ppm (58 %) is within the historical range of this tumour for this strain of male rats at this laboratory when full length studies (104 weeks) were considered (42.9 – 60 %), but outside the historical range of 36.7 – 46.2 % for abbreviated studies (90 – 95 weeks). However, the incidences of this finding in the intermediate dose groups showed no indication of any relationship to dose. Therefore, the slightly higher incidence of this benign tumour, which is well known as a spontaneous age-related lesion in the rat is not considered to be treatment-related. The statistically significantly increased incidence of benign pituitary tumors in females of the 750 ppm dose group (of animals died during the study) is also not considered to be treatment related, as there were no changed in the top dose compared to controls and no related increases in carcinoma were seen in females.

In the skin, incidences of keratoacanthomas in males that received 5 and 5000 ppm achieved statistical significance. If the incidence of keratoacanthoma were considered together with the incidence of the histologically similar benign papilloma of the skin, no significant differences were attained. It is concluded that this fact, together with the lack of a dose-relationship confirms that the tumour is very unlikely to be related to treatment with triticonazole.
Besides these findings observed in the pituitary and skin, the only remarkable observation was an increased incidence of benign follicular cell adenomas noted in the thyroids of 7/49 males treated at 5000 ppm compared to 3/47, 1/30, 0/34 and 0/39 in the 0, 5, 25 and 750 ppm dose groups, respectively. However, this finding was considered very unlikely to be treatment-related because (i) no statistical significance was attained, (ii) there was no dose-relationship and (iii) there were no other associated findings that could suggest a treatment-related effect of triticonazole on the thyroid (e.g. thyroid weights or histopathological changes at interim sacrifices or in the subchronic study). All other tumours were of the types commonly seen in CD rats and occurred with the expected frequency.

Conclusion
The continuous dietary administration of triticonazole to CD rats during lifetime period produced clear evidence of toxicity at 5000 ppm with body weight gains and food efficiency reduced, more specifically in females. Significant treatment-related findings in haematology and clinical chemistry parameters were reported in both sexes. Changes in the eye lens (evident in males at 5000 ppm after 98 weeks of treatment) were considered to be normal age-related changes and not an effect of treatment. Significant treatment-related non-neoplastic lesions were noted in the adrenal cortex (interim sacrifice, females only) and in the liver (terminal sacrifice, females only).
The statistically significantly increased incidences of benign pituitary adenoma in males at 5000 ppm, and of keratoacanthoma of the skin in males treated at 5 or 5000 ppm were considered to be incidental and not indicative of an oncogenic potential.
The NOAEL for this study was considered to be 750 ppm (29.4 – 38.3 mg/kg bw/d) based on decreased bodyweight gain and significant histopathological findings in the liver and adrenals evident at the next higher dose level.

Mouse, 78 weeks feeding study, 0, 15, 150, 1500 ppm (R013143)

Guidelines: This study was conducted according to the US EPA Guideline No. 83-5.
GLP: Yes
Acceptance: The study was considered acceptable in the EU registration process 2003.

Material and method:
Groups of 52 male and 52 female CD-1 mice (source: [redacted]) were treated with triticonazole (batch no. DA646; purity 96.4 – 97.1 %) via diet at dose levels of 0, 15, 150 and 1500 ppm (equivalent to 0, 1.8, 17.4 and 202.2 mg/kg bw/d in males and 0, 2.1, 20.1 and 209.5 mg/kg bw/d in females) for 78 weeks. An additional 16 animals/sex and dose group were sacrificed after 26 weeks of treatment. Test diets were prepared each week; concentrations, stability and homogeneity of the test diets were confirmed periodically by analysis. Animals were observed daily for mortalities, clinical signs and behavioural pattern. All animals were also given a more detailed clinical examination weekly. Body weights were recorded before treatment, at weekly intervals until week 15 and once every two weeks thereafter and at necropsy. Food consumption by each cage was determined at weekly intervals throughout the study period. After 24 weeks of treatment, ophthalmoscopic examination was performed on both eyes of all mice from the control groups and test groups receiving 1500 ppm scheduled for interim sacrifice. Blood smears for haematological investigations were obtained after 50 and 76 weeks of treatment from all animals of the control and high dose treated groups and differential leucocyte counts were performed. In addition, at necrospy blood samples from ten male and ten female mice from each dose group were taken and detailed haematological investigations conducted (haematocrit, haemoglobin concentration, RBC, MCHC, MCV, MCH, WBC total, WBC differential, platelets count). At terminal necropsy, the weights of selected organs (adrenals, brain, heart, kidneys, liver, lungs, spleen, testes, and uterus) were recorded. Histopathology was performed in a full range of tissues from all animals in the control and high dose groups (adrenals, aorta, brain, caecum, colon, duodenum, epididymides, eyes and optic nerve, femur including marrow, gall bladder, heart, ileum, jejunum, kidneys, liver, lungs, lymph nodes, mammary gland, oesophagus, ovaries, pancreas, pituitary, prostate, rectum, salivary glands, sciatic nerve, seminal vesicles, skeletal muscle, skin, spinal cord, spleen, sternum, stomach, testes, thymus, thyroid with parathyroid, trachea, urinary bladder, uterus and vagina), from animals that died or were killed in extremis, and on lungs, liver and kidneys from animals in the low and intermediate dose groups.

Findings:
General observations: There were no treatment-related clinical signs or changes in the incidence of palpable swelling at any dose level. At the end of the treatment period at week 78, the survival rates of males were 63 %, 62 %, 50 % and 58 %, and of females 73 %, 73%, 73 % and 88 % in the control, low, intermediate and high dose groups, respectively. No treatment-related effects on the distribution and timing of premature deaths were reported.

In animals receiving 1500 ppm, lower body weight gains were reported during the majority of the dosing period with a depression of 25 % (males) and 31 % (females) in week 52 compared to controls. The overall body weight gains for the entire dosing period of males and females at 1500 ppm were 85 % and 77 % of control values, respectively. There were no clear effects of treatment on body weight gains of animals treated at 5 or 150 ppm. Food consumption was similar between treated and control groups; however, efficiency of food conversion was slightly lower during the first 14 days of treatment for males at 1500 ppm.
Ophthalmoscopy: There were no findings at the ophthalmoscopic examinations of high dose and control animals after 24 week which could be attributed to treatment. Superficial corneal opacities were evident in 3 males and 3 females receiving 1500 ppm compared to one male and one female in the controls, but this was stated to be a common finding in mice of this strain and the distribution was considered to be incidental.
Haematology: Examination of blood smears after 50 and 76 weeks, and a detailed haematological examination after 77 weeks of treatment did not indicate any effects of treatment. Organ weight analysis revealed significantly higher liver weights (absolute and relative) of males and females receiving 1500 ppm at both the interim and terminal sacrifices. Absolute and relative adrenal weights in this group were also slightly higher than in controls at the interim sacrifice, being statistically significant only for the relative weights in males. No changes of treatment-related changes were observed in adrenal weights at the terminal sacrifice (see Table 5.5-6).

Table 5.5-6: Organ weight changes (mean group values)

Parameter	Dose group level [ppm]							
	Males				Females			
	0	15	150	1500	0	15	150	1500
Adrenals (interim phase – 26 weeks)								
Absolute weights [g]	0.005	0.005	0.005	0.007	0.007	0.008	0.007	0.009
Relative weights [%]	0.0098	0.0095	0.0116	0.0171*	0.0224	0.0261	0.0212	0.0292
Adrenals (terminal phase – 78 weeks)								
Absolute weights [g]	0.006	0.004	0.005	0.004	0.006	0.007	0.006	0.006
Relative weights [%]	0.0129	0.0094	0.0101	0.0098	0.0158	0.0179	0.0173	0.0188
Liver (interim phase – 26 weeks)								
Absolute weights [g]	2.52	2.55	2.44	3.19**	1.82	1.69	1.75	2.08**
Relative weights [%]	5.23	5.19	5.51	7.89**	5.54	5.28	4.98	6.58
Liver (terminal phase - 78 weeks)								
Absolute weights [g]	2.88	2.46*	2.58	3.36*	1.90	1.95	1.95	2.19**
Relative weights [%]	6.05	5.40	5.31	7.65**	4.84	5.12	4.97	6.23**

*p<0.05, **p<0.01

Pathology: There were no macroscopic findings considered treatment-related at the interim phase. At the terminal phase, there was an increased incidence of enlarged livers among males receiving 1500 ppm. In addition, there was a slightly higher incidence of large and dark mesenteric lymph nodes among males of the top dose compared to concurrent controls.
Histopathological examinations of the animals at the interim as well as the terminal phase exhibited no neoplastic findings considered related to treatment with triticonazole. Non-neoplastic findings revealed the liver as the main target organ: At the interim phase, periacinar hepatocytic hypertrophy and centriacinar hepatocytic fatty vacuolation was noted among animals receiving 1500 ppm. The incidences are given in Table 5.5-7.

Table 5.5-7: Group incidences of non-neoplastic findings in liver and mesenteric lymph nodes

Parameter	Dose group level [ppm]							
	Males				Females			
	0	15	150	1500	0	15	150	1500
Liver (interim phase (26 weeks)								
Centriacinar fatty vacuolation	0/16	0/16	0/16	5/14*	0/16	0/16	2/16	9/16***
Periacinar hepatocyte hypertrophy	0/16	0/16	0/16	3/14	0/16	0/16	0/16	0/16
Liver (terminal phase – 78 weeks)								
Centriacinar fatty vacuolation	1/52	0/52	0/52	12/52**	0/52	1/52	0/52	8/52**
Periacinar hepatocyte hypertrophy	0/52	0/52	0/52	0/52	0/52	0/52	0/52	1/52
Mesenteric lymph node (terminal phase - 78 weeks)								
Parafollicular hyperplasia (scheduled sacrifice)	0/31	1/4	1/5	4/29*	1/38	0/7	0/3	1/44
Parafollicular hyperplasia (all animals)	1/49	1/24	1/28	4/46	1/52	0/21	0/16	1/49

*p<0.05, **p<0.01, ***p<0.001

At the terminal phase, there was also a statistically significant increase in the incidence of centriacinar hepatocytic fatty vacuolation in males and females of the top dose groups. In addition, there was an increased incidence of parafollicular hyperplasia of the mesenteric lymph nodes in males that had received 1500 ppm and been killed at termination. For this finding, significance was not reached when animals dying during the study period were included. An increased incidence of amyloidosis was evident in several organs (adrenals, gastro-intestinal tract, kidneys, lymph nodes, spleen thyroid) in males receiving either 150 or 1500 ppm. However, amyloidosis is known to occur spontaneously in aged mice of this strain and its occurrence is not regarded as a finding of toxicological importance.

Conclusion:
It can be concluded that dietary administration of triticonazole to mice up to and including 1500 ppm for 78 weeks showed no evidence on any treatment-related increase in the type or incidence of neoplastic findings in this study suggestive of a carcinogenic effect. At the top dose increased liver weights and increased incidences of fatty vacuolations in both sexes were seen. Based on, reduced body weight gains and clear effects on the liver (including increased organ weights and histopathological findings) the mid dose level of 150 ppm (equivalent to 17.4 [♂] and 20.1 [♀] mg/kg bw/d) is considered to be the NOAEL in this study.

Comparison with CLP criteria:
Two long term studies are available with triticonazole: One combined chronic and carcinogenicity study in rats and one carcinogenicity study in mouse. The terminal sacrifices in the rat chronic studies started already in week 99 compared to the normal sacrifice time of 104 weeks, as the survival was affected in all groups with treatment groups showing higher survival compared to the controls. Significant treatment-related non-neoplastic lesions were noted in the adrenal cortex (interim sacrifice, females only) and in the liver (terminal sacrifice, females only).
The statistically significantly increased incidences of benign pituitary adenoma in males at 5000 ppm, of increased benign follicular cell adenoma incidences in the thyroid of males at 5000 ppm and of keratoacanthoma of the skin in males treated at 5 or 5000 ppm were considered to be incidental and not indicative of an oncogenic potential. In mice the dietary administration of up to 1500 ppm triticonazole did not give evidence of any treatment-related increased in the type or incidence of neoplastic findings. Thus no classification for carcinogenicity is warranted for triticonazole.
No classification with STOT RE is justified, as all treatment-related effects occur at doses of ≥ 200 mg/kg bw in both species, which is above the trigger value for classification.

CA 5.6 Reproductive Toxicity

Studies evaluated in the draft monograph of the Rapporteur Member State Austria of Feb. 2003:

The triticonazole studies - evaluated in the draft monograph of the Rapporteur Member State Austria of February 2003 - consisted of a two-generation study in rats and developmental toxicity studies in rats and rabbits. These studies have been evaluated by European authorities and Austria as Rapporteur Member State and were considered to be acceptable. For the convenience of the reviewer, these are summarized below as extracted from the monograph and the EFSA conclusion. For a better overview the endpoints parental toxicity, reproductive toxicity and developmental toxicity were addressed separately.

Table 5.6-1: Summary of already peer-reviewed reproduction toxicity studies as available in the monograph (2003)

Study	Dosages (mg/kg bw/day)	NOAEL (mg/kg bw/day)	Main adverse effect	Reference and year
Two-generation dietary study, Sprague Dawley CD rats 0, 5, 25, 750, 5000 ppm	M: F0: 0, 0.34, 1.64, 49.35, 350.8; F1: 0.37, 1.82, 56.18, 445.3 mg/kg bw F: 0.32, 1.59, 48.41, 337.6 (gestation F0); *0.33, 1.60, 49.10, 339.08 (F1 gestation)* mg/kg bw Premating F0: 0.37, 1.81, 54.80, 389.3 mg/kg bw; F1 0.43, 2.14, 65.25, 493.8 mg/kg bw *Lactation F0: 0.58, 2.97, 87.99, 502.00 mg/kg bw, F1: 0.46, 2.96, 93.25, 528.05 mg/kg bw*	parental /reproductive: M: 49.35 F: 48.41	Parental effects: • mortalities • decreased weight gain • necropsy findings in adrenals, ovaries and liver Fertility effects: • decreased mating and fertility indices* Litter data: • decreased pup body weight • decreased livebirth and viability indices • no. stillborn pups increased	(R013085)
Developmental toxicity, gavage, Sprague-Dawley CD rat	0, 40, 200, 1000	Maternal toxicity: 40 Developmental toxicity: 200	Maternal toxicity: • body weight gain retarded • liver findings (pale areas) Fetal toxicity: additional 14th ribs	(C018955)

Study	Dosages (mg/kg bw/day)	NOAEL (mg/kg bw/day)	Main adverse effect	Reference and year
Developmental toxicity, gavage, New Zealand White rabbits	0, 5, 25, 50, 75	Maternal toxicity: 5 Developmental toxicity: 5	Maternal toxicity: • decreased body weight • mortalities; clinical signs • abortion Fetal toxicity: • skeletal abnormalites *(variations)*	(C018959)

*in the F1 generation only

The summary of reproductive toxicity studies as given in the monograph is as follows: In the 2-generation reproductive study in rats, distinct parental toxicity was produced at the dietary concentration of 5000 ppm (premature deaths, significant reductions in body weight gain and food consumption as well, and necropsy findings in adrenals, liver and ovary). Adverse effects on reproductive parameters in both generations at 5000 ppm included decreased fertility (F_1), increased pup mortality, decreased pup viability and decreased pup bodyweights and weight gain (F_0 and F_1). These adverse effects on reproductive parameters are considered as consequence of distinct maternal toxicity at this very high dose level, exceeding the maximum tolerated dose. There were no significant parental or reproductive findings at 48.4 mg/kg bw/day which was considered the NOAEL for both parental and reproductive effects as well.
In the teratology study in the rat, there was evidence of slight maternal toxicity at 1000 mg/kg bw/d. There were also incidences of pale areas in the liver at 200 and 1000 mg/kg bw/d indicative of maternal toxicity. Foetal survival and growth was not affected in any dose group. However, there was an increase in the incidence of foetuses with an additional 14th rib or pair of ribs at all dose levels, but this was only outside the historical background range at 1000 mg/kg bw/d. These findings were not regarded as a major malformation and it is considered that no teratogenic effect was observed at any dose level. The NOAEL for maternal toxicity was 40 mg/kg bw/d and for developmental toxicity 200 mg/kg bw/d.
In the oral teratology study in rabbits, marked maternal toxicity (30 % mortality) was noted at 75 mg/kg bw/d with the MTD being exceeded. There was also one treatment-related death at the next lower dose level of 50 mg/kg bw/d. Treatment-related body weight loss during the first two days of treatment and reduced food consumption were observed at the dose level of > 25 mg/kg bw/d. A slight, but not statistically significant increase in both pre- and post-implantation losses were noted at 75 mg/kg bw/d. Skeletal anomalies considered treatment-related were noted at > 25 mg/kg bw/d. However, there was no teratogenic effect observed at any dose level. On the basis of these results, the dose level of 5 mg/kg bw/d can be considered the maternal and the foetal NOAEL.
Based on the available data, the following endpoints were determined during the last Annex I listing of triticonazole.

Reproductive toxicity

Reproduction target / critical effect	decreased fertility indices; reduced viability and growth of pups at 330 mg/kg bw/day, a dose level causing severe parental toxicity
Lowest relevant reproductive NOAEL / NOEL	48.4 mg/kg bw/day for parental and reproduction toxicity
Developmental target / critical effect	foetal effects (increased incidence of additional 14th ribs in rats, increased incidence of skeletal anomalies in rabbits) at maternal toxic dose levels; no evidence of teratogenic potential
Lowest relevant developmental NOAEL / NOEL	rabbit: 5 mg/kg bw/day, developmental and maternal effects

Studies submitted in this AIR3 dossier (not yet peer-reviewed):

There are no new studies available with triticonazole that could affect the overall evaluation for reproductive toxicity.
A rat 2-generation study (CA 5.6.1) was conducted in 1993, prior to the 2001 iteration of the OECD 416, and was missing measurements for oestrus cycling, sperm parameters, implantation sites, some organ weights, gonadal histopathology, evaluation of the physical development of offspring.
However non-adversity with respect to these parameters can be concluded via other means:

- Oestrus cycling: Time to mating unaffected in (CA 5.6.1).
- Implantation sites: Covered in the developmental toxicity study (; CA 5.6.2).
- Some organ weights: 90-day rat study, 2-year chronic toxicity/carcinogenicity study (; CA 5.3.2, ; CA 5.5)
- Gonadal histopathology / Sperm parameters: No abnormalities in 90-day rat study, 2-year chronic toxicity/carcinogenicity study (; CA 5.3.2, ; CA 5.5).
- Evaluation of the physical development of offspring: Bodyweight development and reproductive capability of the F1 generation (, CA 5.6.1).

As such a repeat of the study is not considered likely to provide new information that would impact on the risk assessment of triticonazole. A thorough discussion of effects on the endocrine system will be provided in relevant sections. Teratogenicity studies in rats (; CA 5.6.2) and rabbits (; CA 5.6.2) are available for triticonazole. Both studies were broadly in line with the most recent OECD guideline and a repeat of either study is not considered necessary.

Thus, the conclusion for relevant endpoints for the current re-registration remains as follows:

Reproductive toxicity

Reproduction toxicity

Reproduction target / critical effect	Parental toxicity: (mortality, decreased food consumption and body weight, liver and adrenal histopathology) Reproductive toxicity: decreased fertility and mating index in F1 generation Offspring's toxicity: Reduced viability and reduced growth (corresponding to maternal toxicity and/or self-feeding of offspring)	
Relevant parental NOAEL	750 ppm (corresponding to 48.4 – 65.3 mg/kg bw per day*)	
Relevant reproductive NOAEL	750 ppm (corresponding to 48.4 – 65.3 mg/kg bw per day*)	
Relevant offspring NOAEL	750 ppm (corresponding to 48.4 – 65.3 mg/kg bw per day*)	

*corresponding dose variations for F0 and F1 male and female substance intakes during premating and gestation

Developmental toxicity

Developmental target / critical effect	Rat: Maternal toxicity: (reduced body weight gain, pale areas in liver). Developmental toxicity (variation: additional 14th ribs) Rabbit: Maternal toxicity: (clinical signs, mortality, decreased food consumption and body weight, abortions). Developmental toxicity (precocious ossification of acromion process, increased skeletal variations at excessive maternal toxicity)	
Relevant maternal NOAEL	Rat: 40 mg/kg bw per day Rabbit: 5 mg/kg bw per day	
Relevant developmental NOAEL	Rat: 200 mg/kg bw per day Rabbit: 5 mg/kg bw per day	

CA 5.6.1 Generational studies

Triticonazole - Two Generation reproduction study in rats ([redacted], R013085)

Guidelines: According to US EPA guideline 83-4
Deviations: In comparison to the 2001 iteration of the OECD 416 measurements for oestrus cycling, sperm parameters, implantation sites, some organ weights (brain, liver, kidney, spleen, thyroid), gonadal histopathology, evaluation of the physical development of offspring were missing.
GLP: Yes
Acceptance: The study was considered acceptable in the EU registration process 2003

The non-adversity with respect to the above mentioned parameters can in parts be concluded via:
Oestrus cycling: Time to mating / pre-coital interval unaffected
Gonadal histopathology / Sperm parameters: No abnormalities in the 90-day rat study, 2-year chronic toxicity / carcinogenicity study
Implantation sites: Covered via the developmental toxicity study in rats: No effects on implantation sites and number of corpora lutea
Organ weights: No abnormalities in weights of reproductive organs detected in 90-day rat study, 2-year chronic/carcinogenicity study; no histopathological abnormalities reported at necropsy of reproductive organs
No specific effect on fertility/reproductive performance (only high dose effects based on maternal toxicity)
Sexual maturation: No effect on pre-coital interval

Material and method:
Groups of 28 rats/sex/dose group (strain: Sprague Dawley CRL:CD®BR VAF/plus; source: [redacted]) of the F0 generation received triticonazole (batch no. DA646; purity 97.1 %) via diet at dose levels of 0, 5, 25, 750 and 5000 ppm for 10 weeks before mating, and throughout mating, gestation and lactation period until terminal sacrifice. The rats were paired on a basis of 1 male : 1 female. F1-offspring were culled on day 4 post-partum to give four male and four female offspring per litter where possible. At weaning (day 21 post-partum) 28 male and 28 female F1 pups were selected to form the F1 parental generation. These animals were maintained for 10 weeks and were then paired. The F1 parental animals received diets containing the same concentration of test material as their parents for at least 10 weeks prior to mating, then throughout mating, gestation and lactation of the F2 litters. F1 females were allowed to litter and rear their F2 offspring to weaning. The F1 animals and the F2 pups were then examined at necropsy for abnormalities. The observations included clinical signs (at least twice daily), recording of body weights (weekly during premating; on days 0, 7, 14 and 20 of gestation; on days 0, 4, 7, 14 and 21 of lactation) and food consumption (weekly during premating; days 0 - 4, 4 - 7, 7 - 14 and 14 - 20 of gestation; 0 - 4, 4 - 7, 7 - 10 and 10 – 14 of lactation) and reproductive performance (mating and fertility indices, conception rate, duration of gestation, gestation index, number, sex and weight of live and dead pups/litter and presence of external anomalies at birth; number and individual weight of pups were recorded on days 4, 7, 14 and 21 post-partum).

At necropsy, in addition to macroscopic examination of adult animals (F0 and F1), male and female reproductive organs (epididymides, prostata, seminal vesicles, testes, uterus, vagina and ovaries), liver, pituitary and adrenals were weighed. Microscopic examination was performed on all these tissues from control and high dose group animals, with adrenals also being examined from all dose groups. F1 pups culled on day 4 post-partum and post-weaning offspring not selected for contiuation of the study, and F2 pups were examined macroscopically.

Findings:
Test article intake:
No information on the test compound intake (mg/kg bw/d) is provided in the study report. Achieved daily intakes for both generations at the different segments of the study have been calculated by the notifier (see Table 5.6.1-1).

Table 5.6.1-1: Calculated compound intake in mg/kg bw (using food consumption and body weight data)

	F0	F1	F0	F1	F0	F1
Dose [ppm]	**m/f (pre-mating)**		**f (gestation)**		**f (lactation)**	
5	0.34/0.37	0.37/0.43	0.32	0.33	0.58	0.46
25	1.64/1.81	1.82/2.14	1.59	1.60	2.97	2.96
750	49.35/54.80	56.18/65.25	48.41	49.10	87.99	93.25
5000	350.8/389.3	445.3/493.8	337.64	339.08	502.99	528.05

It is evident, that at the same dietary concentrations of 5000 ppm the actual substance intakes are considerably (about roughly 30%) higher in the male and female F0 generation compared to the F1 generation (350.8 mg/kg bw for F0 males vs 445.4 mg/kg bw for F1 males and 389.3 mg/kg bw for F0 females vs 493.8 mg/kg bw for F1 females during the premating phase.

Mortality and clinical signs:
During gestation, one 5000 ppm F0 female was sacrificed after prolonged parturition (#C79528) and 3 further F0 females receiving 5000 ppm were sacrificed or found dead during late gestation (day 23; #C79549) or lactation (day 7, #C79538 and 9, #C79536). No specific clinical signs were noted before death of these animals, animal #C79536 was thin, hunched, languid. At necropsy histopathological lesions of the adrenal cortex including degeneration of cells, focal acute inflammation and moderate to severe adrenal haemorrhage were observed in these females. It was considered that the four deaths could have been related to treatment. During the last European Review, the increased substance intakes during lactation (being 1.8 and 1.3-fold higher during lactation, than during pre-mating) were considered to be responsible for the observed mortalities in the top dose group.

In addition, a total of 5 premature deaths amongst F0 (1 male at 750 ppm and 1 control female, with lesions of the urinary system seen at necropsy) and F1 parental animals (1 male at 5000 ppm and 2 females at 5 and 750 ppm, respectively, sacrificed due to hindlimb injury and poor health, respectively) occurred but were considered not to be treatment-related. There were no other deaths or clinical signs of toxicity considered related to treatment in any other group amongst F0 and F1 animals.

Body weights:
In F0 parental females at 5000 ppm, bodyweights and weight gains were significantly lower throughout premating, gestation and lactation, compared with control animals (see **Figure 5.6/1**). Also in F0 males receiving 5000 ppm, bodyweight gains were significantly lower over the first two weeks of pre-mating In F1 parental animals at 5000 ppm, body weights and weight gains were significantly lower during pre-mating in males and females, and throughout gestation and lactation for females (see **Figure 5.6/2**).

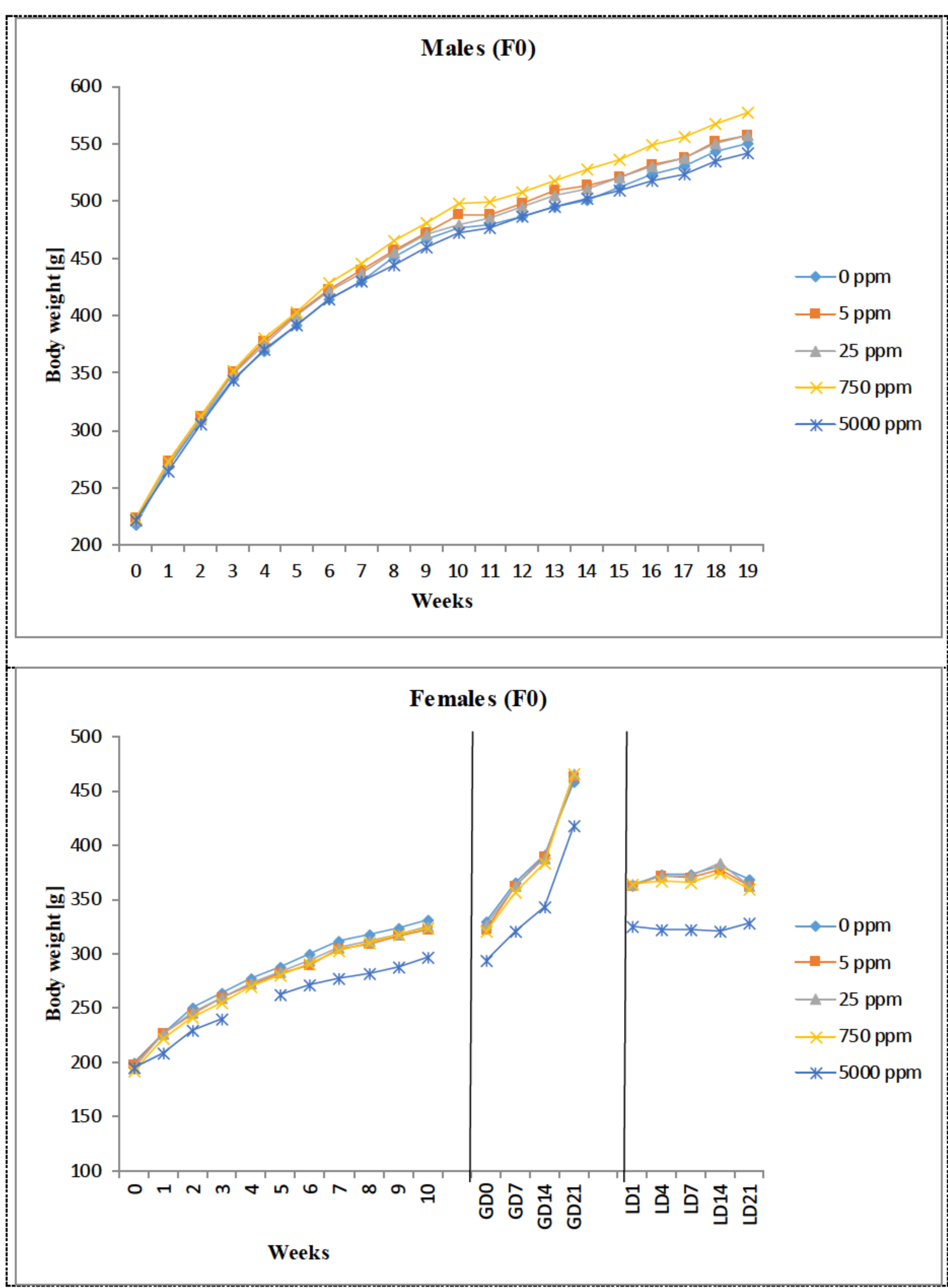
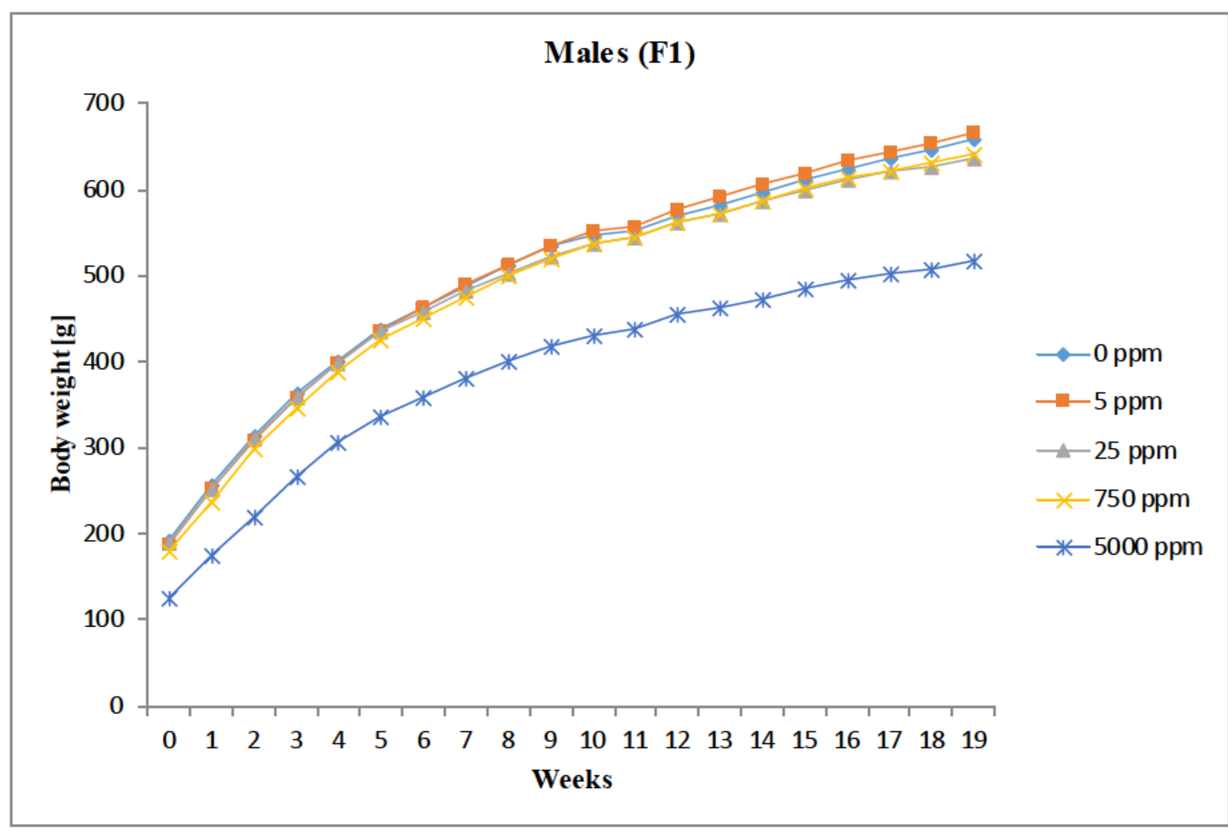


Figure 5.6/1 Body weight development of parental F0 rats



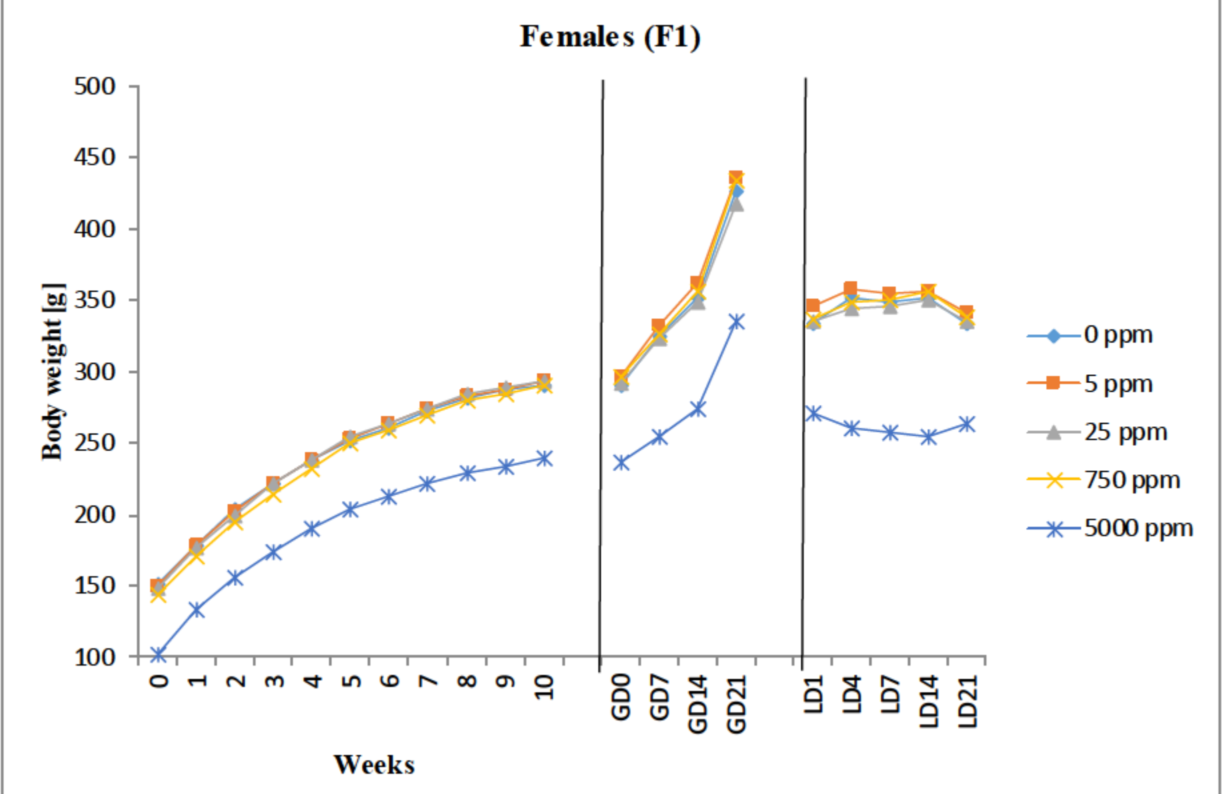


Figure 5.6/2: Body weight development of parental F1 rats

There is an apparent difference in body weight and body weight development of F0 males compared to F1 males (see Fig. 5.6/2 and Table 5.6.1-2), most probably reflecting the higher substance intake of F1 males compared to F0 males, with mean F1 males having -20-35% less weight compared to controls and the F0 males being almost unaffected by treatment with triticonazole. Also the body weight development of F1 females was more affected by treatment with triticonazole than the F0 females.
There was also a significant ($p<0.05$) reduction of body weight and body weight gain among males receiving 750 ppm for the first week of pre-mating. However, these reductions were very slight and considered to be coincidental. Tabulated summaries over the body weight development in males and females is given in the tables below.

Table 5.6.1-2: Mean body weights of F0 and F1 males

Dose groups	0 ppm	5 ppm		25 ppm		750 ppm		5000 ppm	
Study weeks		Body weight [g]	Δ [%]	Body weight [g]	Δ [%]	Body weight [g]	Δ]%]	Body weight [g]	Δ [%]
F0 Generation - Males									
0	217.45	222.26	2.21	220.96	1.61	222.77	2.45	222.08	2.13
1	267.79	272.70	1.83	270.48	1.00	272.14	1.62	263.82	-1.48
2	307.56	312.47	1.60	310.01	0.80	312.91	1.74	304.96	-0.85
3	344.66	350.23	1.62	349.21	1.32	352.08	2.15	342.80	-0.54
4	368.43	378.02	2.60	374.96	1.77	380.02	3.15	370.15	0.47
5	392.23	401.59	2.39	399.70	1.90	403.10	2.77	391.92	-0.08
6	414.95	423.04	1.95	421.96	1.69	428.90	3.36	414.09	-0.21
7	429.94	439.10	2.13	436.70	1.57	445.54	3.63	429.71	-0.05
8	450.71	456.52	1.29	455.55	1.07	465.37	3.25	444.44	-1.39
9	467.05	472.50	1.17	471.27	0.90	481.25	3.04	458.93	-1.74
10	476.82	487.96	2.34	479.81	0.63	497.41	4.32	471.94	-1.02
11	478.90	487.59	1.81	485.15	1.31	498.86	4.17	477.07	-0.38
12	486.73	498.56	2.43	494.44	1.58	507.64	4.30	486.56	-0.03
13	495.72	509.29	2.74	505.36	1.94	518.18	4.53	495.39	-0.07
14	501.20	512.89	2.33	511.33	2.02	527.05	5.16	502.45	0.25
15	511.81	520.16	1.63	519.99	1.60	536.12	4.75	508.60	-0.63
16	523.80	531.64	1.50	530.88	1.35	548.94	4.80	518.03	-1.10
17	530.21	537.78	1.43	537.56	1.39	555.96	4.86	522.80	-1.40
18	542.72	552.23	1.75	550.15	1.37	567.69	4.60	534.06	-1.60
19	549.78	558.00	1.50	557.68	1.44	577.07	4.96	541.21	-1.56
F1 Generation Males									
0	193.29	187.30	-3.10	189.96	-1.72	179.21	-7.28	125.53	-35.06
1	256.66	250.66	-2.34	252.31	-1.69	238.22	-7.18	175.56	-31.60
2	314.48	309.49	-1.59	310.28	-1.34	298.41	-5.11	220.48	-29.89
3	363.95	359.01	-1.36	358.43	-1.52	347.28	-4.58	267.15	-26.60
4	401.90	399.18	-0.68	397.69	-1.05	389.24	-3.15	305.54	-23.98
5	436.67	436.57	-0.02	434.71	-0.45	425.18	-2.63	337.15	-22.79
6	461.54	463.00	0.32	456.90	-1.01	450.00	-2.50	357.64	-22.51
7	488.11	490.24	0.44	483.41	-0.96	475.29	-2.63	380.85	-21.97
8	511.84	512.85	0.20	503.44	-1.64	499.41	-2.43	400.22	-21.81
9	533.85	534.88	0.19	521.90	-2.24	519.40	-2.71	417.11	-21.87
10	546.35	551.49	0.94	537.86	-1.55	536.80	-1.75	429.60	-21.37
11	553.17	558.11	0.89	544.12	-1.64	543.63	-1.72	437.79	-20.86
12	569.01	576.74	1.36	561.98	-1.24	561.74	-1.28	454.34	-20.15
13	582.68	590.50	1.34	571.96	-1.84	572.37	-1.77	462.26	-20.67
14	596.40	605.57	1.54	585.45	-1.84	587.11	-1.56	472.14	-20.84
15	611.08	619.98	1.46	598.62	-2.04	601.33	-1.60	485.88	-20.49
16	622.78	632.96	1.63	610.88	-1.91	613.30	-1.52	495.61	-20.42
17	635.20	643.29	1.27	620.30	-2.35	622.01	-2.08	502.60	-20.88
18	645.84	652.81	1.08	627.18	-2.89	630.47	-2.38	507.74	-21.38
19	658.36	665.25	1.05	635.88	-3.41	641.13	-2.62	517.02	-21.47

Table 5.6.1-3: Mean body weights of F0 and F1 males

Dose groups	0 ppm	5 ppm		25 ppm		750 ppm		5000 ppm	
Study weeks		Body weight [g]	Δ [%]	Body weight [g]	Δ [%]	Body weight [g]	Δ]%]	Body weight [g]	Δ [%]
F0 Generation - Females									
0	200.11	198.57	-0.77	195.74	-2.18	222.77	-3.73	194.89	-2.61
1	226.17	226.57	0.18	226.22	0.02	192.64	-2.00	208.6	-7.77
2	251.25	245.37	-2.34	245.5	-2.29	221.65	-3.66	229.75	-8.56
3	263.41	259.81	-1.37	260.3	-1.18	242.06	-3.23	240.59	-8.66
4	277.4	270.85	-2.36	273.2	-1.51	254.89	-2.43		
5	288.2	281.86	-2.20	283.77	-1.54	270.67	-2.40	261.9	-9.13
6	300.16	289.83	-3.44	294.36	-1.93	281.28	-3.01	271.1	-9.68
7	312.53	303.9	-2.76	306.35	-1.98	291.13	-2.92	277.93	-11.07
8	318.59	309.7	-2.79	311.91	-2.10	303.4	-2.75	282.35	-11.38
9	324.41	316.6	-2.41	317.91	-2.00	309.82	-1.90	288.34	-11.12
10	331.62	322.48	-2.76	325.81	-1.75	318.26	-2.47	296.57	-10.57
GD0	329.35	322.74	-2.01	326.34	-0.91	320.35	-2.73	293.75	-10.81
GD1	365.9	362.87	-0.83	362.34	-0.97	356.71	-2.51	320.81	-12.32
GD14	390.55	389.13	-0.36	388.04	-0.64	383.47	-1.81	343.96	-11.93
GD21	458.16	463.8	1.23	465.68	1.64	466.35	1.79	418.83	-8.58
LD1	329.35	322.74	-0.33	364.5	0.28	364.5	0.28	324.7	-10.67
LD4	365.9	362.87	-0.48	372	-0.24	367.8	-1.37	322.4	-13.54
LD7	390.55	389.13	-0.59	372.3	-0.19	366.1	-1.85	322.1	-13.65
LD14	458.16	463.8	-0.53	383.7	0.87	374.5	-1.55	320.8	-15.67
LD21	329.35	322.74	-1.49	363.4	-1.44	359.2	-2.58	328.5	-10.90
F1 Generation Females									
0	151.48	150.37	-0.73	148.11	-2.22	144.14	-4.85	102.41	-32.39
1	178.42	178.92	0.28	176.46	-1.10	171.05	-4.13	133.6	-25.12
2	203.78	202.94	-0.41	200.02	-1.85	194.93	-4.34	155.51	-23.69
3	222.37	221.15	-0.55	221.21	-0.52	214.27	-3.64	174.22	-21.65
4	238.82	238.82	0.00	237.66	-0.49	232.84	-2.50	190.66	-20.17
5	252.45	253.59	0.45	255	1.01	250.32	-0.84	204.27	-19.08
6	261.12	263.67	0.98	263.67	0.98	259.62	-0.57	213.17	-18.36
7	273.32	273.69	0.14	274.61	0.47	270.13	-1.17	221.09	-19.11
8	281.71	283.11	0.50	284.38	0.95	280.16	-0.55	229.22	-18.63
9	286.95	287.65	0.24	289.55	0.91	284.74	-0.77	234.53	-18.27
10	290.71	293.56	0.98	294.09	1.16	291.13	0.14	239.07	-17.76
GD0	290.8	297.14	2.18	292.14	0.46	296.9	2.10	236.27	-18.75
GD1	324.59	331.83	2.23	324.26	-0.10	326.74	0.66	255.45	-21.30
GD14	352.72	362.3	2.72	348.76	-1.12	355.96	0.92	273.81	-22.37
GD21	426.97	435.33	1.96	418.44	-2.00	434.01	1.65	335.59	-21.40
LD1	334.7	346.2	3.44	335.6	0.27	337.3	0.78	271	-19.03
LD4	351.3	358	1.91	344.7	-1.88	349.2	-0.60	260.1	-25.96
LD7	349.4	355.3	1.69	346.6	-0.80	349.8	0.11	258.1	-26.13
LD14	352.3	357.2	1.39	350.7	-0.45	356.2	1.11	255.3	-27.53
LD21	334.6	341.2	1.97	335.1	0.15	338.3	1.11	264.2	-21.04

Marked decreases in food consumption were reported in F0 females receiving 5000 ppm throughout gestation, and in F1 males and females receiving 5000 ppm during premating, and throughout gestation and lactation, respectively. There were also sporadic reductions in food consumption amongst F1 males receiving 25 and 750 ppm, but there was no consistent dose-related trend.

Effects on fertility:
In the F0 generation, there were no treatment-related effects on mating and fertility index, conception rate (no. pregnant animals/no. mated animals) and gestation index (no. live litters born/no. animals pregnant) at any dose level.
The tables below summarize the reproduction and gestational parameter of F0 and F1 females.

Table 5.6.1-4: Reproduction and gestational parameters of female and male F0 and F1 rats

Parental generation		**F0**				
Dose	[ppm]	0	5	25	750	5000
Animals per dose		28	28	28	28	28
Female fertility						
- placed with males		27	28	28	28	28
- mated	[n]	26	28	28	27	28
- mating index	[%]	96	100	100	96	100
HCD (F_0/F_1: 90-100%)[1]						
- pregnant	[n]	23	27	22	25	28
- **Fertility index**	[%]	85	96	79	89	100
HCD (F0/F1 76 – 96%)[1]						
Male fertility						
- placed with females		27	28	28	28	28
- mated	[n]	26	28	28	27	28
- mating index	[%]	96	100	100	96	100
HCD (F_0/F_1: 90-100%)[1]						
- pregnant	[n]	23	27	22	25	28
- **Fertility index**	[%]	85	96	79	89	100
HCD (F0/F1 76 – 96%)[1]						
Pre coital interval	[days]	2.12	2.81	3.32	2.81	2.61
Duration of gestation	[days]	22.1	22.1	22.2	22.0	22.6*
Females with liveborn		23	27	22	25	26
- **Gestation index**	[%]	100	100	100	100	93
- with stillborn pups	[n]	5	6	2	6	14
- with all stillborn	[n]	0	0	0	0	1
Pups delivered	[n]	335	419	341	411	382
- per dam	[mean n]	14.57	15.52	15.50	16.44	14.15
- liveborn	[n]	315	411	337	403	321
- stillborn	[n]	18	7	2	7	46
- **Live birth index**	[%]	93	98**	99*	98**	82**
Parental generation		**F1**				
Dose	[ppm]	0	5	25	750	5000
Animals per dose		28	28	28	28	28
Female fertility						
- placed with males		28	28	28	27	28
- mated	[n]	28	28	26	27	20**
- mating index	[%]	100	100	93	100	71
HCD (F_1/F_2: 81-100 %)[1]						
- pregnant	[n]	26	28	25	25	18**
- **Fertility index**	[%]	93	100	89	93	64**
HCD (F1/F2 76 – 96%)[1]						

Male fertility						
- placed with females		28	28	28	27	28
- mated	[n]	28	28	26	27	20**
- mating index	[%]	100	100	93	100	71
HCD (F1/F2: 81-100 %)[1]						
- pregnant	[n]	26	28	25	25	18**
- **Fertility index**	[%]	93	100	89	93	64**
HCD (F1/F2 76 – 96%)[1]						
Pre coital interval	[days]	3.07	3.18	2.54	3.38	3.30
Duration of gestation	[days]	22.1	22.2	22.1	22.1	22.6
Females with liveborn		26	28	25	25	16
- **Gestation index**	[%]	100	100	100	100	89
- with stillborn pups	[n]	4	4	2	4	3
- with all stillborn	[n]	0	0	0	0	1
Pups delivered	[n]	381	408	343	388	203
- per dam	[mean n]	14.65	14.57	13.72	15.52	11.94**
- liveborn	[n]	376	401	338	380	178
- stillborn	[n]	4	4	3	7	11
- **Live birth index**	[%]	99	98	99	98	85**

* p ≤ 0.05; ** p ≤ 0.01 (Dunnet-test, two sided or Fisher's exact test, one sided)
Values may not calculate exactly due to rounding of values
[1]Historical control data: DocID C044926

With regard to male and female fertility, there were no changes in fertility observed in the F0 generation, however the mating (no. animals inseminated/no. animals paired) and the fertility index (no. animals pregnant/animals paired) was decreased in the top dose of the F1 generation. 8 out of 28 males did not mate, resulting in a mating index of 71%, being lower than the historical control data (HCD F1/F2: 81 – 100%). Also the fertility index with 64% was below the historical controls (HCD F0/F1 and F1/F2: 76-96%). The two apparently infertile F1 high dose males (#84836, 84845; that mated but did not produce pregnancy) did not show histopathological findings that could explain infertility (testes and epididymides). The most obvious difference between the F0 and the F1 animals is a substantial higher substance intake in the F1 generation (males & females) and an apparently substantially higher systemic toxicity in the F1 animals, expressed as up to 30% lower body weights shown for males of the F1 generation and almost no effects in the F0 generation (see above).

During the last European Peer Review of triticonazole, it was discussed, whether a deficiency of hormonal secretion – produced by ovaries and adrenals – could be responsible for a weak or absent female sexual response, as supposedly indicated by degeneration of the adrenal cortex observed in females treated with 5000 ppm triticonazole. It was further stated, that - in contrast to laboratory animals - human female sexual behavior does not depend entirely upon the direct action of such hormones, but is facilitated by the central nervous system. Comparing the incidences of the F0 females with the F1 females, 22/24 F0 females show degenerative signs in the adrenals cortex compared to only 11/28 affected F1 females. Giant cell formation in the ovary was only seen in 1/24 females from the F0 generation and 2/28 females from the F1 generation. Evaluating the individual histopathological sheets, only 3 out of the 8 F1 females (#C84860, #C84862, #C84871, #C84872, #C84873, #C84877, #C84878, #C84879) treated with 5000 ppm triticonazole, which were not mated, showed adrenal cortex degenerative effects or giant cells (nos. #C84873, #C84879 and #C84878) and only of these animals showed ovary vacuolation and the presence of giant cells (#C84878; see Table 5.6.1-8). The severity of the adrenal findings in females decreases with increasing exposure duration; this was also observed in the rat carcinogenicity study (see Chapter MCA 5.5 and 5.8). Thus, an adrenal or hormone systemic contribution to the observed lower mating and fertility indices is highly unlikely from the available data (see Chapter MCA 5.8).

There were no effects on fertility that were considered treatment-related at 5, 25 or 750 ppm for the both generations.

The mean duration of gestation was slightly, but significantly increased at 5000 ppm (22.6 days compared to 22.1 days in the control group) in the F0 generation. A summary of the gestation times for the F0 and the F1 generations is given in the Table below:

Table 5.6.1-5: Summary of gestation times for the F0 and F1 generations in the 2 generation study in rats

	Number of animals with gestation time (days)									
	F0					F1				
	21	22	23	24	25	21	22	23	24	25
0 (control)	-	20	3	-	-	-	23	3	-	-
5 ppm	3	18	4	1	-	1	21	6	-	-
25 ppm	-	18	4	-	-	2	17	4	-	-
750 ppm	3	19	2	-	-	-	21	3	-	-
5000 ppm	1	11	13	1	1	-	10	5	1	1

The increased mean duration of gestation is due to two females of the 5000 ppm dose group, which had gestation times of 24 (#C79524) and 25 days (#C79528). As mentioned above female no. #C79528 had to be sacrificed moribund after prolonged parturition, however the other female had surviving pups. Prolonged gestation times are a known toxicological features of compounds with aromatase inhibiting properties, however triticonazole has very weak (compared to other azoles) aromatase inhibiting properties (between factors 35 - 50000-fold lower for human aromatase, than positive controls; for details see Chapter MCA 5.8; BASF DocID 2015/1197309). Furthermore, there is a considerable difference between rat and human aromatase inhibition measured for triticonazole, indicating a >20-fold lower potency of triticonazole to inhibit human aromatase compared to rat aromatase, even no complete inactivation of the human aromatase could be achieved by the maximum (technically achievable) concentration of triticonazole.

In addition, triticonazole intake for females was comparable between premating and gestation periods, but during the 7-14 day lactation period when highest values were observed, the compound intake increased due to the increased food consumption representing 1.8 fold and 1.3 fold increases over the pre-mating compound intake values for the F0 and F1 dams respectively. This marked increase in compound intake during the lactation phase has contributed to the strong toxicity observed in dams indicated by a marked decrease in body weight gain and high treatment-related mortality in F0 females (14%), and likely contributed to reduced pup viability at this dose. An apparent increase in the duration of gestation at 5000 ppm are within the range of historical control data, and occurred in the presence of pronounced parental toxicity, which gives some evidence, that this is not a specific endocrine-related effect, but rather occurring secondary to general systemic toxicity. At this dose group, there was no consistent correlation between dam survival or pup deaths and the duration of gestation neither in the F0 nor in the F1-parental generation.

There were no significant differences in conception rate, gestation index and duration of gestation occurred in any dose group.

Litter data:
There were a number of effects on litter parameters at 5000 ppm seen in the F1 and F2 generations. The livebirth index (no. live pups at day 1/no. born pups) and viability index (no. pups alive on day 4/no. liveborn pups) were significantly lower for both F1 and F2 litters at this dose level compared with those of the respective controls whereas the total number of stillborn F1 and F2 pups was increased (see Table 5.6.1-4). In addition, total litter death was noted for four F0 females given 5000 ppm during lactation between days 0 – 4, and the mean number of live pups/litter with live pups on days 0 and 4 (precull) of lactation were all significantly reduced among F2 litters at 5000 ppm. Necropsy of dead pups showed that no milk in the stomach was the only notable finding (see Table 5.6.1-6).

Table 5.6.1-6: Incidence of gross necropsy observations in F1 and F2 pups

Dose [ppm]	0	5	25	750	5000
			F1 pups		
Litters evaluated	22	25	22	25	21
Pups evaluated	217	299	240	307	203
- Live	205	292	238	300	183
- Stillborn	12	7	2	7	20
No milk in stomach	16 (5)	7 (6)	1 (1)	9 (8)	26 (11)
Milk in stomach	1 (1)	3 (2)	2 (2)	3 (2)	1 (1)
Total pup necropsy observations	17	10	5	14	30
- % affected pups/litter	27	32	12	44	62
			F2 pups		
Litters evaluated	26	28	25	25	17
Pups evaluated	335	363	300	339	141
- Live	331	359	297	332	130
- Stillborn	4	4	3	7	11
No milk in stomach	3 (3)	2 (2)	5 (4)	6 (4)	13 (5)
Milk in stomach	2 (1)	1 (1)	1 (1)	2 (2)	2 (2)
Total pup necropsy observations	14	12	13	14	15
- % affected pups/litter	42	25	32	36	35

* $p \leq 0.05$, ** $p \leq 0.01$ (Wilcoxon-test, one-sided)
( ) values in brackets give litter incidence

The effects on pup survival in the 5000 ppm dose group are considered to be directly related to maternal toxicity, as the compound intake is higher in the F1 vs the F0 generation and highest in the lactational phase of the F0 and the F1 dams (substance intake of 502.99 mg/kg bw during lactation vs 389.3 during premating for the F0 dams and 528.05 mg/kg bw during lactation vs. 493.8 mg/kg bw during premating for the F1 dams, see Table 5.6.1-1). Furthermore, there is no individual correlation between the marginally observed extended gestation lengths and pup survival, which further suggest a general systemic effect.

Mean pup bodyweights were significantly lower for male and female F1 and F2 pups at 5000 ppm on days 4, 7, 14 and 21 (see table 5.6.1-7), F2 pups being more affected than F1 pups.

Table 5.6.1-7: Mean pup weights (F1 and F2 litters)

Dose level	Males					Females				
	Day 0	Day 4	Day 7	Day 14	Day 21	Day 0	Day 4	Day 7	Day 14	Day 21
F1 Litters (Body weight [g] (SD))										
0 ppm	6.46 (0.69)	9.94 (1.93)	17.33 (2.74)	36.31 (4.51)	59.25 (6.82)	6.03 (0.58)	9.44 (1.41)	16.10 (2.68)	34.28 (4.35)	54.98 (5.96)
5 ppm	6.55 (0.76)	10.70 (1.57)	17.98 (2.21)	37.07 (3.52)	59.32 (5.27)	6.16 (0.58)	10.16 (1.43)	17.17 (2.29)	35.63 (3.21)	56.77 (4.41)
25 ppm	6.59 (0.64)	10.95 (1.11)	18.43 (1.81)	37.96 (3.01)	61.11 (4.71)	6.17 (0.62)	10.42* (1.33)	17.39 (1.77)	36.21 (2.53)	57.99 (3.93)
750 ppm	6.40 (0.56)	10.47 (1.01)	17.65 (1.42)	36.50 (2.68)	58.35 (4.21)	6.10 (0.51)	10.06 (0.82)	16.81 (1.30)	34.89 (2.46)	55.41 (3.95)
5000 ppm	6.14 (0.81)	9.86 (1.60)	15.21** (2.16)	26.83** (4.10)	41.82** (7.22)	5.85 (0.68)	8.97 (1.51)	14.13** (2.16)	25.54** (4.04)	39.27** (6.66)
F2 Litters (Body weight [g] (SD))										
0 ppm	6.35 (0.49)	10.56 (1.32)	17.32 (1.43)	34.62 (2.27)	56.21 (4.02)	5.94 (0.43)	10.01 (1.30)	16.52 (1.59)	33.60 (2.61)	53.96 (4.08)
5 ppm	6.58 (0.55)	11.12 (1.13)	18.25 (1.99)	36.32* (3.07)	58.57 (4.83)	6.18 (0.49)	10.63 (1.02)	17.55 (1.72)	35.04 (2.50)	56.10 (3.41)
25 ppm	6.38 (0.66)	10.49 (1.65)	17.69 (2.01)	35.95 (2.45)	58.43 (4.63)	6.11 (0.63)	10.21 (1.54)	16.84 (1.86)	34.45 (2.67)	55.38 (4.58)
750 ppm	6.42 (0.55)	10.68 (1.30)	17.55 (1.40)	35.36 (1.68)	57.31 (3.31)	6.07 (0.54)	10.24 (1.19)	16.65 (1.53)	34.17 (2.26)	54.76 (3.89)
5000 ppm	5.71** (0.47)	7.83** (1.35)	12.07** (1.55)	19.81** (1.83)	28.49** (4.01)	5.37** (0.55)	7.16** (0.90)	11.28** (1.16)	18.67** (1.73)	26.66** (3.62)

*p<0.05, **p<0.01

This is in accordance with maternal body weight development (see above), where also the F1 maternal generation showed stronger body weight effects, than the F0 females, which is again reflecting the higher substance intake of the F1 parental vs the F0 parental generation. It is further evident, that the body weight effects increase in severity, when pups start self-feeding (around PND 12; [redacted] BASF DocID 2008/1102837). During the last European Review, the litter effects observed in the top dose only were discussed and assessed as followed: As the reduction in the number of pups born, reduced pup viability and pup weights were observed only in litters from females dosed at 5000 ppm, these effects were considered to be the consequence of maternal toxicity and not considered as specific signs of pup toxicity. Furthermore, no developmental changes were observed in offspring, even, when parental animals were treated at the highest dose. There were no effects on litter data that were considered treatment-related at 5, 25 or 750 ppm for both generations.

Pathology:
At necropsy of parental F0 animals, absolute and relative left adrenal weights were significantly lower and absolute and relative liver weights were significantly higher for F0 females at 5000 ppm compared with controls. Microscopic findings in the adrenals comprised increased incidence and severity of vacuolation in the adrenal cortex for males, and degenerative changes of the adrenal cortex in females (vacuolation, syncytial giant cell formation, deposition of collagen, large pigment laden cells, and minimal inflammation). Similar organ weight changes of liver and adrenals and microscopic findings were observed following necropsy of F1 parental animals with the addition of vacuoles and giant cells present in the ovaries in four females which were considered treatment-related. It is important to note, that only one of the females with ovary pathological effects did not become pregnant or was pregnant and did not deliver pups (#C84878). A summary of the histopathological changes in parental F0 and F1 animals is given in the Table (Table 4.5.1-8) below.

Table 5.6.1-8: Group incidences of histopathological changes in parental F0 and F1 animals of the 2-generation toxicity study in rats

Sex	Males					Females				
Dose [ppm]	0	5	25	750	5000	0	5	25	750	5000
Animals in group	28	28	28	27	28	27	28	28	28	24
F_0 - Generation										
Adrenals # examined	28	28	28	27	28	27	28	28	28	24
- Degeneration, cortical cells characterized by vacuolation and loss of individual cells	0/28	0/28	0/28	0/27	0/28	0/27	0/28	0/28	0/28	22/24
- Vacuolation, cortex	7/28	6/28	4/28	6/27	27/28	0/27	0/28	1/28	1/28	0/24
Ovaries # examined						27	1	6	3	24
-Vacuolation F0						0/27				0/24
-Giant cells F0						0/27				1/24
F_1-Generation										
Adrenals # examined	28	28	28	27	28	27	28	28	28	24
- Degeneration, cortical cells characterized by vacuolation and loss of individual cells	0/28	0/28	0/28	0/28	0/27	0/28	1/27	0/28	0/27	11/28
- Vacuolation, cortex	15/28	9/28	8/28	9/28	27/27	0/28	1/27	0/28	0/27	0/28
Ovaries # examined						27	1	6	3	24
-Vacuolation F1						0/27				4/24*
-Giant cells F1						0/28				2/24*

*overall 4 females were affected (#C84857 and #C84878 showed giant cells and vacuolation, #C84858 and #C84864 showed vacuolation)

There were no treatment-related macroscopic findings present for F1 and F2 weanlings at this dose level. In addition, no treatment-related effects were observed in parental animals given 5, 25 and 750 ppm.

Conclusion:
The NOAEL for both parental toxicity and reproductive effects in this study is 750 ppm (equivalent to 49.35 (♂) and 48.41 (♀) mg/kg bw/d), based on maternal mortality, reduced body weight and necropsy findings in adrenals, liver and ovaries in parental animals, and on significant adverse effects on reproductive parameters and on survival and growth of offspring at 5000 ppm, consistently observed across both generations. The severity of the adrenal effects in females decrease with longer exposure duration (F1 females are less affected than the F0 females). The decreased fertility and mating index observed in the F1 generation at the 5000 ppm dose group and not in the F0 generation is related to considerably higher substance intake and subsequent parental toxicity at that dose. During the premating phase F1 males have substance intakes of 445.3 mg/kg bw compared to 350 mg/kg bw in the F0 generation. F1 males show considerably stronger effects on lower body weight (gain) gain compared to F0 males, demonstrating higher systemic toxicity of F1 vs F0 males. The corresponding values in females are 493.8 mg/kg bw (F1 females) vs 389.3 mg/kg bw in the F0 females. It is a secondary effect. The effects on pup growth correlate with maternal body weight effects and increase in severity, when pups start self-feeding and are also not an indicator for developmental toxicity.
In conclusion, there is evidence that reproductive parameters like female fertility, number of live born pups and pup viability are adversely affected by triticonazole at a very high dose level, exceeding the maximum tolerated dose. However, no effects on reproductive parameters were seen in the absence of maternal toxicity. Therefore, the adverse effects on the reproductive function is the consequence of distinct maternal toxicity.

CA 5.6.2 Developmental toxicity studies

Triticonazole - Teratology study in the rat ([redacted], C018955)

Guidelines: According to US EPA guideline 83-3
Deviations: According to the most recent OECD TG 414, the recommended administration period is covering the whole gestation period and should continue until the day prior to scheduled caesarean section. Otherwise the study conducted in 1991 broadly met the requirements of the 2001 version of the guideline. The study protocol is nevertheless considered to be able to identify potentially azole-induced teratogenicity and a repeat of the study was – also in the interest of animal welfare - considered not needed.
GLP: Yes
Acceptance: The study was considered acceptable in the EU registration process 2003

Material and method:
Groups of 23 presumed pregnant rats (strain: CD of Sprague Dawley origin; source: [redacted]) received triticonazole (batch no YG 2156/1; purity 99.5 %; suspended in 0.5% w/v aqueous methylcellulose) from day 6 to 15 of pregnancy by oral gavage at a constant dosing volume of 10 mL/kg bw. Dose levels were 0 (vehicle control), 40, 200 and 1000 mg/kg bw/d. (These dose levels were based on the results of a preliminary study [reported in summary form only] with dose levels of 0, 50, 250 and 1250 mg/kg bw/d given to groups of 6 pregnant rats.) Dosing suspensions were prepared freshly every day and concentrations were confirmed by analysis.
Observations for mortality and clinical signs were made daily. Body weights were recorded on days 0, 3, 6, 6 – 16, 18 and 20 of gestation, food consumption was measured over two to four day periods on days 3, 6, 9, 12, 16, 18 and 20 of gestation. On gestation day 20, females were subjected to a post-mortem macroscopic examination. The reproductive tract was examined for the number of corpora lutea/ovary, implantation and resorption sites, number and distribution of live and dead fetuses, weight and sex of each fetus and external malformed fetuses. Half of the fetuses from each litter were processed for the examination of skeletal abnormalities and the remainder processed for visceral abnormalities.

Findings:
Preliminary study:
At the top dose level (1250 mg/kg), treatment-related effects included slightly impaired bodyweight gain of females, reduced mean fetal weight and slight increase in placental weight. No effects on these parameters were observed at the dose levels of 50 and 250 mg/kg. It was stated in the summary that there was a low but apparently dosage-related increased incidence of hydronephrosis in fetuses in all three dose groups, but detailed figures were not given. It was mentioned by the study author that in view of the small group sizes and the small number of litters and fetuses affected, the apparent association with treatment was considered to be equivocal.

Main study - maternal effects:
There were no deaths among treated females and no treatment-related clinical signs of toxicity were observed in any dose-group. At the highest dose level, slight maternal toxicity was reflected in marginally, but consistently lower body weight gains (p<0.05) during the treatment period compared to controls, particularly during the later-part of the study, resulting in mean body weight of approx. 96 % of controls at termination (see Table 5.6.2-1).

Table 5.6.2-1: Food consumption and body weight development in rats administered triticonazole from gestation 6 to 15

Dose level [mg/kg bw/day]	0	40	200	1000
Food consumption [g/animal/day]				
Day 0 to 2	25	26	26	25
□%		4.0	4.0	0.0
Day 3 to 5	27	28	28	27
□%		3.7	3.7	0.0
Day 6 to 8	29	29	29	27
□%		0.0	0.0	-6.9
Day 9 to 11	31	31	31	29
□%		0.0	0.0	-6.5
Day 12 to 15	33	33	33	31
□%		0.0	0.0	-6.1
Day 16 to 17	37	37	36	35
□%		0.0	-2.7	-5.4
Day 18 to 19	36	35	35	33
□%		-2.8	-2.8	-8.3
Body weight [g]				
Day 0	226	227	228	225
□%		0.4	0.9	-0.4
Day 6	270	273	272	265
□%		1.1	0.7	-1.9
Day 15	347	351	348	336
□%		1.2	0.3	-3.2
Day 20	437	439	434	419
□%		0.5	-0.7	-4.1
Body weight gain [g]				
Day 0 to 6	44	46	44	40
□%		4.5	0.0	-9.1
Day 6 to 15	77	78	76	71
□%		1.3	-1.3	-7.8
Day 15 to 20	90	88	86	83
□%		-2.2	-4.4	-7.8
Day 0 to 20	211	212	206	194
□%		0.5	-2.4	-8.1

* p < 0.05, ** p < 0.01 (Dunnett test, two-sided)

In addition, food intake was marginally reduced throughout the treatment period at 1000 mg/kg. There were no effects on these parameters at the two lower dose levels. At necropsy, pale areas on the median or lateral liver lobes were noted in 0, 0, 2 and 2 animals at 0, 40, 200 and 1000 mg/kg, respectively (see Table 5.6.2-2).

Table 5.6.2-2: Gross necropsy findings in rats administered triticonazole during gestation days 6 to 15

Dose group	Animal #	Observation
Control	10	raised white areas on surface of spleen
	22	Unilateral hydronephrosis
	23	Uterus distended with yellow fluid
40 mg/kg bw/day	46	Red/brown staining on head/nose/ears
	42	Scab on upper lip
	28	Unilateral hydronephrosis
	28	Unilateral hydroureter
200 mg/kg bw/day	69	Red/brown staining on head/nose/ears
	63, 64	(Punctate) pale areas on surface of median/lateral liver lobes
1000 mg/kg bw/day	81, 96, 99	Red/brown staining on head/nose/ears
	99	Hair-loss on upper fore-limb
	80, 97	(Punctate) pale areas on surface of median/lateral liver lobes

There were no other findings considered treatment-related at terminal necropsy.

Main study - litter data/fetal parameters:

The number of implantations and of the viable young, the extent of pre- and post-implantation losses, and the mean foetal and placental weights gave no indication of any response to treatment in any dose group (see Table 5.6.2-3). The caesarean section data are summarized below:

Table 5.6.2-3: Caesarean section data of rats

Dose level [mg/kg bw/d]	0	40	200	1000
Pregnancy status				
- mated [n]	25	25	25	25
- pregnant [n]	25 (24, one female died on day 8)	24	24	23
conception rate [%]	100	96	96	92
- aborted [n]	0	0	0	0
- dams with viable fetuses [n]	24	24	24	23
- dams with all resorptions [n]	0	0	0	0
- mortality	1	0	0	0
- pregnant terminal sacrifice [n]	24	24	24	23
Cesarean section data[a]				
- Corpora lutea [n]	16.8	17.8	16.7	16.1
- Implantation sites [n]	15.4	16.1	15.7	15.3
- Pre-implantation loss [%]	8.4	10.4	7.6	6.4
- Post-implantation loss [%]	4.6	5.4	5.3	7.7
- Resorptions [n]	0.7	0.9	0.8	1.2
- Early resorptions	0.7	0.9	0.8	1.2
- Late resorptions	0.0	0.0	0.0	0.0
- Dead fetuses [n]	0	0	0	0
- Live fetuses	14.7	15.3	14.9	14.1
- Total live female fetuses [n]	7.0	6.8	7.3	6.6
- Total live male fetuses [n]	7.6	8.4	7.6	7.5
- Percent live females	48	44.8	49	46.9
- Percent live males	52	55.2	51	53.1
Placental weights [g]	0.56	0.53	0.56	0.56
Mean fetal weight [g]	3.74	3.74	3.73	3.77
- males [g]	3.83	3.84	3.84	3.87
- females [g]	3.64	3.61	3.61	3.67

[a] Mean ± SD on litter basis; Statistical evaluation: * $p \le 0.05$; ** $p \le 0.01$ (Dunnett-test, two-sided)

Examination of the foetuses at necropsy showed that the pattern of external gross abnormalities did not suggest any effect of treatment. In addition, no effects of treatment were identified at fetal examination for visceral abnormalities. The incidence of hydronephrosis (unilateral plus bilaterial) was slightly increased in the top dose group (3 % of the fetuses affected) compared with controls (1.1 % of the fetuses affected), but was well within the historical control range (0 – 6.5 %) for these findings in the laboratory.

Examination of the fetuses for skeletal abnormalities revealed a dose-related increase in the incidence of foetuses with an additional thoracolumbar rib (14th rib) or pair of ribs (14/14), slightly exceeding the historical control at 1000 mg/kg bw dose group, but being not statistically signficant. The incidences of unilateral or bilateral 14th rib was slightly higher than the concurrent control values also at 40 and 200 mg/kg, did also not achieve statistical significance and were within the historical background range collected from studies conducted before and after (1991 – 1994) the time, where the present study ran. The incidences of the skeletal findings is summarized in the table below:

Table 5.6.2-4: Incidence of skeletal findings in rats

Dose level [mg/kg]	0	40	200	1000
Litters Evaluated	24	24	24	23
Fetuses Evaluated	352	366	357	324
Live				
Dead				
Individual skeletal findings				
- discrete unossified area in frontal bone				
- Fetal incidence [%]	0.6	0.5	0.5	1.8
HCD: 0.0-2.4				
- Litter incidence [No.]	1	1	1	2
- Affected fetuses/litter (Mean ± SD) [%]	12.50	14.29	11.11	20.53 (11.36)
- incomplete ossification of hyoid bone				
- Fetal incidence [%]	3.9	7.9	5.9	9.6
HCD: 0.0-14.8				
- Litter incidence [No.]	3	9	10	11
- Affected fetuses/ litter (Mean ± SD) [%]	21.43 (6.18)	20.84 (15.41)	13.85 (5.40)	17.70 (7.55)
Ribs 13/14				
- Fetal incidence [%]	11.7	13.2	13.4	16.9
*HCD: 0.0-15.9**				
- Litter incidence [No.]	11	18	13	15
- Affected fetuses/ litter (Mean ± SD) [%]	25.61(9.96)	18.10 (9.06)	22.86 (11.25)	26.08 (14.06)
- Ribs 14/14				
- Fetal incidence [%] *HCD: 1-12**	5.0	9.0	9.7	14.5
- Litter incidence [No.]	3	6	9	9
- Affected fetuses/ litter (Mean ± SD) [%]	34.26 (9.75)	33.23 (11.98)	24.15 (19.27)	37.27 (18.60)
- 14th rib or enlarged rib				
- Fetal incidence [%] HCD: 0.0-8.3*	0.0	0.5	0.5	1.2
- Litter incidence [No.]	0	1	1	2
- Affected fetuses/ litter (Mean ± SD) [%]		10	9.09	15.48 (1.69)

*Historical background data collected in 16 studies conducted approximately up to the time of the present study and in 91 studies conducted between 1991 and 1994.

On the more important litter basis, no conclusive changes are seen between the controls and the treatment groups. When calculating the parameter "affected fetuses/litter", no conclusive difference between the incidences of unilateral or bilaterally 14th ribs between controls and treated groups are noted. This gives some evidence, that the observed non-statistically significant increased fetal incidences are incidental and not treatment related. The litter incidences and affected fetuses/litter incidences of the findings "discrete unossified area in frontal bone" and "incomplete ossification of hyoid bone" were only slightly affected by treatment with triticonazole, however the historical control data on fetal incidences is covering the observed 1.8% of fetuses showing this finding. Overall, there are some doubts on the treatment-relationship of the skeletal findings. Also the observed maternal toxicity could have contributed to very marginally increased incidences in skeletal variations, as there is some evidence in the literature, that increased incidences of supernumerary ribs are related to maternal toxicity and not a specific developmental effect [see KCA 5.6.2/1 1988/1003516].

Conclusion:
The NOAEL for maternal toxicity in this study can be considered to be 40 mg/kg bw/d based on slight reduction in body weight gain and food consumption at 1000 mg/kg bw/d, and incidences of pale areas in the liver of two animals each at 200 and 1000 mg/kg bw/d. Fetal survival and growth was not affected in any dose group. However, there was an apparently increase in the incidence of fetuses with an additional 14th rib or pair of ribs at all dose levels (not regarded as a major malformation), but was only outside the historical range at 1000 mg/kg bw/d. The fetal NOAEL was set at 200 mg/kg bw/d. There was no teratogenic effect observed at any dose level.

Triticonazole - Teratology study in the rabbit ([redacted]; C018959)

Guidelines: According to US EPA guideline 83-3
Deviations: According to the most recent OECD TG 414, the recommended administration period is covering the whole gestation period and should continue until the day prior to scheduled caesarean section. Otherwise the study conducted in 1991 broadly met the requirements of the 2001 version of the guideline. The study protocol is nevertheless considered to be able to identify potentially induced teratogenicity and a repeat of the study was – also in the interest of animal welfare - considered not needed.
GLP: Yes
Acceptance: The study was considered acceptable in the EU registration process 2003

Groups of 20 pregnant does/dose (strain: New Zealand White; source: [redacted]) were treated with triticonazole (batch no.YG2156/1; purity 99.51 %; suspended in 0.5 % w/v methyl cellulose in distilled water) from day 6 to 19 of pregnancy by oral gavage. The dose levels of 0 (vehicle control), 5, 25, 50 and 75 mg/kg bw/d (with a dosing volume of 5 mL/kg) were selected following examination of results in a preliminary range-finding study (not included in the submission). Stability and homogeneity of the test formulation was confirmed periodically by analysis. All animals were examined daily throughout the study for clinical signs and mortality. Body weights of the does were recorded daily and food consumption was recorded over 5-day intervals from day 1 to day 28 of gestation. On day 29 of pregnancy, all females were examined macroscopically for abnormalities, for the presence and number of corpora lutea, implantation sites, resorption sites (early or late) and of live or dead fetuses. Live fetuses were weighed, sexed and examined for external abnormalities in the neck, thorax and abdominal cavities. Following this examination, the fetuses were eviscerated and the heads of one-third of the fetuses in each litter were removed for subsequent examination following random serial sectioning. Torsos and the remaining intact fetuses were processed for skeletal examination using Dawsons Alizarin staining technique.

Findings:
Maternal effects:
There were seven deaths during the study considered treatment-related. One female at 50 mg/kg and 6 females at 75 mg/kg were sacrificed in extremis between day 13 to 18 following marked weight loss, reduced food intake and reduced faecal output, reduced body temperature and red staining in the cage undertray. In addition, abortion in one female receiving 75 mg/kg was also considered treatment-related (see Table 5.6.2-5).

Table 5.6.2-5: Disposition of animals

Dose level [mg/kg bw/d]	0	5	25	50	75
Total number inseminated	**20**	**20**	**20**	**20**	**20**
Deaths	0	1	0	2	6
Not pregnant	0	2	0	2	0
Abortion	0	1	0	0	1
Total litter loss	0	0	2	0	0
Disposition	20	16	18	16	13

Other clinical signs of maternal toxicity (increased respiration rate, reduced faecal output; see Table 5.6.2-6) were noted at 50 and 75 mg/kg.

Table 5.6.2-6: Clinical observations in rabbits administered triticonazole during gestation days 6 to 28

Dose level [mg/kg]	Animal #	Gestation Day	Observation
Control	2 (#472, 485)	9-29	Increased respiration
5 mg/kg	4 (#439, 471, 493, 508)	8, 16-29	Increased respiration
	3 (#442, 473, 508)	16-25, 28-29	Few faeces in undertray
25 mg/kg	2 (#468, 504)	11-15, 19-29	Increased respiration
	6 (#459, 465, 509, 534, 440, 511)	18-29	Few faeces in undertray
50 mg/kg	5 (#425, 443, 467, 470, 499)	11-29	Increased respiration
	7 (#414, 443, 467, 470, 497, 515)	9-19	Few faeces in undertray
75 mg/kg	5 (#257, 423, 494, 502, 488)	7-29	Increased respiration
	15 (257, 423, 444, 466, 479, 494, 502, 505, 516, 445, 448, 455, 488, 521, 541)	7-19	Few faeces in undertray

Dosage-related body weight loss was observed in dams receiving 25, 50 and 75 mg/kg bw/d during the first two days of treatment. Thereafter, body weight gain was essentially similar to controls in all dose groups. Food intake was reduced throughout the treatment period at > 50 mg/kg, and to a marginal extend also at 25 mg/kg. Figures and tables illustrating the food consumption and body weight development are given below.

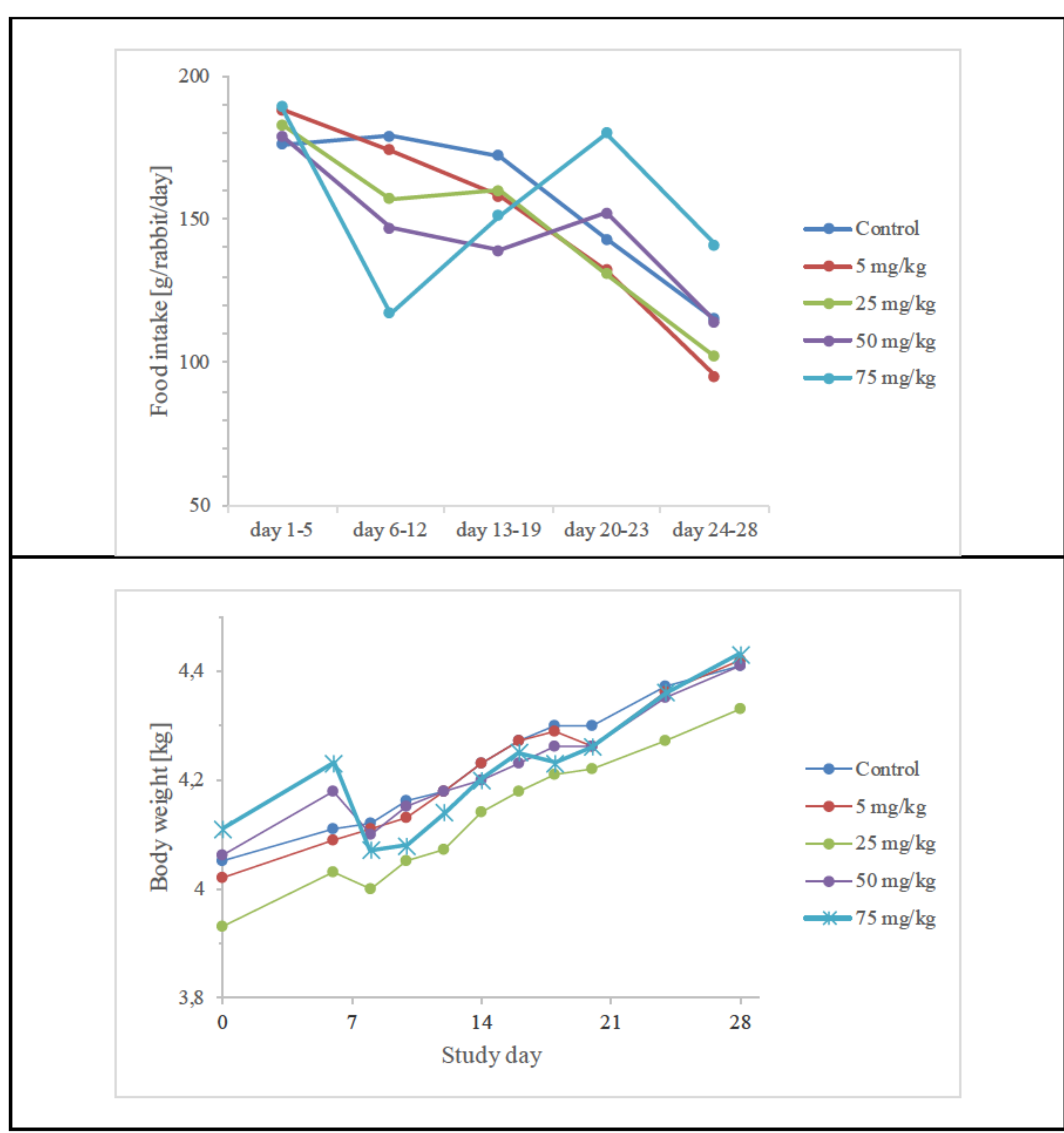


Figure 5.6.2-1: Food consumption and body weight development in rabbits administered triticonazole during gestation days 6 to 28

Table 5.6.2-7: Food consumption and body weight development in rabbits administered triticonazole during gestation days 6 to 28

Dose level [mg/kg]	0	5	25	50	75
Food consumption [g/animal/day][§]					
Day 1-5 □%	176	188 6.8	183 4.0	179 1.7	189 7.4
Day 6-12 □%	179	174 -2.8	157 -12.3	147 -17.9	117 -34.6
Day 13-19 □%	172	158 -8.1	160 -7.0	139 -19.2	151 -12.2
Day 20-23 □%	143	132 -7.7	131 -8.4	152 6.3	180 25.9
Day 24-29 □%	115	95 -17.4	102 -11.3	114 -0.9	141 22.6
Body weight gain [g]					
Day 0-6 □%	0.06	0.07 16.7	0.1 66.7	0.12 100.0	0.12 100.0
Day 6-20 □%	0.19	0.17 -10.5	0.19 0.0	0.08 -57.9	0.03 -84.2
Day 20-28 □%	0.11	0.16 45.5	0.11 0.0	0.15 36.4	0.17 54.5

** $p \leq 0.01$ (Dunnett-test, two sided)

At necropsy on day 29 of gestation, no macroscopic changes considered to be treatment-related were observed in the dams. Triticonazole was clearly too toxic to the rabbit does (with deaths and related abortions) at the two top doses.

Litter data/fetal parameters:
A slight, but not statistically significant increase in pre- and postimplantation losses was observed at 75 mg/kg, but was covered by historical control data. Furthermore, early resorptions were slightly increased in the 75 mg/kg bw test group, only slightly exceeding historical control data. The effects were considered to be related to maternal toxicity and not an indication of a specific developmental toxicity [see KCA 5.6.2/2 2013/1420380] (see Table 5.6.2-8).

Table 5.6.2-8: Pregnancy status and caesarean section data of dams treated with triticonazole during gestation days 6 - 28

Dose level [mg/kg bw/d]		0	5	25	50	75
Pregnancy status						
Females						
- mated	[n]	20	20	20	20	20
- pregnant	[n]	20	17	20	16	14
conception rate	[%]	100	85	100	80	70
- aborted	[n]	0	1	0	0	1
- dams with viable fetuses	[n]	20	16	18	16	13
- dams with all resorptions	[n]	0	0	2	0	0
- mortality		0	1	0	2	6
- pregnant terminal sacrifice	[n]	20	16	18	16	13
Cesarean section data[a]						
- Corpora lutea	[n]	12.7	12.8	12.1	13.6	12.5
- Implantation sites	[n]	10.3	11.6	10.4	11.2	8.9
- Pre-implantation loss *HCD: 8.9-43.0*	[%]	18.9	9.7	14.2	17.9	28.8
- Post-implantation loss *HCD: 4.8-21.7*	[%]	13.6	8.1	7.4	16.2	20.7
- Early resorptions *HCD: 0.1-1.4*	[n]	1.0	0.4	0.6	1.6	1.5
- Late resorptions	[n]	0.5	0.4	0.7	0.3	0.3
- Dead fetuses	[n]	0.0	0.2	0.1	0.0	0.0
- Live fetuses		8.9	10.7	9.7	9.4	7.1
- Total live female fetuses	[n]	3.8	4.6	4.3	4.6	3.6
- Total live male fetuses	[n]	5.2	6.1	5.4	4.8	3.5
- Percent live females		42.1	42.9	44.3	49.3	51.1
- Percent live males		57.9	57.1	55.7	50.7	48.9
Placental weights	[g]	5.1	5.1	5.3	5.2	5.2
Mean fetal weight	[g]	39.8	39.8	39.4	39.1	39.7
- males	[g]	40.7	40.0	39.3	39.5	40.5
- females	[g]	36.3	39.3	39.3	37.9	39.4

All other litter parameter were similar in all groups. In addition, the pattern of incidences of gross abnormalities and visceral anomalies did not indicate any treatment-related effect.

The high dose findings “frontal bone fusion and other major cranial anomalies” and “two or more caudal vertebrae fused and/or reduced, short kinky tail” (see Table below) were not considered to be indicative for a teratogenic response of triticonazole. One fetus from doe no. 89FR444, which showed “frontal bone fusion and other major cranial anomalies” was multiply malformed and should therefore be taken out of the calculation for the specific finding “frontal bone fusion and other major cranial anomalies”. In that case the litter incidence reduces to one and the fetal incidence to 1.6, which is also covered by historical controls, as the given percentages indicate, that there were studies, where at least one fetus was affected. As further considerations, it is important to note, that the doses, where single fetuses (2 for the frontal bone fusion and 2 for the fused caudal vertebrae) was excessively toxic to the does, leading to 58% and 84% lower body weight gains during days 6-20 of gestation (see Table 5.6.2-7 and Fig. 5.6.2/1) and even to maternal deaths. In recent guidelines (e.g. OECD TG 414) it is stated that severe maternal toxicity (e.g. reduced body weight gain > 20%, inanition, moribundity or mortality) should be avoided, since fetal wellbeing is compromised, information on developmental effects may be difficult to interpret and of limited value. It is possible, that maternal circulation was severely affected during specific phases of gestation leading to insufficient oxygen or nutrient supply to the embryo ([redacted]).

There were increased fetal incidences of treatment-related findings at 75 mg/kg consisting of minor abnormalities of the midline anterior cranial bones, rudimentary floating 13th rib, incomplete ossification of metacarpals and phalanges and elongation of the acromion process of the scapula (which is better to say: a precocious ossification of the acromion process) (see Table 5.6.2-9).

Table 5.6.2-9: Incidence of skeletal findings[1)]

Dose level [mg/kg]	0	5	25	50	75
Individual skeletal observations					
Head					
Litters Evaluated[1)] Fetuses Evaluated[1)]	20 124	16 118	18 121	15 103	13 63
- Frontal bone fusion and other major cranial anomalies					
- Fetal incidence [(%)] *HCD: 0.0-1.5*	0.0	0.0	0.0	1.0	3.2 **or 1.6**
- Litter incidence [N]	0	0	0	1	2 **or 1**[2)]
- Affected fetuses/litter (Mean ± SD) [%]	0	0	0	16.67	**14.29**[2)]
Anterior fontanelle extended anteriorly					
- Fetal incidence [(%)] *HCD: 0.0-1.9*	0.8	0.0	0.0	1.0	6.3
- Litter incidence [N]	1	0	0	1	2
- Affected fetuses/litter (Mean ± SD) [%]	33.33	0	0	16.67	47.5 (38.89)
Additional suture in nasal bone					
- Fetal incidence [(%)] *HCD: 0.0-1.2*	0.0	0.0	0.8	0.0	1.6
- Litter incidence [N]	0	0	1	0	1
- Affected fetuses/litter (Mean ± SD) [%]	0	0	16,67	0	25
- Irregular ossification of frontal suture					
- Fetal incidence [(%)] *HCD: 0.0-9.1*	5.6	2.5	1.7	5.8	17.5
- Litter incidence [N]	5	3	2	4	8
- Affected fetuses/litter (Mean ± SD) [%]	22,86 (11.65)	13.69 (1.03)	12.15 (3.03)	27.78 (16.20)	26.91 (12.69)

Dose level [mg/kg]	0	5	25	50	75
- Frontal suture enlarged at fronto-nasal junction					
- Fetal incidence [(%)] *HCD: 0.0-1.9*	0.0	0.0	0.0	0.0	3.2
- Litter incidence [N]	0	0	0	0	1
- Affected fetuses/litter (Mean ± SD) [%]	0	0	0	0	50
- Additional plaque of bone in nasal structure					
- Fetal incidence [(%)] *HCD: 0.0-1.5*	0.0	0.0	0.0	0.0	6.3
- Litter incidence [N]	0	0	0	0	4
- Affected fetuses/litter (Mean ± SD) [%]	0	0	0	0	21.07 (8.50)
- Lachrymal fossa enlarged					
- Fetal incidence [(%)] *HCD: 0.0-3.8*	0.8	0.0	0.0	1.0	4.8
- Litter incidence [N]	1	0	0	1	3[2)]
- Affected fetuses/litter (Mean ± SD) [%]	16.67	0	0	10	19.65[2)] (7.57)
Sternebrae and ribs					
Litters Evaluated[1)]	20	16	18	16	13
Fetuses Evaluated[1)]	178	171	174	150	92
- rudimentary floating 13th rib or ribs					
- Fetal incidence [(%)] *HCD: 0.7-8.5*	3.9	2.3	6.3	3.3	10.9
- Litter incidence [N]	5	3	9	5	6
- Affected fetuses/litter (Mean ± SD) [%]	13.56 (7.75)	10.25 (2.78)	11.74 (5.28)	10.19 (3.93)	20.27 (9.37)
Vertebrae, limbs and girdles					
Litters Evaluated[1)]	20	16	18	16	13
Fetuses Evaluated[1)]	178	171	174	150	92
- Two or more caudal vertebrae fused and/or reduced, short kinky tail					
- Fetal incidence [(%)] *HCD: 0.0-0.8*	0.0	0.0	0.0	0.0	2.2
- Litter incidence [N]	0	0	0	0	2
- Affected fetuses/litter (Mean ± SD) [%]	0	0	0	0	12.7 (2.25)
- One or both acromion process elongated (=precocious ossification of acromion process)					
- Fetal incidence [(%)] *HCD: 0.0-1.0*	1.7	0.0	3.4	5.3	10.9*
- Litter incidence [N]	1	0	4	5	4
- Affected fetuses/litter (Mean ± SD) [%]	7.69	0	20.51 (10.94)	12.25 (4.08)	30.83[2)] (17.46)
- Metacarpals and phalanges incomplete ossified or unossified					
- Fetal incidence [(%)] *HCD: 2.8-26.0*	23.0	12.9	13.8	34.7	38.0
- Litter incidence [N]	12	7	10	11	11
- Affected fetuses/litter (Mean ± SD) [%]	24.41 (19.14)	17.58 (10.11)	18.69 (7.75)	35,26 (20,24)	44.33 (30.71)

*$p<0.05$
1)only two-third of foetuses have been evaluated in the skeletal examinations of the heads (as one-third of the heads underwent a specific investigation, see above), for the other parts of the body, all foetuses were investigated
2)one out of two fetuses of the high dose group (#89FR444) showing the malformation “frontal bone fusion and other major cranial anomalies” and the variation “lachrymal fossa enlarged” was multiply malformed and was therefore not included in the calculation of the affected foetuses/litter percentage

The variations in the midline cranial structures involving mostly the bones and sutures of the anterior cranium and the “rudimentary floating 13th rib or ribs” were only very slightly increased or “affected fetuses/litter values” were close to the concurrent control values and in some of the findings only one or 2 fetuses of the high dose group showed this finding (“additional suture in nasal bone”, frontal suture enlarged at fronto-nasal junction”), leaving some doubts on the treatment relationship (see Table 5.6.2-9). As mentioned above, the maternal toxicity was excessive at the 50 and at the 75 mg/kg bw doses, where some very marginally increased incidences of variations were observed. It is not unlikely, that the maternal toxicity has contributed to the observed increased incidences of variations, due to possibly insufficient nutrient or oxygen supply to the embryo during specific phases of gestation and should therefore not be considered indicative for a specific teratogenic response of triticoanzole.

With regard to the finding “metacarpals and phalanges incomplete ossified or unossified” the litter incidences do not indicate a relationship to treatment, however the fetal incidences and the “affected fetuses/litter” percentages are slightly increased at ≥ 50 mg/kg bw. A relationship to maternal toxicity is more likely, as Cappon et al., also found increased incidences of unossified metatarsals and metacarpals in offspring of feed-restricted New Zealand white rabbits [see KCA 5.6.2/3 2005/1044140]. The fact, that, there is no correlation seen to any potentially corresponding external malformations on the fore- or hind-limbs, or digits in this study, gives further evidence, that this is only a variation with no impact on development.

When compared with control groups, a dose-related increase of elongated acromion process of the scapula was also evident at ≥ 25 mg/kg bw. Concerning the findings of the acromion process of the scapula at the dose levels of 25 and 50 mg/kg, it was stated in the report that the incidences at these dose levels, when compared with concurrent controls, did not approach statistical significance, and considered doubtful whether these differences were the result of treatment with triticonazole. However, considering the clear dose-response in increase with incidences exceeding both concurrent control and historical control range values, relation to treatment can be assumed also at 25 and 50 mg/kg dose levels. The acromion is the lateral processus on the head of the spine of the scapula, which is normally cartilaginous (see Figure 5.6.2/2).

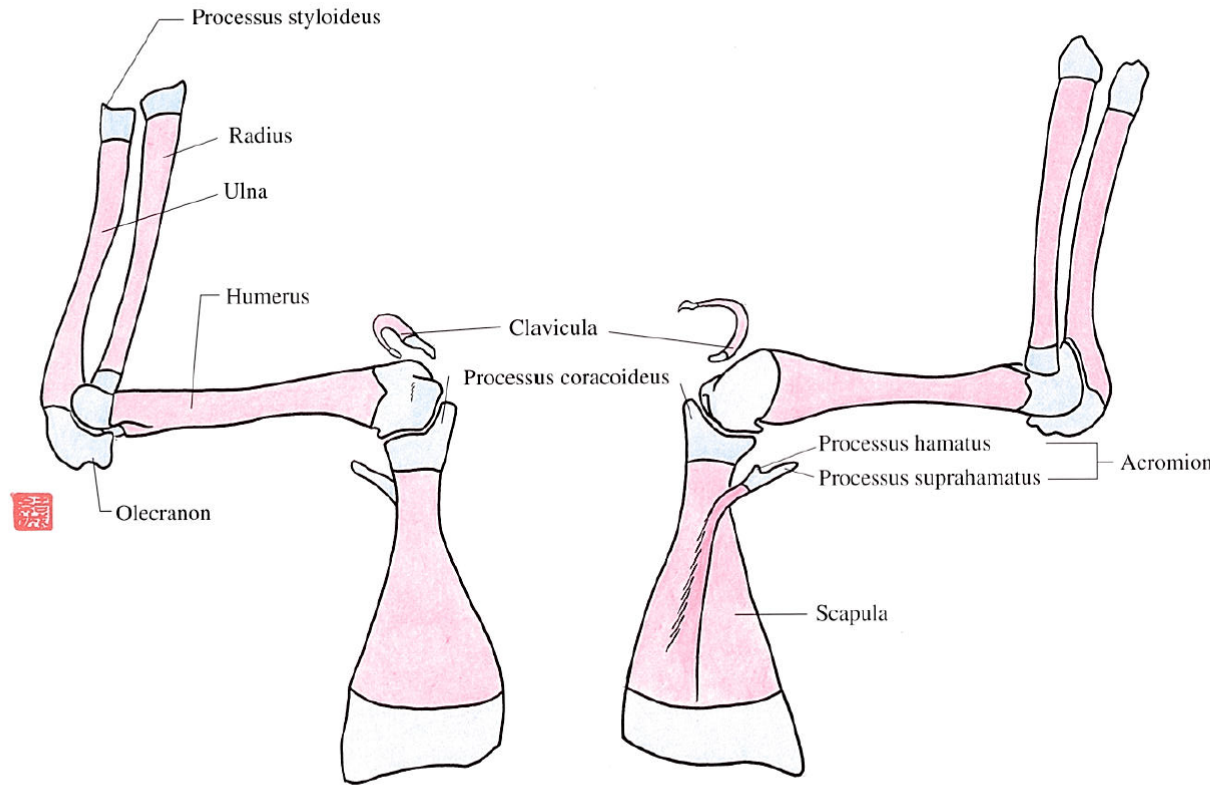


Figure 5.6.2/2: Schematic picture of a rabbit´s scapula (ossified parts are coloured in red, cartilaginous parts are colored in blue)

Reference: Yasuda M, Yuki T (1997) Color atlas of fetal skeleton of the mouse, rat and rabbit

If only the ossified parts of the skeleton is stained – as is the case in this study – the acromion process seems to be elongated, however this has been more accurately be described in the results section of the study as a "precocious ossification of the acromion process". As this part of the skeleton is ossified later in development of the young animals, the increased incidences of precocious ossification of acromion process seen at ≥ 25 mg/kg bw are not considered to affect development or survival of the animals.

Conclusion:
Triticonazole caused body weight losses and decreased food consumption in pregnant rabbits at doses ≥ 25 mg/kg bw/d. Both top doses (50 and 75 mg/kg bw caused excessive maternal toxicity indicated by deaths, abortions, decreased feces and an increased respiration rate. The MTD was clearly exceeded in the top dose group with > 10 % maternal mortality occurring. Concerning fetal findings, a slight increase in both pre- and post-implantation losses was observed at 75 mg/kg bw, which is considered to be related to maternal toxicity. Increased incidences of precocious ossification of acromion process (=elongation of acromion process) were seen at ≥ 25 mg/kg bw/d. The precocious ossification of the acromion process is of low severity, as this part of the scapula is ossified during development of the offspring and an earlier ossification has no impact on survival or quality of life. In the top dose increased incidences of variations of the midline anterior cranial bones, rudimentary floating 13th rib, and reduced/incomplete ossification of metacarpals and phalanges (also at 50 mg/kg bw) were seen at excessive maternal toxic doses. As these variations occur only in the presence of excessive maternal toxicity, they are not indicative of a specific teratogenic response of triticoanzole. There was no teratogenic effect observed at any dose level. The maternal NOAEL is 5 mg/kg bw based on body weight loss and reduced food intake at the next higher dose level. The $NOAEL_{development}$ is 5 mg/kg bw/d.

Report: CA 5.6.2/4
BAS 595 F (Triticonazole) - Chicken embryotoxicity screening test (CHEST)
2010/1177161
Guidelines: none
GLP: no

Executive Summary

Triticonazole (Batch: COD-000601, Purity: 90.3%) was tested in vitro for its embryotoxic potential using the Chicken Embryotoxicity Screening test (CHEST) in concentrations of 0.1, 1 and 10 mg/mL in DMSO. Each concentration and the vehicle control were applied to 8 embryos in 3 independent experiments. After 24-hour exposure, mortality, blood vessel and embryo development, blood vessel coloration and malformation were assessed.
In comparison to the incidence in vehicle treated embryos, the test substance caused no effects on mortality, blood vessel development, embryo development and blood vessels discoloration at all concentration tested. Taking in to account the above described endpoint evaluation, the overall evaluation for embryotoxic potential of the test item was negative.
Based on the results of the present study, triticonazole showed no signs of embryotoxicity in the Chicken Embryotoxicity Screening Test (CHEST) under the conditions reported.

(BASF DocID 2010/1177161)

Remark

The chicken embryotoxicity screening test (CHEST) is performed as an in house screening study. Based on an in house validation study assessing 53 compounds, it was demonstrated, that the CHEST assay could be used as an indicator test for embryotoxicity with an overall concordance of 85%. In a post-validation process, conclusive results were obtained for 68 non-corrosive and non-acute toxic compounds out of 88 compounds tested. In conclusion, the overall concordance of the CHEST assay is 87%.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material Triticonazole (BAS 595 F)

Description:	solid / white
Lot/Batch #:	COD-000601
Purity:	90.3%
Stability of test compound:	not specified
Solvent:	DMSO

B. STUDY DESIGN AND METHODS

1. Dates of experimental work: not specified, finalization date: 13-Sep-2010

2. Experimental procedure:

Fertilized white leghorn chicken eggs (SPF) were pre-incubated for about 48 hours and synchronized for development. The eggs were windowed and 10 µL of the test substance preparation were injected directly below the embryo (*area pellucida*). The study was performed with 3 test substance concentrations (0.1, 1 and 10 mg/mL) and a vehicle control (2% DMSO). Each concentration and the vehicle control were applied to 8 embryos in 3 independent experiments (24 eggs per concentration). The eggs were sealed with tape and re-incubated for 24 hours. The endpoints mortality, blood vessel and embryo development, blood vessel coloration and malformation were assessed. For the qualitative examination of endpoints the findings were compared to the vehicle control data.

II. RESULTS AND DISCUSSION

In comparison to the incidence in vehicle treated embryos, the test substance caused no effects on mortality, blood vessel development, embryo development and blood vessels discoloration at all concentration tested.

Table 5.6.2-10: Mean results of the CHEST endpoints

Endpoint	Dose group [mg/mL]			
	0.1	1	10	Historical control
Mortality				
Finding (%)	0	0	0	4
SD	0	0	0	7
Evaluation				
Run 1	-	-	-	
Run 2	-	-	-	
Run 3	-	-	-	
All runs	-	-	-	
Reduced vessel development				
Finding (%)	4	0	8	14
SD	7	0	7	12
Evaluation				
Run 1	-	-	-	
Run 2	-	-	-	
Run 3	-	-	-	
All runs	-	-	-	
Reduced embryo development				
Finding (%)	0	0	4	2
SD	0	0	7	6
Evaluation				
Run 1	-	-	-	
Run 2	-	-	-	
Run 3	-	-	-	
All runs	-	-	-	
Discolored blood vessel				
Finding (%)	13	0	8	21
SD	13	0	7	14
Evaluation				
Run 1	-	-	-	
Run 2	-	-	-	
Run 3	-	-	-	
All runs	-	-	-	

Taking in to account the above described endpoint evaluation, the overall evaluation for embryotoxic potential of the test item was negative.

III. CONCLUSION

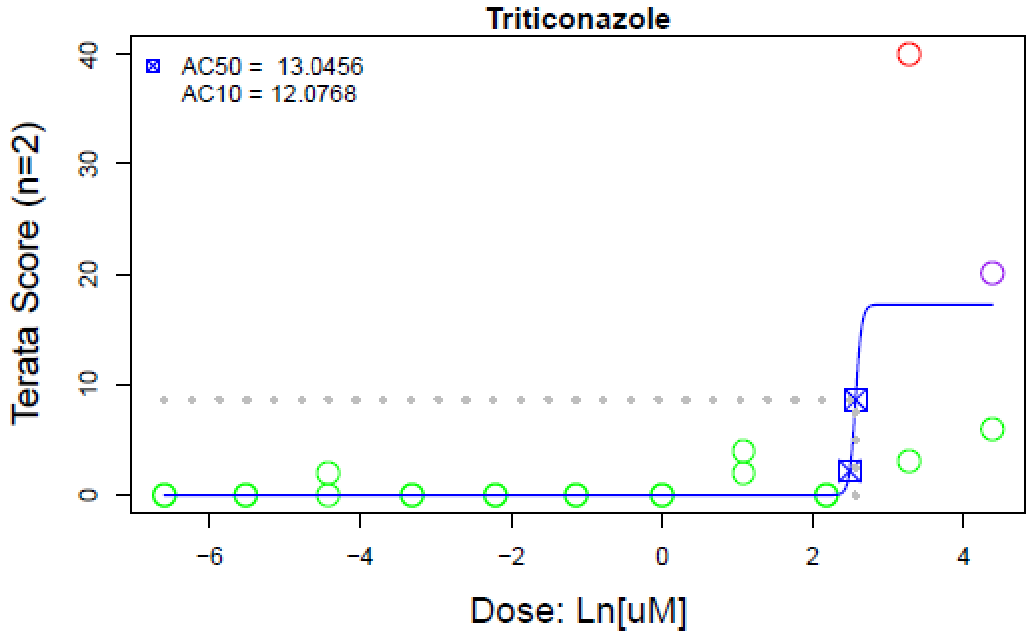
According to the results of the present study, the test substance Triticonazole showed no signs of embryotoxicity in the Chicken Embryotoxicity Screening Test (CHEST) under the conditions reported.

Supplemental information from published literature

Report: CA 5.6.2/5
[redacted]
Zebrafish developmental screening of the ToxCast Phase I chemical library
2012/1368722
Guidelines: none
GLP: no

Executive Summary of the Literature

Zebrafish (Danio rerio) is an emerging toxicity screening model for both human health and ecology. As part of the Computational Toxicology Research Program of the U.S. EPA, the toxicity of the 309 ToxCast™ Phase I chemicals was assessed using a zebrafish screen for developmental toxicity. All exposures were by immersion from 6–8 h post fertilization (hpf) to 5 days post fertilization (dpf); nominal concentration range of 1 nM–80µM. On 6 dpf larvae were assessed for death and overt structural defects. Results revealed that the majority (62%) of chemicals were toxic to the developing zebrafish; both toxicity incidence and potency was correlated with chemical class and hydrophobicity (logP); and inter-and intra-plate replicates showed good agreement. The numerical score groups into lethality (40), non-hatching (20) and malformation index (<20).
A toxicity score of 7 was calculated for triticonazole based on the single concentration study, indicating a positive response. An AC_{50} of 13.05 µM was derived for triticonazole in the dose-response experiment. In conclusion, equivocal effects were observed at high concentrations because of a wide variation of individual scores.



Classification of the study: supplemental information

Report: CA 5.6.2/6
[redacted]
Triazole-induced gene expression changes in the zebrafish embryo
2012/1369002
Guidelines: none
GLP: no

Executive Summary of the Literature

The zebrafish embryo is considered to provide a promising alternative test model for developmental toxicity testing. Most systems use morphological assessment of the embryos, however, microarray analyses may increase sensitivity and predictability of the test by detecting more subtle and detailed responses.

The purpose of the study was to investigate the possibility of relating gene expression profiles of structurally similar chemicals testid in a single concentration, to a complete transcriptomic concentration-response of flusilazole (FLU). Besides triticonazole also hexaconazole, cyproconazole, triadimefon and myclobutanil at equipotent concentrations (based on morphological alterations) have been tested. The gene expression profiles of triticonazole (TTC) was assessed at the highest possible concentration of 100 µM. Compared to the other azoles, this was the highest concentration tested. Zebrafish embryos within the 4- to 64 –cell stage were incubated with triticonazole solution. Cultures were checked for morphology and parallel cultures were used for gene expression analysis.

Triticonazole induced very little morphological effects and showed a low absolute average fold change. It was stated, that the **nonembryotoxic** triticonazole induces a 4.7% decrease in GMS (general morphology score) at 100 µM, the highest concentration testable. Also the absolute average fold change (aaFC) and the expression of all upregulated genes was very low and similar to the low flutamide concentration.

Slight induction (1.2-1.4 fold) was observed for genes of transcriptional, or retinoid metabolism pathways, however to a much lesser degree for triticoanzole compared to the other triazoles. The genese indicative for fatty acid metabolism were also slightly upregulated (roughly 1.2-fold). A higher fold-increase of 1.5 was observed for the steroid biosynthesis pathways, triticonazole showing the highest effect compared to the other triazoles in this test system. It was discussed, that triticoanzole, that the cyp51 expression and steroid biosynthesis regulation is high compred to other other azoles and that together with the low retinol metabolism regulation, it is suggested, that this compound has the least unwanted effects with respect to developmental toxicity. Based on 205 concentrations-response genes correlated to GMS, triticonazole also appeared as the least potent compound.

Conclusion:

Triticonazole is considered to be non-embryotoxic, based on very little morphological changes induced in zebra fish embryos. The results of the gene expression data and the concentration-response genes correlated to GMS (general morphology score) suggest, that triticoanzole has the least unwanted effects – compared to other azoles – with respect to developmental toxicity.

Classification of the study: supplemental information

Report: CA 5.6.2/7
[redacted]
Relative embryotoxicity of two classes of chemicals in a modified zebrafish embryotoxicity test and comparison with their in vivo potencies
2011/1297791
Guidelines: none
GLP: no

Executive Summary of the Literature

The zebrafish embryotoxicity test (ZET) is a fast and simple method to study chemical toxicity after exposure of the complete vertebrate embryo during embryogenesis in ovo. A novel quantitative evaluation method to assess the development of the zebrafish embryo based on specific endpoints in time, the general morphology score (GMS) system was used. Morphological evaluation of the embryos was performed at 72 h post fertilization (hpf). The GMS scoring system used is similar to the one used for rat Whole-embryo-cultures and comprises the normal development of a zebrafish embryo up to 72 hpf (detachment of tail, somite formation, eye development, movement, heartbeat, blood circulation, pigmentation head-body, pigmentation tail, pectoral fin, protruding mouth, hatching). The semiquantitative assessment of specific developmental endpoints supports standardization of the evaluation. An experimental embryo is compared to the reference embryo in the scoring matrix and receives points for each developmental hallmark dependent on its stage of development. All deviations, for instance incomplete detachment of the tail, will result in a lower point score which corresponds to a certain extent of developmental retardation.

Malformations and other teratogenic effects are separately recorded as present or absent according to a list provided (pericardial edema, yolk sac edema, eye edema, malformation of the head, malformation of saccule/otoliths, malformation of tail, malformation of heart, modified chorda structure, scoliosis, rachischisis, yolk deformation) with the effects scored as present or absent.
Zebrafish embryos within the 4- to 32-cell stage were incubated with triticonazole solution. Cultures were checked for morphology 72 h post fertilization. In this study triticonazole (TTC) has been tested and compared with other 1,2,4-triazoles (flusilazole (FLU), hexaconazole (HEX), cyproconazole (CYP), triadimefon (TDF), myclobutanil (MYC)).

For triticonazole benchmark concentrations of 80.5 µM and 40 µM were derived for general morphology and teratogenicity. Triticonazole showed minor effects only in the highest concentration tested and was indicated as the least potent triazole tested for general developmental toxicity. With regard to specific teratogenicity in this test system, triticonazole showed did not cause 100% teratogenicity at the highest concentration tested in contrast to the other compounds (see Figure below).

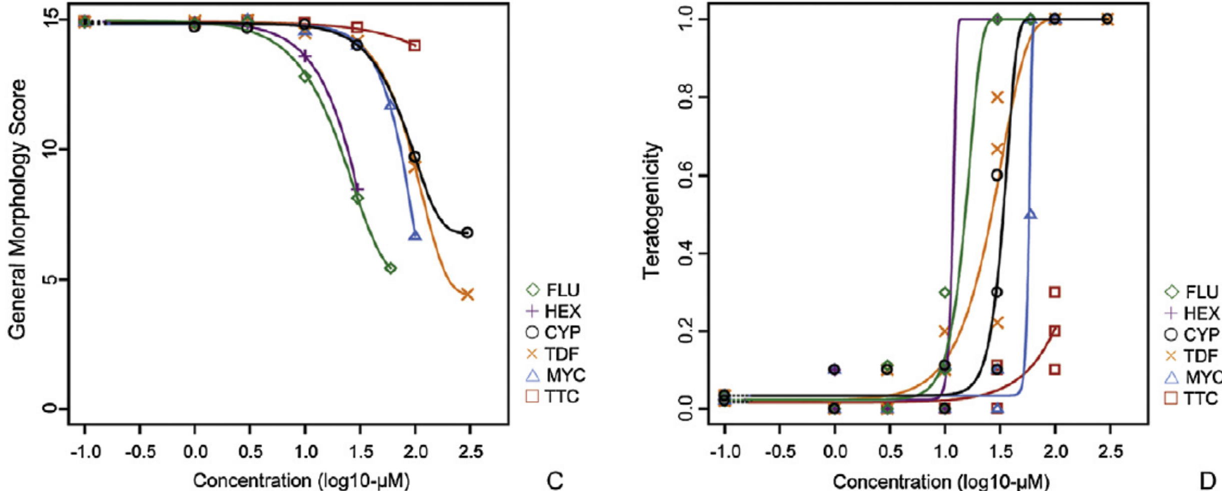


Figure 5.6.2-3: Concentration response curves of general morphology score and teratogenicity (fraction of embryos with at least one teratogenic effect) scored at 72 h post fertilization

Conclusion:
Triticonazole was found to be the least potent triazole with regard to general developmental and to specific teratogenic endpoints in a zebrafish embryotoxicity test, where zebrafish embryos have been evaluated 72 h post post fertilization.

Classification of the study: supplemental information

Report: CA 5.6.2/8
[redacted]
Comparison of the mouse embryonic stem cell test, the rat whole embryo culture and the zebrafish embryotoxicity test as alternative methods for developmental toxicity testing of six 1,2,4-triazoles
2011/1297792
Guidelines: none
GLP: no

Executive Summary of the Literature

Triticonazole was investigated in three widely studied alternative assays for developmental toxicity, the mouse Embryonic Stem cell Test (EST), the Zebrafish Embryotoxicity Test (ZET) and the rat post-implantation Whole Embryo Culture (WEC). Again in this comparative study 5 further 1,2,4-triazoles were tested in all test systems (flusilazole, hexaconazole, cyproconazole, triadimefon, and myclobutanil). For EST an established cell line was used, while the WEC and the ZET used mammalian and non-mammalian embryos, respectively. The results were analyzed using the Benchmark Dose (BMD) approach and compared with known BMD for skeletal variations and developmental lowest effect levels (dLEL) derived from an in vivo study from the ToxREF database (EPA). The rat developmental toxicity study conducted with triticonazole ([redacted]; DocID C018955) has been evaluated.
For Triticonazole benchmark concentrations of 35.8, 272.1 and 80.5 µM were derived for the differentiation of embryonic stem cells into beating cardiomyocytes (EST) and on embryonic development using total and general morphological score (WEC and ZET, respectively), respectively. Compared to the other test triazoles, triticonazole was least potent in the embryonic stem cell test and in the zebrafish assay (see above: Hermsen et al., 2011 and 2012) and together with cyproconazole also least potent in the Whole Embryo Culture test.
Also the in vivo results from rat developmental toxicity studies of the six triazoles have been compared. For triticonazole, the BMD_{10} for skeletal variations was calculated to be 3720 µmol/kg, which is above the highest dose tested in the in vivo rat [Crl:CD(SD)] teratology study [redacted]; DocID C018955). It was stated, that all triazoles with the exception of triticonazole, caused malformations, such as cleft palate, renal malformations and hydrocephaly.

Conclusion:

With exception of WEC (here cyproconazole was least potent followed by triticonazole), EST and ZET assays correctly identified the potency of triticonazole for developmental effects, that were the lowest of six tested 1,2,4-triazole compounds (flusilazole, hexaconazole, cyproconazole, triadimefon, myclobutanil and triticonazole) based on in vivo data.

Classification of the study: supplemental information

Comparison with CLP criteria
2-generation toxicity study
A 2-generation toxicity study was conducted in Sprague Dawley rats using dietary doses of 5, 25, 750 and 5000 ppm triticonazole. The study was performed according to OECD TG 416 (1981) and did not use an appropriate dose adjustment during lactation and early post-weaning in order to account for age- and life-stage-related differences in food consumption. Thus the corresponding doses were highly different between the F0 and the F1 generation and males and females during different life stages: The substance intakes during premating were 0, 0.34, 1.64, 49.35, 350.8 (males, F0), 0, 0.37, 1.82, 56.18, 445.3 (males, F1) mg/kg bw and 0.37, 1.81, 54.80, 389.3 (females F0) mg/kg bw; F1 0.43, 2.14, 65.25, 493.8 (females, F1) mg/kg bw. During gestation, the substance intakes in females were 0.32, 1.59, 48.41, 337.6 (gestation F0); 0.33, 1.60, 49.10, 339.08. During lactation the substance intakes increased to 0.58, 2.97, 87.99, 502.00 (females, F0) mg/kg bw, F1: 0.46, 2.96, 93.25, 528.05 (females, F1) mg/kg bw.
Parental toxicity was observed at 5000 ppm as maternal mortality, reduced body weight and necropsy findings in adrenals, liver and ovaries in parental animals, and on significant adverse effects on reproductive parameters and on survival and growth of offspring at 5000 ppm, consistently observed across both generations. The decreased fertility and mating index observed in the F1 generation at the 5000 ppm dose group and not in the F0 generation is related to considerably higher substance intake and subsequent parental toxicity at that dose. During the premating phase F1 males have substance intakes of 445.3 mg/kg bw compared to 350 mg/kg bw in the F0 generation. F1 males show considerably stronger effects on lower body weight (gain) gain compared to F0 males, demonstrating higher systemic toxicity of F1 vs F0 males. The corresponding values in females are 493.8 mg/kg bw (F1 females) vs 389.3 mg/kg bw in the F0 females. It is a secondary effect. The increased mean gestation lengths (from 22.6 days to 22.1 days) seen at 5000 ppm compared to controls is of borderline significance, as it is within historical controls. However as it is a known effects seen in rats dosed with compounds with aromatase inhibiting properties, it seems to be important to note, that the rat aromatase inhibiting properties of triticonazole are weak (compared to positive controls). Furthermore the binding to human aromatase is >20-fold lower compared to rat aromatase and does not lead to a full inhibition in an available aromatase inhibition assay (see Chapter MCA 5.8) at the maximum achievable triticonazole concentration. Thus the observed borderline effect observed in rats is considered to be of very low human relevance. The effects on pup growth correlate with maternal body weight effects and increase in severity, when pups start self-feeding and are also not an indicator for developmental toxicity.
In conclusion, there is evidence that reproductive parameters like female fertility, number of live born pups and pup viability are adversely affected by triticonazole at a very high dose level, exceeding the maximum tolerated dose. However, no effects on reproductive parameters were seen in the absence of maternal toxicity. Therefore, the adverse effects on the reproductive function is the consequence of distinct maternal toxicity. As such, no classification and labelling of triticonazole for reproductive toxicity for reproduction toxicity is warranted. That was confirmed by EFSA and the ECB.

Rat developmental toxicity study:
A developmental toxicity study in rats used gavage dose levels of 40, 200 and 1000 mg/kg bw. The NOAEL for maternal toxicity in this study can be considered to be 40 mg/kg bw/d based on slight reduction in body weight gain and food consumption at 1000 mg/kg bw/d, and incidences of pale areas in the liver of two animals each at 200 and 1000 mg/kg bw/d. Fetal survival and growth was not affected in any dose group.

However, there was an apparently increase in the incidence of fetuses with an additional 14th rib or pair of ribs at all dose levels (not regarded as a major malformation), but was only outside the historical range at 1000 mg/kg bw/d. The fetal NOAEL was set at 200 mg/kg bw/d. Incidences of additional 14th ribs are known common variations in rats and are not an indication of a specific teratogenicity. A classification for reproduction toxicity is not warranted.

Rabbit developmental toxicity study:
A rabbit developmental toxicity study was conducted at gavage doses of 5, 25, 50 and 75 mg/kg bw in New Zealand rabbits. Triticonazole caused body weight losses and decreased food consumption in pregnant rabbits at doses ≥ 25 mg/kg bw/d. Both top doses (50 and 75 mg/kg bw caused excessive maternal toxicity indicated by deaths, abortions, decreased feces and an increased respiration rate. At the top dose group > 10 % maternal mortality was observed. Concerning fetal findings, a slight increase in both pre- and post-implantation losses was observed at 75 mg/kg bw, which is considered to be related to maternal toxicity. Increased incidences of precocious ossification of acromion process (=elongation of acromion process) were seen at ≥ 25 mg/kg bw/d. The precocious ossification of the acromion process is of low severity, as this part of the scapula is ossified during development of the offspring and an earlier ossification has no impact on survival or quality of life. In the top dose increased incidences of variations of the midline anterior cranial bones, rudimentary floating 13th rib, and reduced/incomplete ossification of metacarpals and phalanges (also at 50 mg/kg bw) were seen at excessive maternal toxic doses. As these variations occur only in the presence of excessive maternal toxicity, they are not indicative of a specific teratogenic response of triticonazole. There was no teratogenic effect observed at any dose level. No classification for reproduction toxicity is warranted.

References

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Tyl RW, Crofton K, Moretto A, Moser V, Sheets LP, Sobotka TJ. (2008; BASF DocID 2008/1102837) Identification and interpretation of developmental neurotoxicity effects: a report from the ILSI Research Foundation/Risk Science Institute expert working group on neurodevelopmental endpoints. Neurotoxicol Teratol. 30(4):349-81.

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CA 5.7 Neurotoxicity Studies

Studies evaluated in the draft monograph of Rapporteur Member State Austria of February 2003:

Triticonazole does not belong to a chemical family for which testing for delayed neurotoxicity is required. However, there was indication of neurotoxicity seen at the top dose in the 1-yr dog study (tremors, ataxia, convulsions), but no microscopic findings in brain, spinal cord or ischiatic nerves were observed. For further clarification, studies on neurotoxicity after acute and repeated oral exposure to rats have been performed: In the acute oral neurotoxicity study in rats, no evidence for neurotoxicity was seen up to dose levels of 2000 mg/kg bw. Also after repeated dose administration via the diet, no neurobehavioral or neuro morphological effects occurred following 13 weeks of continuous exposure. Based on the study results a NOAEL of 10000 ppm (554 – 931 mg/kg bw/d for males and 704 – 937 mg/kg bw/d for females) was derived.

An acute and a subchronic neurotoxicity study in rats are available for triticonazole. These studies have been evaluated by European authorities and Austria as Rapporteur Member State (European Commission Peer Review Program) and were considered to be acceptable. For the convenience of the reviewer, these are summarized below as extracted from the monograph and brief summaries are provided under the respective chapters.

Table 5.7-1: Summary of already peer-reviewed triticonazole neurotoxicity studies as available in the DAR (2003)

Study Dose levels	NOAEL mg/kg bw/d	LOAEL mg/kg bw/d	Effects observed	Reference
Neurotoxicity studies				
Benchmark and time-to-peak neurotoxicity study Crl:CD:BR rat, 0, 50, 1000, 2000 mg/kg bw/d (gavage, MC)	-	-	motor activity slightly increased at 2000 mg/kg bw (time-to-peak 2-3 h) No time-to-peak effect for FOB	; DocID R012965
Acute neurotoxicity study Crl:CD:BR rat, 0, 80, 400, 2000 mg/kg bw/day (gavage, MC)	>2000 mg/kg bw	-	-	; DocID R012968
Subchronic neurotoxicity study, rat Crl:CD:BR rat, 0, 500, 2500, 10000 ppm for 13 weeks (equivalent to ranges of 25 – 47, 130 – 240 and 554 – 931 mg/kg bw/d for males and 32 – 49, 168 –250 and 704 – 937 mg/kg bw/d for females) (diet, MC)	>10000 ppm	-	-	; DocID R012967

Based on the available data, the following endpoint was determined during the last Annex I listing of triticonazole concerning neurotoxicity/delayed neurotoxicity:

Neurotoxicity / Delayed neurotoxicity

Effects observed	no evidence of neurotoxicity after acute (NOAEL > 2000 mg/kg bw) and subchronic (13 week) oral administration (NOAEL > 500 mg/kg bw/d) in rat

Submission of not yet peer-reviewed studies in this AIR3-Dossier:

No new neurotoxicity studies have been performed, and thus no change in the assessment of the endpoint "neurotoxicity/delayed neurotoxicity" is considered.

The following endpoint listing is proposed

Neurotoxicity

Acute neurotoxicity	No evidence of neurotoxicity after acute oral gavage administration of triticonazole to male and female Sprague Dawley rats (NOAEL > 2000 mg/kg bw)
Repeated neurotoxicity	No evidence of neurotoxicity after subchronic dietary (13 week) oral administration to male and female Sprague Dawley rats (NOAEL ≥ 10000 ppm; corresponding to ≥ 554 and 704 mg/kg bw in male and female rats).

CA 5.7.1 Neurotoxicity studies in rodents

Triticonazole – Benchmark and time-to-peak effect neurotoxicity study in rats ; DocID R012965)

Guidelines: According to US EPA guideline 82-7
GLP: Yes
Acceptance: The study was considered scientific valid, but due to limited parameters investigated it was considered only to be additional information in the EU registration process 2003

Material and method:
4 male and 4 female rats (strain: Crl:CD®[SD]BR VAF/plus; source:) received single doses of 0 (vehicle control), 50, 1000 or 2000 mg/kg bw triticonazole (batch no. 9550347; purity 97.2 %, dissolved in 0.5 % w/v methylcellulose in water) by oral gavage at a dose volume of 10 ml/kg (groups 1, 2, 3, 4). An additional group of 8 male rats (group 5) were dosed at 2000 mg/kg bw on the following day to determine the time-to-peak effect.
Animals were observed twice daily for mortalities and clinical signs; individual body weights were recorded on the day of dosing. Neurobehavioural tests, consisting of an abbreviated functional observational battery (during handling, in an open arena, and during manipulations to assess reflexes and physiological parameters) and locomotor activity assessment in a circular open field enclosure for 60 minutes (cumulative counts of photobeam breaks in 10-minute intervals) were done for all animals predose and approx. 2 hours after dosing (groups 1, 2, 3, 4), and approx. 1 and 4 hours postdose for the males in the additional group (four males at each interval). On day 4, the animals were sacrificed and the carcasses were discarded.

Findings:
There were no clear test material-related observations noted during the FOB at any dose level. The FOB results were similar across all groups, both sexes and across the three intervals the animals were assessed. Motor activity appeared higher for males given 2000 mg/kg bw than of the controls especially during the 20 to 40-minute interval of the motor activity session, and appeared to peak between 2 and 4 hours after dosing (highest counts for most of the intervals whilst motor activity session occurring for animals tested 2 hours post-dose).

Conclusion:
Based on the results, dose levels of 80, 400 and 2000 mg/kg bw were recommended for further testing. In addition, a time-to-peak effect for motor activity testing of approx. 2 – 3 hours after dosing was recommended for the acute study. No time-to-peak effect for FOB testing was established.

Triticonazole – Acute neurotoxicity study in rats ([redacted], DocID R012968)

Guidelines: Based on US EPA guideline 81-8
GLP: Yes
Acceptance: The study was considered scientific valid and acceptable during the EU registration process 2003.

Material and method:
Groups of 10 male and 10 female rats (strain: Crl:CD®[SD]BR VAF/plus; source: [redacted]) received single doses of 0 (vehicle control), 80, 400 and 2000 mg/kg bw triticonazole (batch no. 9550347; purity 97.2 %, dissolved in 0.5 % w/v methylcellulose in water) by oral gavage. Animals were observed twice daily for mortalities and clinical signs; individual body weights were recorded on days 1, 8 and 15. All animals were subjected to a functional observational battery (FOB) and an assessment of locomotor activity in a circular open field enclosure for 40 minutes (cumulative counts of photobeam breaks in 10-minute intervals) prior and also at 2 hours, 7 and 14 days after treatment. The FOB included testing of various sensorimotor functions, reflexes, behavioural functions and also body temperature and comprised:

(i) set of observations while the animals were in their home cages;
(ii) set of observations when initially handling the animals;
(iii) set of observations in an open test arena and
(iv) set of observations during manipulation/specific testing. After 15 or 16 days post-treatment, macroscopic neuropathological examination (including measurement of length, width and height of each cerebrum and cerebellum) was performed on all animals. Histopathology of designated nervous tissues (7 sections of the brain; pituitary gland; 3 sections of the spinal cord; cervical, lumbar and trigeminal ganglion; optic, sciatic, tibial and sural nerve), eyes and skeletal muscle was performed, taken from 6 animals/sex in the control and high-dose groups, respectively.

Findings:
Clinical signs and mortality:
There were no significant toxicological findings noted at any dose level. All animals survived the scheduled sacrifice. In addition, body weights and weight gains were unaffected by treatment.

FOB/locomotor activity:
The results obtained were similar across all groups, both sexes and across the testing intervals. Numerous variations were noted but considered of normal biological differences because they occurred in control animals as well as triticonazole treated animals. Of the data evaluated statistically, significant decreases in the number of fecal boli were observed on day 1 for males receiving 2000 mg/kg and in the number of rears and body temperature, resp. on day 8 for males given 2000 mg/kg when compared with controls. These significant differences, however, were not considered to be toxicologically relevant because the statistical differences were either consequences of the variation noted for the control animals or the mean values of the males in these groups were similar to the mean values observed for the same animals predose.

Pathology:
There were no macroscopic or microscopic findings related to the administration of triticonazole observed at any dose level. The only statistical significant finding was a smaller mean brain size for females given 400 and 2000 mg/kg, but the differences from control females were negligible (<1.5 %), were not found in males, and were not associated with any other pathological abnormalities or behavioural abnormalities. Therefore, this finding was not considered biologically relevant or related to triticonazole. The absence of any effects on brain size or morphology in the 13-week study (see below) in females (dosed up to 704 – 937 mg/kg bw) gives further evidence, that the effects found in this acute neurotoxicity study are rather not related to treatment with triticonazole but incidental.

Conclusion:
Based on the results of the study, the NOAEL for acute oral neurotoxicity of triticonazole to rats is greater than 2000 mg/kg bw.

Triticonazole – 13 week dietary neurotoxicity study in rats (; DocID R012967)

Guidelines: According to US EPA guideline 82-7
GLP: Yes
Acceptance: The study was considered scientific valid and acceptable during the EU registration process 2003.

Material and method:
Four groups of 10 rats/sex/dose (strain: Crl:CD®[SD]BR VAF/plus; source:) received triticonazole (batch no. 9550347; purity 97.2 %) at dietary dose levels of 0, 500, 2500 and 10000 ppm for 13 weeks (equivalent to ranges of 25 – 47, 130 – 240 and 554 – 931 mg/kg bw/d for males and 32 – 49, 168 –250 and 704 – 937 mg/kg bw/d for females).
Throughout the study, clinical signs, body weight and food consumption were recorded. Neurotoxicological examinations included a functional observational battery (FOB) and an assessment of locomotor activity in a circular open field enclosure prior and during the 4th, 8th and 13th week of treatment. The functional observational battery comprised of the same 4 sets of observations as described in the acute neurotoxicity study. At terminal necropsy after 13 weeks of treatment, a macroscopic examination was performed on all animals.
Histopathology of designated nervous tissues (7 sections of the brain; pituitary gland; 3 sections of the spinal cord; cervical, lumbar and trigeminal ganglion; optic, sciatic, tibial and sural nerve), eyes and skeletal muscle was performed, taken from 6 animals/sex in the control and high-dose groups, respectively.

Findings:
General observations: All animals survived to the scheduled sacrifice. There were no treatment-related clinical signs noted at any dose level. During the first 4 weeks of treatment, significant lower mean body weight gains were observed among animals receiving 10000 ppm.

FOB/locomotor activity:
The FOB results were similar across all groups, both sexes and at all testing intervals. Variations were observed during all test intervals in the resistance of animals when being removed from the cage, the activity in the open field, and the response of the animals to being touched with a penlight. However, these findings were attributed to normal biological variation because they occurred in control animals as well as in triticonazole-treated animals and the occurrence was unrelated to dose. Of the FOB parameter evaluated statistically, significant differences in the number of rears were noted at week 8 for males given 10000 ppm and at week 13 for females given 500 or 10000 ppm and in the hindlimb grip strength of females given 2500 ppm at week 8. These statistical differences were not considered treatment-related because they were not dose-related and they occurred at week 8 but not at the week 13 interval.
There were no significant differences in the locomotor activity of animals after ingestion of triticonazole.

Pathology:
There were no significant differences in brain size measurements between control and treated animals. In addition, no macroscopic or microscopic findings related to the administration of triticonazole were observed at any dose level.

Conclusion:
Based on the results of the study, the NOAEL for neurotoxicity in rats given diets containing triticonazole is greater than 10000 ppm, corresponding to doses of 554 – 931 mg/kg bw/d for males and 704 – 937 mg/kg bw/d for females.

CA 5.7.2 Delayed polyneuropathy studies

No delayed polyneuropathy studies were performed.

CA 5.8 Other Toxicological Studies

CA 5.8.1 Toxicity studies of metabolites

Studies evaluated in the monograph of the Rapporteur Member State Austria of February 2003:

Some limited toxicological studies were presented for metabolites during the former European assessment of triticonazole.
RPA 406341 (Reg.No. 5059144) (a hydroxylated metabolite of triticonazole found in cereals following seed treatment) and RPA 406203 (Reg.No. 5079359) (cis-isomer of triticonazole; soil photo-metabolite) were of low acute oral toxicity in rats and showed no indication of mutagenic properties when tested in the bacterial reverse mutation assay. It can be concluded that these metabolites are of similar toxicity to triticonazole in terms of acute oral and mutagenic potential (investigated by Ames-test only).

Thus, the following conclusion was drawn in the Annex I listing of triticonazole:

Other toxicological studies	RPA 406341 (plant metabolite and found in rats at low levels) and RPA 406203 (soil metabolite only): Oral LD50 value > 2000 mg/kg bw no evidence of genotoxic activity (Ames test) RPA 402570 (major impurity): Oral and dermal toxicity LD50 values > 2000 mg/kg bw No evidence of genotoxic activity (Ames test) Short term toxicity NOAEL 10 mg/kg bw/day, 14 day rat The impurity was present in the batches used in the toxicity studies.

However, by re-evaluating the residue studies, these compounds (Reg.Nos. 5059144 = RPA 406341 and 5079359 = RPA 406203) were not found in significant amounts in the studies (see Chapter MCA 6.7), on this basis no toxicological assessment was needed and no consumer risk assessment had been performed.

A normal practice for cereal seed treatment as well as storage is the exclusion of light in order to avoid seed germination. Further, treated seeds are also stored in brown paper bags. However, to address a theoretical exposure to the cis-isomer of triticonazole, which can be formed under UV-light (cis-isomer / Z-isomer / RPA 406203 = Reg.No. 5079359) via treatment/handling of triticonazole-treated seeds (exposed to sunlight), a small toxicological data package has been conducted on this metabolite (acute oral toxicity, Ames test and in vitro micronucleus test to be conducted in early 2016).

Regarding the potential for human exposure, based on the results of a non-GLP study – conducted for the purpose of an ecotoxicological assessment of the Z-isomer – no cis-isomer / Z-isomer is present after fresh treatment of seeds with BAS 595 01 F and UV light exposure (BASF DocID 2015/1189076; study results summarized in Chapter MCP 10.7), building up to very small amounts of cis-isomer after a period of 10 days (maximum of 2.4% Z-isomer vs 97.6% E-isomer). Thus – besides genotoxicity testing – no further toxicological studies are considered needed.
With regard to the triazole-derived metabolites, no toxicological assessment has been conducted as that would be beyond the scope of the triticonazole assessment and will be reconsidered based on the outcome of the on-going UK assessment.
RPA 402570 (Reg. No. 5055519) is only found as an impurity of technical triticonazole. The study summaries are therefore provided in Doc J of this dossier.

The list of endpoints (Doc N2) contains the following entry:

Studies performed on metabolites or impurities	No metabolites were found in significant amounts in the residue studies. Cis-isomer/Z-isomer (Reg.No. 5079359 = RPA 406203): Oral LD_{50} value > 2000 mg/kg bw No evidence of genotoxic activity (Ames test; in vitro MNT planned) With regard to the triazole-derived metabolites, no toxicological assessment has been conducted, as that would be beyond the scope of the triticonazole assessment and will be reconsidered based on the outcome of the on-going UK assessment. Studies and assessment performed on the impurities are presented in the confidential part of the Dossier (Doc J)

Acute oral toxicity

Report:	R000127;
Title:	An acute oral toxicity in rats with RPA406203.
Guidelines:	USEPA (=EPA) 81-1
Deviations:	None
GLP	Yes

Material and Methods:
The purpose of this study was to determine the acute LD_{50} of RPA 406203 following a single oral administration.
Groups of 5 fasted male and female Sprague Dawley CD rats (9 and 12-week old male and female, respectively) received a single oral administration of RPA 406203 by gavage at dose levels of 2000 mg/kg body weight. RPA 406203 was suspended in corn oil.
Animals were observed twice during the first day and at least daily thereafter for clinical signs and mortality until death or sacrifice on Day 14. Body weights were recorded prior to dosing, on the day of dosing and weekly thereafter.
At termination of the study, all surviving animals were autopsied and subjected to a macroscopic examination.

Findings:
No treatment-related deaths were observed throughout the study. Clinical signs included, dark material around the facial area and faecal stain. All animals had recovered on Day 4 except with one female which showed hair loss on Day 12. The body weight evolution was normal for all animals.
Gross examination revealed the presence of foci on the lungs of 4 out of 5 male rats. However, the toxicological significance of this finding is unclear since these foci have been observed in untreated animals in the same strain of rats in the performing laboratory.

Table 5.8.1-1: Mortality induced by RPA 406203 in Sprague Dawley CD rats after a single oral administration

Dose (mg/kg)	Male		Female	
	Mortality	Time of death in days (number of rats)	Mortality	Time of death in days (number of rats)
2000	0/5	-	0/5	-

Conclusion:
The acute oral LD_{50} of RPA 406203 (= Reg.No. 5079359) in Sprague Dawley CD rats was greater than 2000 mg/kg bw.

Ames test

Report:	R000129; Lawlor T.E. 1999
Title:	Salmonella - Escherichia coli / Mammalian-microsome reverse mutation assay with a confirmatory assay with RPA406203 - Amended final report.
Guidelines:	OECD 471, (revised draft documents, March 1996) USEPA (=EPA) 84.2
Deviations:	None
GLP	Yes

Material and Methods:
RPA 406203 (batch N° OB0012, purity 99.8 %) was tested for its ability to induce mutation in 4 histidine dependent auxotrophic mutant strains TA98, TA100, TA1535 and TA1537 of *Salmonella typhimurium* and the tryptophan deficient strain WP2 uvrA of *Escherichia coli*. The study consisted of a cytotoxicity range-finding experiment conducted in TA100 and WP2 uvrA followed by two independent experiments, each conducted in the absence and presence of metabolic activation by an Aroclor 1254 induced rat liver post-mitochondrial fraction (S-9). Negative and appropriate positive controls were included in each experiment.

Findings:
- **Cytotoxicity range-finder experiment**

RPA 406203 was dissolved in DMSO and tested from 6.67 to 5000 µg/plate in TA100 and WP2 uvrA.
Cytotoxicity evidenced by a thinning of the background lawn of non-revertant cells and a reduction in revertant colony numbers was observed in TA 100 tester strain at 1000 and 3330 µg/plate in the presence and absence of S9, respectively. Indications of cytotoxicity were noted in WP2 tester strain at 3330 µg/plate in the absence of S9 whereas no cytotoxicity was observed in the presence of S9.
- **Experiment 1 (standard plate incorporation)**

Accordingly, RPA 406203 was tested at from 10.0, 33.3, 100, 333, 1000 and 5000 µg/plate.
The mean numbers of revertant colonies on negative control plates were within acceptable ranges while the mean number of revertant colonies in positive control plates were significantly increased. RPA 406203 treatments produced no statistically significant increases in the mean numbers of revertant colonies in any tested strains both in the absence and presence of S-9.
- **Experiment 2 (standard plate incorporation)**

RPA 406203 was tested at the same final concentrations.
Similarly to Experiment 1, RPA 406203 treatments produced no statistically significant increases in the mean numbers of revertant colonies in any tested strains both in the absence and presence of S-9.

Conclusion:
Following 2 independent experiments that were performed in the absence and presence of a rat liver metabolic activation system, RPA 406203 (= Reg.No. 5079359) did not induce reverse gene mutation in any *Salmonella typhimurium* and *Escherichia coli* strains when tested at dose levels up to 5000 µg/plate.

Table 5.8.1-2: Number of revertant colonies in Salmonella typhimurium strains following treatment with RPA 406203 - Experiment 1

Without metabolic activation					
Treatment and concentration (µg/plate)	**Mean revertants per plate (± SD)** TA 98	TA 100	TA 1535	TA 1537	TA 1538
Solvent control	13 ± 1	76 ± 6	8 ± 4	3 ± 1	8 ± 1
RPA 406203 10	15 ± 5	76 ± 8	9 ± 4	3 ± 1	13 ± 3
RPA 406203 33.3	17 ± 3	80 ± 6	10 ± 2	5 ± 4	13 ± 4
RPA 406203 100	16 ± 4	81 ± 10	11 ± 3	6 ± 2	8 ± 2
RPA 406203 330	17 ± 3	83 ± 6	6 ± 2	4 ± 1	11 ± 3
RPA 406203 1000 sp	14 ± 2	79 ± 11	5 ± 2	3 ± 2	10 ± 2
RPA 406203 5000 mp - *	4 ± 1	63 ± 10	6 ± 4	4 ± 2	9 ± 1
2-nitrofluorene 1	147 ± 25	-	-		
Sodium azide 2	-	735 ± 145	659 ± 1	-	-
ICR-191 2	-	-	-	902 ± 84	-
4-nitroquinoline-N-oxide 1	-	-	-	-	328 ± 47

With metabolic activation					
Treatment and concentration (µg/plate)	**Mean revertants per plate (± SD)** TA 98	TA 100	TA 1535	TA 1537	TA 1538
Solvent control	19 ± 5	95 ± 2	13 ± 4	10 ± 1	14 ± 3
RPA 406203 10	25 ± 3	97 ± 10	10 ± 2	9 ± 2	11 ± 1
RPA 406203 33.3	25 ± 3	105 ± 10	12 ± 1	7 ± 1	14 ± 3
RPA 406203 100	17 ± 2	103 ± 6	12 ± 3	12 ± 4	10 ± 3
RPA 406203 330	22 ± 4	98 ± 7	10 ± 1	9 ± 3	15 ± 2
RPA 406203 1000 sp	20 ± 6	89 ± 7	18 ± 2	6 ± 2	11 ± 3
RPA 406203 5000 mp - *	21 ± 3	108 ± 2	9 ± 2	7 ± 4	18 ± 3
Benzo[a]pyrene 2.5	388 ± 20	-	-	-	-
2-aminoanthracene 2.5	-	622 ± 29	111 ± 8	181 ± 7	-
2-aminoanthracene 25	-	-	-	-	421 ± 27

SD: standard deviation; sp: slight precipitate; mp: moderate precipitate; * : presence of cytotoxicity

Table 5.8.1-3: Number of revertant colonies in Salmonella typhimurium strains following treatment with RPA 406203 - Experiment 2

Without metabolic activation					
Treatment and concentration (µg/plate)	Mean revertants per plate (± SD)				
	TA 98 *	TA 100 *	TA 1535	TA 1537 *	TA 1538
Solvent control	13 ± 5	69 ± 5	9 ± 6	3 ± 1	10 ± 5
RPA 406203 10	15 ± 4	73 ± 6	9 ± 2	6 ± 2	14 ± 1
RPA 406203 33.3	12 ± 3	67 ± 9	11 ± 2	5 ± 2	17 ± 1
RPA 406203 100	17 ± 4	71 ± 17	9 ± 2	5 ± 1	9 ± 4
RPA 406203 330	11 ± 2	83 ± 12	12 ± 4	4 ± 3	10 ± 4
RPA 406203 1000 sp	13 ± 3	63 ± 14	10 ± 0	6 ± 1	9 ± 2
RPA 406203 5000 mp - *	6 ± 4	53 ± 3	7 ± 4	1 ± 1	9 ± 4
2-nitrofluorene 1	196 ± 28	-	-		
Sodium azide 2	-	648 ± 24	594 ± 32	-	-
ICR-191 2	-	-	-	612 ± 70	-
4-nitroquinoline-N-oxide 1	-	-	-	-	374 ± 76

With metabolic activation					
Treatment and concentration (µg/plate)	Mean revertants per plate (± SD)				
	TA 98	TA 100	TA 1535	TA 1537	TA 1538
Solvent control	27 ± 4	97 ± 4	13 ± 3	7 ± 4	14 ± 8
RPA 406203 10	26 ± 7	80 ± 13	13 ± 3	9 ± 3	15 ± 7
RPA 406203 33.3	30 ± 5	85 ± 4	11 ± 2	6 ± 1	17 ± 2
RPA 406203 100	23 ± 4	105 ± 10	14 ± 1	11 ± 5	14 ± 3
RPA 406203 330	24 ± 9	86 ± 7	14 ± 6	7 ± 4	14 ± 7
RPA 406203 1000 sp	22 ± 6	81 ± 11	12 ± 1	6 ± 3	14 ± 5
RPA 406203 5000 mp - *	13 ± 2	75 ± 11	11± 5	5 ± 3	10 ± 4
Benzo[a]pyrene 2.5	447 ± 31	-	-	-	-
2-aminoanthracene 2.5	-	913 ± 66	122 ± 11	157 ± 6	-
2-aminoanthracene 25	-	-	-	-	282 ± 24

SD: standard deviation; sp: slight precipitate; mp: moderate precipitate; * : presence of cytotoxicity

Summary of the toxicological studies of Reg.No. 5079359 = RPA 406203

The cis-isomer / Z-isomer (Reg. No. 5079359 = RPA 406203) of triticonazole, which is a soil metabolite and could be formed under UV-light is of low acute oral toxicity and not genotoxic. This compound was not found in significant amounts in the residue studies.

Submission of not yet peer-reviewed studies in this AIR3-Dossier:

For triticonazole a number of supplementary studies have been conducted since the last Annex I inclusion process in Europe. Specifically these were a YAS and a YES screening assay performed, in order to test the potential androgenic/anti-androgenic and estrogenic/anti-estrogenic mode of action. In addition, an in-vitro aromatase inhibition assay has been conducted in rat and human aromatase to prove that triticonazole is a weak aromatase inhibitor in rats and even less weaker in human aromatase. These mechanistic studies are summarized in detail in Chapter MCA 5.8.3 below. Further, an immunotoxicity study (to support the US registration) and a general pharmacology study (to support Japanese registration) have been conducted and are summarized in detail in Chapter MCA 5.8.2.

CA 5.8.2 Supplementary studies on the active substance

Report: CA 5.8.2/1
[redacted]
BAS 595 F (Triticonazole) - Immunotoxicity study in female Wistar rats - Administration via the diet for 4 weeks
2011/1268148
Guidelines: EPA 870.7800
GLP: yes
(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary
The immunotoxic potential of triticonazole (Batch: COD-001440; Purity: 91.3%) in female Wistar rats was analyzed using dietary dose levels of 0, 500, 1500 and 5000 ppm (corresponding to mean intake levels of 53, 162 and 462 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 triticonazole did not result in systemic toxicity in the test groups 500 and 1500 ppm. However, in the high dose group (5000 ppm) a significantly lower body weight (about 9% less as compared to the control) after 14 days of treatment, and a significantly lower body weight gain (about 33% less as compared to the control) after study days 10 and 14 were observed. None of the immunotoxicologically relevant parameters mentioned above were affected by treatment with triticonazole up to the highest dose level tested. However, absolute and relative liver weights were significantly increased in the 5000 ppm treatment group.
Concurrent treatment with positive control substance, cyclophosphamide monohydrate (CPA, 4.5 mg/kg bw/day) induced clear signs of immunotoxicity in any parameter tested, demonstrating the reliability of the test system under the study conditions employed.

Based on the obtained results it can be concluded that triticonazole does not bear an immune-modulatory/immunotoxic potential under the conditions of this study. The NOAEL for the immunotoxicologically relevant endpoints was determined to be 5000 ppm corresponding to 462 mg/kg bw/day. The NOAEL for systemic toxicity was 1500 ppm corresponding to 162 mg/kg bw/day in female Wistar rats.

(BASF DocID 2011/1268148)

I. MATERIAL AND METHODS

1. Test Material:	Triticonazole (BAS 595 F)
Description:	solid/ white
Lot/Batch #:	COD-001440
Purity:	91.3%
Stability of test compound:	The test substance was stable over the study period (Expiry date February 11, 2014).
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 (Sigma-Aldrich) the positive control substance was stable over the study period (Expiry date Sep. 2012).
Vehicle for CPA:	Drinking water
4. Test animals:	
Species:	Rat
Strain:	Crl:WI(Han)
Sex:	Female (more sensitive gender based on previously performed repeated dose studies)
Age:	34 ± 1 days at delivery; approx. 42 ± 1 days at start of administration
Reason for the selection:	The rat is a frequently used laboratory animal, and there is comprehensive experience with this animal species. Moreover, the rat has been proposed as a suitable animal species by the OECD and the EPA for this type of study.
Weight at dosing:	133.3 ± 1.2 g
Source:	

Acclimation period:	7 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 cm²) cages (TECNIPLAST, Hohenpeißenberg, Germany). Dust-free wooden bedding was used in this study. Wooden gnawing blocks (Type NGM E-022; Abedd® Lab. and Vet. Service GmbH, Vienna, Austria) were used for environmental enrichment.
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: 19-Apr-2011 - 28-Oct-2011
(In life dates: 27-Apr-2011 (start of administration) to 26-May-2011 (necropsy))

2. Animal assignment and treatment:
The animals were assigned to the treatment groups by means of computer generated randomization list based on body weights.
Triticonazole was administered to groups of 8 female rats at dietary concentrations of 0, 500, 1500 and 5000 ppm over a period of 4 weeks. Control animals received the ground diet only. Additionally, 8 female mice were treated orally (gavage) with 4.5 mg/kg bw/day Cyclophosphamide monohydrate (CPA; positive control substance for immunotoxicity). 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.
Six days before necropsy (day 23), 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 triticonazole 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 5.8.2-1: Results of homogeneity and concentration control analysis of triticonazole in rodent diet

Nominal Dose level [ppm]	Sampling/ Analysis	Concentration Mean ± SD# [ppm]	Mean of nominal concentration [%]	Relative standard deviation [%]
500	Apr. 25, 2011/ May 09, 2011	556.9 ± 22.9	111.4	4.1
1500	"/"	1662.7 ± 88.6	110.8	5.3
5000	"/"	5467.3 ± 179.4	109.3	3.3

= Values may not calculate exactly due to rounding of figures

Relative standard deviations of the homogeneity of the triticonazole samples were quite low in the range of 3.3 to 5.3%, which indicates the homogenous distribution of triticonazole in the diet preparations. The actual (mean) average test-substance concentrations were in the range of 109.3 to 111.4% of the nominal concentrations confirming the correctness of the concentrations. No test substance was found in the control samples.

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 7 and 32 days when stored at room temperature and deep frozen, respectively.
The actual CPA concentrations were in the range of 97.3 to 100.5% of the nominal concentration confirming the correctness of the concentration.

Table 5.8.2-2: 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	Jan. 28, 2010/ Mar. 01, 2010	0.0435	100.5
0.045	Apr. 26, 2011/ Jul. 26, 2011	0.0438	97.3

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 5.8.2-3: 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 5.8.2-4: 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 5.8.2-5: 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 by daily visual inspection of the water bottles for any overt changes in volume.

4. Body weight and body weight change:

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 twice weekly thereafter. The difference between the body weight on the respective day of weighing and the body weight on day 0 was calculated as body weight change

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 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 under internal laboratory quality control conditions and in a randomized sequence to assure reliable test results.

Anti-SRBC IgM ELISA:

- The Anti SRBC IgM ELISA of Life Diagnostics Inc. (cat no. 4200-2) was performed according to manufacturer instructions.
- Each serum sample was applied to the ELISA in two dilutions, i.e. 1:500 and 1:1000 (positive control group samples: 1:50 and 1:100).
- OD values of the sample dilutions outside of the linear range of the standard curve were repeated with an appropriate further dilution.
- 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. Animals that died within the test substance application period were necropsied as soon as possible after their death. The following weights were determined for all animals sacrificed at scheduled dates and the organs or tissues were fixed in 4% buffered formaldehyde solution:

1. Anesthetized animals (all gross lesions fixed in formaldehyde)
2. Liver
3. Spleen
4. Thymus

From the liver, each one slice of the Lobus dexter medialis and the Lobus sinister lateralis was fixed in Carnoy's solution and embedded in paraplast.
No histopathological examinations were performed.

II. RESULTS AND DISCUSSION

A. OBSERVATIONS

1. Clinical signs of toxicity

No clinical signs of toxicity were observed in animals treated with Triticonazole at any dose level. Two of eight animals (Nos. 35 and 36) treated with CPA showed signs of general poor condition like moderately labored respiration and slight piloerection.

2. Mortality

Animal No. 35 of the positive control group was found dead and cannibalized on study days 25. Animal No. 36 of the same test group was found dead on study day 28. Both animals showed clinical findings on the day before premature death. The premature deaths of these animals were likely due to treatment (gavage error). However, a relation to treatment with CPA was possible.

B. FOOD AND DRINKING WATER CONSUMPTION, AND COMPOUND INTAKE

No test substance-related findings were observed for the food and water consumption.
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 5.8.2-6: Calculated intake of Triticonazole

Test group	Concentration in the diet (ppm)	Mean daily test-substance intake (mg/kg bw/day)
		Females
1	500	53
2	1500	162
3	5000	462

C. BODY WEIGHT AND BODY WEIGHT GAIN

Body weight in animals of test group 3 (5000 ppm) was reduced (-5% to -7% from day 7 until day 28, with a maximum of -8.9% compared to control animals on day 14 [see Table 5.8.2-7]. Body weight change values in animals of test group 3 (5000 ppm) were reduced after the first study week and reached a significant maximum reduction of -32% on study days 10 and 14 [see Table 5.8.2-8]. These finding occurred in the absence of a similar pattern in mean food consumption, and therefore, were considered to be treatment-related, direct adverse systemic effects of Triticonazole.
At dietary concentrations of 500 and 1500 ppm, there were no treatment-related effects on body weight and body weight gain. The significant deviation of the mean body weight gain between study days 0-3 at 500 ppm, was assessed as being incidental.

Body weights of animals treated with CPA not significantly reduced from day 7 until day 28, with a maximum of -5.7% on day 24.
Body weight change of animals treated with CPA was not significantly decreased from day 7 until day 28, with a maximum of -16% on day 24.

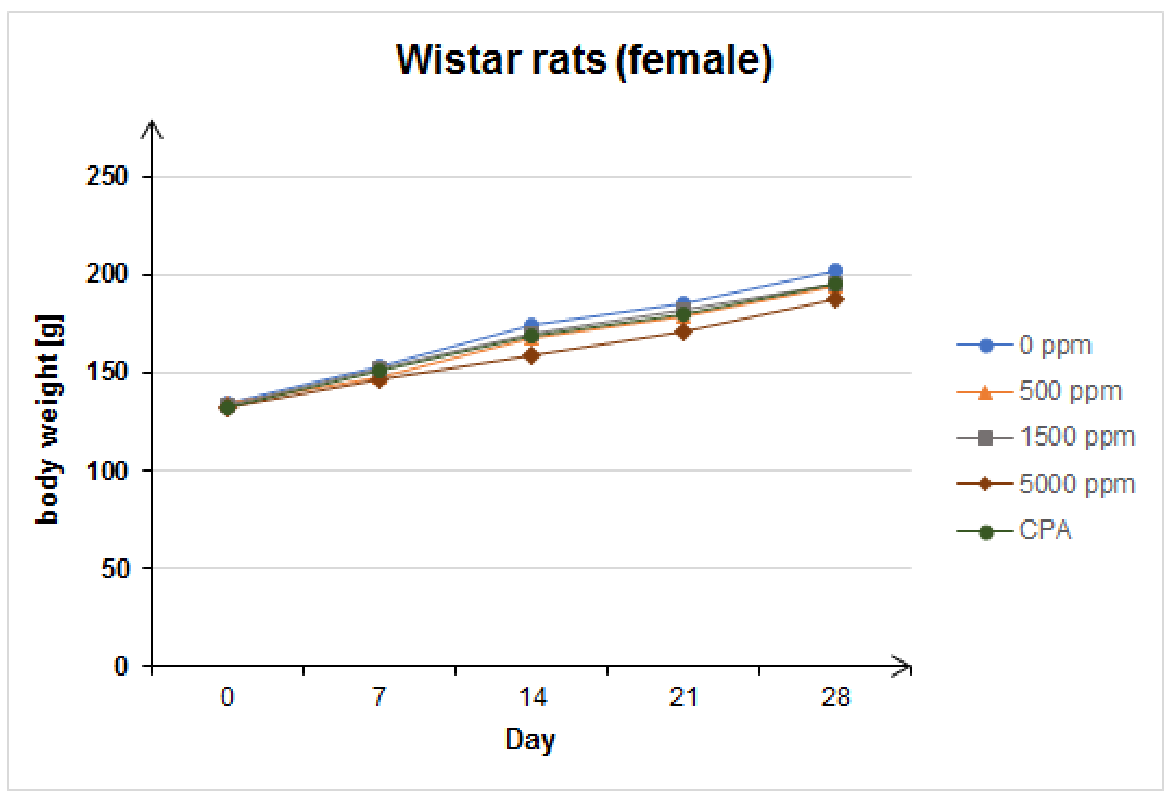


Figure 5.8.2-1:Body weight development of rats administered Triticonazole or Cyclophosphamide for 28 days

Table 5.8.2-7: Mean body weight of rats administered Triticonazole or Cyclo-phosphamide for 28 days

Treatment	Triticonazole				CPA
Dose level	0 ppm	500 ppm	1500 ppm	5000 ppm	4.5 mg/kg bw/day
Body weight [g]					
Day 0	134.6	134.3	132.9	131.8	132.7
SD	6.4	6.9	6.7	6.9	6.0
Δ% (compared to control)	-	-0.2	-1.2	-2.1	-1.4
Day 3	143.2	133.8	143.2	141.6	142.5
SD	7.1	7.6	8.6	8.5	9.3
Δ% (compared to control)	-	-6.6	0.0	-1.1	-0.5
Day 7	153.8	147.6	151.6	146.8	150.9
SD	7.1	9.0	11.0	9.8	9.9
Δ% (compared to control)	-	-4.1	-1.3	-4.6	-1.9
Day 10	161.1	157.0	159.3	149.9	158.1
SD	9.8	9.5	11.8	12.0	8.2
Δ% (compared to control)	-	-2.6	-1.1	-6.9	-1.9
Day 14	173.9	168.2	170.4	**158.4***	168.8
SD	10.1	8.8	10.0	13.4	9.4
Δ% (compared to control)	-	-3.2	-2.0	-8.9	-2.9
Day 24	193.2	184.5	185.7	177.4	182.2
SD	12.9	8.5	9.0	19.1	12.7
Δ% (compared to control)	-	-4.5	-3.9	-8.2	-5.7
Day 28	202.2	194.3	195.6	187.5	195.2
SD	11.5	9.4	9.8	19.0	12.1
Δ% (compared to control)	-	-3.9	-3.3	-7.3	-3.5

*: $p \leq 0.05$

Table 5.8.2-8: Mean body weight gain of rats administered Triticonazole or Cyclophosphamide for 28 days

Treatment	Triticonazole				CPA
Dose level	0 ppm	500 ppm	1500 ppm	5000 ppm	4.5 mg/kg bw/day
Body weight gain [g]					
Day 0 →3	8.6	**-0.5****	10.3	9.8	9.7
SD	2.2	3.6	5.1	3.4	5.9
Δ% (compared to control)	-	-105.9	19.0	13.9	12.7
Day 0 →7	19.3	13.3	19.0	15.0	18.2
SD	4.1	4.3	7.1	4.0	6.0
Δ% (compared to control)	-	-31.1	-1.3	-22.0	-5.5
Day 0 →10	26.5	22.7	26.4	**18.2***	25.3
SD	6.5	3.8	9.1	5.8	4.5
Δ% (compared to control)	-	-14.5	-0.4	-31.5	-4.4
Day 0 →14	39.3	33.9	37.5	**26.7****	36.0
SD	7.4	3.0	7.1	7.8	6.7
Δ% (compared to control)	-	-13.7	-4.6	-32.1	-8.2
Day 0 →24	58.6	50.1	52.8	**45.6***	49.4
SD	12.4	4.9	6.2	13.3	10.8
Δ% (compared to control)	-	-14.4	-9.9	-22.1	-15.7
Day 0 →28	67.6	60.0	62.7	**55.7***	62.0
SD	10.7	4.2	6.5	13.0	9.9
Δ% (compared to control)	-	-11.3	-7.3	-17.6	-8.3

*: p≤0.05, **: p≤0.01

D. IMMUNOLOGICAL ANALYSES

1. Analysis of the primary T-cell dependent antibody response (TDAR)

Six days after immunization, no changes in the SRBC IgM titres were found in female rats treated with the test substance, whereas the SRBC titres were significantly lower in rats of test group 4 (CPA, positive control group) [see Table 5.8.2-9].

Table 5.8.2-9: Analysis of the specific primary (IgM) immune response to SRBC in rats treated with Triticonazole or Cyclophosphamide for 28 days

Treatment	Triticonazole				CPA
Dose [ppm]	0	500	1500	5000	
[mg/kg bw/day]		53	162	462	4.5
Specific IgM Titer (U/mL)					
Mean	42129	25692	40858	43540	**15456****
SD	16647	9511	27242	25949	5824
Median	38297	27973	39414	42298	16622

* $p \leq 0.05$; ** $p \leq 0.01$ (Kruskal-Wallis and Wilcoxon-test. two sided)

G. NECROPSY

1. Terminal body weight and organ weights

The absolute and relative mean weights of spleen and thymus of animals in test groups 50, 1500, and 5000 ppm showed no 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 5.8.2-10: Mean absolute and relative organ weights of female rats treated with Triticonazole or Cyclophosphamide for at 28 days

		Triticonazole				CPA
Dose [ppm]		**0**	**500**	**1500**	**5000**	
[mg/kg bw/day]			**53**	**162**	**462**	**4.5**
Terminal bodyweight	[g]	184.81	176.83	179.06	169.63	176.2
	SD	12.12	9.12	9.47	17.18	10.36
	[% of control]	100	96	97	92	95
Liver, absolute	[g]	5.13	5.12	5.30	**6.02****	5.15
	SD	0.34	0.33	0.40	0.74	0.89
	[% of control]	100	100	103	**117**	100
Liver, relative	[%]	2.78	2.90	**2.96***	**3.56****	2.91
	SD	0.11	0.12	0.18	0.32	0.36
	[% of control]	100	104	**107**	**128**	105
Spleen, absolute	[g]	0.45	0.45	0.42	0.45	**0.27****
	SD	0.05	0.06	0.05	0.06	0.02
	[% of control]	100	99	93	99	**59**
Spleen, relative	[%]	0.24	0.25	0.24	0.27	**0.15****
	SD	0.02	0.03	0.02	0.04	0.01
	[% of control]	100	103	96	109	**62**
Thymus, absolute	[mg]	475.25	453.13	477.63	449.88	**316.83****
	SD	64.44	91.38	22.37	36.30	61.36
	[% of control]	100	95	100	95	**67**
Thymus, relative	[%]	0.26	0.26	0.27	0.27	**0.18****
	SD	0.03	0.05	0.01	0.04	0.04
	[% of control]	100	100	104	104	**70**

* $p \leq 0.05$; ** $p \leq 0.01$ (Kruskal-Wallis and Wilcoxon-test, two sided)

The absolute and relative liver weight of animals treated with 5000 ppm was significantly increased by 17% ($p<0.01$) and 28% ($p<0.01$), respectively, and was regarded as treatment-related. In animals of the 1500 ppm dose group, only the relative liver weight was marginally, but statistically significantly increased by 7% ($p<0.05$). This finding was not regarded as treatment-related, as the overall mean increase in weight was very small and the absolute weights were not increased.

2. Gross pathology

The enlarged liver of two animals in the 5000 ppm test group was regarded as treatment-related. All other gross lesions observed were considered incidental and not related to treatment.
Animal Nos. 35 and 36 of the positive control group (CPA) died spontaneously without any signs of relationship to the treatment. However, a treatment-relation could not excluded totally.

III.CONCLUSIONS

Under the conditions of the study, Triticonazole did not reveal any signs of immunotoxicity when administered via the diet over a period of 4 weeks to female Wistar rats. The NOAEL for the immunotoxicity was determined to be 5000 ppm (462 mg/kg bw/day; highest dose tested). The NOAEL for systemic toxicity was set to 1500 ppm (162 mg/kg bw/day), based on treatment-related changes (reduced body weight (gain) and increased absolute and relative liver weights) in the next higher dose group (5000 ppm).
The oral administration of the positive control substance Cyclophosphamide (4.5 mg/kg bw/day) led to findings indicative of immunotoxicity by TDAR assay. 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 female Wistar rats.

Report:	CA 5.8.2/2 [redacted] General pharmacology study of BAS 595 F 2014/8000175
Guidelines:	JMAFF No 12 Nosan No 8147, Pharmacology studies (2-2-1) of Data Requirements for Supporting Registration of Pesticides, MAFF 25-Shouan-630 (May 2013)
GLP:	yes (certified by Ministry of Agriculture, Forestry and Fisheries of Japan, Japan)

Executive Summary

Triticonazole (Batch: COD-001440; Purity: 91.3%) was administered to groups of 5 female rats and mice in concentrations of 0, 200, 600 and 2000 mg/kg bw orally by gavage with an administration volume of 1 mL/100 g bw. Control animals received 0.5% MC solution. The observation of general behavior was performed with mice as well as with rats prior to the administration and at 0.5, 1, 2, 3, 6 and 24 hours after the administration. The observations of the respiratory-cardiovascular systems were performed only with rats and included respiratory rate and blood pressure and heart rate measurements.

Numbers of defecation in mice of the 600 and 2000 mg/kg bw groups were significantly lower than that of the control group 6 hours after test item administration. However, this finding was not observed at 24 hours and no similar effects were observed in rats. Therefore, this change was judged to be not a test substance-related effect. No other treatment-related effects in other parameters of general behavior were observed in mice and rats.

There were no substance-induced significant differences in the respiratory rate and tidal volume in any treatment groups compared with control group at each time point.
Blood pressure observations revealed significantly higher or showed a tendency to higher systolic blood pressures (SBP), diastolic blood pressures (DBP), and mean blood pressure (MBP) at 2000 mg/kg bw 1 and 2 hours after administration. These changes were assessed to be test substance-related effects. However, normal blood pressure was noted 3 hours after administration, so that these high values were considered to be transient.
Heart rate (HR) was not affected by the test substance treatment in rats.
Based on the results of this study, triticonazole did not affect the general behavior of mice and rats. In the cardiorespiratory system, no effects were noted on the respiratory parameters and heart rate in rats. Blood pressure was elevated transitory in rats treated with 2000 mg triticonazole/kg bw.

(BASF DocID 2014/8000175)

I. MATERIAL AND METHODS

1. Test Material:	Triticonazole (BAS 595 F)
Description:	solid (powder) / white
Lot/Batch #:	COD-001440
Purity:	91.3%
Stability of test compound:	The test substance was stable over the study period (Expiry date February 11, 2014).
2. Vehicle control:	0.5% (w/v) methyl cellulose (MC) 400 solution
3. Test animals:	
Species:	Mouse / Rat
Strain:	Crlj:CD1(ICR) / Crl:CD(SD)
Sex:	Female mice (more sensitive gender based on results of a preliminary test) / Female rats (more sensitive gender based on previously performed acute oral toxicity study)
Age:	5 / 6 weeks at delivery; approx. 6 / 7 weeks at start of administration
Reason for the selection:	The rat and mouse are frequently used laboratory animals, and there is comprehensive experience with these animal species.
Weight at dosing:	19.3 to 22.4 g / 132.0 to 172.9 g
Source:	
Acclimation period:	5 days
Diet:	commercial diet (CRF-1), ad libitum
Water:	Tap water, ad libitum
Housing:	animals were housed individually in metal mesh cages (mouse: 10 x 19.6 x 13 cm; rat: 19.7 x 26.3 x 18 cm (WDH)).
Environmental conditions:	
Temperature:	20 - 26 °C
Humidity:	35 - 70 %
Air changes:	at least 12/hour
Photo period:	12 h light / 12 h dark (07:00 - 19:00 / 19:00 - 07:00)

B. STUDY DESIGN

1. Dates of experimental work: 01-Oct-2013 - 23-Oct-2013
(In life dates: 02-Oct-2013 (start of administration) to 22-Oct-2013)

2. Animal assignment and treatment:
The animals were assigned to the treatment groups by means of computer generated randomization list based on body weights.
Triticonazole was administered to groups of 5 female rats and mice in concentrations of 0, 200, 600 and 2000 mg/kg bw orally by gavage with an administration volume of 1 mL/100 g bw. Control animals received 0.5% MC solution.
After the experiments, the surviving and unused (rejected animals from the group assignment) animals were transferred to Animal Care Section.

3. Test substance preparation:
Vehicle
0.5% (w/v) MC solution was prepared by weighing the required amount of methyl cellulose 400 (e.g. 2.5 g for 500 mL preparation) and dispersing in warm distilled water for injection (about 2/3 of final preparation volume). This water dispersion was stirred under room temperature to dissolve dispersoid, and was diluted to the required volume with distilled water for injection. The prepared vehicle was stored at 4°C and used within 14 days after preparation.

Test substance
The weighed amount of test substance for 200 mg/mL stock solution was corrected using the purity (correction factor: 1.10 [100%/91.3%]), and ground well in an agate mortar with agate pestle and mixed with a small volume of vehicle. Then, this suspension and the wash of the mortar were transferred into a graduated cylinder. The suspension was diluted to the required volume with the vehicle to give a stock solution of 200 mg/mL was serially diluted to make 60 and 20 mg/mL dosing solutions, for the 2000, 600 and 200 mg/kg bw dose groups, respectively for an application volume of 10 mL/kg.

4. Statistics:
Means and standard deviations of each test group were calculated for several parameters. First, the raw data were analysed by Bartlett's test for comparison of homogeneity of variances. Homogeneous data were analysed by Dunnett's multiple comparison test for significant differences between the control group and each test substance-treated group. Heterogeneous data by Bartlett's test were subjected to Steel's test for significant differences between the control group and each test substance -treated group. Significance of differences were two-sided 5% in Bartlett's test, and two-sided 5% and 1% in other tests.

C. METHODS

1. General behavior

The observation of general behavior was performed with mice as well as with rats.

According to the Irwin's Comprehensive Observation Assessment, the animals were observed for the presence and frequency of the symptoms listed below prior to the administration and at 0.5, 1, 2, 3, 6 and 24 hours after the administration of the dosing solution. At each observation time point, animals were observed for behavioral parameters for 2 minutes in the polycarbonate cage (34.5 x 40.3 x 17.7 cm (WDH)), then the items of reflex and muscle tone were examined.

The observed parameters were:

- Body position
- Behavior
- Autonomic nervous system
- Reflex and muscle tone

2. Effects on respiratory-cardiovascular system

The observations of the respiratory-cardiovascular systems were performed only with rats.

Respiratory rate

The respiratory movement was recorded on sampling/analyzing software (PowerLab/4S, ADInstuments) with a measuring device (Whole body plethysmography, emka Technologies), under food and water deprivation, for 30 minutes before administration and form just after to 6 hours after the administration.

Respiratory rate (resp. times/minute) and tidal volume (VT, mL) were measured prior to and 0.5, 1, 2, 3 and 6 hours after administration. The changes from the values before administration were calculated. Theses parameter were calculated from the 1-minute interval data at each measuring point using sampling/analyzing software (PowerLab).

Blood pressure and heart rate

The systolic blood pressure (SBP), diastolic blood pressure (DBP), mean blood pressure (MBP) and heart rate (HR) were measured with a non-invasive manometer (BP-98A, Softron) before and at 1, 2, 3 and 6 hours after administration of the dosing solutions. Rats were warmed for about 15 minutes before measurement using the warming equipment set at 38°C. The animals were measured 3-times at each time point, and the mean value was calculated. The changes from the values before administration were also calculated.

II. RESULTS AND DISCUSSION

A. GENERAL BEHAVIOR

1. Mice

Numbers of defecation in the 600 and 2000 mg/kg bw groups were significantly lower than that of the control at 6 hours after test item administration. However, this finding was not observed at 24 hours after administration and suppressed changes were not found in any time point of observation. In addition, there were no similar effects on the observation of general behavior in rats. Therefore, this change was judged to be not a test substance-related effect. No other treatment-related effects in other parameters were observed in mice.

2. Rats

There were no abnormal clinical signs in any of the test substance-treated groups. Moreover, there were no significant differences in the numbers of appearance of grooming, yawn, urination and defecation between the test substance-treated groups and control groups.

B. RESPIRATORY-CARDIOVASCULAR SYSTEM

1. Respiratory rate

There were no significant differences in the respiratory rate or the values of change from the value before administration of this parameter between the treatment groups and control group at each time point.

There were no significant differences in the tidal volume between the treatment groups and control group at each time point. The value of change from the value before administration of tidal volume in the 600 mg/kg group was significantly higher than that of control at 1-hour time point after administration.

Table 5.8.2-11:Effect of Triticonazole on tidal volume (VT) in rats

Test substance		**Vehicle (control)**	**Triticonazole**		
Dose [mg/kg p.o.]		**10 mL/kg**	**200**	**600**	**2000**
No. of animals used		5	5	5	5
Tidal volume (change from before administration)					
Before	[mL]	1.00	1.02	1.00	0.75
	SD	0.32	0.15	0.20	0.31
0.5h	[mL]	1.12 (0.12)	0.96 (-0.06)	1.42 (0.42)	1.32 (0.57)
	SD	0.36 (0.12)	0.12 (0.07)	0.27 (0.18)	0.36 (0.32)
1h	[mL]	0.94 (-0.06)	0.95 (-0.08)	1.48 **(0.48)***	1.11 (0.37)
	SD	0.30 (0.12)	0.17 (0.14)	0.32 (0.16)	0.30 (0.15)
2h	[mL]	0.85 (-0.15)	1.13 (0.11)	1.16 (0.16)	1.12 (0.37)
	SD	0.24 (0.17)	0.14 (0.15)	0.31 (0.15)	0.41 (0.13)
3h	[mL]	0.95 (-0.05)	1.06 (0.04)	1.24 (0.23)	0.98 (0.23)
	SD	0.26 (0.15)	0.20 (0.13)	0.34 (0.15)	0.30 (0.04)
6h	[mL]	0.98 (-0.02)	0.93 (-0.09)	1.16 (0.15)	0.96 (0.21)
	SD	0.26 (0.17)	0.21 (0.12)	0.21 (0.08)	0.29 (0.11)

* $p \leq 0.05$ (Dunnett-test, two sided)

2. Blood pressure and heart rate

When compared with the control group, statistically significant higher SBP values and higher values of changes from the values before administration were noted in the 600 mg/kg bw group at 1 hour after administration and in the 2000 mg/kg group at 1 and 2 hours after administration. Significant higher DBP values were noted in the 600 mg/kg bw group at 2 hours after administration and in the 2000 mg/kg group at 1 and 2 hours after administration. Significant higher values of changes of DBP form the values before administration were noted in the 200 and 2000 mg/kg groups at 1 hour after administration.
Significant higher values of changes of MBP from the values before administration were noted in the 200, 600 and 2000 mg/kg bw groups at 1 hour after administration.
The differences of blood pressure in the 200 and 600 mg/kg bw groups were less than 10 mmHg, and there were no changes in the MBP values throughout this study. Therefore, these changes were assessed to be not test substance-related effects.
In the 2000 mg/kg bw group, the SBP, DBP and MBP values were significantly higher or tended to be high at 1 and 2 hours after administration. These changes in the 2000 mg/kg bw group were suggested to be test substance-related effects. However, normal blood pressure was noted at 3 hours after administration, so that these high values were considered to be transient.

Table 5.8.2-12: Effect of Triticonazole on blood pressure in rats

Test substance		Vehicle (control)	Triticonazole		
Dose [mg/kg bw p.o.]		**10 mL/kg**	**200**	**600**	**2000**
No. of animals used		5	5	5	5
Systolic blood pressure (change from before administration)					
Before	[mmHg]	101.9	103.5	102.1	103.2
	SD	2.2	2.2	3.7	4.6
1h	[mmHg]	99.3 (-2.7)	106.2 (2.7)	**111.7**# **(9.6***)	116.2 (**13.0****)
	SD	3.7 (2.7)	3.4 (3.3)	0.6 (3.4)	5.3 (3.5)
2h	[mmHg]	95.7 (-6.3)	108.6 (5.1)	110.5 (8.4)	**121.9**** **(18.7***)
	SD	5.1 (4.2)	6.0 (6.5)	2.3 (5.9)	7.0 (6.2)
3h	[mmHg]	104.5 (2.5)	110.1 (6.6)	110.5 (8.4)	108.3 (5.1)
	SD	7.7 (7.3)	5.0 (5.6)	3.1 (4.7)	4.6 (4.9)
6h	[mmHg]	101.7 (-0.3)	109.5 (6.1)	101.9 (-0.1)	109.6 (6.4)
	SD	2.8 (4.9)	4.0 (4.3)	3.3 (5.2)	3.5 (1.7)
Diastolic blood pressure (change from before administration)					
Before	[mmHg]	79.5	77.5	82.8	80.6
	SD	4.8	2.9	3.2	3.6
1h	[mmHg]	77.0 (-2.5)	86.7 (**9.3***)	89.2 (6.4)	**91.3* (10.7*)**
	SD	4.6 (3.4)	1.7 (2.8)	2.1 (2.2)	5.5 (3.6)
2h	[mmHg]	77.3 (-2.1)	85.6 (8.1)	**91.1*** (8.3)	**91.6*** (11.0)
	SD	1.9 (3.2)	3.8 (4.4)	1.8 (4.6)	5.4 (7.2)
3h	[mmHg]	83.5 (4.0)	90.0 (12.6)	91.8 (8.9)	86.3 (5.7)
	SD	7.1 (5.0)	4.6 (5.4)	2.2 (3.5)	3.5 (6.4)
6h	[mmHg]	80.9 (1.4)	90.0 (12.5)	83.3 (0.5)	84.3 (3.7)
	SD	3.5 (6.7)	4.3 (4.8)	1.7 (3.6)	3.9 (2.5)
Mean blood pressure (change from before administration)					
Before	[mmHg]	86.9	86.1	89.1	88.1
	SD	3.7	2.6	3.3	3.8
1h	[mmHg]	84.2 (-2.7)	93.1 (**7.0***)	96.6 (**7.5***)	**99.5* (11.4**)**
	SD	4.2 (2.5)	2.1 (2.3)	1.5 (2:3)	5.2 (2.9)
2h	[mmHg]	83.5 (-3.4)	93.2 (7.1)	97.4 (8.3)	**101.6*** (13.5)
	SD	2.8 (2.2)	4.4 (5.0)	2.0 (5.0)	5.9 (6.8)
3h	[mmHg]	90.3 (3.5)	96.3 (10.2)	89.3 (0.2)	92.6 (4.6)
	SD	7.2 (5.5)	4.6 (5.4)	2.5 (3.8)	3.5 (5.8)
6h	[mmHg]	87.7 (0.8)	96.3 (10.2)	89.3 (0.2)	92.6 (4.6)
	SD	2.9 (5.8)	4.1 (4.4)	2.1 (4.0)	3.8 (1.9)

* $p \leq 0.05$; ** $p \leq 0.01$ (Dunnett-test, two sided); # $p \leq 0.05$ (Steel-test, two-sided)

There were no significant differences in the HR value or the value of change of it from the value before administration at each time point between test substance-treated groups and control group.

III. CONCLUSIONS

Based on the results of this study, triticonazole did not affect the general behavior of mice and rats. In the cardiorespiratory system, no effects were noted on the respiratory parameters in rats. Blood pressure was elevated transitory in rats treated with 2000 mg triticonazole/kg bw.

Report: CA 5.8.2/3
Rotroff D.M. et al., 2010a
Xenobiotic-metabolizing enzyme and transporter gene expression in primary cultures of human hepatocytes modulated by toxcast chemicals
2010/1233112
Guidelines: none
GLP: no

Executive Summary

This study is part of the EPA ToxCast program. Primary human hepatocyte cultures as model system were used to characterize the concentration- and time-response of the 320 ToxCast chemicals for changes in expression of genes regulated by nuclear receptors. Fourteen gene targets were monitored in quantitative nuclease protection assays: six representative cytochromes P-450, four hepatic transporters, three Phase II conjugating enzymes, and one endogenous metabolism gene involved in cholesterol synthesis. These gene targets are sentinels of five major signalling pathways: AhR, CAR, PXR, FXR, and PPARalpha. Besides gene expression, the relative potency and efficacy of these chemicals to modulate cellular health and enzymatic activity were assessed. Results demonstrated that the culture system was an effective model of chemical-induced responses by prototypical inducers such as phenobarbital and rifampicin. Gene expression results identified various ToxCast chemicals that were potent or efficacious inducers of one or more of the 14 genes, and potent to interfere with the 5 nuclear receptor signalling pathways. Significant relative risk associations with rodent in vivo chronic toxicity effects are reported for the five major receptor pathways. These gene expression data are being incorporated into the larger ToxCast predictive modelling effort.
Triticonazole was active in the following NVS_ADME assays: rCYP1A1, hCYP2C18, rCYP3A1, rCYP2D2, rCYP2C11, rCYP3A2, rCYP2B1, hCYP2C19, hCYP1A1, rCYP2A2.
Expression of genes that were induced by triticonazole were linked to AhR, CAR, and PXR. In conclusion, these results together with the results of the transactivation studies (Shah et al., 2011a) should be used as supporting screening information only. Further, as triticonazole did not induce liver tumors, these results are of minor relevance for the hazard assessment of triticonazole.

Classification of study: Supplementary information

Report: CA 5.8.2/4
Shah I. et al., 2011a
Using nuclear receptor activity to stratify hepatocarcinogens
2011/1295091
Guidelines: none
GLP: no

Executive Summary

This study is part of the EPA ToxCast program. The authors investigated the effects of 309 environmental pesticides on nuclear receptors using primary human hepatocytes, HepG2 cells transfected with a multiple reporter transcription unit (MRTU) library consisting of 48 transcription factor binding sites, cis reporter gene assays and cell free and cell based cytochrome P450 assays. The resulting data was used to calculate an aggregate scaled activity score for different nuclear receptors (CAR, PXR, PPAR, AhR, SR, RXR), which was then correlated to lesion progression data extracted from EPA's pesticide database.
Triticonazole, together with Flutolanil and Oxyfluorfen, was grouped into category VII A; group A indicates, that the compound can activate the nuclear receptors AhR, CAR and PXR, but only produces mild or no lesions. The rationale for this grouping is largely based on the activation of the reporter genes.

Triticonazole was tested positive amongst others in the following activation assays: ATG_PXR_TRANS_up, ATG_PXRE_CIS, ATG_PPRE_CIS, Tox21_AR_LUC_MDAKB2_Agonist. However, as triticonazole did not induce liver tumors, these results are of minor relevance for the hazard assessment of triticonazole. Triticonazole was not associated with the activation in other assays targeting estrogen receptor.

Classification of study: Supplementary information

CA 5.8.3 Endocrine disrupting properties

Triticonazole is not falling under the interim criteria for endocrine disruption, outlined in the EU Directive 1107/2009, as it is not classified for reproduction toxicity or carcinogenicity. It is also not to be classified for reproduction toxicity or carcinogenicity as outlined in the chapters MCA 5.5 and 5.6 of this dossier.

As triticonazole is an azole, the – probably to be expected - azole-specific toxicity is evaluated in more detail in this chapter. Furthermore, the adrenal specific toxicity – seen in the rat (13-week, chronic, and 2-generation toxicity study) and dog studies (52-week) with triticonazole are also presented and discussed in more detail in this chapter. Further, the specific endocrine mechanistic studies conducted with triticonazole (either by the notifier or published in the literature) are discussed in this chapter and assessed in the context of the available in vivo studies in Sprague-Dawley rat, CD-1 mice, and in Beagle dogs.

Report: CA 5.8.3/1
Woitkowiak C., 2012a
BAS 595 F (Triticonazole) - Testing for potential androgenic and antiandrogenic activity using the YAS-assay [AR] (Yeast androgen screening)
2012/1276019
Guidelines: none
GLP: no

Executive Summary

BAS 595 F (Triticonazole; Batch: COD-001440; purity 91.3%) was tested to assess an androgenic and/or anti-androgenic activity by using the Yeast Androgen Screening Assay (YAS-Assay) with the hAR yeast strain. Two independent experiments were carried out. Vehicle (DMSO) and positive controls for androgenic effects (5α-dihydrotestosterone: 10 pM - 1 µM) and antiandrogenic effects (5α-dihydrotestosterone, 5 nM; hydroxyflutamide, 10 µM) were included into the experiment. BAS 595 F was tested at concentrations from 100 pM up to 100 µM. No precipitation was observed up to the highest concentration tested. Clear cytotoxicity was observed at 1µM. An increase in the androgen receptor dependent enzyme expression (colour development) was not observed. An inhibition of the androgen effect compared to 5 nM 5α-dihydrotestosterone was also not observed.
Under the experimental conditions of the study, BAS 595 F (Triticonazole) did not exert androgenic or anti-androgenic effects in the Yeast Androgen Screening (YAS) assay using the hAR yeast strain.

(BASF DocID 2012/1276019)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material BAS 595 F (Triticonazole)

Description:	Solid, white
Lot/Batch #:	COD-001440
Purity:	91.3% (tolerance ± 1.0%)
Stability of test compound:	NA
Solvent used:	Dimethylsulfoxide (DMSO)

2. Control Materials:

Vehicle control:	DMSO 1% (v/v)
Positive control compounds:	Androgenic control: 5α-dihydrotestosterone Antiandrogenic control: 5α-dihydrotestosterone combined with hydroxyflutamide

3. Test organisms: Yeast cells (*Saccharomyces cerevisiae*) have been stably transformed with a gene encoding the human androgen receptor (hAR), which is constitutively expressed. Additionally, these cells are stably transformed with a reporter gene plasmid, containing an androgen response element and the *LacZ* gene, which encodes the reporter enzyme β-galactosidase.

B. TEST PERFORMANCE:

1. Dates of experimental work: 10-Sep-2012 to 21-Sep-2012

2. Final test substance concentrations:

Final test substance concentrations: 10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} and 10^{-4} mol/L

2. Test method:

A deep-frozen (-80°C) yeast stock culture was thawed at room temperature, inoculated in growth medium and incubated for pre-culture (24-72 h) and growth medium was exchanged after 72 h before use. Of the preculture, optical density (OD) was determined at 690 nm. For preparation of the test culture, 0.50 mL of the pre-culture with an OD of 1.0 was transferred into 50 mL fresh culture medium including 0.5 mL chromogenic substrate CPRG (chlorophenol red-β-D-galactopyranoside).
The study was carried out in 96-well microtiter plates in which 2 µL of different test substance solutions had been pipetted. 200 µL of the test culture was added to each well. The plates was sealed with breathable tape and incubated until measurement of the OD.

3. Controls:

Each experiment includes a negative control (vehicle control) and positive controls for the verification of the detection of androgenic and anti-androgenic activity in the yeast cells.

Negative controls / Vehicle controls
The vehicle control contains 2 µL of the vehicle used for the test substance. The final concentration of the vehicle in the culture medium will be 1% (v/v).

Positive controls
Androgenic control:
5α-dihydrotestosterone
Final concentrations: 10^{-11}, 10^{-10}, $5*10^{-9}$, 10^{-9}, 10^{-8}, 10^{-7}, and 10^{-6} mol/L

Anti-androgenic Control:
5α-dihydrotestosterone combined with hydroxyflutamide
Final concentrations:
$5x10^{-9}$ mol/L (5α-dihydrotestosterone)/ $1x10^{-5}$ mol/L (hydroxyflutamide)

The stability of the selected positive controls is well-defined under the chosen culture conditions since they are well established reference endocrine disruptors.

4. Evaluation/Assessment

4.1 Endocrine activity

After 48 h (±4 h) incubation, absorbance of the plates is measured at 570 nm (colour development, androgen receptor dependent enzyme expression) and 690 nm (turbidity due to growth of the yeast). Evaluation is performed by calculating the difference of the measured ODs at the two wavelengths (absorption at 570 nm - absorption at 690 nm). For the 4 replicates per concentration the median, minimum and maximum value are presented in a diagram. The findings of the 1st experiment were confirmed in an additional assay. The concentrations and test conditions were the same as in the first investigation.

4.2 Cytotoxicity

Cytotoxicity of the test substance is indicated by decrease of the yeast growth (measurement of the turbidity at 690 nm). For the evaluation of anti-androgenic activity only nontoxic test substance concentrations are taken into consideration.

4.3 Acceptance criteria

Generally, the experiment is considered valid, if the following criteria are met:

- The positive controls induced an agonistic / antagonistic effect within the range of the historical control data.
- The concentration $5x10^{-9}$ mol/L 5α-dihydrotestosterone achieved at least 40 percent of the maximum effected androgen receptor dependent enzyme expression of the positive control (colour development) based on the experiment.
- The vehicle control did not show colour development at 570 nm.

4.4 Assessment criteria

Generally, cytotoxicity (decrease of the yeast growth) is considered for data interpretation especially in the case of anti-androgenic activity.

A test substance is generally considered non-androgenic in this assay, if:

- Androgen receptor dependent enzyme expression was within the historical negative control range under all experimental conditions in two experiments carried out independently.

The test substance is considered as androgenic in this assay, if:

- A concentration-dependent and reproducible increase of the androgen receptor dependent enzyme expression (colour development) by at least 20% compared to the vehicle control was observed.
- If a concentration-dependent and reproducible increase of the androgen receptor dependent enzyme expression (colour development) by at least 10% and less than 20% compared to the vehicle control was observed, the test substance is considered to be slightly androgenic.

The test substance is considered as anti-androgenic in this assay, if:

- A concentration-dependent and reproducible inhibition of the androgenic effect compared to $5x10^{-9}$ mol/L 5α-dihydrotestosterone (partly or total suppression of expected colour development, without signs for cytotoxicity) by at least 20% was observed compared to $5x10^{-9}$ mol/L 5α-dihydrotestosterone alone.
- If a concentration-dependent and reproducible inhibition of the androgenic effect compared to $5x10^{-9}$ mol/L 5α-dihydrotestosterone (partly or total suppression of expected colour development, without signs for cytotoxicity) by at least 10% but less than 20% compared to $5x10^{-9}$ mol/L 5α-dihydrotestosterone alone was observed, the test substance is considered to be slightly anti-androgenic.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

No analytical determination of the test substance solutions was performed. No test substance precipitation was found.

B. ENDOCRINE ACTIVITY

Androgenicity:

An increase in the androgen receptor dependent enzyme expression (colour development) was not observed.

Anti-androgenicity:

Inhibition of the androgen effect in comparison to hydroxyflutamide was not observed.

C. CYTOTOXICITY OF THE TEST SUBSTANCE

Due to clear cytotoxicity observed at a concentration of 10^{-6} mol/L, an evaluation of the androgenic/anti-androgenic potential of the test substance at this concentration was not possible.

Androgen receptor-dependent enzyme expression

% absorption	200, 180, 160, 140, 120, 100, 80, 60, 40, 20, 0
concentration [mol/L]	1,E-12, 1,E-10, 1,E-08, 1,E-06, 1,E-04

—□— Dihydrotestosterone —◆— BAS 595 F (Triticonazole) +Dihydrotestosterone [5nmol/L]

Figure 5.8.3-1: Androgen receptor-dependent enzyme expression; Normalized median (% absorption calculated relative to the absorbance values obtained for 10-8 M dihydrotestosterone)

III. CONCLUSIONS

Under the experimental conditions of the study, BAS 595 F (Triticonazole) did not exert androgenic or anti-androgenic effects in the Yeast Androgen Screening (YAS) assay using the hAR yeast strain.

Report: CA 5.8.3/2
Woitkowiak C., 2012b
BAS 595 F (Triticonazole) - Testing for potential estrogenic and antiestrogenic activity using the YES-assay [ERa] (Yeast estrogen screening)
2012/1276018
Guidelines: none
GLP: no

Executive Summary
BAS 595 F (Triticonazole; Batch: COD-001440; purity 91.3%) was tested to assess an estrogenic and/or anti-estrogenic activity by using the Yeast Estrogen Screening Assay (YES-Assay) with the hERα yeast strain. Two independent experiments were carried out. Vehicle (DMSO) and positive controls for estrogenic effects (17β-estradiol: 1 pM - 1 µM) and anti-estrogenic effects (17β-estradiol, 1 nM; 4-hydroxytamoxifen, 1 µM) were included into the experiment. BAS 595 F was tested at concentrations from 100 pM up to 100 µM. No precipitation was observed up to the highest concentration tested. Clear cytotoxicity was observed at concentrations of 10 µM onwards. An increase in the estrogen receptor dependent enzyme expression (colour development) was not observed. Furthermore, no inhibition of the estrogen effect compared to 1 nM 17β-estradiol was observed.
Under the experimental conditions of the study, BAS 595 F (triticonazole) did not exert estrogenic or anti-estrogenic effects in the Yeast Estrogen Screening (YES) assay using the hERα yeast strain.
(BASF DocID 2012/1276018)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

1. Test Material	BAS 595 F (Triticonazole)
Description:	Solid, white
Lot/Batch #:	COD-001440
Purity:	91.3% (tolerance ± 1.0%)
Stability of test compound:	NA
Solvent used:	Dimethylsulfoxide (DMSO)

2. Control Materials:

Vehicle control:	DMSO 1% (v/v)
Positive control compounds:	Estrogenic control: 17β-estradiol
	Anti-estrogenic control: 17β-estradiol combined with 4-hydroxytamoxifen

3. Test organisms: Yeast cells (*Saccharomyces cerevisiae*) have been stably transformed with a gene encoding the human estrogen receptor α (hERα), which is constitutively expressed. Additionally, these cells are stably transformed with a reporter gene plasmid, containing an estrogen response element and the *LacZ* gene, which encodes the reporter enzyme β-galactosidase. The hERα yeast strain was obtained from "Technische Universität Dresden", Prof. Dr. G. Vollmer on 11 Feb 2010.

B. TEST PERFORMANCE:

1. Dates of experimental work: 09-Sep-2012 to 26-Sep-2012

2. Test substance concentrations:

Final test substance concentrations: 10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} and 10^{-4} mol/L

3. Test method:

A deep-frozen (-80°C) yeast stock culture was thawed at room temperature, inoculated in growth medium and incubated for pre-culture (24-72 h) and growth medium was exchanged after 72 h before use. Of the pre-culture, optical density (OD) was determined at 690 nm. For preparation of the test culture, 0.50 mL of the pre-culture with an OD of 1.0 was transferred into 50 mL fresh culture medium including 0.5 mL chromogenic substrate CPRG (chlorophenol red-β-D-galactopyranoside).

The study was carried out in 96-well microtiter plates in which 2 µL of different test substance solutions had been pipetted. 200 µL of the test culture was added to each well. The plates was sealed with breathable tape and incubated until measurement of the OD.

4. Controls:

Each experiment includes a negative control (vehicle control) and positive controls for the verification of the detection of estrogenic and anti-estrogenic activity in the yeast cells.

Negative controls / Vehicle controls

The vehicle control contains 2 µL of the vehicle used for the test substance. The final concentration of the vehicle in the culture medium will be 1% (v/v).

Positive controls
Estrogenic control:
17β-estradiol (dissolved in ethanol)
Final concentrations: 10^{-12}, 10^{-11}, 10^{-10}, 10^{-9}, 10^{-8}, 10^{-7} and 10^{-6} mol/L

Anti-estrogenic Control:
17β-estradiol combined with 4-hydroxytamoxifen (dissolved in DMSO)
Final concentrations:
$1x10^{-9}$ mol/L (17β-estradiol)/ $1x10^{-6}$ mol/L (4-hydroxytamoxifen)

The stability of the selected positive controls is well-defined under the chosen culture conditions since they are well established reference endocrine disruptors.

5. Evaluation/Assessment

5.1 Endocrine activity

After 48 h (±4 h) incubation, absorbance of the plates is measured at 570 nm (colour development, estrogen receptor dependent enzyme expression) and 690 nm (turbidity due to growth of the yeast). Evaluation is performed by calculating the difference of the measured ODs at the two wavelengths (absorption at 570 nm - absorption at 690 nm). For the 4 replicates per concentration the median, minimum and maximum value are presented in a diagram. The findings of the 1st experiment were confirmed in an additional assay. The concentrations and test conditions were the same as in the first investigation.

5.2 Cytotoxicity

Cytotoxicity of the test substance is indicated by decrease of the yeast growth (measurement of the turbidity at 690 nm). For the evaluation of anti-estrogenic activity only nontoxic test substance concentrations are taken into consideration.

5.3 Acceptance criteria

Generally, the experiment is considered valid, if the following criteria are met:

- The positive controls induced an agonistic / antagonistic effect within the range of the historical control data.
- The concentration $1x10^{-9}$ mol/L 17β-estradiol achieved at least 40 percent of the maximum effected androgen receptor dependent enzyme expression of the positive control (colour development) based on the experiment.
- The vehicle control did not show colour development at 570 nm.

5.4 Assessment criteria

Generally, cytotoxicity (decrease of the yeast growth) is considered for data interpretation especially in the case of anti-estrogenic activity.

A test substance is generally considered non-estrogenic in this assay, if:

- Estrogen receptor dependent enzyme expression was within the historical negative control range under all experimental conditions in two experiments carried out independently.

The test substance is considered as estrogenic in this assay, if:

- A concentration-dependent and reproducible increase of the estrogen receptor dependent enzyme expression (colour development) by at least 20% compared to the vehicle control was observed.
- If a concentration-dependent and reproducible increase of the estrogen receptor dependent enzyme expression (colour development) by at least 10% and less than 20% compared to the vehicle control was observed, the test substance is considered to be slightly estrogenic.

The test substance is considered as anti-estrogenic in this assay, if:

- A concentration-dependent and reproducible inhibition of the estrogenic effect compared to $1x10^{-9}$ mol/L 17β-estradiol (partly or total suppression of expected colour development, without signs for cytotoxicity) by at least 20% was observed compared to $1x10^{-9}$ mol/L 17β-estradiol alone.
- If a concentration-dependent and reproducible inhibition of the estrogenic effect compared to $1x10^{-9}$ mol/L 17β-estradiol (partly or total suppression of expected colour development, without signs for cytotoxicity) by at least 10% but less than 20% compared to $1x10^{-9}$ mol/L 17β-estradiol alone was observed, the test substance is considered to be slightly anti-estrogenic.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

No analytical determination of the test substance solutions was performed. No test substance precipitation was found.

B. ENDOCRINE ACTIVITY

Estrogenicity:

An increase in the androgen receptor dependent enzyme expression (colour development) was not observed.

Anti-estrogenicity:

A reproducible inhibition of the androgen effect compared to $1x10^{-9}$ mol/L 17β-estradiol (partly or total suppression of expected colour development) was not observed.

C. CYTOTOXICITY OF THE TEST SUBSTANCE

At 10^{-6} mol/L a moderate reduction of optical density compared to the control value was observed, and clear cytotoxicity of the test substance was noticed at a concentration of 10^{-5} mol/L onwards.

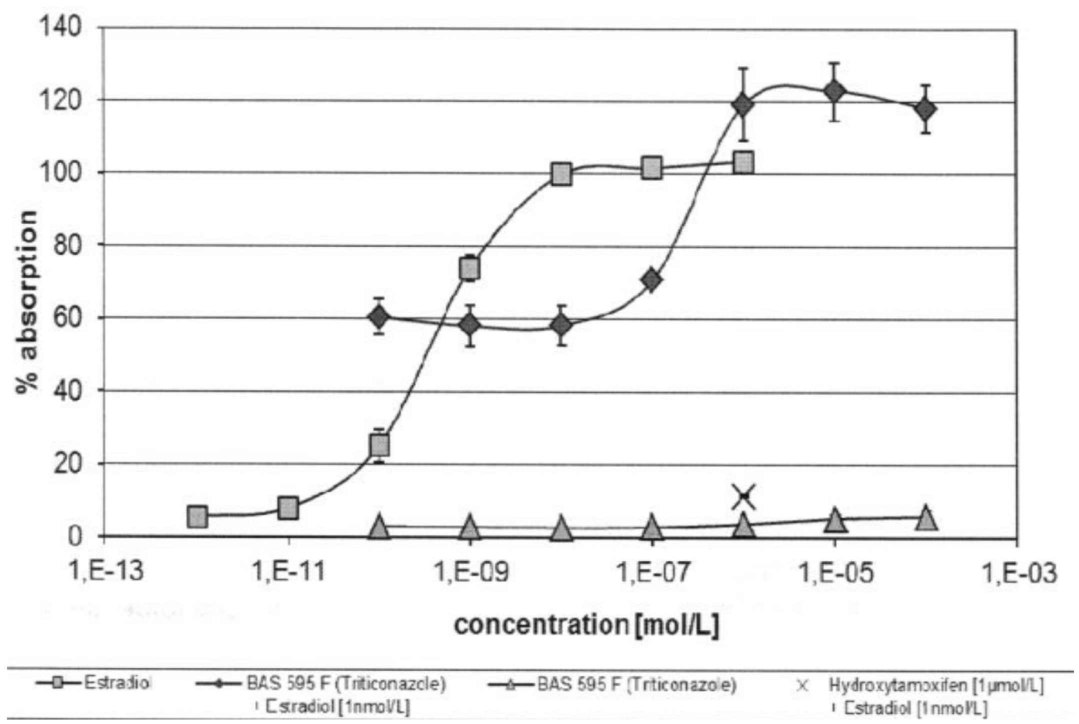


Figure 5.8.3-2: Estrogen receptor-dependent enzyme expression; Normalized median (% absorption calculated relative to the absorbance values obtained for 10^{-8} M 17β-estradiol)

III. CONCLUSIONS

Under the experimental conditions of the study, BAS 595 F (Triticonazole) did not exert estrogenic or anti-estrogenic effects in the Yeast Estrogen Screening (YES) assay using the hERα yeast strain.

Report: CA 5.8.3/3
Mentzel T., 2015a
Triticonazole (BAS 595 F) - Human and rat recombinant aromatase assay
2015/1197309
Guidelines: EPA 890.1200
GLP: no
(certified by <none>)

Executive Summary

Triticonazole (98.8% pure, batch: SZBB349XV) was tested in vitro for its 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 a full inhibition of rat and human aromatase activities was achieved. Also for the test substance a full inhibition of rat aromatase was achieved using a concentration of 100 µM. In contrast no complete inactivation is found for human aromatase enzyme for which a mean activity of about 30 % was found at maximal concentration. Despite this incomplete inhibition half maximal inhibition could be calculated for all compounds. The resulting human aromatase IC_{50} values was 44 µM for triticonazole. The resulting rat aromatase IC_{50} value was 1.8 µM for triticonazole. Thus, the test substance-induced aromatase inhibition was more pronounced for the rat enzyme by a factor of >20.

(BASF DocID 2015/1197309)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

Test item:	Triticonazole
Description:	Solid, powder
Lot/Batch #:	SZBB349XV
Purity:	98.8%

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 09-June-2015

2. Test substance preparation:
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.
In addition to a macroscopic inspection also microscopic analysis has been done to examine solubility limitations at high compound concentrations. Compounds have been diluted in an identical volume and buffer as for the assay performed using a 96 well plate which allows microscopic analysis.

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) as described by Stresser et al., 2000. After addition of the test compounds (in all 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. Also data handling and interpretation is adopted to this guideline. However, 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-ethylhexyl)phthalate) have been carried out not only in one, but in each 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 is 7.69 and 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 significantly diverging 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 (Carroll & Ruppert, 1988). 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 between parameters obtained at different days or for identical plates were detected using a t-test. Significance levels were adjusted using a Bonferroni correction. In cases when parameter estimates for the IC_{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.

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 (Ritz & Strebig 2015).

II. RESULTS AND DISCUSSION

A. Human Aromatase Inhibition

Triticonazole was tested up to 100 µM, as precipitation was observed at higher concentrations. Triticonazole showed aromatase inhibition on the human enzyme with an IC_{50} of 44 µM.

Table 5.8.3-1: Mean human aromatase IC_{50} values

Test item	human aromatase IC50 [M]	
	mean	SE
Test substance		
Triticonazole	4.40×10^{-5}	3.01×10^{-5}
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 other azole compounds tested within this study this corresponds to an about 50 000 to 20000 fold difference for the inhibition of human aromatase compared to letrozole and econazole. Compared to other known aromatase inhibitors we find 3200 fold differences compared to 4-OH ASDN and a 35 fold difference compared to fenarimol. Accordingly triticonazole has a potency for inhibition of the human aromatase enzyme, which is several magnitudes below the weakest inhibitory substance analyzed here.

B. Rat Aromatase Inhibition

For the inhibition of the rat aromatase the half maximal concentration is 1.80×10^{-6} M, however at this concentration not a full inhibition for the aromatase was found.

Table 5.8.3-2: Mean rat aromatase IC_{50} values

Test item	rat aromatase IC_{50} [M]	
	mean	SE
Test substance		
Triticonazole	1.8×10^{-6}	2.59×10^{-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

In addition to the measured differences in IC_{50} values between the tested compounds, triticonazole also has a different potency on human and rat aromatase. The half maximal inhibitory concentration for triticonazole on rat Cyp19 is by a factor >20 fold below the IC_{50} for the inhibition on human aromatase. Accordingly, triticonazole is a stronger inhibitor for rat aromatase than for human aromatase enzyme.

Table 5.8.3-3: Pairwise comparison of IC_{50} values of triticonazole and positive controls for human and rat aromatase inhibition

Test substance	fold difference (human/rat)	Standard error
Triticonazole	24.38	17.06
4-OH ASDN	0.39	0.07
Econazole-nitrate	1.44	0.20
Fenarimol	7.05	1.29
Letrozole	0.59	0.07

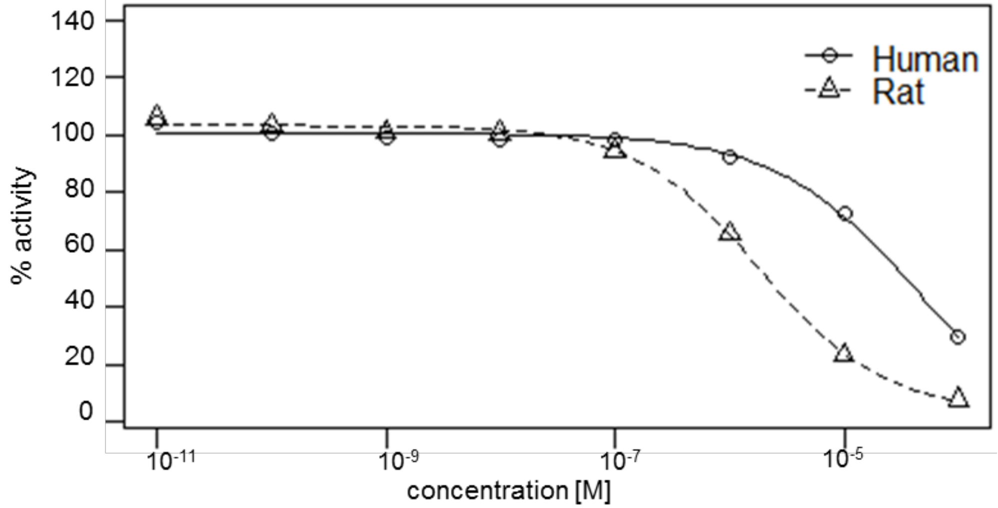


Figure 5.8.3-3: Comparison of triticonazole induced inhibition of rat and human aromatases

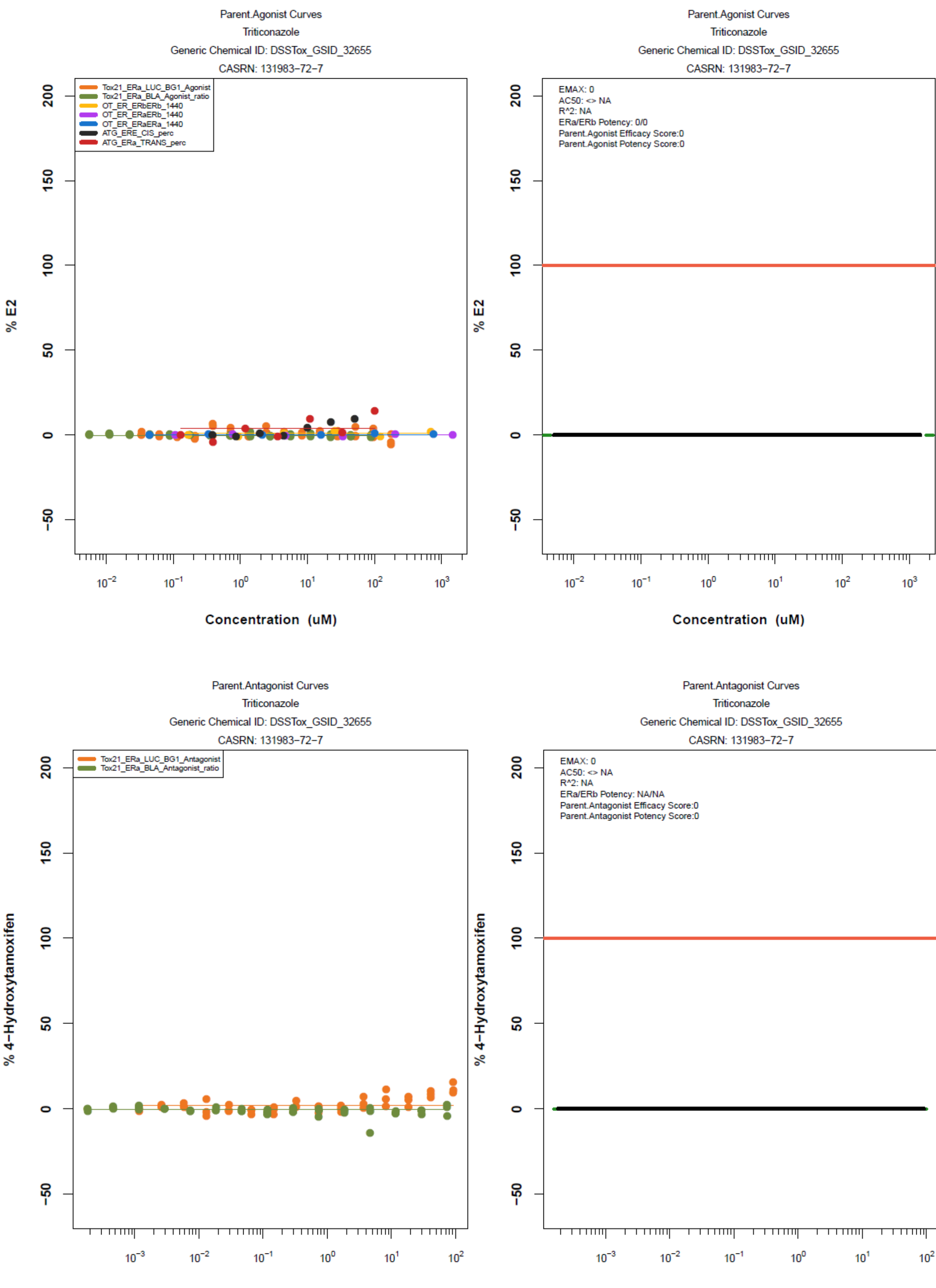
III. CONCLUSION

In this study the IC50 value of triticonazole to inhibit rat and human aromatase has been determined. Triticonazole had an IC50 value for rat aromatase of 1.8 x 10-6 [M]. The IC50 value for human aromatase was found to be 4.40 x 10-5 [M]. Compared to a number of positive controls (4-OH-ASDN, Econazole-nitrate, Fenarimol, Letrozol) the inhibiting properties of triticonazole for human aromatase were between 35 - 50000-fold lower, when IC50 values – measured in the same test runs – were compared.
Comparing the rat and human aromatase inhibiting properties of triticonazole it has been found, that the IC50 values were >20-fold different, giving evidence, that triticonazole was 20-fold less potent to inhibit human aromatase compared to rat aromatase. No complete inactivation was found for human aromatase enzyme for which a mean activity of about 30% was found at the maximum technically achievable concentration of triticonazole (100 µM).

Report:	CA 5.8.3/4 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) signaling, 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 signaling 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 triticonazole is relevant and thus the results are described in the following figures. In summary, triticonazole is negative for estrogen receptor (ER) signaling endpoints, namely binding, agonist, antagonist and cell growth responses. The ER Interaction Score was found to be 0 for triticonazole. Therewith, triticonazole is one of the 82% chemicals which did not display indications of interacting with the ER signaling pathway.


Parent.Agonist Curves
Triticonazole
Generic Chemical ID: DSSTox_GSID_32655
CASRN: 131983−72−7
Tox21_ERa_LUC_BG1_Agonist
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)
EMAX: 0
AC50: <> NA
R^2: NA
ERa/ERb Potency: 0/0
Parent.Agonist Efficacy Score:0
Parent.Agonist Potency Score:0
Parent.Antagonist Curves
Tox21_ERa_LUC_BG1_Antagonist
Tox21_ERa_BLA_Antagonist_ratio
% 4−Hydroxytamoxifen
ERa/ERb Potency: NA/NA
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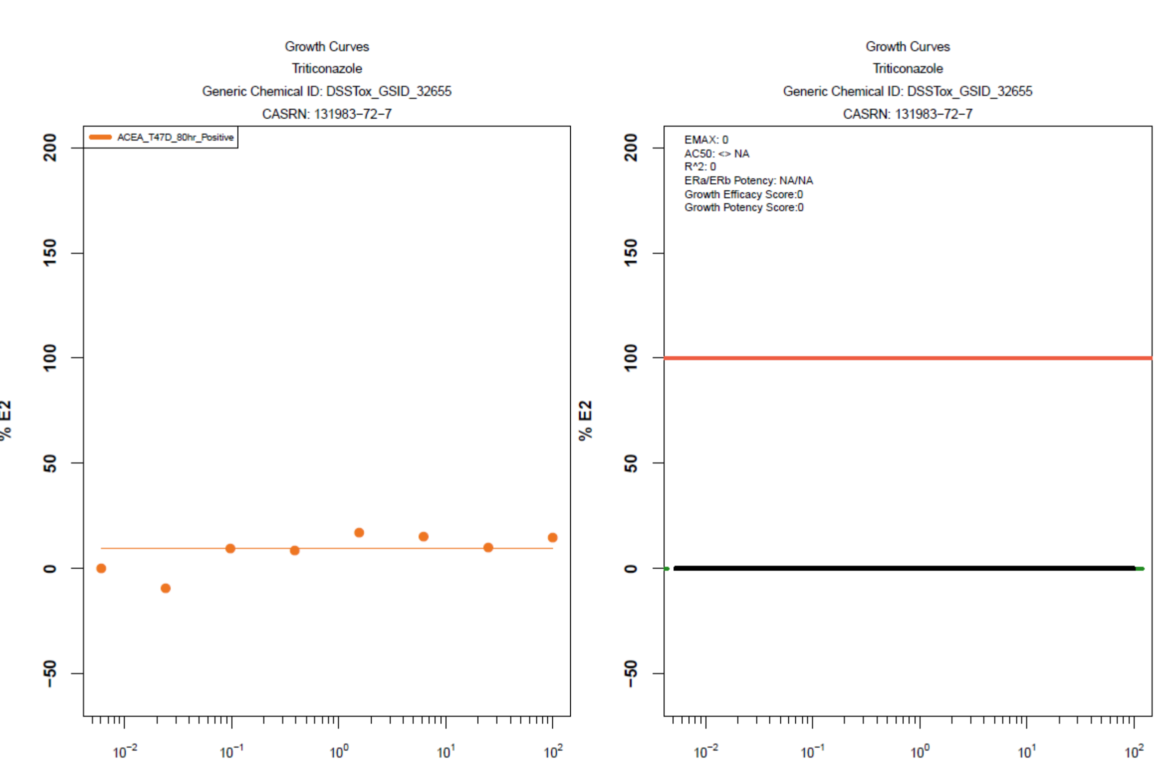
Parent.Antagonist Potency Score:0



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 signaling 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. Triticonazole was one of them and found to be zero for the binding group, agonist group, antagonist group, growth group and the ER Interaction Score and would be therefore of low priority for additional ER testing.

Classification of the study: supplementary information

Report:	CA 5.8.3/5 Reif D.M. et al., 2010a Endocrine profiling and prioritization of environmental chemicals using ToxCast data 2010/1231552
Guidelines:	none
GLP:	no

Executive Summary of the Literature

This publication describes a profiling tool developed on the ToxCast database to prioritize chemicals with regard to endocrine disruption evaluation/testing as a decision support tool. Thus this priorization tool was applied also to triticonazole being part of the ToxCast program. The priorization tool focused on estrogen, androgen and thyroid pathways and thus incorporated those screening assays of the ToxCast program considered relevant for putative endocrine profiles. In addition it incorporated external molecular pathway databases i.e. Kyoto Encyclopedia of Genes and Genomes (KEGG), Ingenuity software and the Online Mendelian Inheritance in Men repository. The tests in which triticonazole showed an activity are the same as described in the publication of Shah et al. 2011 (see M-CA 5.8.2). The current evaluation of the results can be seen on EPA's dashboard (http://actor.epa.gov/dashboard/). The so-called ToxPi profile for triticonazole (supplementary information) indicates activity in LogP and XME/ADME path, and medium activity in TR (thyroid receptor), predicted Caco-2, AR (androgen receptor) and other NR (nuclear receptor), as well as low activity in KEGG pathways. The publication does not provide an absolute ranking but in visual comparison to activity alerts for other compound the ranking of activities for triticonazole is moderate. No linkage between triticonazole and other endpoints is provided.

The results of the ToxCAST program are difficult to assess in the context of a regulatory assessment, as this analysis does not take into account that multiple assays exist for the same pathway or endpoint within the ToxCast program. In principle a chemical acting on any given pathway should score at multiple assays associated with that endpoint. Individual hits should be disregarded in the context of a weight of evidence. Another factor not taken into account is that assay results achieved at cytotoxic concentrations have low reliability. Using the EPA dashboard it is apparent, that positive activity calls were largely seen at cytotoxic concentration. Furthermore different assays for the indicated outcome did not show concordance. Further the observed in vitro activities should be assessed together with the available toxicological data package.

A more detailed description of the assays conducted under the ToxCAST program with triticonazole can be found at the EDSP21 Dashboard (EDSP21 Dashboard Endocrine Disruption Screening Program for the 21st Century (http://actor.epa.gov/edsp21/#chemical/131983-72-7))

Triticonazole was subjected to an assay set for the androgen receptor (AR - 11 assays existing, but triticonazole was only tested in 10 of the 11 assays), for the estrogen receptor (ER - 18 assays existing, but triticonazole was only tested in 16 assays) and the thyroid receptor (ThR - 4 assays existing, but triticonazole was only tested in 3 assays).

The AR assay set consists of the following assays:

- ATG AR TRANS_up
- NVS_NR_cAR
- NVS NR hAR
- NVS_NR_rAR (triticonazole not tested)
- OT AR ARELUC AG_1440
- OT_AR_ARSRC1_0480
- OT_AR_ARSRC1_0960
- Tox21_AR_BLA_Agonist_ratio
- Tox21_AR_BLA_Antagonist_ratio
- Tox21_AR_LUC_MDAKB2_Agonist
- Tox21_AR_LUC_MDAKB2_Antagonist

The ER assay set consists of the following 18 assays

- ACEA_T47D_80hr_Positive
- ATG_ERE_CIS_up
- ATG Era TRANS up
- NVS_NR_bER (triticonazole not tested)
- NVS_NR_hER (triticonazole not tested)
- NVS_NR_mERa
- OT_ER_ERaERa_0480
- OT ER ERaERa 1440
- OT_ER_ERaERb_0480
- OT ER ERaERb 1440
- OT_ER_ERbERb_0480
- OT ER ERbERb 1440
- OT_Era_EREGFP_0120
- OT Era EREGFP 0480
- Tox21_ERaBLA_Agonist_ratio
- Tox21_ERa_BLA_Antagonist_ratio
- Tox21_Era_LUC_BG1_Agonist
- Tox21_Era_LUC_BG1_Antagonist

ThR assay set consists of 4 assays

- ATG THRa1 TRANS up
- NVS_NR_hTRa (triticonazole not tested)
- Tox21 TR LUC GH3 Agonist
- Tox21_TR_LUC_GH3_Antagonist

Triticonazole was inactive in all assays for the estrogen receptor (ER) or the thyroid hormone receptor (ThR). In some of the assays for the androgen receptor, triticonazole was responsive. The respective AC50s, half-maximal activity concentrations, are summarized in the table below together with a description of the respective assay:

Table 5.8.3-4: AC50 concentration of triticonazole in selected AR assays

Assay	AC50 µM]	Description of the assay
NVS_NR_cAR*	0.6743	NVS_NR_cAR was analyzed in the positive direction using measured readouts from the NVS_NR_cAR assay. This biochemical assay uses recombinantly expressed chimpanzee AR protein in a single radioligand binding design with Lysate-based radiodetection technology. Activity values from this assay component endpoint suggests that the chemical exposure for 72 hours resulted in loss-of-signal between the AR receptor binding its ligand.
NVS_NR_hAR*	0.9097	NVS_NR_hAR was analyzed in the positive direction using measured readouts from the NVS_NR_hAR assay. This cell-based assay uses wild type protein extracted from LnCAP** human leydig cells, in a single radioligand binding design with Lysate-based radiodetection technology. Activity values from this assay component endpoint suggests that the chemical exposure for 20 hours resulted in loss-of-signal between the AR receptor binding its ligand.
OT_AR_ARSRC1_0480	13.8876	OT_AR_ARSRC1_0480 was analyzed in the positive direction using measured readouts from the cell-based OT AR ARSRC1 0480 assay. In this assay, recombinantly transfected HEK293T, a human kidney cell line, was exposed to chemical for 8 hours in a single-readout protein fragment complementation assay design and measured by fluorescence technology to understand changes to the protein dimerization through the human AR and its coupling protein, SRC1. Activity values from this assay component endpoint provide gain-of-signal chemical activity.
OT_AR_ARSRC1_0960	15.7639	OT_AR_ARSRC1_0960 was analyzed in the positive direction using measured readouts from the cell-based OT AR ARSRC1 0960 assay. In this assay, recombinantly transfected HEK293T, a human kidney cell line, was exposed to chemical for 16 hours in a single-readout protein fragment complementation assay design and measured by fluorescence technology to understand changes to the protein dimerization through the human AR and its coupling protein, SRC1. Activity values from this assay component endpoint provide gain-of-signal chemical activity.

Tox21_AR_LUC_-MDAKB2_Agonist	97.567	Tox21_AR_LUC_MDAKB2_Agonist was analyzed in the positive direction using measured readouts from the cell-based Tox21_AR_LUC_MDAKB2_Agonist assay. In this assay, MDA-kb2, a human breast cell line, was exposed to chemical for 24 hours in a single-readout luciferase induction design and measured by luminescence technology to understand changes to the regulation of gene expression through the human AR and androgen response element (ERE). Activity values from this assay component endpoint provide gain-of-signal chemical activity as an androgen receptor agonist.

*The abbreviation NVS indicate the supplier of the assay: Novascreen
**LnCAP cells are androgen-sensitive human prostate adenocarcinoma cells derived from the left supraclavicular lymph node metastasis from a 50-year-old Caucasian male in 1977.

Conclusion of the applicant:
The different hits on different liver targets are not surprising, as the primary target organ of triticonazole is the liver. However, no liver tumors were developed in long term studies in rodents. The observed medium activity on the thyroid receptor is considered to be of low relevance, as the thyroid is no target organ after triticonazole treatment in rats, mice and dogs. Following the more recent EPA evaluation of the triticonazole results (http://actor.epa.gov/edsp21/#chemical/131983-72-7), no alert on the thyroid receptor was identified. The AC_{50} concentrations of triticonazole determined in the OT AR ARSRC1 0480, OT AR ARSRC1 0480 and Tox21_AR_LUCMDAKB2_Agonist assays are relatively high (>10^{-5} M) and indicate an only weak activity in the respective assays. In the two Novascreen assays (NVS NR cAR and NVS_NR_hAR) a certain activity of triticonazole resulting in a loss-of-signal between the AR receptor binding its ligand has been detected in these in vitro assays with chimpanzee recombinant AR and wild type human protein isolated from an androgen-sensitive human prostate adenocarcinoma cell line (LnCAP), resulting in AC_{50} values of 0.67 – 0.97 µM. With regard to the moderate androgen receptor activity seen in the ToxCAST, a more detailed discussion is provided below, including the results of a further literature evaluated (see Roelofs et al., 2014a; BASF DocID 2014/1326753) and the negative YAS assay conducted by the notifier (Woitkowiak, 2012a, BASF DocID 2012/1276019).

Classification of study: Supplementary information

Report: CA 5.8.3/6
Roelofs M.J.E. et al., 2014a
Conazole fungicides inhibit Leydig cell testosterone secretion and androgen receptor activation in vitro
2014/1326753
Guidelines: none
GLP: no

Executive Summary of the Literature

In this study, the potential (anti-)androgenic effects of ten conazoles were assessed and mutually compared with existing data. Amongst others effects of triticonazole (TRIT) were examined using murine Leydig (MA-10) cells and human T47D-ARE cells stably transfected with an androgen responsive element and a firefly luciferase reporter gene. The other tested azoles were cyproconazole (CYPRO), fluconazole (FLUC), flusilazole (FLUS), hexaconazole (HEXA), myclobutanil (MYC), penconazole (PEN), prochloraz (PRO), tebucoanzole (TEBU), and triadimefon (TRIA).

The basal testosterone secretion by MA-10 cells (Leydig cells) was assessed after a 48-h exposure to 10 µM of the individual conazoles. This concentration did not significantly affect MA-10 cell viability, however these data were not shown. TRIT inhibited testosterone secretion by MA-10 cells by 44%. As shown in the figure below, triticonazole was medium potent compared to the other azoles tested.

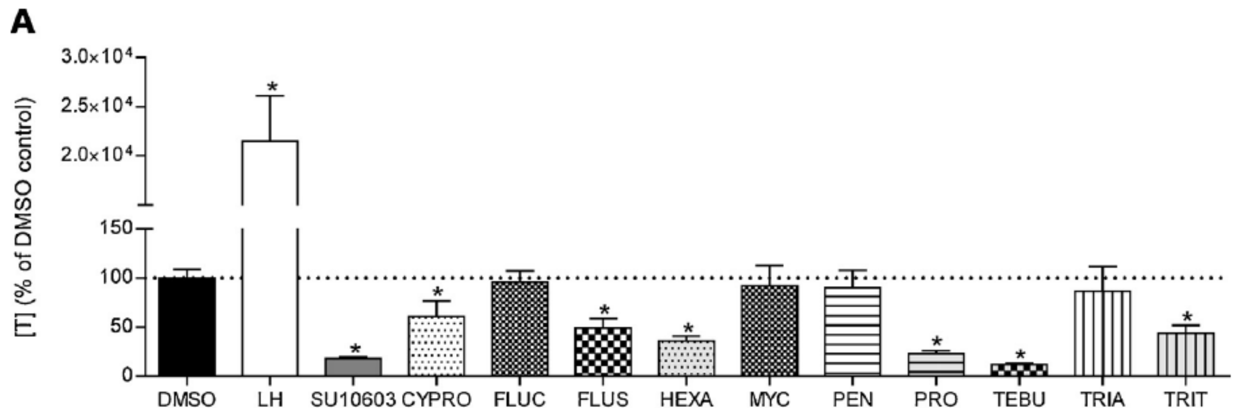


Figure 5.8.3-4: Testosterone secretion by MA-10-cells after a 48-h exposure to DMSO control, LH, the Cyp17 inhibitor SU10603, or one of the 10 tested conazoles (TRIT = triticonazole)

To further explore the nature of T secretion inhibition by MA-10 cells after exposure to certain conazole fungicides, ROS formation was considered as a possible cause for deterioration of Leydig cell function resulting in decreased T secretion. Triticonazole did not have an effect on ROS production, as shown in the figure below.

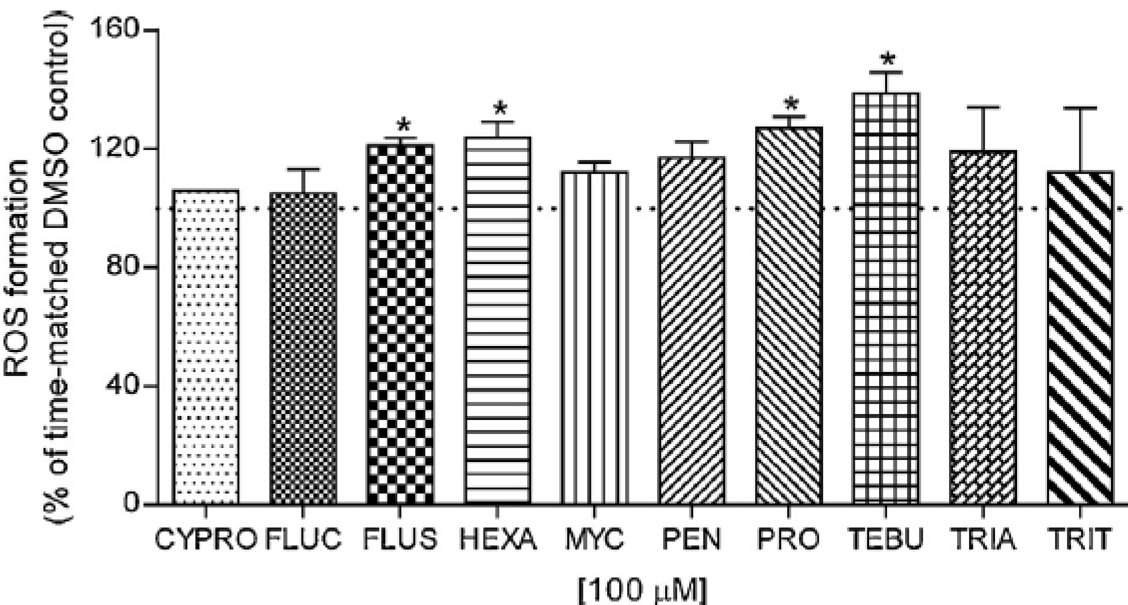


Figure 5.8.3-5: Reactive oxygen species (ROS) formation by MA-10 cells after a 48-h exposure to each of the ten selected conazoles

In a further assay androgen receptor (AR) activation was determined for the conazoles. The assays used an AR reporter gene assay. T47D-ARE cells were exposed to non-cytotoxic concentrations of the tested compounds. Testosterone (T) activated the AR in a concentration-dependent manner with an EC50 of 13.6 nM. Exposure to conazoles did not significantly affect AR activation.

Next, cells were exposed to 20nM T in combination with concentration ranges of the selected conazoles or the AR antagonist flutamide (FLUT). FLUT concentration-dependently decreased AR activation with an IC50 value of 7.0 µM. TRIT concentration-dependently inhibited T-induced AR activation with an IC_{50} of 1.07E-05 (see table below):

Table 5.8.3-5: Androgen receptor reporter gene assays outcome expressed as half maximal effect or inhibitor concentrations (EC/IC50 [M])

Compound	EC/IC_{50} [M]	$BMR_{25\%FLUT}$ [M]	REP
T	1.36E−08	n.a.	n.a.
FLUT	7.02E−06	1.98E−06	1.00
CYPRO	1.36E−05	5.25E−06	0.38
FLUC	n.a.	n.a.	n.a.
FLUS	1.19E−05	5.49E−07	3.61
HEXA	2.32E−05	7.64E−06	0.26
MYC	7.15E−05	7.06E−05	0.03
PEN	1.71E−05	5.54E−06	0.36
PRO	1.17E−05	9.43E−06	0.21
TEBU	2.55E−05	9.01E−06	0.22
TRIA	3.21E−05	1.60E−05	0.12
TRIT	1.07E−05	7.80E−06	0.25

Conclusion:
Triticonazole leads to decreased testosterone secretion in murine Leydig cell (MA-10) in vitro, when the MA-10 cells are exposed to 10 µM triticonazole (10^{-5} [M]) for 48 hours. It was medium potent compared to other azoles tested. There was no indication for an ROS formation in this test system. In an androgen receptor reporter gene assay an IC50 of 1.07E-05 for androgen receptor inhibition was determined for triticonazole. It is to be mentioned, that the concentration of 10 µM are high concentrations, which are probably not reached systemically.

Classification of study: Supplementary information

Assessment of the available potentially endocrine-related endpoints determined in studies with triticonazole

Summary of the aromatase inhibition properties of triticonazole compared to other azoles

The aromatase inhibiting properties of triticonazole have been determined by measuring the IC50 values for rat and human aromatase (see above, DocID 2015/1197309). Triticonazole had an IC_{50} value for **rat aromatase** of 1.8 x 10^{-6} [M]. The IC_{50} value for **human aromatase** was found to be 4.40 x 10^{-5} [M]. Compared to a number of positive controls (4-OH-ASDN, Econazole-nitrate, Fenarimol, Letrozol) – measured in the same test runs - the inhibiting properties of triticonazole for human aromatase were between 35- and 50000-fold lower, when IC_{50} values were compared. Furthermore, there is a considerable difference between rat and human aromatase inhibition, as shown in the study summary above (give DocID 2015/1197309); namely a factor of > 20 has been found for the IC_{50} values between rat and human, indicating a 20-fold lower potency of triticonazole to inhibit human aromatase compared to rat aromatase. It has been found that even no complete inactivation of the human aromatase could be achieved by the maximum (technically achievable) concentration of triticonazole.

Table 5.8.3-2 below presents IC_{50} data for human recombinant aromatase taken from Trösken et al., 2004 (except the figure for triticonazole which is the value derived for human aromatase, as shown above). Data for human pharmaceuticals used as clinical fungal steroid inhibitors (antimycotics) is shown, as is data for two cytostatic conazoles, which specifically exploit aromatase inhibition in the treatment of human breast cancer. As might be expected, these are some of the most potent aromatase inhibitors of all those examined, and certainly the agricultural fungicides. Some of the human pharmaceuticals used as antimycotics (e.g. clotimazole), have aromatase IC_{50} up to 3 orders of magnitude lower than triticonazole, but are considered appropriate for regular human use.

Table 5.8.3-6: Recombinant human aromatase (Cyp19) inhibition (IC_{50}) by azole fungicides and drugs

Azole	Aromatase IC_{50} (µM)
Fungicides	
Prochloraz (I)	0.047
Flusilazole	0.055
Imazalil (I)	0.072
Penconazole	0.85
Epoxiconazole	1.44
Propiconazole	3.2
Tebuconazole	5.8
Cyproconazole	8.5
Triadimenol	12.6
Triadimefon	17.5
Triticonazole	44

Human pharmaceuticals	
Antimycotics	
Bifonazole	0.019
Miconazole	0.064
Clotrimazole	0.11
Ketoconazole	5.6
Itraconazole	>70* (33% inhibition)
Voriconazole	>140* (40% inhibition)
Fluconazole	>140* (16% inhibition)
Cytostatics	
Fadrozole	0.0076
Letrozole	0.015

The IC_{50} values (except triticonazole) were derived from Trösken et al (2004).

Of the human pharmaceutical antimycotic azoles only EPARs (European Public Assessment Reports) are available for voriconazole and posaconazole. For voriconazole and posaconazole (not examined by Trösken et al, 2004), no human adverse events that might be interpreted as endocrine in nature, could be located.

Summary of the results of developmental screening studies conducted with triticonazole compared

Triticonazole has been investigated in three different in vitro screening studies used to screen for developmental toxicity: The Zebrafish embryo test, the embryonic stem cell test (EST) and the whole embryo culture test in rat. Compared to flusilazole, hexaconazole, cyproconazole, triadimefon and myclobutanil, triticonazole was least potent in the EST and in the ZET (for more details please refer to Chapter MCA 5.6 of this dossier).

Comparison of the results of the in vitro studies with the in vivo results

The EFSA Panel on plant protection products and their residues (PPR Panel) have published a scientific opinion to test possible methodologies to assess cumulative effects from the triazole group (EFSA, 2009). As common effects of triazole toxicity, increased incidences of craniofacial malformations and hepatic toxicity (mouse liver tumors) have been identified. Further, late resorptions, extended gestation lengths, parturition difficulties and dystocia have been found to occur in rat toxicological studies conducted with compounds, which have aromatase-inhibiting properties. Most of the mentioned effects are observable in the 2-generation toxicity study (extended gestation lengths, parturition difficulties and dystocia) and the developmental toxicity study in rats (resorptions, craniofacial malformations) conducted with triticonazole. Although this study was conducted according to the old OECD TG 416 (1981), the relevant parameters mentioned above were investigated endpoints in the available study with triticonazole (BASF DocID R013085, see Chapter MCA 5.6).

At the top dose of the 2-generation toxicity study slightly statistically significantly increased extended gestation lengths with mean value of 22.6 days were observed in the F0 generation only at the top dose compared to 22.1 days in the control group. The mean gestation duration was not affected in the F1 generation. An overview over the gestation lengths is given in the following table:

Table 5.8.3-7: Summary of gestation times for the F0 and F1 generations in the 2-generation study in rats

	Number of animals with gestation time (days)									
	F0					F1				
	21	22	23	24	25	21	22	23	24	25
0 (control)	-	20	3	-	-	-	23	3	-	-
5 ppm	3	18	4	1	-	1	21	6	-	-
25 ppm	-	18	4	-	-	2	17	4	-	-
750 ppm	3	19	2	-	-	-	21	3	-	-
5000 ppm	1	11	13	1	1	-	10	5	1	1

The increased mean duration of gestation is due to two females of the 5000 ppm dose group, which had gestation times of 24 (#C79524) and 25 days (#C79528). As mentioned in Chapter MCA5.6 female no. #C79528 had to be sacrificed moribund after prolonged parturition, however the other female had surviving pups. Although prolonged gestation length is a known toxicological effect of aromatase inhibitors, the effects seen with triticonazole are considered to be of borderline significance, as the aromatase inhibiting properties are so low in rats and the substance intake during early lactation is significantly higher compared to the premating phase, which might have contributed to the strong toxicity observed in dams indicated by a marked decrease in body weight gain and high treatment-related mortality in F0 females (14%), and likely contributed to reduced pup viability at this dose. The apparent increase in the duration of gestation at 5000 ppm is within the range of historical control data, and occurred in the presence of pronounced parental toxicity, which gives some evidence, that this could also not be a specific endocrine-related effect, but occur secondary to general systemic toxicity. At this dose group, there was no consistent correlation between dam survival or pup deaths and the duration of gestation neither in the F0 nor in the F1-parental generation.

In the rat developmental toxicity study, which was dosed up to the limit dose of 1000 mg/kg bw, there was no evidence for increased incidences of (early or late) resorptions or of reduced numbers of offspring. Also no treatment-related craniofacial malformations were seen at any of the doses (see Chapter MCA 5.6).
According to the subchronic and chronic studies liver is the major target organ in all species (rats, dogs and mice), but there were no increased incidences of liver tumors detected in mice (or rats) at the terminal sacrifice.

Conclusion

Triticonazole has aromatase inhibiting properties. However, the determined IC_{50} in rats is 1.8 x 10^{-6} [M], which is factor 35 – 50000-fold below the IC_{50} values of the positive controls (see BASF DocID 2015/1197309). The effects on prolonged gestation is of borderline significance (mean days of gestation in the top dose of the F0 dams is 22.6 vs 22.1 days in the controls), as the values are within the historical controls, and they occur at considerable maternal toxicity, as the substance intake during this specific phase of the study was considerably higher compared to the other phases of the study (no dose adjustment has been conducted in this study); more specifically, the substance intake in the top dose during the early lactation was 1.8-fold higher compared to the premating phase for the F0-generation (see Table 5.8.3-8). At this dose group, there was no consistent correlation between dam survival or pup deaths and the duration of gestation neither in the F0 nor in the F1-parental generation.

Triticonazole has no effects in rat developmental toxicity studies – dosed up to 1000 mg/kg bw -; it more specifically does not induce increased incidences of resorptions, or decreases number of offspring, and does not induce craniofacial malformations. No liver tumors are induced by triticonazole. Also a comparison of triticonazole with 5 other azoles in the zebrafish embryo test and the embryonic stem cell test showed that triticonazole was the least potent azole tested.

With regard to human relevance of the described findings, there was a considerable difference determined in the potency of triticonazole to inhibit the rat aromatase vs. the human aromatase, being 20-fold less potent to human aromatase compared to rat aromatase.

Evaluation of the adrenal effects seen in rat and dog studies conducted with triticonazole

As triticonazole is showing adrenal toxicity in rat and dog studies, the following studies are investigated in more detail, in order to conclude on the adversity and on the possible mode of action of adrenal toxicity. For this purpose the notifier had conducted a pathological review [see KCA 5.8.3/7 2015/1197310] of the adrenal slides, which is shown below while presenting the results of the different studies.

Table 5.8.3-8: Studies, in which adrenal toxicity was seen after repeated dose triticonazole administration

Study	Species	Doses	Reference
4-week study	Dog	0, 10, 30, 300 mg/kg bw	R013014
52-week study	Dog	0, 2.5, 25, 150 mg/kg bw	R013082
13-week study	Rat	0, 23, 250, 12500, 25000 ppm (corresponds to 2.0, 19.8, 1117.0, 2309.3 mg/kg bw in males and 2.2, 22.3, 1183.5, 2368.8 mg/kg bw in females	R013029
2-generation toxicity study	Rat	0, 5, 25, 750, 5000 ppm (corresponds to M: F0: 0.34, 1.64, 49.35, 350.8 mg/kg bw, F1: 0.37, 1.82, 56.18, 445.3 mg/kg bw and F: F0 premating: 0.37, 1.81, 54.80, 389.3 mg/kg bw, F0 gestation: 0.32, 1.59, 48.41, 337.6 mg/kg bw; F1 premating: 0.43, 2.14, 65.25, 493.8 mg/kg bw25,	R013085
Combined oncogenicity and toxicity study	Rat	0, 5, 25, 750, 5000 ppm (corresponds to 0.2, 1.0, 29.4, 203.6 mg/kg bw in males and 0.3, 1.3, 38.3, and 286.6 mg/kg bw in females)	R013100

4-week study in dogs
Adrenal weights were not affected via treatment with triticonazole. There were no treatment-related pathology findings seen in the adrenal glands of treated dogs. This assessment was confirmed by a pathological peer-review conducted in 2015 (BASF DocID 2015/1197310).

52-weeks study in dogs
The relative adrenal weights were statistically significantly increased at 150 mg/kg bw (top dose) in male and female Beagle dogs. In the original report, it was found, that the incidence and severity of zona fascicularis (=zona fasciculata) vacuolation was increased in males and females at 150 mg/kg bw (see Table below).

Table 5.8.3-9: Incidence and severity of vacuolation, zona fasciculata, multifocal in animals killed after 52 weeks of treatment – original data evaluation

	Males				**Females**			
Dosage (mg/kg/day)	0	2.5	25	150	0	2.5	25	150
Zona fascicularis (=zona fasciculata): vacuolation								
minimal	1	0	1	1	0	0	0	0
slight	0	1	1	3	0	0	1	3
Total	1	1	2	4	0	0	1	4

After re-evaluation of the slides, it was confirmed that there was a greater incidence and/or severity of multifocal, vacuolation in the zona fasciculata of both sexes given 150 mg/kg bw compared to controls. At 25 mg/kg bw, there was a slight but clear increase in severity and incidence of the finding in males but, in females, the difference was marginal. In animals given 2.5 mg/kg bw the severity and incidence was considered comparable to that of controls (see Table below):

Table 5.8.3-10: Incidence and severity of vacuolation, zona fasciculata, multifocal in animals killed after 52 weeks of treatment – re-examination of data (BASF DocID 2015/1197310)

	Males				**Females**			
Dosage (mg/kg/day)	0	2.5	25	150	0	2.5	25	150
Zona fasciculata: vacuolation								
minimal	2	1	1	1	2	2	2	0
slight	0	1	3	1	1	0	0	2
moderate	0	0	0	2	0	0	1	2
Total	2	2	4	4	3	2	3	4

The differences in incidence and severity between the original data and re-examination can largely be attributed to variation in the level of reporting between pathologists. Essentially the results were very similar. It has been summarized by the reviewing pathologist, that the morphological appearance of the adrenal tissue is consistent with that described by Sato et al., 2012 and McInnes et al., 2011 (references see BASF DocID 2015/1197310) as a spontaneously occurring background lesion in laboratory Beagle dogs.

13-week rat study
The doses administered in this study consisted of two relatively low doses (25 and 250 ppm) and two doses exceeding the limit doses (12500 and 25000 ppm corresponding to 1117.0/1183.5 mg/kg bw and 2309.3/2368.8 mg/kg bw). No consistent adrenal weight changes were observed in the treatment groups compared to the controls, however the pathological investigations showed increased incidences of cortical fatty vacuolation in males and increased incidences of adrenal cortex degeneration in females.

Table 5.8.3-11: Group incidences of histopathological changes in the adrenals of male and female rats after 13-week dietary administration to triticonazole

	Males					Females				
Dose group	0 ppm	25 ppm	250 ppm	12500 ppm	25000 ppm	0 ppm	25 ppm	250 ppm	12500 ppm	25000 ppm
Degeneration of zona reticularis	0/10	1/10	0/10	0/10	0/10	0/10	0/10	0/9	9/10***	10/10***
Cortical fatty vacuolation	2/10	2/10	3/10	10/10	10/10	0/10	0/10	1/9	4/10	10/10
slight	2/10	2/10	3/10	3/10	2/10			1/9	3/10	8/10
Mild				3/10	1/10					
Moderate				2/10	2/10				1/10	2/10
Marked				2/10	4/10					
Severe					1/10					

***p<0.001

Re-examination confirmed the presence of minimal to marked degenerative changes in the adrenal glands of female animals given 12500 and 25000 ppm and the absence of treatment-related findings in females given 250 ppm. The appearance of treatment-related lesions in females differed significantly from that seen in treated males. The two top doses were dosed above the limit dose of 1000 mg/kg bw and are clearly considered to be too high and were not used again in any of the following studies in rats.

Two-generation toxicity study in rats

In the 2-generation toxicity study the top dose administered to rats was 5000 ppm, which corresponds to a dose of roughly 340 – 530 mg/kg bw depending on the sex, the generation and life-stage of the treated rats. Table 5.8.3-8 gives an overview over the achieved substance intakes during the different stages of the study:

Table 5.8.3-12: Calculated compound intake in mg/kg bw (using food consumption and body weight data)

	F0	F1	F0	F1	F0	F1
Dose [ppm]	**m/f (pre-mating)**		**f (gestation)**		**f (lactation)**	
5	0.34/0.37	0.37/0.43	0.32	0.33	0.58	0.46
25	1.64/1.81	1.82/2.14	1.59	1.60	2.97	2.96
750	49.35/54.80	56.18/65.25	48.41	49.10	87.99	93.25
5000	350.8/389.3	445.3/493.8	337.64	339.08	502.99	528.05

Females had lower absolute and relative adrenal weights at the 5000 ppm dose group and showed degenerative effects in histopathology. An overview over the severity and the incidence of the adrenal findings is given in the table below:

Table 5.8.3-13: Group incidences of histopathological changes in parental F0 and F1

Sex	Males					Females				
Dose [ppm]	0	5	25	750	5000	0	5	25	750	5000
Animals in group	28	28	28	27	28	27	28	28	28	24
F0 – Generation										
Adrenals # examined	28	28	28	27	28	27	28	28	28	24
- Degeneration, cortical cells characterized by vacuolation and loss of individual cells	0/28	0/28	0/28	0/27	0/28	0/27	0/28	0/28	0/28	22/24
- Vacuolation, cortex	7/28	6/28	4/28	6/27	27/28	0/27	0/28	1/28	1/28	0/24
F1-Generation										
- Degeneration, cortical cells characterized by vacuolation and loss of individual cells	0/28	0/28	0/28	0/28	0/27	0/28	1/27	0/28	0/27	11/28
- Vacuolation, cortex	15/28	9/28	8/28	9/28	27/27	0/28	1/27	0/28	0/27	0/28

The peer-reviewing pathologist stated that there was good correlation with the findings recorded by the original study pathologist. Further it was said that the lesion in the adrenal gland of females given 5000 ppm were essentially identical to that seen in the 13-week study except that the presence of degenerating corticocytes was less frequent but secondary mononuclear inflammatory cell infiltrates, in particular the appearance of macrophage "giant cells" and to a lesser extent collagen deposition were more prevalent. The 'giant cells' were interpreted to be macrophage syncytial or foreign-body giant cells engaged in the ingestion of breakdown products from degenerate corticocytes. Several of them contained small intracytoplasmic inclusions of amorphous material. This is most probably due to the differences in the doses between the 13-week study (> 1000 mg/kg bw) and the 2-generation toxicity study (340 – 530 mg/kg bw). It was confirmed, that there were no findings at 750 ppm.

It is further evident that the F0 females were more severely affected, compared to the F1 parental generation, with 22/24 females showing cortical cell degeneration in the F0 females and 11/28 F1 females being affected. Considering the fact, that the F1 generation was treated with a higher triticonazole dose (see Table 5.8.3-8) and they were treated over a longer time period (including in-utero and lactational exposure), it seems evident that the body adapts to the adrenal effects. This can also be concluded via the decreasing severity of the adrenal effects in the chronic rat study with increasing exposure duration. The high dose animals of the terminal sacrifice show very low incidences of adrenal findings compared to the interim sacrifices (see below).

Chronic and combined carcinogenicity study in rats

The top dose administered in the chronic rat study was 5000 ppm, this corresponded to mean doses of 203.6 and 286 mg/kg bw in male and female rats respectively. Each 15 animals of each sex and dose group were sacrificed at the interim time points 26 and 53 weeks, the remaining animals were pathologically investigated at the terminal sacrifice (after roughly 2 years). No changes in adrenal weights were observed at none of the investigation time points. The group incidences of the histopathological findings in adrenals at the different sacrifice time points are tabulated below:

Table 5.8.3-14: Group incidences of non-neoplastic findings in adrenals at the interim and the terminal sacrifices (terminals and decedents)

Findings	Dose group level [ppm]									
	Male					Female				
	0	5	25	750	5000	0	5	25	750	5000
Adrenals (26 weeks)										
Multinucleated cells (Z. f.)	0/15	0/15	0/15	0/15	0/15	0/15	0/15	0/15	0/15	9/15***
Cortical fatty vacuolation	4/15	2/15	2/15	3/15	7/15	0/15	0/15	1/15	0/15	1/15
Adrenals (53 weeks)										
Multinucleated cells (Z. f.)	0/14	-	-	-	0/14	0/15	0/15	0/14	0/14	3/14
Chronic inflammation (Z. f.)	0/14	-	-	-	0/14	0/15	0/15	0/14	0/14	4/14*
Cortical fatty vacuolation	1/14	-	-	-	3/14	1/15	0/15	0/14	0/14	0/14
Adrenals (terminal)										
Multinucleated cells (Z. f.)	0/50	0/35	0/42	0/40	0/50	0/50	0/46	0/48	0/46	3/50[1)]
Chronic inflammation (Z. f.)	0/50	0/35	0/42	0/40	0/50	0/50	0/46	0/48	0/46	1/50
Cortical fatty vacuolation	11/50	10/35	7/42	10/40	13/50	8/50	4/46	4/48	6/46	11/50

*p<0.05, ***p<0.001; Z.f.: Zona fasciculate

[1)]the reviewing pathologist stated, that there was no clear evidence of treatment-related degenerative changes in females were observed (see BASF DocID 2015/1197310)

The reviewing pathologist stated that for females assigned to the 26 week sacrifice the presence of multinucleated cells, again interpreted as macrophage giant cells, was confirmed. However, within the area of the junction between reticularis and fasciculata 9/15 females given 5000 ppm were considered to have minimal degenerative changes similar to, but less severe than, those observed in the multigeneration and 13 week studies. Minimal mononuclear inflammatory cell infiltrates, increased pigment deposition and minimal or mild hypertrophy of the reticularis were also present in these animals. Thus the same lesion was observed at this sacrifice as in previous studies but at a much reduced severity.

Further at the 53 week sacrifice re-examination confirmed the presence of multinucleated cells in 3/14 animals. In addition, after careful examination at high power, minimal degenerative changes at the junction between reticularis and fasciculata were considered to be present in only 3/14 females given 5000 ppm. Ten of the fourteen females, from the same dosage group, had minimal or mild hypertrophy of the zona reticularis.

In females assigned to the terminal sacrifice it was noticeable that the incidence of the common spontaneous background changes of cortical hypertrophy and cortical haemorrhagic degeneration (encompassing the more usual twin diagnoses of cystic degeneration and telangectasis) were more prevalent in controls than females given 5000 ppm. No clear evidence of treatment-related degenerative changes were observed. It is noteworthy to mention, that neither an evidence for treatment-related cortical adenocarcinoma (incidences in M: 0//50, 0/35, 0/42, 0/40, 0/50; F: 1/50, 1/46, 0/48, 1/46, 0/50 for controls, 5, 25, 750, 5000 ppm), cortical adenoma (incidences in M: 0//50, 1/35, 0/42, 0/40, 1/50; F: 3/50, 1/46, 0/48, 0/46, 3/50 for controls, 5, 25, 750, 5000 ppm), nor for medullary phaeochromocytoma (incidences in M: 5/50, 6/35, 5/42, 3/40, 4/50; F: 1/50, 2/46, 0/48, 0/46, 1/50 for controls, 5, 25, 750, 5000 ppm).

Conclusion:
For the adrenal degenerative findings in females, there is a clear tendency to adaptation seen in the lifetime study in rats, as the number and percentages of affected females decreased with study duration. At the terminal sacrifice a treatment-relationship for the adrenal findings is even considered to be questionable. This is further supported by the fact, that no increased incidences for adrenal adenoma or carcinoma or phaochromocytoma was observed.

Overall conclusion on the adrenal findings

Dog

The only possible treatment-related finding in the adrenal glands of dogs was a higher than control incidence and/or severity of 'vacuolation, zona fasciculata' in both sexes given 150 mg/kg/day for 52 weeks. Dogs dosed at 300 mg/kg/day for four weeks showed no treatment-related findings. The term 'vacuolation', without stipulation of distribution or textual comment, can be used to describe several conditions with different etiologies within the adrenal glands. However, in this study, the lesions were all consistent in morphology and distribution with the focal or multifocal, commonly observed and spontaneously occurring finding described by McInnes et al (2011) and Sato et al (2012) (references see BASF DocID 2015/1197310). Neither of the publications gives an indication of the underlying mechanism for the finding. Interpretation of its significance is difficult; there was a slight exacerbation of a background finding, whether it arose by chance or due to administration of the test item is impossible to judge with the information available. In support of the former is the absence of any changes in the 4 week study at twice the dosage. On the other hand, there was an increase in mean absolute and body-weight relative adrenal weights recorded for animals given 150 mg/kg/day, although it is considered unlikely, given the focal nature and extent of the lesions seen at the highest dosage, that they would have been responsible for the weight increases. In any event it is doubtful, if this minor exacerbation in a spontaneous finding would have significance for humans.

Male rats

For the rat studies, there was a clear sex difference in the pathological response to triticonazole seen. Male rats, given the test item at 5000 ppm or greater over 13 weeks or in the multigeneration study (F0 and F1 generations) had diffuse cortical vacuolation of, principally, the zona fasciculata although at higher dosage the zona reticularis and, to a lesser extent, the zona glomerulosa was also involved. Accurate definition of the term was given by Dr Isaacs in his bridging report (see Chapter MCA 5.3) as: coarse droplet vacuolation of corticocytes in the zona fasciculata. The vacuoles are mainly concentrated in the inner area of the zona fasciculata and in more severe cases in the zona reticularis. The pathologist responsible for examination of the multigeneration study gave a similar description. This morphological appearance is usually associated with impaired steroidogenesis resulting in excess storage of unmetabolised steroid precursors (Capen et al, 1991), (Greaves, P, 2000), (Rosol et al, 2001) (references see BASF DocID 2015/1197310). It was notable that no significant differences were recorded in vacuolation of the cortex in males from the chronic oncogenicity and toxicity study either after 26 or 53 weeks or at terminal sacrifice suggesting possible tolerance to the test item-related effect. Of note was that there were no consistent changes in adrenal weights in the rat studies. Increases in adrenal weights are a consistent and reliable indicator of the action of adrenocorticotrophic hormone (ACTH) stimulation (Harvey & Sutcliffe, 2010).

Stress increases ACTH secretion (Buckingham, 2008) but so does a direct acting adrenocortical toxicant that inhibits steroidogenesis (e.g. aminoglutethimide, Akana et al., 1983), resulting in increased ACTH due to loss of feedback regulation. Akana et al., 1983 had found, that direct acting adrenal toxicants, that block steroidogenesis and glucocorticoid production can increase the adrenal weights by more the 100% after only 3 days of treatment (Akana et al., 1983b). The lack of large increases in adrenal weight/hypertrophy supports the suggestion that there is adequate glucocorticoid competency.

Female rats

In contrast to males, the principal test-item-related finding in females was degeneration of corticocytes at the junction between the zona fasciculata and zona reticularis in females given 5000 ppm or greater. Minor differences in nomenclature between studies were largely due to the levels of reporting of multinucleated cells, collagen deposition, fibrosis, inflammatory infiltrates and pigment deposition. Findings that were all considered secondary to the principal finding. A good description of the treatment-related changes was given by the study pathologist of the multigeneration study in his text. As in the males, it was noticeable that, as the period of exposure to the test item increased, the degree of change reduced, until in females from the terminal sacrifice of the oncogenicity and chronic toxicity study no evidence of test item-related degeneration could be discerned, again suggesting some form of tolerance was involved. With regard to possible mechanism the lesion seen in females most closely resembled vacuolar and granular degeneration of the zona reticularis and zona fasciculata due to mitochondrial vacuolation and dilatation of the smooth endoplasmic reticulum (Capen et al, 1991), (Capen C.C. (2001) (references see BASF DocID 2015/1197310). Degeneration of any adrenal zone is not considered to be a feature of a stress response (Harvey and Sutcliffe, 2010). The zona reticularis, in some mammalian species, has a role in weak androgen production, however certain species lack this zone (e.g. marmoset, Kaspareit et al., 2009) and it has no vital function as do the other adrenocortical zones. There is little known on the consequences of a hypofunction, as sex steroids (androgens) are derived from other sources. The mechanism and significance of degeneration in the zone fasciculata and reticularis, reported in the rat studies is unclear, however a recovery and an adaptation to the effect can reasonably be assumed by the results of the 2-generation toxicity study (F1 females less affected, than F0 females) and in the chronic rat study (incidences of affected females decrease with exposure duration). Further no increased tumor incidences, especially for phaochromocytoma, adrenal cortex adenoma or carcinoma were observed. The lack of large increases in adrenal weight/hypertrophy supports the suggestion that there is adequate glucocorticoid competency.

Other potentially relevant endocrine modes of actions

Triticonazole was negative in YES and YAS activity assays (did not inhibit or activate human oestrogen or androgen receptors), and so cannot exert its influence as a mimic or antagonist. Based on the literature evaluations, there was no evidence for estrogenicity or antiestrogenicity based on the results of the Tox 21 testing (Rotroff et al., 2014a, BASF DocID 2014/1323273. Decreased testosterone levels have been determined in an in vitro murine Leydig cell assay (MA-10). In the same publication (Roelofs et al., 2014, BASF DocID 2014/1326753) triticonazole was found to have androgen receptor inhibiting properties with an IC_{50} of $1.07E^{-05}$ [M], as determined in an androgen receptor reporter gene assay. At the same time, triticonazole is negative in the YAS assay, as shown above (Woitkowiak, 2012a, BASF DocID 2012/1276019).

With regard to an antiandrogenic mode of action, the results of the in vivo studies in rodents do not suggest an antiandrogenic response, as there were no conclusive changes on male reproductive organ weights – a known sensitive parameter for antiandrogenicity. There were especially no increased incidences of Leydig cell tumors seen in the chronic rat studies. The decreased prostate weights seen at the top dose of the 52-week dog study is an isolated finding in dogs and of unknown relevance, as there were no related histopathological findings seen in the prostate, thus the function of the prostate does not seem to be affected by treatment with triticonazole. There is no respective findings on organ weights or histopathology on male reproductive organs (prostate, seminal vesicles, epididymides, testes) in rats or mice in any of the relevant studies in Sprague-Dawley rat or CD-1 mice.

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CA 5.9 Medical Data

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 on triticonazole. 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 triticonazole exposure have not been observed.

CA 5.9.2 Data collected on humans

No human cases of intoxication or poisoning deriving from triticonazole are known to BASF SE.

CA 5.9.3 Direct observations

Neither data on exposure of the general public nor epidemiologic studies on triticonazole are available for BASF SE, nor is BASF SE aware on any epidemiologic studies performed by third parties.

CA 5.9.4 Epidemiological studies

See above

CA 5.9.5 Diagnosis of poisoning (determination of active substance, metabolites), specific signs of poisoning, clinical tests

Not known (see below).

CA 5.9.6 Proposed treatment: first aid measures, antidotes, medical treatment

See safety data sheet. No specific antidote known.

CA 5.9.7 Expected effects of poisoning

The acute toxicity of triticonazole is very low with LD_{50} values after oral and dermal exposure of > 2000 mg/kg bw. The LC_{50} value was greater than 5.61 mg/L, also indicating very low acute toxicity. In addition, non-specific clinical signs were observed following acute or subchronic and chronic exposure. Under these prerequisites, no specific clinical signs are expected from acute or accidental exposure to humans.

Triticonazole

Document M-CA, Section 6

RESIDUES IN OR ON TREATED PRODUCTS, FOOD AND FEED AND PLANT METABOLISM

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Version history[1]

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[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

An overview of metabolites identified during consumer safety studies is given below.

Table 6-1: Notations of parent and metabolites of triticonazole

Substance/ Metabolite Code	Reg. No	Synonyms	CAS-No	Compound found in	Structure/Name
Triticonazole M595F000	4378513	BAS 9318 F RPA 400727	131983-72-7	Livestock (goat) Plant Fish Rot Crop	(*RS*)-(*E*)-5-(4-chlorobenzylidene)-2,2-dimethyl-1-(1*H*-1,2,4-triazol-1-ylmethyl)cyclopentanol
cis-diol M595F001	5079285	RPA 404766	none	Plant Rot Crop	AND Enantiomer (1RS,2E,3SR)-2-(4-chlorobenzylidene)-5,5-dimethyl-1-(1H-1,2,4-triazol-1-ylmethyl)-1,3-cyclopentanediol
trans-diol M595F002	5059144	RPA 406341 AE 0540093	none	Plant Rot Crop	AND Enantiomer (1RS,2E,3RS)-2-(4-chlorobenzylidene)-5,5-dimethyl-1-(1H-1,2,4-triazol-1-ylmethyl)-1,3-cyclopentanediol

Table 6-1: Notations of parent and metabolites of triticonazole

Substance/ Metabolite Code	Reg. No	Synonyms	CAS-No	Compound found in	Structure/Name
M595F005	5079247	RPA 404886	none	Livestock (goat) Rot Crop	AND Enantiomer (1RS,5E,2RS)-5-(4-chlorobenzylidene)-2-hydroxymethyl-2-methyl-1-(1H-1,2,4-triazol-1-ylmethyl)cyclopentanol
M595F006	5079450	RPA 406972	none	Livestock (goat)	(3E)-3-(4-chlorobenzylidene)-2-hydroxy-2-(1H-1,2,4-triazol-1-ylmethyl)-1-methylcyclopentanecarboxylic acid
1,2,4-Triazole M595F009	87084	BF 480-16 M20 AE C500859	288-88-0	Livestock (goat)	1,2,4-(1H)-triazole
M595F010	none	none	none	Livestock (goat)	none

Table 6-1: Notations of parent and metabolites of triticonazole

Substance/ Metabolite Code	Reg. No	Synonyms	CAS-No	Compound found in	Structure/Name
M595F013	5079288	RPA 407922	none	Rot Crop	(RS)-(E)-5-(4-chloro-3-hydroxybenzylidene)-2,2-dimethyl-1-(1H-1,2,4-triazol-1-ylmethyl)cyclopentanol
M595F014	5079359	RPA 406203 Photometabo lite Z isomer of parent	none	Rot Crop (soil only)	(1RS, Z)-5-(4-chlorobenzylidene)-2, 2-dimethyl-1-((1H)- 1,2,4-triazol-1-ylmethyl)-cyclopentan-1-ol

CA 6.1 Storage stability of residues

For the active substance triticonazole, data on the stability of residues were reviewed during the Annex I inclusion process. Under the 91/414/EEC framework, storage stability of triticonazole was demonstrated for at least 12 months in stored frozen samples of cereal grain and straw. No further residue stability studies are required.

The following information is copied from the Conclusion on the peer review of triticonazole (EFSA Scientific Report (2005) 22, 1-69):

Stability of residues (Annex IIA, point 6 introduction, Annex IIIA, point 8 introduction)

	Stable for 12 months (maize – grain; wheat – grain and straw)

CA 6.2 Metabolism, distribution and expression of residues

CA 6.2.1 Metabolism, distribution and expression of residues in plants

In context of the previous submission for Annex I inclusion, three plant metabolism studies in cereal crops were evaluated, which are still considered to be valid.

Report:	**R000502; Parsons R.G., Ayliffe J.M., John A.E., Lowden P., McMillan-Staff S. (1998b)**
Title:	Fungicides: RPA 400727-[14C-Triazole]: Field Study in Spring Cereals
Guidelines:	USEPA (=EPA) 171-4(a)
GLP	Yes

Report:	**R012989; Doble M.L., Jones M.K., Lowden P., Parsons R.G., McMillan-Staff S. (1997a)**
Title:	Final report: Fungicides: RPA 400727 [14C-Phenyl]: Field Study on Winter Cereals
Guidelines:	USEPA (=EPA) 171-4(a)
GLP	Yes

Report:	**C021046; Oddy A., (2002)**
Title:	[^{14}C]-Triticonazole: Metabolism in Barley Following Seed Treatment
Guidelines:	European Commission Directive 96/68/EC (21st October 1996) Section 6.1 Metabolism, Distribution and Expression of Residues in Plants
GLP	Yes

The metabolism of triticonazole was studied in wheat and barley after seed treatment at rates corresponding to 12.5 to 744 g as/ha, both using phenyl-labeled and triazole-labeled triticonazole.

After treatment with triticonazole ^{14}C-labeled in the phenyl ring, only low total radioactive residues were found in grains at harvest (<0.001 – 0.05 mg/kg). Triticonazole formed the majority of the residue, ranging from 19 – 33% of the recovered radioactivity. Triticonazole was also the largest radioactive component detected in plants (15 – 63%), in chaff (6.4 – 18%) and in straw (28 – 35%). Several hydroxylated metabolites of triticonazole exceeded 10% of the recovered radioactivity in one or more matrices: RPA 406780/RPA 404766, RPA 404886 and RPA 406341.

After treatment with triticonazole ^{14}C-labeled in the triazole ring, the pattern of observed metabolism was rather different. The majority of the recovered radioactivity in nearly all plant parts investigated consisted of numerous polar and natural compounds (15 – 88% in plants; 91% in ears; 91 – 93% in grains; 18 – 52% in straw; 51% in chaff). These compounds are derived through the incorporation of fragments of the triazole ring into polar natural products. Intact 1,2,4-triazole was not identified. Parent triticonazole was also detected, as were three of the hydroxylated metabolites: RPA 406780, RPA 404766 and RPA 404886.

The studies were adequate to conclude that the metabolism of triticonazole occurs by hydroxylation, with separation and destruction of the triazole moiety, leading to incorporation of the triazole-derived material into polar natural products. All metabolism studies were overdosed and do not reflect the intended use pattern. In common with other triazole fungicides, triticonazole has the potential to generate the so-called “triazole-derived metabolites” (TDMs): triazole, triazole alanine, triazole acetic acid, though for triticonazole none of these metabolites were identified in significant amounts.

Nevertheless, in order to provide a metabolism study matching the current GAP, and to completely address any potential issues arising from the TDMs, a new metabolism study in wheat after seed-treatment with triticonazole was conducted.

Report: CA 6.2.1/1
Williams D., 2015a
Interim report: Metabolism of 14C-BAS 595 F with two labels in spring wheat after seed treatment
2014/1090812

Guidelines: OECD 501, EPA 860.1000: EPA Residue Chemistry Test Guidelines, EPA 860.1300: Nature of the Residue in Plants Livestock, PMRA Residue Chemistry Guidelines Section 97.2 Nature of the Residue - Plants - Livestock (Canada), JMAFF No 12 Nosan No 8147

GLP: yes
(certified by Swiss Federal Office of Public Health, Berne, Switzerland)

Executive Summary

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test Material:** phenyl-U-C14-triticonazole and triazole-3(5)-C14-triticonazole
   Description: carbon-14 phenyl ring labelled (5.86 MBq/mg) and carbon-14 triazole-3(5) ring labelled (6.52 and 6.44 MBq/mg) triticonazole
   Lot/Batch #: ^{14}C-BAS 595 F (phenyl): 866-1501
   ^{14}C-BAS 595 F (triazole-3(5)): 867-1301 and 867-1401 (two batches of the Triazole label were pooled and applied in a single application)
   Purity: ^{14}C-BAS 595 F (phenyl): 99.4% (radiochemical)
   ^{14}C-BAS 595 F (triazole-3(5)): 99.3% and 98.8% (radiochemical)
   CAS#: 131983-72-7
   Development code: BAS 595 F
   Stability of test compound: Storage stability investigations were not performed (storage advice according to the analysis certificates of the radiolabelled test items: “keep in freezer (approx. -18°C) or cooler”)

2. **Test Commodity:** Cereals
   Crop: Wheat
   Type: Spring wheat
   Variety: Monsoon
   Botanical name: *Triticum aestivum L.*
   Crop parts(s) or processed Commodity: Forage, Hay, Straw, Grain
   Sample size: Forage: 632.0 g (phenyl) and 720.3 g (triazole)
   Hay: 167.2 g (phenyl) and 191.3 g (triazole)
   Straw: 486.6 g (phenyl) and 610.0 g (triazole)
   Grain: 185.1 g (phenyl) and 326.9 g (triazole)

3. **Soil:** A sandy loam soil was used. The soil physicochemical properties are described below (see Table 6.2.1-1).

B. STUDY DESIGN AND METHODS

1. Test procedure

Two experiments were performed using either phenyl-^{14}C-labelled or triazole-3(5)-^{14}C-labelled triticonazole. Spring wheat seeds were treated with a mixture of ^{14}C-labelled and unlabelled BAS 595 F at a nominal rate of 13.5 g as/ha. Actual application rates were 11.5 g as/ha for [phenyl-^{14}C] BAS 595 F (P-Label) corresponding to 85.2 % of nominal and 11.7 g as/ha for [triazole-3(5)-^{14}C]-BAS 595 F (T-Label), corresponding to 86.8 % of nominal.

Figure 6.2.1-1: Structural formula of ^{14}C-BAS 595 F labelled at the phenyl ring

Figure 6.2.1-2: Structural formula of ^{14}C-BAS 500 F labelled at the triazole-3(5) ring

The treated seeds were sown into plastic containers and filled with loamy sand soil (Spaniergrund; USDA scheme: sandy loam). The cultivation of the wheat took place under controlled climatic conditions without the influence of rain, in a greenhouse. The maintenance of the crop was performed in accordance with normal agricultural practice. Physical and chemical properties of sandy loam soil are given in Table 6.2.1-1.

Table 6.2.1-1: Soil characteristics: soil used for sowing

Parameters		Soil
Name		Spaniergrund
Sampling location		Neukirchen 08459, Germany approximate coordinates: 50°47'N 12°21'E
Batch		Spaniergrund 201104
Date of collection		April 13, 2011
Sampling depth	[cm]	0–25
Texture (USDA)		Sandy loam [b]
Particle size analyses:		
- Clay (< 0.002 mm)	[%]	14.69 [a]
- Silt (0.002 - 0.05 mm)	[%]	31.09 [a]
- Sand (> 0.05 mm)	[%]	54.22 [a]
Water Holding Capacity:		
- at pF 2	[g water/100 g soil]	30.43 [a]
pH (0.01 M $CaCl_2$)		7.09 [a]
pH (water)		7.52 [a]
Organic carbon	[%]	1.41 [a]
Nitrogen	[%]	0.15 [a]
C/N-ratio		9.4 [b]
Organic matter	[%]	2.43 [b]
Cation Exchange Capacity	[mmol/100 g soil]	11.20 [a]

[a] Parameters determined by AgroLab AG, 6037 Root, Switzerland (non-GLP).
[b] Parameters determined by IES Ltd.
[c] Organic matter (OM) and C/N ratio were calculated as follows:
% OM = 1.724 × % organic carbon
C/N ratio = % organic carbon / % nitrogen

Wheat was harvested for both labels at three intervals; the first harvest (forage) was 7 weeks (50 DAA) after sowing treated seeds, the second harvest (hay) was 9 weeks (65 DAA) after sowing treated seeds and the final (maturity, straw and grain) harvest was 18 weeks (134 DAA) after sowing treated seeds. At each harvest whole wheat plants were cut using scissors approximately 5 cm above the soil surface

2. Description of analytical procedures

Forage was cut into smaller pieces with scissors then frozen and homogenized with liquid nitrogen in a food processor then stored frozen (approximately -18°C) until extraction. The hay and the mature wheat harvests were sampled using the same method but allowed to air-dry before freezing and further processing. The air dried mature wheat was separated by hand into ears and straw. The grain was separated from the ears by hand with the assistance of air and the resulting husks and chaff were combined with the straw sample. The straw and grain were homogenized with grinding mills.

The total radioactive residues (TRR) were determined by direct combustion analysis of small aliquots of homogenized sample material. The sample material was combusted by means of a sample oxidizer. The $^{14}CO_2$ was trapped by an absorption and scintillation liquid, and the collected radioactivity was measured by liquid scintillation counting. Aliquots of liquid samples were mixed with a sufficient volume of a suitable scintillator prior to measurement.

In order to determine the background radioactivity, untreated samples of forage, hay and mature wheat (straw and grain) were combusted under the same conditions. This resulted in a mean measured background activity of 96 dpm/g for forage, 550 dpm/g for hay and 134 dpm/g for straw and grain.

The homogenized samples were extracted with acetonitrile and water (forage: only acetonitrile). Subsequently the extracts were combined, adjusted to a defined volume, and aliquots of this combined extracts were used for LSC measuring. In case of HPLC and TLC analyses samples were extracted as described above, combined and concentrated.

The confirmation of the identification of the test item and radioactive components was performed by HPLC co-chromatography with non-radiolabelled test item and reference standards. An aliquot of concentrated extract was admixed with the non-radiolabelled test and reference items. UV-absorbance and radioactivity was recorded for HPLC analysis of these aliquots as well. The time delay between the UV-absorbance and radio-detectors was compensated by a parameter set in the software. Chromatographic correspondence was assessed by comparison of the UV-trace with the unlabelled test item/reference standard and the associated ^{14}C-trace. In the case of ^{14}C labelled reference items, separate solutions were run and the retention time observed in the radio-detector matched with peaks observed in the concentrated plant extracts.

TLC analysis was used for confirmatory chromatographic profiling of wheat extracts. The identification of the compounds detected in the extracts was performed by TLC co-chromatography with non-radiolabelled test item and reference items and also the ^{14}C labelled reference items. For TLC co-chromatography non-labelled test item and reference item were co-applied with the extract as well as separate spots on the same plate. After developing the plates, the non-labelled compounds were detected using a UV lamp (at 254 nm) and the positions of (UV) visible bands were marked with a pencil. Chromatographic correspondence was assessed by comparison of the ^{14}C-labelled spots of the extract with the spots of the unlabelled test item loaded as a separate spot. In the case of ^{14}C labelled reference items, no co-application was conducted and matching was solely performed via comparison of separate spots run on the same plate.

II. RESULTS AND DISCUSSION

A. TOTAL RADIOACTIVE RESIDUES (TRRs)

The levels of total radioactive residues (TRR) of triticonazole (and related compounds) found in wheat forage, hay, straw and grain are reported in Table 6.2.1-2.

Table 6.2.1-2: Total radioactive residues in wheat samples

Wheat matrix	Sampling Interval (DAA[1])	TRR determined [mg/kg]	TRR calculated [2] [mg/kg]
Phenyl Label (P-Label)			
Forage	49	0.048	0.047
Hay	63	0.207	0.225
Straw	126	0.249	0.217
Grain	126	0.002	not applicable
Triazole Label (T-Label)			
Forage	49	0.047	0.048
Hay	63	0.183	0.191
Straw	126	0.182	0.204
Grain	126	0.036	0.038

1) DAA = Days After Application
2) TRR was calculated as the sum of ERR (extraction with acetonitrile and water) + RRR

The Total Radioactive Residue (TRR) was calculated by summarizing the Extractable Radioactive Residues (ERR) and the Residual Radioactive Residue (RRR) after solvent extraction. The calculated TRR of forage was 0.047 mg/kg (P-Label) and 0.048 mg/kg (T-Label). The calculated TRR of hay was 0.225 mg/kg (P-Label) and 0.191 mg/kg (T-Label). The calculated TRR of straw was 0.217 mg/kg (P-Label) and 0.204 (T-Label). The calculated TRR of T-label grain was 0.038 mg/kg. Results from direct combustion of P-label grain were <0.002 mg/kg therefore no extractions were performed. For all matrices (except P-Label grain) the calculated TRR was set to 100 % TRR.

B. EXTRACTION, CHARACTERIZATION AND IDENTIFICATION OF RESIDUES

1. Extraction of residues

The extractability of the wheat matrices with acetonitrile and/or acetonitrile:water mixtures is summarized in Table 6.2.1-3.
For forage matrices the total extractability was 87.2 % TRR for both P-Label and T-Label. The major part of the radioactivity was extracted with acetonitrile (P-Label: 66.0 % TRR and T-Label: 63.4 % TRR) and the remaining radioactivity was subsequently released by extraction with acetonitrile:water mixtures (P-Label: 21.2 % TRR and T-Label: 23.8 % TRR).
For hay, straw and grain matrices all the extractable radioactivity was obtained with acetonitrile:water mixtures. Extractability of hay (79.1 % TRR for P-label and 75.9 % TRR for T-Label) was comparable to that of straw (77.7 % TRR for P-label and 76.8 % TRR for T-Label). The extractability of T-Label grain was lower (66.4 %). The radioactivity levels determined for the P-Label grain by direct combustion were low enough (<0.002 mg/kg) that no extraction was required.

Additionally, the TRR was measured by direct combustion analysis followed by LSC. The measured TRR of both matrices showed no major differences to the calculated TRR values.

Table 6.2.1-3: Extractability of radioactive residues in wheat samples

Matrix	Acetonitrile extract		Acetonitrile/water extracts		ERR[2)]		RRR		TRR calculated [1]
	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	
Phenyl Label (P-Label)									
Forage	0.031	66.0	0.010	21.2	0.041	87.2	0.006	12.8	0.047
Hay	n.a.	n.a.	0.178	79.1	0.178	79.1	0.047	20.7	0.225
Straw	n.a.	n.a.	0.168	77.7	0.168	77.7	0.048	22.3	0.217
Grain	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Triazole Label (T-Label)									
Forage	0.030	63.4	0.011	23.8	0.041	87.2	0.006	12.8	0.048
Hay	n.a.	n.a.	0.145	75.9	0.145	75.9	0.046	24.1	0.191
Straw	n.a.	n.a.	0.157	76.8	0.157	76.8	0.048	23.3	0.204
Grain	n.a.	n.a.	0.026	66.4	0.026	66.4	0.013	33.5	0.038

[1)] TRR was calculated as the sum of ERR + RRR
[2)] Extractable Radioactive Residue (ERR) calculated as sum of acetonitrile and/or acetonitrile:water extracts
n.a. not applicable (not conducted)

2. Identification, characterization and quantitation of extractable residues

For all matrices, identification was based on co-chromatography and comparison of the retention times with those of the labelled and unlabelled reference items (HPLC retentions times and TLC Rf values). Reversed phase HPLC was used as the primary quantification method, with confirmation of major metabolites by normal phase TLC. In the majority of cases HPLC method LC03 was used as the primary quantification method. Exceptionally, HPLC method LC02 was used to quantify components in T-Label straw and T-label grain extracts due the better resolving power for polar metabolites.

Forage

The combined, concentrated acetonitrile:water extracts of immature wheat (forage) of both labels were analyzed with HPLC method LC03 for quantification of the radioactive residues. Analysis of the same extract with TLC method 1 confirmed the general metabolite pattern and co-chromatography experiments confirmed the assignation of BAS 595 F. The parent compound (BAS 595 F) accounted for 65.3 % TRR (0.031 mg/kg) and 62.7 % TRR (0.030 mg/kg) in the P-label and T-label extracts respectively. For the P-Label, analysis with HPLC method LC03 showed the presence of two unknown metabolites both of which were detected at levels below ≤0.005 mg/kg. For the T-Label, analysis with HPLC method LC03 showed the presence of the same two unknown metabolites both of which were detected at levels below ≤0.004 mg/kg and additionally, a polar metabolite detected at 0.004 mg/kg. Co-chromatography experiments using ^{14}C labelled reference items confirmed the assignation of this as R9 (Triazole-alanine).

Hay

The combined, concentrated acetonitrile:water extracts of hay of both labels were analyzed with HPLC method LC03 for quantification of the radioactive residues. Analysis of the same extract with TLC method 1 confirmed the general metabolite pattern.

HPLC with co-chromatography using reference standards allowed the assignation of parent (BAS 595 F; 24.9 % TRR, 0.056 mg/kg), and the uncleaved hydroxy metabolites R1 (Reg. No.: 5079288; 7.1 % TRR, 0.016 mg/kg), R2 (Reg. No.: 5079285; 9.6 % TRR, 0.022 mg/kg), R3 (Reg. No.: 4710773, 6.9 % TRR, 0.015 mg/kg) in the P-Label hay extract. These assignations were confirmed by means of TLC. Analysis with HPLC method LC03 also showed the presence of five unknown metabolites occurring at levels above 0.010 mg/kg (between 0.010-0.020 mg/kg) but under 10 % of the TRR (4.4 % to 8.8 % TRR).

In the T-label hay extract the degradation pattern was slightly more complex with parent (BAS 595 F) accounting for only 17.2 % TRR (0.033 mg/kg). The identification of parent in the T-label extract was additionally verified by LC/MS. The most major metabolite detected showed co-chromatography with the ^{14}C labelled reference standard of the triazole derivative R9 (Triazole-alanine) and accounted for 0.021 mg/kg (11.1 % TRR). The identification of this component was additionally verified by LC/MS. HPLC (LC03) with co-chromatography using reference standards allowed the assignation of the uncleaved hydroxy metabolites R1 (Reg. No.: 5079288; 3.4 % TRR, 0.007 mg/kg) and R2 (Reg. No.: 5079285; 5.2 % TRR, 0.010 mg/kg) refer to Table 6.2.1-5. These assignations were confirmed by means of TLC. Analysis with HPLC method LC03 also showed the presence of five unknown metabolites, three of which occurred at levels above 0.010 mg/kg (between 0.010 to 0.023 mg/kg). The levels detected were under 10 % of the TRR with one exception which was a metabolite at 29.5 min accounting for 0.023 mg/kg (12.1 % TRR). This component is currently being investigated by LC/MS. Two minor unknown metabolites were also detected, none of which singularly accounted for levels >0.01 mg/kg (0.007 to 0.008 mg/kg, 3.8 to 4.3 % TRR).

Straw

The combined concentrated acetonitrile:water extract of mature wheat (straw) treated with the P-Label was analyzed with HPLC method LC03 for quantification of the radioactive residues. Analysis of the same extract with TLC method 1 confirmed the general metabolite pattern. Additionally, co-chromatography experiments confirmed the assignation of parent (BAS 595 F) and the uncleaved, hydroxyl metabolites R1 (Reg. No.: 5079288; 3.8 % TRR, 0.008 mg/kg), R2 (Reg. No.: 5079285; 3.2 % TRR, 0.007 mg/kg) and R5 (Reg. No.: 5079286; 3.3 %, 0.007 mg/kg). The parent compound (BAS 595 F) accounted for 20.3 % TRR (0.044 mg/kg). The presence of two unknown metabolites occurring at levels above 0.010 mg/kg (0.011 and 0.018 mg/kg) but under 10 % of the TRR (4.9 % and 8.5 %) was also observed. A maximum of eight minor unknown metabolites were additionally detected in the extract, none of which singularly accounted for levels >0.01 mg/kg.

The combined concentrated acetonitrile:water extract of mature wheat (straw) treated with the T-Label was analyzed with HPLC method LC02 for quantification of the radioactive residues. HPLC Method LC03 did not resolve the supplied radiolabelled triazole-derivative reference standards (R7, R8, R9 and R11). The triazole-derivatives were more significant in the T-label than in the P-Label straw extract and HPLC method LC02 was therefore used for the primary quantification method, the values obtained for parent were comparable for the two methods. Co-chromatography with reference standards using HPLC method LC02 showed the presence of parent (BAS 595 F; 14.0 % TRR, 0.029 mg/kg), the demethylated metabolite R3 (Reg. No.: 4710773; 8.3 % TRR, 0.017 mg/kg), the hydroxylated metabolite R4 (Reg. No.: 5079247, 9.7 % TRR, 0.020 mg/kg) and the triazole derivative R9 (Triazol-alanine; 4.2 % TRR, 0.009 mg/kg). These assignations were confirmed using TLC method 1. Four unknown metabolites occurring at levels slightly above 0.010 mg/kg (between 0.011 to 0.024 mg/kg) were observed. The levels detected were under 10 % of the TRR with one exception, a metabolite eluting at 25.1 min accounting for 0.024 mg/kg (11.9 % TRR). This unknown component was investigated by LC/MS. One additional minor unknown metabolites was also detected which singularly accounted for levels <0.01 mg/kg.

The results of the analysis of wheat matrices are summarized in Table 6.2.1-4 (phenyl-label) and Table 6.2.1-5 (triazole-label).

Table 6.2.1-4: Summary of identified components in wheat matrices treated with ^{14}C BAS 595 F (phenyl-label)

Metabolite	Structure	P-Label Forage[1]		P-Label Hay[2]		P-Label Straw[3]	
		mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
Parent (BAS 595F)		0.031	65.3	0.056	24.9	0.044	20.3
R1 (Reg. No.: 5079288)		-	-	0.016	7.1	0.008	3.8
R2 (Reg. No.: 5079285)		-	-	0.022	9.6	0.007	3.2
R3 (Reg. No.: 47010773)		-	-	0.015	6.9	-	-
R4 (Reg. No.: 5079247)		-	-	-	-	-	-
R5 (Reg. No.: 5079286)		-	-	-	-	0.007	3.3
R9 (Reg. No.: 270412) Triazole-alanine						0.007	3.4
Sum		*0.031*	*65.3*	*0.109*	*48.5*	*0.073*	*34.0*

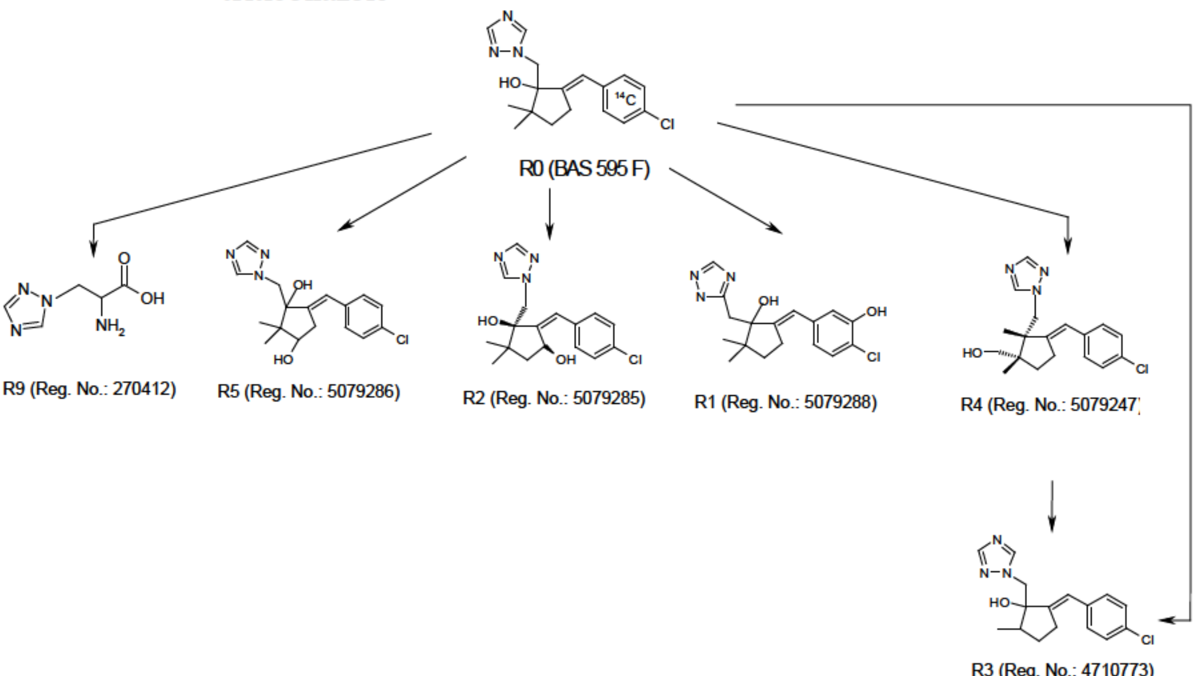
Table 6.2.1-5: Summary of identified components in wheat matrices treated with ^{14}C BAS 595 F (triazole-label)

Metabolite	Structure	T-Label Forage[4]		T-Label Hay[5]		T-Label Straw[6]		T-Label Grain[7]	
		mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
Parent (BAS 595F)		0.030	62.7	0.033	17.2	0.029	14.0		
R1 (Reg. No.: 5079288)		-	-	0.007	3.4	0.009	4.2		
R2 (Reg. No.: 5079285)		-	-	0.010	5.2	-	-		
R3 (Reg. No.: 47010773)		-	-	-	-	0.017	8.3		
R4 (Reg. No.: 5079247)		-	-	-	-	0.020	9.7		
R9 (Reg. No.: 270412) Triazole-alanine		0.004	8.9	0.021	11.1	0.009	4.2	0.015	37.5
Sum		*0.034*	*71.6*	*0.071*	*36.9*	*0.084*	*40.4*	*0.015*	*37.5*

3. Proposed metabolic pathway

The following metabolic pathway has been generated based on the outcome of this study dealing with the metabolism in wheat matrices after seed treatment with triticonazole.

Figure 6.2.1-3: Proposed metabolic pathway in wheat after seed treatment with triticonazole



4. Storage stability

In order to demonstrate the storage stability of the principal crop extracts (stored at approximately -18°C) during the interim period between initial and final analysis, chromatographic profiles obtained initially were compared with profiles of the same extracts (acetonitrile:water extract pools of T-label forage and straw) obtained at the completion of analysis. Comparison of initial and final radio-component profiles showed that no significant changes in the profiles had occurred during the interim period of storage. The only difference is that R9 was not seen in the S-Label straw during the final analysis, it was however present in the T-Label Forage showing that R9 was indeed stable during extract storage. The absence of R9 in the straw may be due to matrix effects.
Additionally, contrasting plant tissues (T-label forage and straw) were re-extracted and concentrated using the same conditions and procedures as those used for initial analysis in order to demonstrate the storage stability of the crop tissues (stored at approximately -18°C). Comparison of initial and storage stability extract radio-component profiles showed that no significant changes in the profiles had occurred during the interim period of storage and the plant tissue can be considered stable for the duration of the study.

III. CONCLUSION

After a single application of either [phenyl-^{14}C] or [triazole-3(5)-^{14}C]-(BAS 595 F) on wheat seeds (actual application rate ranging from 11.5 to 11.7 g a.s. / ha), highest amounts of BAS 595 F residues were detected in hay and straw samples (0.191 to 0.225 mg/kg), where as residues in forage and grain were significantly lower (0.038 to 0.047 mg/kg). The parent compound (BAS 595 F) was the major component identified in forage (62.7 to 65.3 % TRR), whereas in straw, significantly smaller portions of BAS 595 F were detected (14.0 to 20.0 % TRR) and no quantifiable BAS 595 F residues were detected in grain samples. Hence, BAS 595 F was extensively metabolised in mature wheat, particularly wheat grain.

For all matrices, soluble radioactive residues were predominantly extracted with acetonitrile:water mixtures ranging from 62.5 % TRR (grain) to 87.2% TRR (forage). Quantitative HPLC analysis and confirmatory TLC analysis of combined, concentrated acetonitrile:water extracts detected the di-hydroxy metabolites R5 (Reg. No.: 5079286) and R2 (Reg. No.: 5079285), the hydroxy metabolites R1 (Reg. No.: 5079288), R4 (Reg. No.: 5079247), as well as metabolite R3 (Reg. No.: 4710773). The triazole derivatives R9 (Triazole alanine) and traces of R8 (Triazole lactate, confirmed by LC/MS) were detected in the T-Label extracts. One major unknown metabolite in the T-Label hay extract was detected at levels 0.023 mg/kg (12.1 % TRR). This component is currently being investigated by LC/MS. Additionally, an unknown metabolite accounting for 0.024 mg/kg (11.9 % TRR) was detected in the T-label straw. This unknown component is also investigated by LC/MS. The principal residue in wheat grain was R9 (triazole-alanine, which may also contain some triazole-lactate, as shown by LC/MS of the T-Label straw sample).

Driselase treatment of hay and straw RRR, solubilized between 2.7 to 4.0 % of the TRR (0.006 to 0.008 mg/kg) of the residues, leaving the final RRR of the samples at 17.7-24.1% TRR (0.038 to 0.042 mg/kg) indicating that the RRR after solvent extraction is likely to be associated with non-carbohydrate components, such as proteins. Hot water solubilisation of T-Label grain RRR released 23.1 % TRR (0.009 mg/kg) of T-Label grain resulting in a final RRR of the grain was 10.4 % (0.004 mg/kg) showing the RRR of grain comprised relatively high levels (> 50% RRR) of soluble starch associated radioactivity and/or metabolites.

The study demonstrated that BAS 595 F is initially metabolised via hydroxylation to an array of closely related dihydroxy, and hydroxylated metabolites. The major degradation pathway was thus hydroxylation. Cleavage of the cyclopentane moiety resulted in the formation of Triazole derivative metabolites.

Comparison of initial and final radio-component profiles showed that no significant changes in the profiles had occurred during the interim period of storage. The re-analysis of the T-Label Forage showed the stability of R9 (Triazole-alanin) during extract storage. The absence of R9 in the straw may be due to matrix effects.

Radio-component profiles obtained from re-extraction, work up and analysis of contrasting plant tissues (T-label forage and straw) demonstrated the storage stability of the crop tissues for the duration of the study.

CA 6.2.2 Poultry

No further metabolism study was performed in poultry, since the metabolite patterns in livestock did not differ significantly.

CA 6.2.3 Lactating ruminants

No studies on the metabolism, distribution and expression of residues in livestock were submitted for Annex 1 inclusion of triticonazole. Since at that time no residues above the limit of determination were expected to be found in possible feed items, studies were regarded as not necessary. Residues of triticonazole and its metabolites in crop commodities fed to animals are low, but some residues are detectable (see CA 6.3.1) in early-stage green plant material (BBCH 11-23), which could be grazed. In whole plants without roots (which might be used as forage or fodder), no residues above the LOQ (0.01 mg/kg) were determined. Nevertheless, these young plants might potentially contain residues of triticonazole and also of the TDMs. To address the possible occurrence of residues in feed, and because the general potential for TDMs arising from triazole fungicides to occur in animal commodities is a current regulatory concern, a metabolism study was conducted in the lactating goat.

Report:	CA 6.2.3/1 [redacted] The metabolism of 14C-BAS 595 F in the lactating goat 2014/1090814
Guidelines:	EPA 860.1300: Nature of the Residue in Plants Livestock, EEC 91/414 (7030(VI/95 Rev. 3), OECD Test Guideline 503 - Metabolism in livestock
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:** Triticonazole (Reg. No. 4378513)
   Description: [Triazole-3(5)-^{14}C]-Carbon-14 labeled, unlabeled BAS 595 F
   Lot/Batch #: ^{14}C-BAS 595 F: 867-1301 (6.52 MBq/mg)
   BAS 595 F: L76-154
   Purity: ^{14}C-BAS 595 F: 92.3% (chemical), 99.3% (radiochemical)
   BAS 595 F: 98.6% (chemical)
   CAS#: 131983-72-7
   Development code: not applicable
   Stability of test compound: The test item was stable over the test period

2. **Test Animal**
   Species: Lactating goat
   Variety: Saanen cross Toggenburg
   Gender: Female
   Age: not reported
   Weight at dosing: 77 kg (study day -1)
   Number of animals: 1

   Acclimation period: 11 days
   Diet: Hay was offered *ad libitum* throughout the acclimatization and dosing periods. Additionally, the goat was offered a ration (500 g twice daily) of commercially available non-medicated concentrate. Throughout the acclimatization period and dose period the goat was offered Dodson and Horrell Goat Mix.
   Water: Water, *ad libitum*
   Housing: On assignment to study, the goat was individually housed in a pen. On the day prior to first dose administration, the goat was transferred to a metabolism cage with a mesh floor
   Environmental conditions -
   Temperature: 15-18 °C
   Humidity: 21-47%
   Air changes: not reported
   Photoperiod: 16 h light/ 8 h dark cycle for the majority of the study

B. STUDY DESIGN AND METHODS

1. Dosing regime

Oral: Amount of dose:	12 mg/kg feed/day (nominal)
Food consumption:	0.05 - 2.644 kg feed/day (dry weight)
Vehicle:	Orally in a gelatin capsule via dosing gun
Timing:	Once daily
Duration:	7 days

2. Sample collection

Milk collection:	Twice daily (AM and PM)
Excreta collection:	Daily
Interval from last dose to sacrifice:	3 h
Tissues harvested & analyzed:	Liver, both kidneys, omental fat, renal fat, subcutaneous fat, flank muscle, loin muscle, gastrointestinal tract and contents and bile carcass

3. Test system

The metabolism and distribution of Triticonazole was investigated in a single lactating goat following a repeated oral administration of [Triazole-3(5)-^{14}C]-BAS 595 F (Reg. No. 4378513) at the dose level of 12 mg/kg feed for 7 consecutive days. The mean achieved daily dose administered was 22.2 mg/kg food consumed (dry weight equivalent) by the goat. The dose formulation was prepared by mixing ^{14}C and non-labeled BAS 595 F in the ratio 2:5, leading to an actual specific activity of 1.87 MBq/mg.
The test substance was administered orally in a gelatin capsule via dosing gun. Details of the study design are summarized in Table 6.2.3-1. The structural formula of the labeled 14C-BAS 595 F is given in Figure 6.2.3-1 respectively.

Figure 6.2.3-1: Structural formula of labeled ^{14}C-BAS 595 F

Table 6.2.3-1: Dosing of Lactating Goat with ^{14}C-BAS 595 F

Test item	Number of animals	Treatment days	Nominal daily dose mg/kg feed intake	Actual daily dose mg/kg feed intake	Sacrifice time [hour]
[Triazole-3(5)-14C]-BAS 595 F	1	7	12	22.2	3

4. Sampling and Storage

Urine and feces were collected in time intervals of 24 hours for seven days. In addition, following removal of the goat for sacrifice, any urine and feces remaining in the metabolism cage was collected. The goat was milked twice daily (AM and PM) throughout the acclimatization and study period. On day 5, milk was separated into cream and skim milk. The following tissues were dissected from the lactating goats 3 h after the last administration: Liver, both kidneys, omental fat, renal fat, subcutaneous fat, flank muscle, loin muscle, gastrointestinal tract and contents and bile carcass. All samples were stored at ca -20 °C.

5. Description of analytical procedures

Prior to analysis milk samples from days 4, 5, and 6 were combined as one pooled sample. As there were no significant differences between the different types of muscle the flank and loin muscle were pooled in a ratio 1:2 to produce a single composite sample.

TRR combusted:
For the determination of the TRR combusted, homogenized sample material was weighed and combusted by means of a sample oxidizer. The $^{14}CO_2$ evolved during combustion was trapped by an absorption liquid, and the collected radioactivity was measured by liquid scintillation counting (LSC).

Extraction:
Muscle, liver and kidney were extracted using combinations of the following solvents: methanol, methanol:water (4:1 v/v), methanol:water (3:7 v/v). Milk was extracted three times with methanol. The combined results of extractions are referred to as extractable radioactive residues (ERR). The radioactivity in extracts was determined by LSC analysis.
Extracts containing significant amounts of radioactivity were proportionately combined and concentrated. Where necessary, any fat in the sample extracts was partitioned out using a non-polar solvent (e.g. hexane). The radioactivity content of the concentrated extract was determined by LSC analysis.
Residual Radioactive Residue (RRR) was analyzed for radioactivity content by combustion analysis followed by LSC.

Quantification and Identification of Residues:
HPLC analysis was carried out for relevant samples with a sufficient level of radioactivity. The quantification of metabolites and the confirmation of metabolite assignments in sample extracts were determined by HPLC Method 3 and Method 4. The columns used were Imtakt Scherzo SSC18 (150 x 4.6 mm, 3 µm) and Phenomenex Inertsil Phenyl (250 x 4.6 mm, 5 µm). Both eluent systems consisted of 2 mobile phases (method 3: A: 0.1%, TFA acid in MQ-water, B: 0.1%, TFA acid in Acetonitrile; method 4: A: 2% acetic acid in MQ-water; B: 2% acetic acid in acetonitrile). The eluents were used applying gradient elution. The identity of radiolabeled components was based on co-chromatography with authentic reference items.
Tissue and milk extracts were analyzed using the TLC method to aid the identification of the polar metabolites. To confirm the identification of polar metabolites in extracts of muscle and milk, HPLC method 5 (Luna Hilic-Column (250 x 4.6 mm, 5 µm) and two eluents (A: 100 mM Ammonium Formate pH 3.3. + 0.1% formic acid, B: 2% formic acid in acetonitrile) were used.
Tissue, excreta and bile extracts were analyzed by HPLC-MS/MS to identify components which did not correspond to a reference standard and to confirm the assignments made using co-chromatography.

Isomerization:
In order to investigate the isomer ratio of BAS 595 F and M595F006 (Reg. No. 5079450) these components were isolated with HPLC Method 3 from the liver extract. The resulting BAS 595 F and Reg. No. 5079450 fractions were analyzed by HPLC Method 6 and 7, respectively. An aliquot of the dose solution was also analyzed by HPLC Method 6 to determine the initial isomer ratio of the test item. (HPLC method 6: Column: Chiral PAK IC (150 x 4.6 mm, 5 µm), Eluent: Acetonitrile + 0.1% diethyl amine; HPLC method 7: Column: Chiral PAK IC (150 x 4.6 mm, 5 µm), Eluent: MQ-water + 0.2% formic acid:Acetonitrole (60:40 v/v)).

II. RESULTS AND DISCUSSION

General observations
The goat remained in good general health throughout the acclimatization and dosing periods of the study.

A. TOTAL RADIOACTIVE RESIDUES (TRRs)

Approximately 81.5% of the total dose was recovered, the majority of which was present almost equally between feces (36.5%) and urine (29.7%). There was also a large proportion present in the GI tract contents (7.9%) and relatively low proportions recovered in the cage wash (3.4%). Radioactivity associated with edible portions (milk and tissues) accounted for ≤ 1% of the administered dose. The recovery for the labeled ^{14}C- BAS 595 F is shown in Table 6.2.3-2.

Table 6.2.3-2: Recovery of Radioactivity after Dosing of Lactating Goat with [^{14}C]-BAS 595 F

Sample	% Administered Dose Recovered from 001F
Urine	29.7
Faeces	36.5
Cage Wash	3.4
Milk	0.1
Kidneys	< 0.1
Liver	0.7
Plasma	< 0.1
GI contents and Rinsings	7.9
GI tract	3.1
Bile	0.1
Total	**81.5**

The radioactive residues in milk, muscle and fat were very low and accounted for a maximum of 0.026 mg/kg. Residues in milk had reached steady state within 5 days. The plateau concentration accounted for approximately 0.019 mg/kg. The ratio of residues associated with the skimmed milk and cream was determined in a representative 24 h composite sample from the plateau and shown to be 1.2:1.
The residues in the other edible matrices accounted for 1.028 mg/kg (liver) and 0.394 mg/kg (kidney). The total radioactive residues of the labeled substance are summarized in Table 6.2.3-3 for tissue and bile samples and in Table 6.2.3-4 for milk samples.

Table 6.2.3-3: Total Radioactive Residues in Tissues and Bile after dosing of Lactating Goat with [^{14}C]-BAS 595 F

Matrix	Total radioactive residue [mg/kg]
Bile	23.592
Liver	1.028
Kidneys	0.394
Muscle: Loin	0.022
Muscle: Flank	0.026
Fat: Subcutaneous	0.008
Fat: Omental	0.005
Fat: Renal	0.007

Table 6.2.3-4: Total Radioactive Residues (TRR) in 24h Milk Samples after dosing of Lactating Goat with [^{14}C]-BAS 595 F

Timepoint (day)	Total radioactive residue[1]	
	[%]	[mg/kg]
1	0.007	0.008
2	0.013	0.012
3	0.018	0.015
4	0.018	0.019
5	0.017	0.019
6	0.020	0.023
7	n.a.	n.a.

[1] 24 h Milk Sample data calculated from PM and AM milk collections.

B. EXTRACTION, CHARACTERIZATION AND IDENTIFICATION OF RESIDUES

1. Extractability

Milk

The extractability of milk was high. The milk sample was extracted with methanol, resulting in 100.0% TRR (0.023 mg/kg) in the extract. Less than LOQ was present in the Residual Radioactive Residue (RRR), which was determined by combustion analysis. Aliquots of the extracts containing significant radioactivity (95.6% TRR; 0.022 mg/kg) were proportionately combined and concentrated. During concentration, the extract was partitioned against hexane to remove fat resulting in 12.4% TRR (0.003 mg/kg) partitioning into the hexane fraction. Following concentration of the aqueous fraction the precipitated protein fraction was further extracted and the extracts added back to the concentrated extract, resulting in a final concentrated extract containing 64.4% TRR (0.015 mg/kg). A protein precipitate formed during concentration accounted for 0.1% TRR (<0.001 mg/kg).

Liver

The homogenized liver sample was extracted with methanol and methanol/water mixtures, resulting in extraction of 98.7% TRR (1.062 mg/kg). The RRR was analyzed by combustion analysis and contained 1.4% TRR (0.015 mg/kg). Aliquots of the extracts containing significant radioactivity (98.0% TRR) were proportionately combined and partitioned against hexane resulting in an aqueous soluble fraction containing 98.6% TRR. The extract was concentrated, resulting in a sample which contained 91.8% TRR (0.988 mg/kg) and was analyzed by HPLC and TLC.

Kidney

The homogenized kidney sample was extracted with methanol and methanol/water mixtures, resulting in extraction of 99.5% TRR (0.426 mg/kg). The Residual Radioactive Residue was analyzed by combustion analysis and contained 0.5% TRR (0.002 mg/kg). Aliquots of the extracts containing significant radioactivity (98.7% TRR) were proportionately combined and partitioned against hexane resulting in an aqueous soluble fraction containing 92.3% TRR. The extract was concentrated, resulting in a sample which contained 87.3% TRR (0.375 mg/kg) and was analyzed by HPLC and TLC.

Muscle

The pooled and homogenized muscle sample was extracted with methanol and methanol/water mixtures, resulting in extraction of 100.0% TRR (0.025 mg/kg). Less than LOQ was present in the Residual Radioactive Residue which was determined by combustion analysis. Aliquots of the extracts containing significant radioactivity (99.9% TRR) were proportionately combined and partitioned against hexane resulting in an aqueous soluble fraction containing 99.1% TRR. The extract was concentrated, resulting in a sample which contained 84.8% TRR (0.021 mg/kg) and was analyzed by HPLC.

A summary of the extraction behavior is given in Table 6.2.3-5.

Table 6.2.3-5: Extractability of Goat Matrices after dosing of Lactating Goats with [^{14}C]-BAS 595 F

Matrix	TRR mg/kg (% TRR)	ERR mg/kg (% TRR)	RRR mg/kg (% TRR)	Recovery %
Liver	1.076 (100%)	1.062 (98.7%)	0.015 (1.4%)	100.1%
Milk	0.023 (100%)	0.023 (100.0%)	< LOQ (< LOQ)	100.0%
Kidney	0.429 (100%)	0.426 (99.5%)	0.002 (0.5%)	100.0%
Muscle	0.025 (100%)	0.025 (99.9%)	< LOQ (< LOQ)	99.9%

TRR: Total Radioactive Residue
ERR: Extractable Radioactive Residue
RRR: Residual (= non-released) Radioactive Residue

2. Identification, characterization and quantitation of extractable residues

Milk:
Analysis of milk extract led to a pattern of 4 peaks one of which was identified as M595F009 (Reg. No.87084; 0.020 mg/kg; 86.2% TRR). The other peaks were not identified and individually accounted for less than 0.001 mg/kg (4.6% TRR).

A summary of identified and characterized ^{14}C-residues in milk is shown in Table 6.2.3-6. The structure of the identified metabolite is shown in Table 6.2.3-10.

Table 6.2.3-6: Summary of Identified and Characterized ^{14}C-Residues extracted from Milk after dosing of Lactating Goat with [^{14}C]-BAS 595 F

Designation	Extract: Methanol Extract 0.022 mg/kg, 94.4 % TRR		Total	
	mg/kg	% TRR	mg/kg	% TRR
Identified				
M595F009 (Reg. No.87084)	0.020	86.2	0.020	86.2
Total Identified in Extractable Radioactivity			**0.020**	**86.2**
Characterized				
Unknown at t_R ca 2 min			< 0.001	2.0
Unknown at t_R ca 28 min			< 0.001	1.6
Unknown at t_R ca 36 min			0.001	4.6
Total Characterized in Extractable Radioactivity			**0.001**	**8.2**
Total Identified and Characterized			**0.021**	**94.4**
Final Residue			**< LOQ**	**< LOQ**
Grand Total			**0.021**	**94.4**

Liver:
Analysis of liver extract led to a pattern of 22 peaks. The residues were identified as unchanged BAS 595 F (0.157 mg/kg; 14.6% TRR) and M595F006 (Reg. No. 5079450) (0.251 mg/kg; 23.4% TRR). The other peaks did not correspond to reference standards. One peak was identified as M595F010 from mass spectral analysis and accounted for 0.219 mg/kg (20.4% TRR). The other peaks were not identified and individually accounted for less than 0.041 mg/kg (3.8% TRR). Following analysis by TLC, M595F009 (Reg. No. 87084) was identified as a minor metabolite accounting for 0.030 mg/kg (2.8% TRR).

A summary of identified and characterized ^{14}C-residues in liver is shown in Table 6.2.3-7. The structures of identified metabolites are presented in Table 6.2.3-10.

Table 6.2.3-7: Summary of Identified and Characterized ^{14}C -Residues extracted from Liver after dosing of Lactating Goat with [^{14}C]-BAS 595 F

Designation	Extract Methanol Extract 0.988 mg/kg, 91.8 % TRR		Total	
	mg/kg	% TRR	mg/kg	% TRR
Identified				
M595F010	0.219	20.4	0.219	20.4
M595F006 (Reg. No. 5079450)	0.251	23.4	0.251	23.4
BAS 595 F (Reg. No. 4378513)	0.157	14.6	0.157	14.6
Total Identified in Extractable Radioactivity			**0.627**	**58.4**
Characterized				
Unknown at t_R ca 4 min			0.021	1.9
Unknown at t_R ca 24 min			0.007	0.7
Unknown at t_R ca 26 min			0.016	1.5
Unknown at t_R ca 27 min			0.017	1.5
Unknown at t_R ca 28 min			0.022	2.1
Unknown at t_R ca 31 min			0.041	3.8
Unknown at t_R ca 32 min			0.040	3.7
Unknown at t_R ca 33 min			0.024	2.2
Unknown at t_R ca 33.5 min			0.013	1.2
Unknown at t_R ca 34 min			0.006	0.6
Unknown at t_R ca 36 min			0.032	3.0
Unknown at t_R ca 37 min			0.026	2.4
Unknown at t_R ca 39 min			0.027	2.5
Unknown at t_R ca 40 min			0.003	0.3
Unknown at t_R ca 43 min			0.008	0.7
Unknown at t_R ca 44 min			0.037	3.4
Unknown at t_R ca 45 min			0.009	0.8
Unknown at t_R ca 47 min			0.005	0.5
Unknown at t_R ca 63 min			0.006	0.6
Total Characterized in Extractable Radioactivity			**0.360**	**33.4**
Total Identified and Characterized in Extractable Radioactivity			**0.987**	**91.8**
Final Residue			**0.015**	**1.4**
Grand Total			**1.002**	**93.2**

Kidney:
Analysis of kidney extract led to a pattern of 13 peaks. The major peak was identified as M595F006 (Reg No.5079450) (0.244 mg/kg; 56.8% TRR) and unchanged BAS 595 F was identified as a minor component (0.004 mg/kg; 0.8% TRR). The other peaks did not correspond to reference standards and remained unidentified individually accounting for less than 0.025 mg/kg (5.9% TRR). Following analysis by TLC, M595F009 (Reg. No. 87084) was identified as a minor metabolite accounting for 0.020 mg/kg (5.2% TRR).

A summary of identified and characterized ^{14}C-residues in kidney is shown in Table 6.2.3-8. The structures of identified metabolites are presented in Table 6.2.3-10.

Table 6.2.3-8: Summary of Identified and Characterized ^{14}C-Residues extracted from Kidney after dosing of Lactating Goat with [^{14}C]-BAS 595 F

Designation	**Extract**		**Total**	
	Methanol Extract 0.375 mg/kg, 87.3 % TRR			
	mg/kg	**% TRR**	**mg/kg**	**% TRR**
Identified				
M595F006 (Reg. No.5079450)	0.244	56.8	0.244	56.8
BAS 595 F (Reg. No. 4378513)	0.004	0.8	0.004	0.8
Total Identified in Extractable Radioactivity			**0.248**	**57.6**
Characterized				
Unknown at t_R ca 4 min			0.024	5.6
Unknown at t_R ca 23 min			0.003	0.8
Unknown at t_R ca 24 min			0.011	2.7
Unknown at t_R ca 25 min			0.014	3.2
Unknown at t_R ca 27 min			0.011	2.6
Unknown at t_R ca 27.5 min			0.008	1.7
Unknown at t_R ca 29 min			0.025	5.9
Unknown at t_R ca 30 min			0.008	1.9
Unknown at t_R ca 31 min			0.009	2.0
Unknown at t_R ca 41 min			0.006	1.5
Unknown at t_R ca 41.5 min			0.008	1.8
Total Characterized in Extractable Radioactivity			**0.127**	**29.7**
Total Identified and Characterized			**0.375**	**87.3**
Final Residue			**0.002**	**0.5**
Grand Total			**0.377**	**87.8**

Composite Muscle:
Analysis of muscle extract led to a pattern of 9 peaks one of which was identified as M595F009 (Reg. No.87084) (0.014 mg/kg; 57.5% TRR). Two other components were identified as M595F006 (Reg. No.5079450) (0.004 mg/kg; 14.6% TRR) and unchanged BAS 595 F (0.001 mg/kg; 2.6% TRR). The other peaks did not correspond to reference standards and remained unidentified individually accounting for less than 0.001 mg/kg (3.2% TRR).

A summary of identified and characterized ^{14}C-residues in muscle is shown in Table 6.2.3-9. The structures of identified metabolites are presented in Table 6.2.3-10.

Table 6.2.3-9: Summary of Identified and Characterized ^{14}C-Residues extracted from Muscle after dosing of Lactating Goat with [^{14}C]-BAS 595 F

Designation	Extract		Total	
	Methanol Extract 0.021 mg/kg, 84.8 % TRR			
	mg/kg	% TRR	mg/kg	% TRR
Identified				
M595F009 (Reg. No 87084)	0.014	57.5	0.014	57.5
M595F006 (Reg. No 5079450)	0.004	14.6	0.004	14.6
BAS 595 F (Reg. No.4378513)	0.001	2.6	0.001	2.6
Total Identified in Extractable Radioactivity			**0.019**	**74.7**
Characterized				
Unknown at t_R ca 23 min			< 0.001	1.3
Unknown at t_R ca 24 min			< 0.001	0.8
Unknown at t_R ca 27 min			< 0.001	1.0
Unknown at t_R ca 29 min			0.001	3.2
Unknown at t_R ca 32 min			< 0.001	1.0
Unknown at t_R ca 35 min			0.001	2.8
Total Characterized in Extractable Radioactivity			**0.002**	**10.1**
Total Identified and Characterized			**0.021**	**84.8**
Final Residue			**< LOQ**	**< LOQ**
Grand Total			**0.021**	**84.8**

Chiral Analysis
The ratio of the isomers of BAS 595 F was ca 1:1 in the dose solution and ca 3:2 in the liver extract. The ratio of the isomers of M595F006 (Reg. No. 5079450) were ca 1:1 in the isolate from the liver extract.

Table 6.2.3-10: Summary of Identified Residues in Goat Tissue and Milk

Metabolites		Matrices			
Metabolite code, Reg No of reference substance	**Structural Formula**	**Milk** **mg/kg (% TRR)**	**Liver** **mg/kg (% TRR)**	**Kidney** **mg/kg (% TRR)**	**Composite Muscle mg/kg (% TRR)**
Triticonazole BAS 595 F (Reg. No. 4378513)		n.d.	0.157 (14.6)	0.044 (0.8)	0.001 (2.6)
1,2,4-Triazole M595F009 (Reg. No.87084)		0.020 (86.2)	0.030 (2.8)[1]	0.020 (5.2)[1]	0.014 (57.5)
M595F010		n.d.	0.219 (20.4)	n.d	n.d
M595F006 (Reg. No. 5079450)		n.d.	0.251 (23.4)	0.244 (56.8)	0.004 (14.6)

n.d. = not detected
[1] Identified and quantified by TLC for liver and kidney

3. Proposed metabolic pathway

The proposed metabolic pathway of BAS 595 F in the lactating goat is provided in Figure 6.2.3-2. BAS 595 F was extensively metabolized in the lactating goat. The unchanged parent was not detected in samples of milk or muscle and was found in portions below 0.8% TRR in kidney. There was a greater proportion of BAS 595 F found in the liver which accounted for 14.6% TRR. The main component in extracts of liver and kidney was M595F006 (Reg. No. 5079450), formed by oxidation of the cyclopentane alkyl side chain to yield a carboxyl group. This component was also identified in composite muscle at lower proportions (14.6% TRR, 0.004 mg/kg). Metabolite M595F010 was also found in the liver in significant proportions. The main component in extracts of milk and composite muscle was M595F009 (Reg. No. 87084), formed by the cleavage of the triazole moiety from the parent compound. M595F009 (Reg. No. 87084) was also identified as a minor component in the liver and kidney.

Thus metabolic transformations occurred

- mainly via oxidation followed by glucuronide conjugation and
- Cleavage of the triazole moiety

A proposed metabolic pathway for BAS 595 F in lactating goats is given in Figure 6.2.3-2.

Figure 6.2.3-2: Proposed Biotransformation Pathway for [14C]-BAS 595 F in the Lactating Goat

BAS 595 F
Reg. No. 4378513
M595F009
Reg. No. 87084
M595F006
Reg. No.5079450
M595F005
Reg. No. 5079247
M595F010

4. Storage stability

Initial analyses of the tissue, milk and excreta extracts were carried out within 6 months of sacrifice. The original concentrated and reconstituted extracts from milk, liver and kidney (stored at -20°C) were analyzed seventeen months after their initial analysis. The profiles are comparable to the initial profiles obtained showing stability in the sample extracts following long term storage at -20°C.
Subsamples of the liver and kidney tissues and a subsample of milk (stored at -20°C) were re-extracted thirteen (milk) or eighteen (liver and kidney) months after the original extraction and analysis was carried out. The profiles of the kidney and milk extracts are comparable to the initial profiles obtained showing stability in the milk and kidney tissue following long term storage at -20°C.
The profile of the extract obtained from liver tissue following long term storage (stored at -20°C) was not quantitatively comparable to the initial liver extract profile obtained.
M595F010, the glucuronide present in the liver sample was shown to degrade in the tissue during long term storage and this was accompanied by an increase in M595F006 (Reg No. 5079450). The conjugate however is stable in the liver extract following long term storage at -20°C as demonstrated by the reanalysis of the initial extract seventeen months later. The extract from the re-extracted milk sample and liver and kidney tissues were not used for quantification. The extracts obtained following re-extraction of the liver and kidney were used for investigation of the storage stability only, while the extract obtained from milk following re-extraction was also used to aid metabolite identification.

III. CONCLUSION

The mean daily dose administered was 22.2 mg [Triazole-3(5)-^{14}C]-BAS 595 F per kg food consumed (dry weight equivalent).
The radioactive residues in milk, muscle and fat were very low and accounted for a maximum of 0.026 mg/kg. Residues in milk had reached steady state within 5 days. The plateau concentration accounted for approximately 0.019 mg equiv/kg.
The residues in the other edible matrices accounted for 1.028 mg/kg (liver) and 0.394 mg/kg (kidney).
Approximately 81.5% of the total dose was recovered, the majority of which was present almost equally between feces (36.5%) and urine (29.7%). There was also a large proportion present in the GI tract contents (7.9%) and relatively low proportions recovered in the cage wash (3.4%). Radioactivity associated with edible portions (milk and tissues) accounted for ≤ 1% of the administered dose.
The extractability of milk was high (100.0% TRR), equivalent to 0.023 mg/kg. The extractability of the edible tissues was also high, ranging from 98.7% (liver) to 99.9% (muscle) of the TRR.
BAS 595 F (Reg. No. 4378513) was extensively metabolized in the lactating goat. The unchanged parent was not detected in samples of milk or muscle and was found in portions below 0.8% TRR in kidney.
There was a greater proportion of BAS 595 F found in the liver which accounted for 14.6% TRR.
The main component in extracts of liver and kidney was M595F006 (Reg. No. 5079450), formed by oxidation of the cyclopentane alkyl side chain to yield a carboxyl group. This component was also identified in composite muscle at lower proportions (14.6% TRR, 0.004 mg/kg). M595F010 was also found in the liver in significant proportions. The main component in extracts of milk and composite muscle was M595F009 (Reg. No. 87084), formed by the cleavage of the triazole moiety from the parent compound. M595F009 (Reg. No. 87084) was also identified as a minor component in the liver and kidney.
Thus metabolic transformations occurred
- **mainly via oxidation followed by glucuronide conjugation and**
- **Cleavage of the triazole moiety.**

CA 6.2.4 Pigs

No metabolism study was performed in pigs, since the metabolite patterns in rodents (rats) and ruminants (goats) did not differ significantly.

CA 6.2.5 Fish

According to Commission Regulation 283/2013, metabolism studies in fish may be required where the plant protection product is used in crops whose parts or products, also after processing, are fed to fish and where residues in feed may occur from the intended applications. Green forage does not form part of fish diets and since there are no detectable residues in grain or straw, a fish metabolism study is not required.

CA 6.3 Magnitude of residues trials in plants

Triticonazole is registered in multiple crops belonging to different EU crop groups. Within this dossier residue data are only provided for the representative uses supporting the renewal of approval. The FS formulation BAS 595 01 F (25 g/L triticonazole) has been selected as representative formulation.

Consequently, in this dossier section the relevant data for the following crop are summarized:

- Wheat (BAS 595 01 F)

The studies provided below for wheat have not been evaluated within the MRL re-evaluation process according to Reg. 396/2005, Art. 12. Consequently they are not considered as peer-reviewed.

All studies submitted have been done with racemic mixture as obtained from synthesis and the enantiomers were not separated during analytical phase (Analytical methods in M-CA Section 4). Regular chromatography columns used in these studies detect Triticonazole as one peak and no information in the raw data allows quantification of the enantiomers separately. Moreover Triticonazole is used for seed treatment only and is a non-systemic compound, so the residue levels in the different plant commodities are very limited as described in this section. At harvest there are no detectable residues in straw and grain, therefore a separation of enantiomers was not seen as necessary.

CA 6.3.1 Cereals

Crop residue data from 23 field studies on wheat, barley and rye (40 trial sites conducted in Germany, Denmark, Italy, Spain and Greece) were submitted for Annex I inclusion of triticonazole. All 40 trials (20 in northern Europe and 20 in southern Europe) match the intended use as a seed treatment for cereals. At harvest, no residues at or above the LOQ (0.01 mg/kg) were detected in any of the grain samples. In context of the existing residue studies only residues of triticonazole were determined. In view of the current concern over possible occurrence of the common TDMs, a new set of residue trials was conducted. Eight trials with triticonazole applied as a seed treatment to wheat at a rate of 6.25 g as/100 kg seed based on a seeding rate of 180 kg/ha were performed in Europe (four in northern Europe and four in Southern Europe) in the growing season 2012/2013. This number of trials is adequate to support use on all cereal crops, since the use is a seed treatment. A second year of residue trials was conducted in 2013/2014 to evaluate possible residues in feed items (plants at different growth stages, fodder, forage).

Table 6.3.1-1: Summary of the critical GAP for the proposed use in cereals for BAS 595 01 F

Crop	Outdoor/ Protected	Growth stage (BBCH)	Maximum number of applications	Minimum application interval (days)	Application Method	Maximum		Minimum PHI (days)
						Rate* (g as/ha)	Water (L/ha)	
Wheat	O	BBCH 00 Spring and autumn	1	-	Seed treatment	12.5 * based on 5 g as/100 kg seed, 250 kg seed/ha	Used undiluted or diluted with water at a max ratio of 1:5 (prod:water)	n.a.

n.a. not applicable

Table 6.3.1-2: Number of residue trials conducted per geographical region and vegetation period

Crop	Vegetation period	Number of trials					Reference
		EU North	Country	EU South	Country	Total	
Wheat	1994	4	DE	-	-	4	C016577
Wheat	1995	2	DE	-	-	2	C016014 C017676
Wheat	1995-1996	2	DK	-	-	2	R003472
Barley	1994	4	DE	-	-	4	C016581
Barley	1995	2	DE	-	-	2	C016580
Barley	1995	2	DK	-	-	2	C015362
Barley	1996	2	DK	-	-	2	C015364
Rye	1995-1996	2	DK	-	-	2	R003473
Wheat	1995-1996	-	-	1	IT	1	R003257
Wheat	1993-1994	-	-	1	IT	1	C017252
Wheat	1994	-	-	1	IT	1	C017316
Wheat	1994-1995	-	-	1	IT	1	C014706
Wheat	1994-1995	-	-	2	IT	2	R002862
Wheat	1995-1996	-	-	1	GR	1	R013164
Wheat	1993-1994	-	-	2	ES	2	C017251
Wheat	1994-1995	-	-	1	IT	1	C014710
Wheat	1994-1995	-	-	2	IT	2	R002860
Wheat	1995-1996	-	-	1	IT	1	R003235
Barley	1993-1994	-	-	1	IT	1	C017317
Barley	1993-1994	-	-	1	ES	1	C017659
Barley	1994-1995	-	-	2	IT	2	C014712
Barley	1995-1996	-	-	1	GR	1	R013162
Barley	1995-1996	-	-	2	IT	2	C014734
Total number of trials per region (old trials, evaluated in context of Annex I inclusion)		**20**		**20**	**Total number of trials (old trials, evaluated in context of Annex I inclusion)**	**40**	
Wheat	2012-2013	4	DE (2x), FR, UK	4	FR, GR, IT, ES	8	6.3.1/1
	2013-2014	4	DE, NL, FR, UK	4	FR, GR, IT, ES	8	6.3.1/2
Total number of trials per region (new trials)		**8**		**8**	**Total number of trials (new trials)**	**16**	

Report: CA 6.3.1/1
Martin T., 2015a
Study on the residue behavior of Triticonazole after seed treatment (with BAS 595 01 F) on wheat under field conditions in Germany, United Kingdom, France (North and South), Greece, Italy and Spain, 2012-2013
2014/1043281

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

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test Material:** Triticonazole BAS 595 01 F
   Description: BAS 595 01 F: Triticonazole (BAS 595 F), 25.0 g/L nominal content, FS
   Lot/Batch #: 84210
   Purity: not reported
   CAS#: 131983-72-7
   Development code: not applicable
   Spiking levels: 0.01 - 1.0 mg/kg (whole plant w/o roots), 0.01 - 0.50 mg/kg (grain and straw)

2. **Test Commodity:** Cereals
   Crop: Wheat
   Type: not applicable
   Variety: ESTEROS, KWS CHAMSIN
   Botanical name: *Triticum* L.
   Crop parts(s) or processed Commodity: Whole plant w/o roots, grain, straw
   Sample size: Whole plant w/o roots: ≥ 1.0 kg, grain: ≥ 1.0 kg, straw: ≥ 0.5 kg

1. Test procedure

During the 2012-2013 growing season, a total of 8 field trials were conducted in different representative growing areas in Germany, United Kingdom, France (North and South), Greece, Italy and Spain to determine the residue level of triticonazole (BAS 595 F) in wheat (whole plant no roots, grain and straw) after seed treatment.

Each field trial consisted of one untreated (plot 1) and one treated plot (plot 2). Seeds for plot 2 were treated using formulation BAS 595 01 F (25 g/L of BAS 595 F, FS) at a rate equivalent to 6.25 g as per 100 kg seed. Seeds for plot 1 remained untreated. The seeding rate for both plots was 180 kg/ha (plot 1 seeded before plot 2), the application rate at plot 2 was 11.25 g as/ha.

In trials L120653, L120654, L120659 and L120660 specimens of whole plant (no roots) were collected at 11-12 BBCH, 13-14 BBCH and 49 BBCH and specimens of grain and straw were collected at 89 BBCH. While the plant samples collected at BBCH 11/12 and 13/14 are considered not considered relevant as feed item for livestock, the BBCH 49 samples are considered relevant to be fed to animals (forage and fodder).

In trials L120655, L120656, L120657 and L120658 specimens of whole plant (no roots) were collected at 49 BBCH and specimens of grain and straw were collected at 89 BBCH.

The samples were stored deep frozen until analysis. The maximum storage intervals from harvest until last measurement were 311 days (Triticonazole samples) and 597 days (Triazole metabolite samples).

Table 6.3.1-3: Application and sampling details for trials conducted in 2012-2013

Region	No. of trials	No. of appl.	F, P, or G	Test item	Active substance	Application		Target timing	
						Rate as (g as/ha)	Water vol. (L/ha)	Application	Sampling (DALA)[1]
Northern and Southern Europe	8	1	F	BAS 595 01 F (FS)	BAS 595 F	11.25	n.a.	n.a.	whole plant (no roots): 11-12 BBCH 13-14 BBCH 49 BBCH (forage and fodder) grain / straw: 89 BBCH

[1] days after last application
n.a. not applicable

2. Description of analytical procedures

All wheat specimens were analyzed for triticonazole according to BASF analytical method No L0106/01 (= method no. 562/0):
BAS 595 F is extracted with a mixture of methanol and water. An aliquot of the extract is centrifuged and partitioned against dichlormethane. The final determination of BAS 595 F is performed by HPLC-MS/MS. The limit of quantitation (LOQ) of the method for BAS 595 F is 0.01 mg/kg.
The mean recovery results for triticonazole were between 76 and 99% (overall recovery: 87.9%, overall RSD: 15.7%) for wheat whole plant w/o roots, grain and straw at fortification levels between 0.01 and 1.0 mg/kg.

The results of procedural recovery experiments are summarized in the following table:

Table 6.3.1-4: Summary of recoveries for triticonazole on wheat

Matrix	Fortification level (mg/kg)	Summary recoveries		
		n	Average (%)	RSD (%)
BASF analytical method No L0106/01 (=562/0)		**Triticonazole (FS)**		
Wheat / Whole Plant (no roots)	0.01, 0.10, 1.0	3	99.3	13.8
Wheat / Grain	0.01, 0.10	2	82.4	not applicable
Wheat / Straw	0.01, 0.10	2	76.3	not applicable
	Overall:	**7**	**87.9**	**15.7**

The wheat specimens were analyzed for the triazole metabolites 1,2,4-triazole, triazolylalanine, triazole acetic acid and triazole lactic acid according to BASF method No. L0170/02.
The metabolites 1,2,4-triazole, triazolylalanine, triazole acetic acid and triazole lactic acid were extracted with methanol/water (80/20, v/v). An aliquot of the extract was filtered, concentrated to an aqueous reminder and cleaned-up by a simple dispersive C18-SPE step. The final determination was performed by LC-DMS/MS/MS. Residues were quantified using isotopically labelled internal standards. The limit of quantitation (LOQ) of the method for BAS 595 F is 0.01 mg/kg.

The mean recovery results for the triazole metabolite were between 77 and 112% (overall recoveries: 90.3-105%%, overall RSDs: 16.7-20.8%) for wheat whole plant w/o roots, grain and straw at fortification levels between 0.01 and 0.5 mg/kg.

The results of procedural recovery experiments are summarized in the following table:

Table 6.3.1-5: Summary of recoveries for triazole metabolites on wheat

Matrix	Fortification level (mg/kg)	Summary recoveries		
		n	Average (%)	RSD (%)
BASF analytical method No L0170/02		**1,2,4-Triazole**		
Wheat / Whole Plant (no roots)	0.01, 0.10	4	106	27.9
Wheat / Grain	0.01, 0.10	2	104	not applicable
Wheat / Straw	0.01, 0.10, 0.50	3	104	11.8
Overall:		**9**	**105**	**18.8**
BASF analytical method No L0170/02		**Triazolalanine**		
Wheat / Whole Plant (no roots)	0.01, 0.10	4	103	17.1
Wheat / Grain	0.01, 0.10	2	77.1	not applicable
Wheat / Straw	0.01, 0.10, 0.50	3	82.5	13.3
Overall:		**9**	**90.3**	**20.5**
BASF analytical method No L0170/02		**Triazole Acetic acid**		
Wheat / Whole Plant (no roots)	0.01, 0.10	4	100	18.1
Wheat / Grain	0.01, 0.10	2	97.7	not applicable
Wheat / Straw	0.01, 0.10, 0.50	3	83.2	10.1
Overall:		**9**	**94.0**	**16.7**
BASF analytical method No L0170/02		**Triazole Lactic Acid**		
Wheat / Whole Plant (no roots)	0.01, 0.10	4	112	16.8
Wheat / Grain	0.01, 0.10	2	99.1	not applicable
Wheat / Straw	0.01, 0.10, 0.50	3	78.0	8.3
Overall:		**9**	**97.8**	**20.8**

II. RESULTS AND DISCUSSION

The triticonazole residues in the whole plant (no roots) specimens taken at BBCH 11-12 ranged between 0.13 and 0.64 mg/kg. At BBCH 13-14, triticonazole residues ranged between < 0.01 and 0.18 mg/kg and at BBCH 49 (forage and fodder) residues were below the limit of quantitation (0.01 mg/kg). The triticonazole residues in the straw and grain samples taken at BBCH 89 were below the limit of quantitation (0.01 mg/kg). No residues of triticonazole at or above the limit of quantitation (0.01 mg/kg) were detected in the untreated specimens of this study.

The range of residues for the triazole metabolites in the matrices of concern are shown below (Table 6.3.1-7).

A summary of residues is presented in Table 6.3.1-6 (Triticonazole) and Table 6.3.1-7 (Triazole metabolites). An overview on the triazole metabolite residues in the untreated samples is given in Table 6.3.1-8. Details are shown in Table 6.3.1-9, Table 6.3.1-10, Table 6.3.1-11 and Table 6.3.1-12.

Table 6.3.1-6: Summary of triticonazole residues in the treated wheat specimens after application of BAS 595 01 F

Crop	Year	Appl.	DALA[1]	Growth stage (BBCH)	Residues found (mg/kg)	
					Matrix	BAS 595 F
Wheat	2012-2013	BAS 595 01 F (FS)	20-51	11-12	whole plant[2]	0.13 - 0.64
			30-71	13-14	whole plant[2]	<0.01 - 0.18
			59-100	49 (forage and fodder)	whole plant[2]	< 0.01
			105-170	89	grain	< 0.01
			105-170	89	straw	< 0.01

[1] days after last application
[2] no roots

Table 6.3.1-7: Summary of triazole metabolite residues in the treated wheat specimens after application of BAS 595 01 F

Crop	Year	Appl.	DALA[1]	Growth stage (BBCH)	Residues found (mg/kg)				
					Matrix	1,2,4-Triazole (T)	Triazolalanine (TA)	Triazole Acetic Acid (TAA)	Triazole Lactic Acid (TLA)
Wheat	2012-2013	BAS 595 01 F (FS)	20-51	11-12	whole plant[2]	<0.01	0.034 – 0.13	0.01 – 0.023	0.031 – 0.071
			30-71	13-14	whole plant[2]	<0.01	0.015 – 0.049	<0.01	0.030 – 0.075
			59-100	49 (forage and fodder)	whole plant[2]	<0.01	< 0.01 - 0.054	< 0.01 - 0.036	0.016 – 0.084
			105-170	89	grain	<0.01	< 0.01 - 0.023	< 0.01 - 0.078	< 0.01 - 0.066
			105-170	89	straw	<0.01	< 0.01 - 0.36	< 0.01 - 0.13	<0.01

[1] days after last application, [2] no roots

Table 6.3.1-8: Summary of triazole metabolite residues in the untreated wheat specimens

Crop	Year	Appl.	DALA[1]	Growth stage (BBCH)	Residues found (mg/kg)				
					Matrix	1,2,4-Triazole (T)	Triazolalanine (TA)	Triazole Acetic Acid (TAA)	Triazole Lactic Acid (TLA)
Wheat	2012-2013	BAS 595 01 F (FS)	20-51	11-12	whole plant[2]	<0.01	0.020 – 0.13	< 0.01 - 0.023	0.021 – 0.062
			30-71	13-14	whole plant[2]	<0.01	0.016 – 0.063	<0.01	0.037 – 0.052
			59-100	49 (forage and fodder)	whole plant[2]	<0.01	< 0.01 - 0.044	< 0.01 - 0.028	0.010 – 0.062
			105-170	89	grain	<0.01	< 0.01 - 0.020	< 0.01 - 0.052	< 0.01 - 0.042
			105-170	89	straw	<0.01	< 0.01 - 0.15	< 0.01 - 0.088	<0.01

[1] days after last application
[2] no roots

Table 6.3.1-9: Residues of triticonazole in wheat after one application of BAS 595 01 F (FS) in Northern Europe

Study details		Crop	Country	Formulation application rate (g a.s./ha)[0)	GS [2) BBCH	DALA[1)	Residues found (mg/kg)	
							Matrix	Triticonazole
Study code:	407785			BAS 595 01 F	n.a.	21	Wh. plant*	0.64
DocID:	2014/1043281			FS		31	Wh. plant*	0.11
Trial No:	L120653	Wheat	Germany	1 x 11.25		72	Wh. plant*	< 0.01
GLP:	Yes					129	Grain	< 0.01
Year:	2013					129	Straw	< 0.01
Study code:	407785			BAS 595 01 F	n.a.	51	Wh. plant*	0.13
DocID:	2014/1043281			FS		71	Wh. plant*	< 0.01
Trial No:	L120654	Wheat	United Kingdom	1 x 11.25		100	Wh. plant*	< 0.01
GLP:	Yes					170	Grain	< 0.01
Year:	2013					170	Straw	< 0.01
Study code:	407785			BAS 595 01 F	n.a.	94	Wh. plant*	< 0.01
DocID:	2014/1043281			FS		163	Grain	< 0.01
Trial No:	L120655	Wheat	France (North)	1 x 11.25		163	Straw	< 0.01
GLP:	Yes							
Year:	2013							
Study code:	407785			BAS 595 01 F	n.a.	68	Wh. plant*	< 0.01
DocID:	2014/1043281			FS		136	Grain	< 0.01
Trial No:	L120656	Wheat	Germany	1 x 11.25		136	Straw	< 0.01
GLP:	Yes							
Year:	2013							

[1] Days after last application
[2] At last application
* Whole plants without roots, n.a. not applicable
_ underlined values are used for MRL calculation

Table 6.3.1-10: Residues of triazole metabolites in wheat after one application of BAS 595 01 F (FS) in Northern Europe

Study details		Crop	Country	Formulation application rate (g a.s./ha)[0]	GS [2] BBCH	DALA[1]	Residues found (mg/kg)				
							Matrix	T	TA	TAA	TLA
Study code:	407785	Wheat	Germany	BAS 595 01 F	n.a.	21	Wh. plant*	<0.01	0.13	0.023	0.044
DocID:	2014/1043281			FS		31	Wh. plant*	<0.01	0.035	<0.01	0.030
Trial No:	L120653			1 x 11.25		72	Wh. plant*	<0.01	0.023	<0.01	0.030
GLP:	Yes					129	Grain	<0.01	0.063	0.043	<0.01
Year:	2013					129	Straw	<0.01	<0.01	0.018	0.023
Study code:	407785	Wheat	United Kingdom	BAS 595 01 F	n.a.	51	Wh. plant*	<0.01	0.034	0.012	0.071
DocID:	2014/1043281			FS		71	Wh. plant*	<0.01	0.015	<0.01	0.035
Trial No:	L120654			1 x 11.25		100	Wh. plant*	<0.01	0.013	<0.01	0.021
GLP:	Yes					170	Grain	<0.01	0.026	0.013	<0.01
Year:	2013					170	Straw	<0.01	<0.01	<0.01	<0.01
Study code:	407785	Wheat	France (North)	BAS 595 01 F	n.a.	94	Wh. plant*	<0.01	<0.01	<0.01	0.016
DocID:	2014/1043281			FS		163	Grain	<0.01	0.050	0.027	<0.01
Trial No:	L120655			1 x 11.25		163	Straw	<0.01	<0.01	0.011	<0.01
GLP:	Yes										
Year:	2013										
Study code:	407785	Wheat	Germany	BAS 595 01 F	n.a.	68	Wh. plant*	<0.01	<0.01	<0.01	0.023
DocID:	2014/1043281			FS		136	Grain	<0.01	0.052	0.021	<0.01
Trial No:	L120656			1 x 11.25		136	Straw	<0.01	<0.01	0.014	<0.01
GLP:	Yes										
Year:	2013										

[1] Days after last application
[2] At last application
* Whole plants without roots, n.a. not applicable

Table 6.3.1-11: Residues of triazole metabolites in wheat in untreated samples in Northern Europe

Study details		Crop	Country	Formulation application rate (g a.s./ha)[0]	GS [2] BBCH	DALA[1]	Residues found (mg/kg)				
							Matrix	T	TA	TAA	TLA
Study code:	407785	Wheat	Germany	BAS 595 01 F FS 1 x 11.25	n.a.	21	Wh. plant*	<0.01	0.13	0.023	0.033
DocID:	2014/1043281					31	Wh. plant*	<0.01	0.063	<0.01	0.037
Trial No:	L120653					72	Wh. plant*	<0.01	0.023	<0.01	0.031
GLP:	Yes					129	Grain	<0.01	0.15	0.086	<0.01
Year:	2013					129	Straw	<0.01	<0.01	0.038	0.042
Study code:	407785	Wheat	United Kingdom	BAS 595 01 F FS 1 x 11.25	n.a.	51	Wh. plant*	<0.01	0.030	<0.01	0.062
DocID:	2014/1043281					71	Wh. plant*	<0.01	0.018	<0.01	0.040
Trial No:	L120654					100	Wh. plant*	<0.01	0.013	<0.01	0.017
GLP:	Yes					170	Grain	<0.01	0.035	0.016	<0.01
Year:	2013					170	Straw	<0.01	<0.01	<0.01	<0.01
Study code:	407785	Wheat	France (North)	BAS 595 01 F FS 1 x 11.25	n.a.	94	Wh. plant*	<0.01	<0.01	<0.01	0.011
DocID:	2014/1043281					163	Grain	<0.01	0.042	0.015	<0.01
Trial No:	L120655					163	Straw	<0.01	<0.01	<0.01	<0.01
GLP:	Yes										
Year:	2013										
Study code:	407785	Wheat	Germany	BAS 595 01 F FS 1 x 11.25	n.a.	68	Wh. plant*	<0.01	0.010	<0.01	0.015
DocID:	2014/1043281					136	Grain	<0.01	0.066	0.023	<0.01
Trial No:	L120656					136	Straw	<0.01	0.010	0.016	<0.01
GLP:	Yes										
Year:	2013										

[1] Days after last application
[2] At last application
* Whole plants without roots, n.a. not applicable

Table 6.3.1-12: Residues of triticonazole in wheat after one application of BAS 595 01 F (FS) in Southern Europe

Study details		Crop	Country	Formulation application rate (g a.s./ha)	GS [2) BBCH	DALA[1)	Residues found (mg/kg)	
							Matrix	Triticonazole
Study code:	407785	Wheat	France (South)	BAS 595 01 F	n.a.	98	Wh. plant*	< 0.01
DocID:	2014/1043281			FS		161	Grain	≤ 0.01
Trial No:	L120657			1 x 11.25		161	Straw	< 0.01
GLP:	Yes							
Year:	2013							
Study code:	407785	Wheat	Greece	BAS 595 01 F	n.a.	59	Wh. plant*	< 0.01
DocID:	2014/1043281			FS		105	Grain	≤ 0.01
Trial No:	L120658			1 x 11.25		105	Straw	< 0.01
GLP:	Yes							
Year:	2013							
Study code:	407785	Wheat	Italy	BAS 595 01 F	n.a.	23	Wh. plant*	0.20
DocID:	2014/1043281			FS		36	Wh. plant*	0.087
Trial No:	L120659			1 x 11.25		71	Wh. plant*	< 0.01
GLP:	Yes					123	Grain	≤ 0.01
Year:	2013					123	Straw	< 0.01
Study code:	407785	Wheat	Spain	BAS 595 01 F	n.a.	20	Wh. plant*	0.52
DocID:	2014/1043281			FS		34	Wh. plant*	0.18
Trial No:	L120660			1 x 11.25		84	Wh. plant*	< 0.01
GLP:	Yes					153	Grain	≤ 0.01
Year:	2013					153	Straw	< 0.01

[1] Days after last application
[2] At last application
* Whole plants without roots, n.a. not applicable
_ underlined values are used for MRL calculation

Table 6.3.1-13: Residues of triazole metabolites in wheat after one application of BAS 595 01 F (FS) in Southern Europe

Study details		Crop	Country	Formulation application rate (g a.s./ha)[0]	GS [2] BBCH	DALA [1]	Residues found (mg/kg)				
							Matrix	T	TA	TAA	TLA
Study code:	407785	Wheat	France (South)	BAS 595 01 F	n.a.	98	Wh. plant*	< 0.01	0.052	0.016	0.044
DocID:	2014/1043281			FS		161	Grain	< 0.01	0.24	0.13	<0.01
Trial No:	L120657			1 x 11.25		161	Straw	< 0.01	<0.01	0.078	0.066
GLP:	Yes										
Year:	2013										
Study code:	407785	Wheat	Greece	BAS 595 01 F	n.a.	59	Wh. plant*	< 0.01	<0.01	<0.01	0.033
DocID:	2014/1043281			FS		105	Grain	< 0.01	<0.01	<0.01	<0.01
Trial No:	L120658			1 x 11.25		105	Straw	< 0.01	<0.01	<0.01	<0.01
GLP:	Yes										
Year:	2013										
Study code:	407785	Wheat	Italy	BAS 595 01 F	n.a.	23	Wh. plant*	<0.01	0.046	<0.01	0.031
DocID:	2014/1043281			FS		36	Wh. plant*	<0.01	0.027	<0.01	0.043
Trial No:	L120659			1 x 11.25		71	Wh. plant*	<0.01	0.054	0.036	0.084
GLP:	Yes					123	Grain	<0.01	0.18	0.12	<0.01
Year:	2013					123	Straw	<0.01	0.023	0.062	0.028
Study code:	407785	Wheat	Spain	BAS 595 01 F	n.a.	20	Wh. plant*	<0.01	0.044	<0.01	0.052
DocID:	2014/1043281			FS		34	Wh. plant*	<0.01	0.049	<0.01	0.075
Trial No:	L120660			1 x 11.25		84	Wh. plant*	<0.01	0.021	<0.01	0.018
GLP:	Yes					153	Grain	<0.01	0.36	0.089	<0.01
Year:	2013					153	Straw	<0.01	<0.01	0.024	0.035

[1] Days after last application
[2] At last application
* Whole plants without roots, n.a. not applicable

Table 6.3.1-14: Residues of triazole metabolites in wheat in untreated samples in Southern Europe

Study details		Crop	Country	Formulation application rate (g a.s./ha)[0]	GS [2] BBCH	DALA[1]	Residues found (mg/kg)				
							Matrix	T	TA	TAA	TLA
Study code:	407785	Wheat	France (South)	BAS 595 01 F	n.a.	98	Wh. plant*	< 0.01	< 0.01	< 0.01	0.015
DocID:	2014/1043281			FS		161	Grain	< 0.01	0.038	0.017	< 0.01
Trial No:	L120657			1 x 11.25		161	Straw	< 0.01	< 0.01	0.014	0.012
GLP:	Yes										
Year:	2013										
Study code:	407785	Wheat	Greece	BAS 595 01 F	n.a.	59	Wh. plant*	< 0.01	< 0.01	< 0.01	0.025
DocID:	2014/1043281			FS		105	Grain	< 0.01	< 0.01	< 0.01	< 0.01
Trial No:	L120658			1 x 11.25		105	Straw	< 0.01	< 0.01	< 0.01	< 0.01
GLP:	Yes										
Year:	2013										
Study code:	407785	Wheat	Italy	BAS 595 01 F	n.a.	23	Wh. plant*	<0.01	0.040	< 0.01	0.021
DocID:	2014/1043281			FS		36	Wh. plant*	<0.01	0.025	< 0.01	0.052
Trial No:	L120659			1 x 11.25		71	Wh. plant*	<0.01	0.044	0.028	0.062
GLP:	Yes					123	Grain	<0.01	0.11	0.088	< 0.01
Year:	2013					123	Straw	<0.01	0.020	0.052	0.020
Study code:	407785	Wheat	Spain	BAS 595 01 F	n.a.	20	Wh. plant*	<0.01	0.020	<0.01	0.036
DocID:	2014/1043281			FS		34	Wh. plant*	<0.01	0.016	<0.01	0.040
Trial No:	L120660			1 x 11.25		84	Wh. plant*	<0.01	<0.01	<0.01	0.010
GLP:	Yes					153	Grain	<0.01	0.076	0.018	<0.01
Year:	2013					153	Straw	<0.01	<0.01	<0.01	<0.01

[1] Days after last application
[2] At last application
* Whole plants without roots, n.a. not applicable

III. CONCLUSION

The triticonazole residues in the whole plant (no roots) specimens taken at BBCH 11-12 ranged between 0.13 and 0.64 mg/kg. At BBCH 13-14, triticonazole residues ranged between < 0.01 and 0.18 mg/kg and at BBCH 49 residues were below the limit of quantitation (0.01 mg/kg). The triticonazole residues in the straw and grain samples taken at harvest maturity (BBCH 89) were below the limit of quantitation (0.01 mg/kg).
Samples were also analyzed for the triazole metabolites 1,2,4-triazole, triazolylalanine, triazole acetic acid and triazole lactic acid.
No residues of 1,2,4-triazole were detected in any of the treated samples analyzed.
Residues of triazolylalanine ranged in whole plant (no roots) and straw between <0.01 mg/kg and 0.054 mg/kg, in grain residues up to 0.36 mg/kg were determined.
Residues of triazole acetic acid ranged in whole plant (no roots) and straw between <0.01 mg/kg and 0.078 mg/kg, in grain residues up to 0.13 mg/kg were determined.
Residues of triazole lactic acid ranged in whole plant (no roots), straw and grain between <0.01 mg/kg and 0.084 mg/kg.
In the untreated samples no residues of triticonazole and 1,2,4-triazole above the LOQ (0.01 mg/kg) were found.
Residues of triazolylalanine ranged in untreated whole plant (no roots), straw and grain between <0.01 mg/kg and 0.15 mg/kg.
Residues of triazole acetic acid ranged in untreated whole plant (no roots), straw and grain between <0.01 mg/kg and 0.088 mg/kg.
Residues of triazole lactic acid ranged untreated whole plant (no roots), straw and grain between <0.01 mg/kg and 0.062 mg/kg.

Report: CA 6.3.1/2
Martin T., 2015b
Study on the residue behaviour of Triticonazole after seed treatment (with BAS 595 01 F) on wheat under field conditions in Germany, France (North and South), Netherlands, United Kingdom, Greece, Italy and Spain, 2013-2014
2014/1090813
Guidelines: 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), EEC 7525/VI/95 rev. 9 (March 2011)
GLP: yes
(certified by ENAC, Entidad Nacional de Acreditación, Madrid Spain)

Executive Summary

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test Material:** Triticonazole BAS 595 01 F
Description: BAS 595 01 F: Triticonazole (BAS 595 F), 25.0 g/L nominal content, FS
Lot/Batch #: FRE-000978
Purity: not reported
CAS#: 131983-72-7
Development code: not applicable
Spiking levels: 0.01 - 1.0 mg/kg (whole plant w/o roots), 0.01 - 0.10 mg/kg (grain and straw)

2. **Test Commodity:** Cereals
Crop: Wheat
Type: not applicable
Variety: ESTEROS, KWS CHAMSIN
Botanical name: *Triticum* L.
Crop parts(s) or processed Commodity: Whole plant w/o roots, grain, straw
Sample size: Whole plant w/o roots: ≥ 0.5 kg, grain: ≥ 1.0 kg, straw: ≥ 0.5 kg

1. Test procedure

During the 2013-2014 growing season, a total of 8 field trials were conducted in different representative growing areas in Germany, United Kingdom, France (North and South), Netherlands, Greece, Italy and Spain to determine the residue level of triticonazole (BAS 595 F) in wheat (whole plant no roots, grain and straw) after seed treatment. Each field trial consisted of one untreated (plot 1) and one treated plot (plot 2). Seeds for plot 2 were treated using formulation BAS 595 01 F (25 g/L of BAS 595 F, FS) at a rate equivalent to 6.25 g as per 100 kg seed. Seeds for plot 1 remained untreated. The seeding rate for both plots was 180 kg/ha (plot 1 seeded before plot 2), the application rate at plot 2 was 11.25 g as/ha. Specimens of whole plant (no roots) were collected at 12-13 BBCH, 21-23 BBCH and 59 BBCH and specimens of grain and straw were collected at 89 BBCH.

The samples were stored deep frozen until analysis. The maximum storage interval from harvest until extraction was 275 days.

Table 6.3.1-15: Application and sampling details for trials conducted in 2013-2014

Region	No. of trials	No. of appl.	F, P, or G	Test item	Active substance	Application		Target timing	
						Rate as (g as/ha)	Water vol. (L/ha)	Application	Sampling (DALA)[1]
Northern and Southern Europe	8	1	F	BAS 595 01 F (FS)	BAS 595 F	11.25	n.a.	n.a.	whole plant (no roots): 12-13 BBCH 21-23 BBCH 59 BBCH (forage and fodder) grain / straw: 89 BBCH

[1] days after last application
n.a. not applicable

2. Description of analytical procedures

All wheat specimens were analyzed for triticonazole according to BASF analytical method No 562/0 (adapted for triticonazole):
BAS 595 F is extracted with a mixture of methanol and water. An aliquot of the extract is centrifuged and partitioned against dichlormethane. The final determination of BAS 595 F is performed by HPLC-MS/MS. The limit of quantitation (LOQ) of the method for BAS 595 F is 0.01 mg/kg.
The mean recovery results for triticonazole were between 103 and 108% (RSD: 2.8 - 7.9%) for wheat whole plant, grain and straw at fortification levels between 0.01 and 1.0 mg/kg.

The results of procedural recovery experiments are summarized in the following table:

Table 6.3.1-16: Summary of recoveries for triticonazole on wheat

Matrix	Fortification level (mg/kg)	Summary recoveries		
		n	Average (%)	RSD (%)
BASF analytical method No 562/0		**Triticonazole (FS)**		
Wheat / Whole Plant (no roots)	0.01, 0.10, 1.0	7	103	6.0
Wheat / Grain	0.01, 0.10	6	108	2.8
Wheat / Straw	0.01, 0.10	6	104	7.9
	Overall:	**19**	**105**	**5.9**

II. RESULTS AND DISCUSSION

The triticonazole residues in the whole plant (no roots) specimens taken at BBCH 12-13 ranged between 0.052 and 0.46 mg/kg. At BBCH 21-23, triticonazole residues ranged between < 0.01 and 0.064 mg/kg and at BBCH 59 residues were below the limit of quantitation (0.01 mg/kg). The triticonazole residues in the straw and grain samples taken at BBCH 89 were below the limit of quantitation (0.01 mg/kg). No residues of triticonazole at or above the limit of quantitation (0.01 mg/kg) were detected in the untreated specimens of this study.

A summary of residues is presented in Table 6.3.1-17. Details are shown in Table 6.3.1-18 and Table 6.3.1-19.

Table 6.3.1-17: Summary of triticonazole residues in the treated wheat specimens after application of BAS 595 01 F

Crop	Year	Appl.	DALA[1]	Growth stage (BBCH)	Residues found (mg/kg)	
					Matrix	BAS 595 F
Wheat	2013-2014	BAS 595 01 F (FS)	15-47	12-13	whole plant[2]	0.052 - 0.46
			29-67	21-23	whole plant[2]	< 0.01 - 0.064
			72-104	59 (forage and fodder)	whole plant[2]	< 0.01
			124-147	89	grain	< 0.01
			124-147	89	straw	< 0.01

[1] days after last application
[2] no roots

Table 6.3.1-18: Residues of triticonazole in wheat after one application of BAS 595 01 F (FS) in Northern Europe

Study details		Crop	Country	Formulation application rate (g a.s./ha)[0]	GS [2] BBCH	DALA[1]	Residues found (mg/kg)	
							Matrix	Triticonazole
Study code:	721605	Wheat	Germany	BAS 595 01 F FS 1 x 11.25	n.a.	32	Wh. plant*	0.134
DocID:	2014/1090813					46	Wh. plant*	0.023
Trial No:	L130814					86	Wh. plant*	< 0.01
GLP:	Yes					142	Grain	< 0.01
Year:	2014					142	Straw	< 0.01
Study code:	721605	Wheat	France (N)	BAS 595 01 F FS 1 x 11.25	n.a.	28	Wh. plant*	0.202
DocID:	2014/1090813					50	Wh. plant*	0.028
Trial No:	130815					85	Wh. plant*	< 0.01
GLP:	Yes							
Year:	2014							
Study code:	721605	Wheat	Netherlands	BAS 595 01 F FS 1 x 11.25	n.a.	15	Wh. plant*	0.462
DocID:	2014/1090813					29	Wh. plant*	0.020
Trial No:	L130816					72	Wh. plant*	< 0.01
GLP:	Yes					126	Grain	< 0.01
Year:	2014					126	Straw	< 0.01
Study code:	721605	Wheat	UK	BAS 595 01 F FS 1 x 11.25	n.a.	30	Wh. plant*	0.259
DocID:	2014/1090813					49	Wh. plant*	0.028
Trial No:	L130817					83	Wh. plant*	< 0.01
GLP:	Yes					139	Grain	< 0.01
Year:	2014					139	Straw	< 0.01

[1] Days after last application
[2] At last application
* Whole plants without roots
_ underlined values are used for MRL calculation

Table 6.3.1-19: Residues of triticonazole in wheat after one application of BAS 595 01 F (FS) in Southern Europe

Study details		Crop	Country	Formulation application rate (g a.s./ha)	GS [2] BBCH	DALA[1]	Residues found (mg/kg)	
							Matrix	Triticonazole
Study code:	721605	Wheat	France (S)	BAS 595 01 F FS 1 x 11.25	n.a.	47	Wh. plant*	0.052
DocID:	2014/1090813					67	Wh. plant*	< 0.01
Trial No:	L130818					97	Wh. plant*	< 0.01
GLP:	Yes					147	Grain	< 0.01
Year:	2014					147	Straw	< 0.01
Study code:	721605	Wheat	Greece	BAS 595 01 F FS 1 x 11.25	n.a.	33	Wh. plant*	0.097
DocID:	2014/1090813					49	Wh. plant*	< 0.01
Trial No:	L130819					87	Wh. plant*	< 0.01
GLP:	Yes					124	Grain	< 0.01
Year:	2014					124	Straw	< 0.01
Study code:	721605	Wheat	Italy	BAS 595 01 F FS 1 x 11.25	n.a.	35	Wh. plant*	0.064
DocID:	2014/1090813					48	Wh. plant*	< 0.01
Trial No:	L130820					104	Wh. plant*	< 0.01
GLP:	Yes					136	Grain	< 0.01
Year:	2014					136	Straw	< 0.01
Study code:	721605	Wheat	Spain	BAS 595 01 F FS 1 x 11.25	n.a.	22	Wh. plant*	0.446
DocID:	2014/1090813					39	Wh. plant*	0.064
Trial No:	L130821					79	Wh. plant*	< 0.01
GLP:	Yes					134	Grain	< 0.01
Year:	2014					134	Straw	< 0.01

[1] Days after last application
[2] At last application
* Whole plants without roots
_ underlined values are used for MRL calculation

III. CONCLUSION

The triticonazole residues in the whole plant (no roots) specimens taken at BBCH 12-13 ranged between 0.052 and 0.46 mg/kg. At BBCH 21-23, triticonazole residues ranged between < 0.01 and 0.064 mg/kg and at BBCH 59 residues were below the limit of quantitation (0.01 mg/kg). The triticonazole residues in the straw and grain samples taken at BBCH 89 were below the limit of quantitation (0.01 mg/kg).

Supplemental Trials

Table 6.3.1-20: Summary of Triticonazole Residues from Field Trials in Cereals. Northern Europe - Supplemental Data

Report-No.; Location incl Postal code; Country	Crop Variety	Date of (a) 1) Sowing S or Planting P 2) Flowering 3) Harvest	Method of treatment	Formulation application rate (g/100 kg of seed)	Dates of treatment(s) or number of treatments and last date	Growth stage at last treatment or date	Matrix	Triticonazole Residues (mg/kg)	PHI (days)	Remarks:
Doc. No: C016577 WTA REGION 10/94-593 Decline Curve 27234 Winkelsett Niedersachsen Germany	Common wheat Nandu	1) 29.03.1994 S 2) - 3) 17.05.1994 - 01.08.1994	Seed treatment	EXP80525 FS (12.5 g/l Triticonazole and 150 g/L Guazatine) 4.8	28.02.1994	00 Dry seed (caryopsis)	Shoots Grains Straw	< 0.05 < 0.01 < 0.05	49 125 125	Anal. method: AR92-92 Analytical principle: GC-NPD (shoot) GC-MSD (grain & straw) LOQ Grains: 0.01 mg/kg LOQ Shoots: 0.05 mg/kg LOQ Straw: 0.05 mg/kg
Doc. No: C016577 WTA REGION 30/94-593 Decline Curve 59457 Oberbergstraße Nordrhein - Westfalen Germany	Common wheat Nandu	1) 16.04.1994 S 2) - 3) 13.05.1994 - 08.08.1994	Seed treatment	EXP80525 FS (12.5 g/l Triticonazole and 150 g/L Guazatine) 4.8	28.02.1994	00 Dry seed (caryopsis)	Shoots Grains Straw	0.080 < 0.01 < 0.05	27 114 114	Anal. method: AR92-92 Analytical principle: GC-NPD (shoot) GC-MSD (grain & straw) LOQ Grains: 0.01 mg/kg LOQ Shoots: 0.05 mg/kg LOQ Straw: 0.05 mg/kg
Doc. No: C016577 WTA REGION 50/94-593 Decline Curve 67294 Mauchenheim Rheinland - Pfalz Germany	Common wheat Nandu	1) 11.03.1994 S 2) - 3) 06.05.1994 - 01.08.1994	Seed treatment	EXP80525 FS (12.5 g/l Triticonazole and 150 g/L Guazatine) 4.8	28.02.1994	00 Dry seed (caryopsis)	Shoots Grains Straw	0.11 < 0.01 < 0.05	56 143 143	Anal. method: AR92-92 Analytical principle: GC-NPD (shoot) GC-MSD (grain & straw) LOQ Grains: 0.01 mg/kg LOQ Shoots: 0.05 mg/kg LOQ Straw: 0.05 mg/kg
Doc. No: C016577 WTA REGION 60/94-593 Decline Curve 99834 Gerstungen Thüringen Germany	Common wheat Nandu	1) 22.04.1994 S 2) - 3) 24.05.1994 - 05.08.1994	Seed treatment	EXP80525 FS (12.5 g/l Triticonazole and 150 g/L Guazatine) 4.8	28.02.1994	00 Dry seed (caryopsis)	Shoots Grains Straw	< 0.05 < 0.01 < 0.05	32 105 105	Anal. method: AR92-92 Analytical principle: GC-NPD (shoot) GC-MSD (grain & straw) LOQ Grains: 0.01 mg/kg LOQ Shoots: 0.05 mg/kg LOQ Straw: 0.05 mg/kg

Report-No.; Location incl Postal code; Country	Crop Variety	Date of (a) 1) Sowing S or Planting P 2) Flowering 3) Harvest	Method of treatment	Formulation application rate (g/100 kg of seed)	Dates of treatment(s) or number of treatments and last date	Growth stage at last treatment or date	Matrix	Triticonazole Residues (mg/kg)	PHI (days)	Remarks:
Doc. No: C016014 **Doc. No: C017676** WTA REGION 20/95-682 Harvest study 24623 Großenaspe Schleswig-Holstein Germany	Common wheat Nandu	1) 04.04.1995 S 2) - 3) 18.05.1995 - 04.08.1995	Seed treatment	EXP80525D FS (12.5 g/l Triticonazole and 150 g/L Guazatine) 4.8	24.02.1995	00 Dry seed (caryopsis)	Shoots Grains Straw	< 0.05 < 0.01 < 0.05	44 122 122	Anal. method: AR92-92 Analytical principle: GC-NPD (shoot) GC-MSD (grain & straw) LOQ Grains: 0.01 mg/kg LOQ Shoots: 0.05 mg/kg LOQ Straw: 0.05 mg/kg
Doc. No: C016014 **Doc. No: C017676** WTA REGION 60/95-682 Harvest study 37242 Bad Sooden Hessen Germany	Common wheat Nandu	1) 14.03.1995 S 2) - 3) 12.05.1995 - 11.08.1995	Seed treatment	EXP80525D FS (12.5 g/l Triticonazole and 150 g/L Guazatine) 4.8	24.02.1995	00 Dry seed (caryopsis)	Shoots Grains Straw	< 0.05 < 0.01 < 0.05	59 150 150	Anal. method: AR92-92 Analytical principle: GC-NPD(shoot) GC-MSD(grain & straw) LOQ Grains: 0.01 mg/kg LOQ Shoots: 0.05 mg/kg LOQ Straw: 0.05 mg/kg
Doc. No: R003472 95-691DK1 Decline Curve 18DK-5500 Rojleskovvej Middelfart Denmark	Common wheat Marabu	1) 17.10.1995 S 2) - 3) 20.08.1996	Seed treatment	EXP80525D FS (12.5 g/l Triticonazole and 150 g/L Guazatine) 5	12.10.1995	00 Dry seed (caryopsis)	Seeds Shoots Shoots Shoots Shoots ear Straw Grains	41 0.22 0.35 0.42 < 0.015 < 0.015 < 0.015 < 0.01	0 27 34 48 274 274 308 308	Anal. method: AR92-92 Analytical principle: GC-NPD (shoot) GC-MSD (grain & straw) LOQ Grains: 0.01 mg/kg LOQ Seeds: - LOQ Shoots: 0.015 mg/kg LOQ Straw: 0.015 mg/kg LOQ ear: 0.015 mg/kg
Doc. No: R003472 95-691DK2 Decline Curve 5500 Hyllehoj I Middelfart Denmark	Common wheat Marabu	1) 17.10.1995 S 2) - 3) 23.08.1996	Seed treatment	EXP80525D FS (12.5 g/l Triticonazole and 150 g/L Guazatine) 5	12.10.1995	00 Dry seed (caryopsis)	Seeds Shoots Shoots Shoots Shoots ear Straw Grains	41 0.20 0.31 0.40 < 0.015 < 0.015 < 0.015 < 0.003	0 27 34 48 274 274 311 311	Anal. method: AR92-92 Analytical principle: GC-NPD (shoot) GC-MSD (grain & straw) LOQ Grains: 0.003 mg/kg LOQ Seeds: - LOQ Shoots: 0.015 mg/kg LOQ Straw: 0.015 mg/kg LOQ ear: 0.015 mg/kg

Report-No.; Location incl Postal code; Country	Crop Variety	Date of (a) 1) Sowing S or Planting P 2) Flowering 3) Harvest	Method of treatment	Formulation application rate (g/100 kg of seed)	Dates of treatment(s) or number of treatments and last date	Growth stage at last treatment or date	Matrix	Triticonazole Residues (mg/kg)	PHI (days)	Remarks:
Doc. No: C016581 WTA REGION 10/94-595 Decline Curve 27234 Winkelsett Niedersachsen Germany	Barley Gimpel	1) 29.03.1994 S 2) - 3) 17.05.1994 - 01.08.1994	Seed treatment	EXP80524 FS (12.5 g/l Triticonazole and 125 g/L Iprodione) 4.8	28.02.1994	00 Dry seed (caryopsis)	Shoots Grains Straw	< 0.05 < 0.01 < 0.05	49 125 125	Anal. method: AR92-92 Analytical principle: GC-NPD (shoot) GC-MSD (grain & straw) LOQ Grains: 0.01 mg/kg LOQ Shoots: 0.05 mg/kg LOQ Straw: 0.05 mg/kg
Doc. No: C016581 WTA REGION 20/94-595 Decline Curve 24623 Großenaspe Schleswig-Holstein Germany	Barley Gimpel	1) 31.03.1994 S 2) - 3) 17.05.1994 - 03.08.1994	Seed treatment	EXP80524 FS (12.5 g/l Triticonazole and 125 g/L Iprodione) 4.8	28.02.1994	00 Dry seed (caryopsis)	Shoots Grains Straw	< 0.05 < 0.01 < 0.05	46 125 125	Anal. method: AR92-92 Analytical principle: GC-NPD (shoot) GC-MSD (grain & straw) LOQ Grains: 0.01 mg/kg LOQ Shoots: 0.05 mg/kg LOQ Straw: 0.05 mg/kg
Doc. No: C016581 WTA REGION 50/94-595 Decline Curve 67294 Mauchenheim Rheinland - Pfalz Germany	Barley Gimpel	1) 11.03.1994 S 2) - 3) 06.05.1994 - 25.07.1994	Seed treatment	EXP80524 FS (12.5 g/l Triticonazole and 125 g/L Iprodione) 4.8	28.02.1994	00 Dry seed (caryopsis)	Shoots Grains Straw	< 0.05 < 0.01 < 0.05	56 136 136	Anal. method: AR92-92 Analytical principle: GC-NPD (shoot) GC-MSD (grain & straw) LOQ Grains: 0.01 mg/kg LOQ Shoots: 0.05 mg/kg LOQ Straw: 0.05 mg/kg
Doc. No: C016581 WTA REGION 60/94-595 Decline Curve 99834 Gerstungen Thüringen Germany	Barley Gimpel	1) 22.04.1994 S 2) - 3) 24.05.1994 - 05.08.1994	Seed treatment	EXP80524 FS (12.5 g/l Triticonazole and 125 g/L Iprodione) 4.8	28.02.1994	00 Dry seed (caryopsis)	Shoots Grains Straw	< 0.05 < 0.01 < 0.05	32 105 105	Anal. method: AR92-92 Analytical principle: GC-NPD (shoot) GC-MSD (grain & straw) LOQ Grains: 0.01 mg/kg LOQ Shoots: 0.05 mg/kg LOQ Straw: 0.05 mg/kg

Report-No.; Location incl Postal code; Country	Crop Variety	Date of (a) 1) Sowing S or Planting P 2) Flowering 3) Harvest	Method of treatment	Formulation application rate (g/100 kg of seed)	Dates of treatment(s) or number of treatments and last date	Growth stage at last treatment or date	Matrix	Triticonazole Residues (mg/kg)	PHI (days)	Remarks:
Doc. No: C016580 WTA REGION 20/95-681 Decline Curve 24623 Großenaspe Schleswig-Holstein Germany	Barley Alexis	1) 04.04.1995 S 2) - 3) 18.05.1995 - 04.08.1995	Seed treatment	EXP80524 FS (12.5 g/l Triticonazole and 125 g/L Iprodione) 4.8	24.02.1995	00 Dry seed (caryopsis)	Shoots Grains Straw	< 0.05 < 0.01 < 0.05	44 122 122	Anal. method: AR92-92 Analytical principle: GC-NPD (shoot) GC-MSD (grain & straw) LOQ Grains: 0.01 mg/kg LOQ Shoots: 0.05 mg/kg LOQ Straw: 0.05 mg/kg
Doc. No: C016580 WTA REGION 60/95-681 Decline Curve 37242 Bad Sooden Hessen, Germany	Barley Alexis	1) 14.03.1995 S 2) - 3) 12.05.1995 - 03.08.1995	Seed treatment	EXP80524 FS (12.5 g/l Triticonazole and 125 g/L Iprodione) 4.8	24.02.1995	00 Dry seed (caryopsis)	Shoots Grains Straw	< 0.05 < 0.01 < 0.05	59 142 142	Anal. method: AR92-92 Analytical principle: GC-NPD (shoot) GC-MSD (grain & straw) LOQ Grains: 0.01 mg/kg LOQ Shoots: 0.05 mg/kg LOQ Straw: 0.05 mg/kg
Doc. No: C015362 24 96 01 01 Decline Curve Study No: 95-690 DK-5500 Hyllehoj Middelfart Denmark	Barley Alexis	1) 26.04.1995 S 2) 12.07.1995 3) 22.08.1995	Seed treatment	EXP80524 FS (12.5 g/l Triticonazole and 125 g/L Iprodione) 5	25.04.1995	00 Dry seed (caryopsis)	Seeds Shoots Shoots Shoots shoot,without ear ear Straw Grains	49 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.01	0 21 28 73 115 115 150 150	Anal. method: AR92-92 Analytical principle: GC-NPD (shoot) GC-MSD (grain & straw) LOQ Grains: 0.01 mg/kg LOQ Seeds: mg/kg LOQ Shoots: 0.05 mg/kg LOQ Straw: 0.05 mg/kg LOQ ear: 0.05 mg/kg LOQ shoot,without ear: 0.05 mg/kg

Report-No.; Location incl Postal code; Country	Crop Variety	Date of (a) 1) Sowing S or Planting P 2) Flowering 3) Harvest	Method of treatment	Formulation application rate (g/100 kg of seed)	Dates of treatment(s) or number of treatments and last date	Growth stage at last treatment or date	Matrix	Triticonazole Residues (mg/kg)	PHI (days)	Remarks:
Doc. No: C015362 24 96 01 02 Decline Curve Study No: 95-690 DK-5500 Vejlbyvej 43 Middelfart Denmark	Barley Alexis	1) 26.04.1995 S 2) 12.07.1995 3) 22.08.1995	Seed treatment	EXP80524 FS (12.5 g/l Triticonazole and 125 g/L Iprodione) 5	25.04.1995	00 Dry seed (caryopsis)	Seeds Shoots Shoots Shoots shoot,without ear ear Straw Grains	26 0.066 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.01	0 21 28 73 115 115 150 150	Anal. method: AR92-92 Analytical principle: GC-NPD (shoot) GC-MSD (grain & straw) LOQ Grains: 0.01 mg/kg LOQ Seeds: mg/kg LOQ Shoots: 0.05 mg/kg LOQ Straw: 0.05 mg/kg LOQ ear: 0.05 mg/kg LOQ shoot,without ear: 0.05 mg/kg
Doc. No: C015364 96701DK1 Decline Curve Study No: 96-701 18 DK-5500 Rojleskovvej Middelfart Denmark	Barley Alexis	1) 25.04.1996 S 2) - 3) 27.08.1996	Seed treatment	EXP80524 FS (12.5 g/l Triticonazole and 125 g/L Iprodione) 5	25.04.1996	00 Dry seed (caryopsis)	Seeds Shoots Shoots Shoots Shoots ear Straw Grains	42 0.13 0.071 < 0.05 < 0.015 < 0.015 < 0.015 < 0.003	0 20 28 42 77 77 124 124	Anal. method: AR92-92 Analytical principle: GC-NPD (shoot) GC-MSD (grain & straw) LOQ Grains: 0.003 mg/kg LOQ Seeds: 1 mg/kg LOQ Shoots: 0.015 mg/kg LOQ Straw: 0.015 mg/kg LOQ ear: 0.015 mg/kg
Doc. No: C015364 96701DK2 Decline Curve Study No: 96-701 5500 Vejlbyvej 43 Middelfart Denmark	Barley Alexis	1) 29.04.1996 S 2) - 3) 27.08.1996	Seed treatment	EXP80524 FS (12.5 g/l Triticonazole and 125 g/L Iprodione) 5	25.04.1996	00 Dry seed (caryopsis)	Seeds Shoots Shoots Shoots Shoots ear Straw Grains	54 < 0.05 0.051 < 0.015 < 0.015 < 0.015 < 0.015 < 0.003	0 21 29 42 73 73 120 120	Anal. method: AR92-92 Analytical principle: GC-NPD (shoot) GC-MSD (grain & straw) LOQ Grains: 0.003 mg/kg LOQ Seeds: 1 mg/kg LOQ Shoots: 0.015 mg/kg LOQ Straw: 0.015 mg/kg LOQ ear: 0.015 mg/kg

Report-No.; Location incl Postal code; Country	Crop Variety	Date of (a) 1) Sowing S or Planting P 2) Flowering 3) Harvest	Method of treatment	Formulation application rate (g/100 kg of seed)	Dates of treatment(s) or number of treatments and last date	Growth stage at last treatment or date	Matrix	Triticonazole Residues (mg/kg)	PHI (days)	Remarks:
Doc. No: R003473 95-692DK1 Decline Curve 18DK-5500 Rojleskovvej Middelfart Denmark	Common rye Dominator	1) 17.10.1995 S 2) - 3) 20.08.1996	Seed treatment	EXP80525D FS (12.5 g/l Triticonazole and 150 g/L Guazatine) 5	12.10.1995	00 Dry seed (caryopsis)	Seeds Shoots Shoots Shoots Shoots Shoots ear Straw Grains	48 0.32 0.25 0.33 < 0.015 < 0.015 < 0.015 < 0.015 < 0.003	 0 27 34 48 216 266 266 308 308	Anal. method: AR92-92 Analytical principle: GC-NPD (shoot) GC-MSD (grain & straw) LOQ Grains: 0.003 mg/kg LOQ Seeds: 0.5 mg/kg LOQ Shoots: 0.015 mg/kg LOQ Straw: 0.015 mg/kg LOQ ear: 0.015 mg/kg
Doc. No: R003473 95-692DK2 Decline Curve 5500 Hyllehoj I Middelfart Denmark	Common rye Dominator	1) 17.10.1995 S 2) - 3) 23.08.1996	Seed treatment	EXP80525D FS (12.5 g/l Triticonazole and 150 g/L Guazatine) 5	12.10.1995	00 Dry seed (caryopsis)	Seeds Shoots Shoots Shoots Shoots Shoots ear Straw Grains	42 0.37 0.35 0.36 < 0.015 < 0.015 < 0.015 < 0.015 < 0.003	0 27 34 48 217 267 267 311 311	Anal. method: AR92-92 Analytical principle: GC-NPD (shoot) GC-MSD (grain & straw) LOQ Grains: 0.003 mg/kg LOQ Seeds: 0.5 mg/kg LOQ Shoots: 0.015 mg/kg LOQ Straw: 0.015 mg/kg LOQ ear: 0.015 mg/kg

Table 6.3.1-21: Summary of Triticonazole Residues from Field Trials in Cereals. Southern Europe - Supplemental Data

Report-No.; Location incl Postal code; Country	Crop Variety	Date of (a) 1) Sowing S or Planting P 2) Flowering 3) Harvest	Method of treatment	Formulation application rate (g/100 kg of seed)	Dates of treatment(s) or number of treatments and last date	Growth stage at last treatment or date	Matrix	Triticonazole Residues (mg/kg)	PHI (days)	Remarks:
Doc. No: R003257 96604BO1 Harvest study Study No: 96-604 Voghera PV Italy	Durum wheat Brindur	1) 25.10.1995 S 2) - 3) 27.06.1996	Seed treatment	EXP80525D FS (12.5 g/l Triticonazole and 150 g/L Guazatine) 5	20.10.1995	00 Dry seed (caryopsis)	Grains Straw	< 0.01 < 0.05	-> 246 -> 246	Anal. method: AR96-93 Analytical principle: GC-NPD LOQ Grains: 0.01 mg/kg LOQ Straw: 0.05 mg/kg
Doc. No: R013126 94640BO1 Harvest study Study No: 94-640 Corticella Italy	Common wheat Simeto	1) 29.11.1993 S 2) - 3) 30.06.1994	Seed treatment	EXP80523A DS (25 g/kg Triticonazole) 5	29.11.1993	00 Dry seed (caryopsis)	Grains Straw	< 0.01 < 0.05	-> 213 -> 213	Analytical principle: GC-FPD or GC-TID LOQ Grains: 0.01 mg/kg LOQ Straw: 0.05 mg/kg

Report-No.; Location incl Postal code; Country	Crop Variety	Date of (a) 1) Sowing S or Planting P 2) Flowering 3) Harvest	Method of treatment	Formulation application rate (g/100 kg of seed)	Dates of treatment(s) or number of treatments and last date	Growth stage at last treatment or date	Matrix	Triticonazole Residues (mg/kg)	PHI (days)	Remarks:
Doc. No: C017316 94643BO1 Harvest study Study No: 94-643 Corticella Italy	Durum wheat Grazia	1) 04.03.1994 S 2) - 3) 06.07.1994	Seed treatment	EXP80524 FS (12.5 g/l Triticonazole and 125 g/L Iprodione) 5	04.03.1994	00 Dry seed (caryopsis)	Grains Straw	< 0.01 < 0.05	-> 124 -> 124	Anal. method: AR96-93 Analytical principle: GC-NPD LOQ Grains: 0.01 mg/kg LOQ Straw: 0.05 mg/kg
Doc. No: C014706 95606BO1 Harvest study Study No: 95-606 Corticella BO Italy	Durum wheat Simeto	1) 30.11.1994 S 2) - 3) 07.07.1995	Seed treatment	EXP80524 FS (12.5 g/l Triticonazole and 125 g/L Iprodione) 5	30.11.1994	00 Dry seed (caryopsis)	Grains Straw	< 0.01 < 0.05	-> 219 -> 219	Anal. method: AR96-93 Analytical principle: GC-NPD LOQ Grains: 0.01 mg/kg LOQ Straw: 0.05 mg/kg
Doc. No: R002862 95610BO1 Harvest study Study No: 95-610 Corticella, BO; Italy	Durum wheat Cirillo	1) 30.11.1994 S 2) - 3) 07.07.1995	Seed treatment	EXP80525C FS (12.5 g/l Triticonazole and 150 g/L Guazatine) 5	30.11.1994	00 Dry seed (caryopsis)	Grains Straw	< 0.01 < 0.05	219 219	Anal. method: AR96-93 Analytical principle: GC-NPD LOQ Grains: 0.01 mg/kg LOQ Straw: 0.05 mg/kg
Doc. No: R002862 95610BO2 Harvest study Study No: 95-610 Corticella, BO; Italy	Durum wheat Simeto	1) 30.11.1994 S 2) - 3) 07.07.1995	Seed treatment	EXP80525C FS (12.5 g/l Triticonazole and 150 g/L Guazatine) 5	30.11.1994	00 Dry seed (caryopsis)	Grains Straw	< 0.01 < 0.05	219 219	Anal. method: AR96-93 Analytical principle: GC-NPD LOQ Grains: 0.01 mg/kg LOQ Straw: 0.05 mg/kg

Report-No.; Location incl Postal code; Country	Crop Variety	Date of (a) 1) Sowing S or Planting P 2) Flowering 3) Harvest	Method of treatment	Formulation application rate (g/100 kg of seed)	Dates of treatment(s) or number of treatments and last date	Growth stage at last treatment or date	Matrix	Triticonazole Residues (mg/kg)	PHI (days)	Remarks:
Doc. No: R013164 96601GR1 Harvest study Study No: 96-601 Avlon Greece	Common wheat	1) 15.02.1996 S 2) - 3) 16.07.1996	Seed treatment	EXP80523A DS (25 g/kg Triticonazole) 5	26.10.1995	00 Dry seed (caryopsis)	Grains	< 0.01	-> 152	Anal. method: GC-NPD or TSD LOQ Grains: 0.01 mg/kg
Doc. No: C017251 94670SE1 Harvest study Study No: 94-670 Torre de la reina Sevilla, Spain	Common wheat Aragon	1) 01.12.1993 S 2) - 3) 08.06.1994	Seed treatment	EXP80472A FS (25 g/l Triticonazole) 5 Water = 10 l/t	05.11.1993	00 Dry seed (caryopsis)	Grains Straw	< 0.01 < 0.01	-> 189 -> 189	Anal. method: GC-FPD or GC-TID LOQ Grains: 0.01 mg/kg LOQ Straw: 0.01 mg/kg
Doc. No: C017251 94670SE2 Harvest study Study No: 94-670 Santa Ollala, Spain	Common wheat Cajeme	1) 20.12.1993 S 2) - 3) 30.06.1994	Seed treatment	EXP80472A FS (25 g/l Triticonazole) 5 Water = 10 l/t	05.11.1993	00 Dry seed (caryopsis)	Grains Straw	< 0.01 < 0.05	-> 192 -> 192	Anal. method: GC-FPD or GC-TID LOQ Grains: 0.01 mg/kg LOQ Straw: 0.05 mg/kg
Doc. No: C014710 95607BO1 Harvest study Study No: 95-607 Corticella BO Italy	Common wheat Spada	1) 30.11.1994 S 2) - 3) 07.07.1995	Seed treatment	EXP80524 C FS (12.5 g/l Triticonazole, 125 g/l Iprodione) 5	30.11.1994	00 Dry seed (caryopsis)	Grains Straw	< 0.01 < 0.05	-> 219 -> 219	Anal. method: AR96-93 Analytical principle: GC-NPD LOQ Grains: 0.01 mg/kg LOQ Straw: 0.05 mg/kg

Report-No.; Location incl Postal code; Country	Crop Variety	Date of (a) 1) Sowing S or Planting P 2) Flowering 3) Harvest	Method of treatment	Formulation application rate (g/100 kg of seed)	Dates of treatment(s) or number of treatments and last date	Growth stage at last treatment or date	Matrix	Triticonazole Residues (mg/kg)	PHI (days)	Remarks:
Doc. No: R002860 95609BO1 Harvest study Study No: 95-609 Corticella BO Italy	Common wheat Tremie	1) 30.11.1994 S 2) - 3) 07.07.1995	Seed treatment	EXP80525 C FS (12.5 g/l Triticonazole, 150 g/l Guazatine) 5	30.11.1994	00 Dry seed (caryopsis)	Grains Straw	< 0.01 < 0.05	219 219	Anal. method: AR96-93 Analytical principle: GC-NPD LOQ Grains: 0.01 mg/kg LOQ Straw: 0.05 mg/kg
Doc. No: R002860 95609BO2 Harvest study Study No: 95-609 Corticella BO Italy	Common wheat Centauro	1) 30.11.1994 S 2) - 3) 07.07.1995	Seed treatment	EXP80525 C FS (12.5 g/l Triticonazole, 150 g/l Guazatine) 5	30.11.1994	00 Dry seed (caryopsis)	Grains Straw	< 0.014 < 0.05	219 219	Anal. method: AR96-93 Analytical principle: GC-NPD LOQ Grains: 0.014 mg/kg LOQ Straw: 0.05 mg/kg
Doc. No: R003235 96603BO1 Harvest study Study No: 96-603 Conselice RA Italy	Common wheat Centauro	1) 23.10.1995 S 2) - 3) 19.06.1996	Seed treatment	EXP80525D FS (12.5 g/l Triticonazole, 150 g/l Guazatine) 5	20.10.1996	00 Dry seed (caryopsis)	Grains Straw	< 0.01 < 0.05	-> 240 -> 240	Anal. method: AR96-93 Analytical principle: GC-NPD LOQ Grains: 0.01 mg/kg LOQ Straw: 0.05 mg/kg

Report-No.; Location incl Postal code; Country	Crop Variety	Date of (a) 1) Sowing S or Planting P 2) Flowering 3) Harvest	Method of treatment	Formulation application rate (g/100 kg of seed)	Dates of treatment(s) or number of treatments and last date	Growth stage at last treatment or date	Matrix	Triticonazole Residues (mg/kg)	PHI (days)	Remarks:
Doc. No: C017317 94648BO1 Harvest study Study No: 94-648 Corticella Italy	Barley Perga	1) 29.11.1993 S 2) - 3) 30.06.1994	Seed treatment	EXP80524 A FS (12.5 g/l Triticonazole, 125 g/l Iprodione) 5	29.11.1993	00 Dry seed (caryopsis)	Grains Straw	< 0.01 < 0.05	-> 213 -> 213	Anal. method: AR96-93 Analytical principle: GC-NPD LOQ Grains: 0.01 mg/kg LOQ Straw: 0.05 mg/kg
Doc. No: C017659 94671SE1 Harvest study Study No: 94-671 Santa Ollala Toledo, Spain	Barley Georgia	1) 20.12.1993 S 2) - 3) 30.06.1994	Seed treatment	EXP80472 A FS (25 g/l Triticonazole) 5 water = 1 l/t.	05.11.1993	00 Dry seed (caryopsis)	Grains Straw	< 0.01 < 0.05	-> 192 -> 192	Anal. method: GC-FPDor GC-TID LOQ Grains: 0.01 mg/kg LOQ Straw: 0.05 mg/kg
Doc. No: C014712 95608BO1 Harvest study Study No: 95-608 Corticella, BO, Italy	Barley Kelibia	1) 30.11.1994 S 2) - 3) 07.07.1995	Seed treatment	EXP80524 C FS (12.5 g/l Triticonazole, 125 g/l Iprodione) 5	30.11.1994	00 Dry seed (caryopsis)	Grains Straw	< 0.01 < 0.05	-> 219 -> 219	Anal. method: AR96-93 Analytical principle: GC-NPD LOQ Grains: 0.01 mg/kg LOQ Straw: 0.05 mg/kg
Doc. No: C014712 95608BO2 Harvest study Study No: 95-608 Corticella, BO, Italy	Barley Plaisant	1) 30.11.1994 S 2) - 3) 07.07.1995	Seed treatment	EXP80524 C FS (12.5 g/l Triticonazole, 125 g/l Iprodione) 5	30.11.1994	00 Dry seed (caryopsis)	Grains Straw	< 0.01 < 0.05	-> 219 -> 219	Anal. method: AR96-93 Analytical principle: GC-NPD LOQ Grains: 0.01 mg/kg LOQ Straw: 0.05 mg/kg

Report-No.; Location incl Postal code; Country	Crop Variety	Date of (a) 1) Sowing S or Planting P 2) Flowering 3) Harvest	Method of treatment	Formulation application rate (g/100 kg of seed)	Dates of treatment(s) or number of treatments and last date	Growth stage at last treatment or date	Matrix	Triticonazole Residues (mg/kg)	PHI (days)	Remarks:
Doc. No: R013162 96602GR1 Harvest study Study No: 96-602 Avlon; Greece	Barley	1) 15.02.1996 S 2) - 3) 16.07.1996	Seed treatment	EXP80523A DS FS (25 g/kg Triticonazole) 5	26.10.1995	00 Dry seed (caryopsis)	Grains	< 0.01	-> 152	Anal. method: GC-NPD or TSD LOQ Grains: 0.01 mg/kg
Doc. No: C014734 96605BO1 Harvest study Study No: 96-605 Voghera, PV, Italy	Barley Plaisant	1) 25.10.1995 S 2) - 3) 27.06.1996	Seed treatment	EXP80524 C FS (12.5 g/l Triticonazole, 125 g/l Iprodione) 5	20.10.1995	00 Dry seed (caryopsis)	Grains Straw	< 0.01 < 0.05	-> 246 -> 246	Anal. method: AR96-93 Analytical principle: GC-NPD LOQ Grains: 0.01 mg/kg LOQ Straw: 0.05 mg/kg
Doc. No: C014734 96605BO2 Harvest study Study No: 96-605 R&D/CRLD/AN/DBE/97 16147 Falconara, AN, Italy	Barley Kelibia	1) 25.10.1995 S 2) - 3) 22.06.1996	Seed treatment	EXP80524 C FS (12.5 g/l Triticonazole, 125 g/l Iprodione) 5	20.10.1995	00 Dry seed (caryopsis)	Grains Straw	< 0.01 < 0.05	-> 241 -> 241	Anal. method: AR96-93 Analytical principle: GC-NPD LOQ Grains: 0.01 mg/kg LOQ Straw: 0.05 mg/kg

CA 6.4 Feeding studies

In comparison to the dietary burden calculations conducted by EFSA in context of the MRL review according to Article 12 (EFSA Scientific Report (2009) 277, 1-23) no new input values (STMR, HR) were derived for cereal matrices. In grain, all residues were below the LOQ (0.01 mg/kg) and also in harvestable forage (whole plant without root) no residues above the LOQ were determined. In 16 trials throughout Europe, at BBCH 49 and 59 in specimens of "whole plant without root" the triticonazole residues were always <0.01 mg/kg (see chapter CA 6.3).
Based on the calculations, the trigger of 0.1 mg/kg DM (dry matter) was not exceeded for livestock (dairy ruminants, meat ruminants, poultry and pigs).
Additionally, the results of the newly conducted goat metabolism study (see CA 6.2) indicate, that no relevant residues will occur in edible animal tissue taking into account the residue levels in potential feedingstuffs obtained at the 1x dose rate.
Therefore, no feeding studies are required (7031/VI/95 rev. 4, 22/7/96, Appendix G).

CA 6.4.1 Poultry

A poultry feeding study is not required, see explanation in chapter CA 6.4.

CA 6.4.2 Ruminants

A ruminants feeding study is not required, see explanation in chapter CA 6.4.

CA 6.4.3 Pigs

A feeding study in pigs is not needed.

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. Green rest of plants do not form part of fish diets and since there are also no detectable residues in cereal grain or straw, a fish feeding study is not required.

CA 6.5 Effects of Processing

CA 6.5.1 Nature of the residue

As quantifiable residues of triticonazole are not expected in cereal grains, there is no need to investigate the effect of industrial and/or household processing.
However, current guidance requires that a study on the nature of the residue in processed commodities (high-temperature hydrolysis study) is required in any case where residues at or above 0.01 mg/kg may be found. In view of this low threshold, and to provide information on the fate of any possible residues in cereal commodities to be processed, a high-temperature hydrolysis study was conducted (BASF No. 2013/1135885). The study was not reviewed during the Annex I inclusion process and is included in this dossier.

Report: CA 6.5.1/1
Adam D., 2013a
14C-Triticonazole (BAS 595 F): Stimulated processing - Hydrolysis at 90°C, 100°C and 120°C
2013/1135885
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)

Executive Summary

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test Material:** BAS 595 F (Triticonazole, Reg No. 4378513)
   Description: Triazole-3(5)-^{14}C labeled BAS 595 F, specific radioactivity 6.52 MBq/mg
   Lot/Batch #: 867-1301
   Purity: Radiochemical purity: 99.3% (93.6% re-determined), chemical purity: 92.3%
   CAS#: 131983-72-7
   Development code: not applicable

B. STUDY DESIGN AND METHODS

1. Test procedure

The hydrolytic stability of ^{14}C-triticonazole (BAS 595 F) was investigated in sterile aqueous acetate buffer solutions at three pH values and temperatures in order to simulate processing practice.

Figure 6.5.1-1: Structural formula of triazole-3(5)-^{14}C labeled BAS 595 F

The study was performed at pH 4, 5 and 6 at temperatures of 90°C, 100°C and 120°C for 20 or 60 minutes, respectively. The range of hydrolytic conditions used, represent the processes of pasteurisation, baking/brewing/boiling and sterilisation. Buffer solutions containing the radiolabelled test item at an initial concentration of 0.251 mg/L were incubated in closed high pressure stainless steel vessels placed in an autoclave at the desired temperature. At time 0 and after 20 or 60 minutes of incubation, duplicate samples per pH value were taken, measured for total radioactivity and analyzed for the parent compound and eventual degradates. The conditions for the hydrolysis study are shown in Table 6.5.1-1.

Table 6.5.1-1: Simulated conditions for the hydrolysis study

Temperature (°C)	Time (min)	pH	Process Represented	Remarks
90	20	4	Pasteurisation	In closed high pressure stainless steel vessels using autoclave
100	60	5	Baking, Brewing, Boiling	In high pressure stainless steel vessels using autoclave
120	20	6	Sterilisation	In closed high pressure stainless steel vessels using autoclave

2. Description of analytical procedures

Samples were measured for total radioactivity by Liquid Scintillation Counting (LSC).
HPLC was the primary analytical method used to determine the amount of the parent and degradation products in the samples. The column used was a Phenomenex Luna C18, the eluent system consisted of 2 mobile phases (A: water with 0.1% trifluoroacetic acid, B: acetonitrile with 0.1% trifluoroacetic acid), which were used applying gradient elution.
TLC was the secondary analytical method used to confirm the identity of the parent and degradation products in the samples. The following solvent system was used: Chloroform/Methanol/Water/Formic acid (77/20/2/1; v/v/v/v).

II. RESULTS AND DISCUSSION

1. TOTAL RADIOACTIVE RESIDUES (TRRs)

Individual recoveries of radioactivity during the respective incubation periods represented 99.0% at pH 4 at 90°C (20 min). Corresponding values for pH 5 at 100°C (60 min) and pH 6 at 120°C (20 min) ranged from 104.4% to 104.9% and from 101.8% to 104.3%, respectively. The results are summaries in Table 6.5.1-2.

Table 6.5.1-2: Balance of radioactivity

Sample	Replicate	Incubation time (min)					
		pH 4, 90°C		pH 5, 100°C		pH 6, 120°C	
		0	20	0	60	0	20
Radioactivity in buffer solution	A	98.5	99.0	98.2	104.4	99.7	104.3
	B	101.5	99.0	101.8	104.9	100.3	101.8
	Mean	100.0	99.0	100.0	104.7	100.0	103.1

Values are given in percent of applied radioactivity (AR)

2. Identification, characterization and quantitation of extractable residues

At pH 4 and 90°C:
^{14}C-BAS 595 F was shown to be stable to hydrolysis at pH 4 and 90°C, representing a mean amount of 95.3% of the radioactivity in the solution after 20 minutes of incubation in the buffer solution. One other peak above the Limit of Detection (LOD) of 0.5% AR was detected: Metabolite M2, which represented 3.2% of AR. The distribution pattern of radioactivity at pH 4 and 90°C is shown in Table 6.5.1-3.

Table 6.5.1-3: Distribution pattern of radioactivity at pH 4 at 90°C, incubation time 20 min.

Substance	Replicate	Incubation time (min)	
		0	20
Triticonazole	A	95.4	94.5
	B	97.8	96.2
	mean	96.6	**95.3**
M1	A	0.8	0.9
	B	0.9	*
	mean	0.8	0.4
M2	A	2.3	3.6
	B	2.9	2.8
	mean	2.6	**3.2**
M3	A	*	*
	B	*	*
	mean	*	*

*not detected or below detection limit
Values are given in percent of applied radioactivity (AR)
Since M2 was detected under all three incubation conditions, as well as in the non-incubated samples and application solution, its appearance was correlated with an impurity of the application solution.

At pH 5 and 100°C:
^{14}C BAS 595 F represented 101.7% of the solution radioactivity after 60 minutes of incubation at pH 5 and 100°C and was shown to be stable under the mentioned conditions. One other peak above the Limit of Detection (LOD) of 0.5% AR was detected: Metabolite M2, which represented 2.9% of AR. The distribution pattern of radioactivity at pH 5 and 100°C is shown in Table 6.5.1-4.

Table 6.5.1-4: Distribution pattern of radioactivity at pH 5 at 100°C, incubation time 60 min.

Substance	Replicate	Incubation time (min)	
		0	60
Triticonazole	A	95.2	101.3
	B	98.6	102.1
	mean	96.9	**101.7**
M1	A	*	*
	B	*	*
	mean	*	*
M2	A	3.0	3.0
	B	3.2	2.8
	mean	3.1	**2.9**
M3	A	*	*
	B	*	*
	mean	*	*

*not detected or below detection limit
Values are given in percent of applied radioactivity (AR)

At pH 6 and 120°C:
At pH 6 at 120°C, the test item was shown to be stable as well. ^{14}C BAS 595 F represented a mean amount of 99.3% of the solution radioactivity after 20 minutes of incubation in the buffer solution. One other peak above the Limit of Detection (LOD) of 0.5% AR was detected: Metabolite M2, which represented 2.8% of AR. The distribution pattern of radioactivity at pH 6 and 120°C is shown in Table 6.5.1-5.

Table 6.5.1-5: Distribution pattern of radioactivity at pH 6 at 120°C, incubation time 20 min.

Substance	Replicate	Incubation time (min)	
		0	20
Triticonazole	A	96.9	101.1
	B	97.2	97.6
	mean	97.0	**99.3**
M1	A	*	*
	B	*	0.9
	mean	*	0.4
M2	A	2.8	3.3
	B	3.1	2.3
	mean	3.0	**2.8**
M3	A	*	*
	B	*	0.9
	mean	*	0.5

*not detected or below detection limit
Values are given in percent of applied radioactivity (AR)

4. Storage stability

No degradation of the test item was observed in time 0 samples, thereby proving the stability during treatment procedure.

III. CONCLUSION

^{14}C-triticonazole (BAS 595 F) was hydrolytically stable in sterile buffer solution pH 4 at a temperature simulating pasteurisation (90°C) after 20 minutes, at pH 5 at 100°C after 60 minutes (simulating baking/brewing/boiling) and at pH 6 at 120°C after 20 minutes simulating sterilisation.

CA 6.5.2 Distribution of the residue in inedible peel and pulp

Not relevant for the intended uses in cereals.

CA 6.5.3 Magnitude of residues in processed commodities

The residue studies show residues in grain to be < 0.01 mg/kg, and therefore industrial processing studies are not required.

CA 6.6 Residues in Rotational Crops

CA 6.6.1 Metabolism in rotational crops

A metabolism study in rotational crops was evaluated previously for Annex I inclusion of triticonazole. The possible uptake of soil-derived triticonazole residues into succeeding crops was investigated after application of triticonazole to bare soil at a rate 286 g as/ha (approximately 20 x the intended application rate). Edible parts of plants from the first rotation (30 day plant-back) contained total radioactive residues of 0.23 mg/kg (radish roots), 0.048 mg/kg (lettuce leaves) and 0.003 mg/kg (wheat grain). Total radioactive residues declined with the planting interval and the major component of the recovered radioactivity was unchanged triticonazole.

The uptake and metabolism of triticonazole residues in succeeding crops are adequately understood and no further data are required.

CA 6.6.2 Magnitude of residues in rotational crops

In the framework of the inclusion into Annex I according to Directive 91/414/EEC, five additional field trials on the magnitude of the residue in rotational crops were evaluated. After sowing of seed treated wheat no triticonazole residues < 0.01 mg/kg in crops planted after harvest of wheat (protein peas, sugar beet roots, sunflower seed, oilseed rape and grains of wheat) were detected. No new studies have been performed.

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 established. It is not limited to the intended use in wheat.

Table 6.7.1-1: Residue definition - triticonazole

End-Point	Active Substance: Triticonazole	
	EU agreed endpoints (EFSA Scientific Report (2005) 33, 1-69))	Residue definitions proposed in the context of this dossier
Residue definition in plant matrices for risk assessment	Triticonazole	Triticonazole
Residue definition in plant matrices for monitoring	Triticonazole	Triticonazole
Residue definition in animal matrices for risk assessment	not required/not proposed	not required/not proposed
Residue definition in animal matrices for monitoring	not required/not proposed	not required/not proposed

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 triticonazole (mg/kg) as of July 20, 2015 (source: DG SANTE website http://ec.europa.eu/sanco_pesticides/public/index.cfm?event =substance.resultat&s=1). * indicates the lower limit of analytical determination.

Table 6.7.2-1: EU MRLs set for the uses of triticonazole

Code number	Groups and examples of individual products to which the MRLs apply (a)	Triticonazole (mg/kg)
100000	. FRUITS, FRESH or FROZEN; TREE NUTS	0.01*
110000	. Citrus fruits	0.01*
110010	. Grapefruits	0.01*
110020	. Oranges	0.01*
110030	. Lemons	0.01*
110040	. Limes	0.01*
110050	. Mandarins	0.01*
110990	. Others (2)	0.01*
120000	. Tree nuts	0.01*
120010	. Almonds	0.01*
120020	. Brazil nuts	0.01*
120030	. Cashew nuts	0.01*

Code number	Groups and examples of individual products to which the MRLs apply (a)	Triticonazole (mg/kg)
120040	. Chestnuts	0.01*
120050	. Coconuts	0.01*
120060	. Hazelnuts/cobnuts	0.01*
120070	. Macadamias	0.01*
120080	. Pecans	0.01*
120090	. Pine nut kernels	0.01*
120100	. Pistachios	0.01*
120110	. Walnuts	0.01*
120990	. Others (2)	0.01*
130000	. Pome fruits	0.01*
130010	. Apples	0.01*
130020	. Pears	0.01*
130030	. Quinces	0.01*
130040	. Medlars	0.01*
130050	. Loquats/Japanese medlars	0.01*
130990	. Others (2)	0.01*
140000	. Stone fruits	0.01*
140010	. Apricots	0.01*
140020	. Cherries (sweet)	0.01*
140030	. Peaches	0.01*
140040	. Plums	0.01*
140990	. Others (2)	0.01*
150000	. Berries and small fruits	0.01*
151000	. (a) grapes	0.01*
151010	. Table grapes	0.01*
151020	. Wine grapes	0.01*
152000	. (b) strawberries	0.01*
153000	. (c) cane fruits	0.01*
153010	. Blackberries	0.01*
153020	. Dewberries	0.01*
153030	. Raspberries (red and yellow)	0.01*
153990	. Others (2)	0.01*
154000	. (d) other small fruits and berries	0.01*
154010	. Blueberries	0.01*
154020	. Cranberries	0.01*
154030	. Currants (black, red and white)	0.01*
154040	. Gooseberries (green, red and yellow)	0.01*
154050	. Rose hips	0.01*
154060	. Mulberries (black and white)	0.01*
154070	. Azaroles/Mediterranean medlars	0.01*
154080	. Elderberries	0.01*
154990	. Others (2)	0.01*
160000	. Miscellaneous fruits with	0.01*
161000	. (a) edible peel	0.01*
161010	. Dates	0.01*
161020	. Figs	0.01*
161030	. Table olives	0.01*
161040	. Kumquats	0.01*
161050	. Carambolas	0.01*
161060	. Kaki/Japanese persimmons	0.01*
161070	. Jambuls/jambolans	0.01*
161990	. Others (2)	0.01*

Code number	Groups and examples of individual products to which the MRLs apply (a)	Triticonazole (mg/kg)
162000	. (b) inedible peel, small	0.01*
162010	. Kiwi fruits (green, red, yellow)	0.01*
162020	. Litchis/lychees	0.01*
162030	. Passionfruits/maracujas	0.01*
162040	. Prickly pears/cactus fruits	0.01*
162050	. Star apples/cainitos	0.01*
162060	. American persimmons/Virginia kaki	0.01*
162990	. Others (2)	0.01*
163000	. (c) inedible peel, large	0.01*
163010	. Avocados	0.01*
163020	. Bananas	0.01*
163030	. Mangoes	0.01*
163040	. Papayas	0.01*
163050	. Granate apples/pomegranates	0.01*
163060	. Cherimoyas	0.01*
163070	. Guavas	0.01*
163080	. Pineapples	0.01*
163090	. Breadfruits	0.01*
163100	. Durians	0.01*
163110	. Soursops/guanabanas	0.01*
163990	. Others (2)	0.01*
200000	. VEGETABLES, FRESH or FROZEN	0.01*
210000	. Root and tuber vegetables	0.01*
211000	. (a) potatoes	0.01*
212000	. (b) tropical root and tuber vegetables	0.01*
212010	. Cassava roots/manioc	0.01*
212020	. Sweet potatoes	0.01*
212030	. Yams	0.01*
212040	. Arrowroots	0.01*
212990	. Others (2)	0.01*
213000	. (c) other root and tuber vegetables except sugar beets	0.01*
213010	. Beetroots	0.01*
213020	. Carrots	0.01*
213030	. Celeriacs/turnip rooted celeries	0.01*
213040	. Horseradishes	0.01*
213050	. Jerusalem artichokes	0.01*
213060	. Parsnips	0.01*
213070	. Parsley roots/Hamburg roots parsley	0.01*
213080	. Radishes	0.01*
213090	. Salsifies	0.01*
213100	. Swedes/rutabagas	0.01*
213110	. Turnips	0.01*
213990	. Others (2)	0.01*
220000	. Bulb vegetables	0.01*
220010	. Garlic	0.01*
220020	. Onions	0.01*
220030	. Shallots	0.01*
220040	. Spring onions/green onions and Welsh onions	0.01*
220990	. Others (2)	0.01*

Code number	Groups and examples of individual products to which the MRLs apply (a)	Triticonazole (mg/kg)
230000	. Fruiting vegetables	0.01*
231000	. (a) solanacea	0.01*
231010	. Tomatoes	0.01*
231020	. Sweet peppers/bell peppers	0.01*
231030	. Aubergines/eggplants	0.01*
231040	. Okra/lady's fingers	0.01*
231990	. Others (2)	0.01*
232000	. (b) cucurbits with edible peel	0.01*
232010	. Cucumbers	0.01*
232020	. Gherkins	0.01*
232030	. Courgettes	0.01*
232990	. Others (2)	0.01*
233000	. (c) cucurbits with inedible peel	0.01*
233010	. Melons	0.01*
233020	. Pumpkins	0.01*
233030	. Watermelons	0.01*
233990	. Others (2)	0.01*
234000	. (d) sweet corn	0.01*
239000	. (e) other fruiting vegetables	0.01*
240000	. Brassica vegetables (excluding brassica roots and brassica baby leaf crops)	0.01*
241000	. (a) flowering brassica	0.01*
241010	. Broccoli	0.01*
241020	. Cauliflowers	0.01*
241990	. Others (2)	0.01*
242000	. (b) head brassica	0.01*
242010	. Brussels sprouts	0.01*
242020	. Head cabbages	0.01*
242990	. Others (2)	0.01*
243000	. (c) leafy brassica	0.01*
243010	. Chinese cabbages/pe-tsai	0.01*
243020	. Kales	0.01*
243990	. Others (2)	0.01*
244000	. (d) kohlrabies	0.01*
250000	. Leaf vegetables, herbs and edible flowers	0.01*
251000	. (a) lettuces and salad plants	0.01*
251010	. Lamb's lettuces/corn salads	0.01*
251020	. Lettuces	0.01*
251030	. Escaroles/broad-leaved endives	0.01*
251040	. Cresses and other sprouts and shoots	0.01*
251050	. Land cresses	0.01*
251060	. Roman rocket/rucola	0.01*
251070	. Red mustards	0.01*
251080	. Baby leaf crops (including brassica species)	0.01*
251990	. Others (2)	0.01*
252000	. (b) spinaches and similar leaves	0.01*
252010	. Spinaches	0.01*
252020	. Purslanes	0.01*
252030	. Chards/beet leaves	0.01*
252990	. Others (2)	0.01*

Code number	Groups and examples of individual products to which the MRLs apply (a)	Triticonazole (mg/kg)
253000	. (c) grape leaves and similar species	0.01*
254000	. (d) watercresses	0.01*
255000	. (e) witloofs/Belgian endives	0.01*
256000	. (f) herbs and edible flowers	0.01*
256010	. Chervil	0.01*
256020	. Chives	0.01*
256030	. Celery leaves	0.01*
256040	. Parsley	0.01*
256050	. Sage	0.01*
256060	. Rosemary	0.01*
256070	. Thyme	0.01*
256080	. Basil and edible flowers	0.01*
256090	. Laurel/bay leave	0.01*
256100	. Tarragon	0.01*
256990	. Others (2)	0.01*
260000	. Legume vegetables	0.01*
260010	. Beans (with pods)	0.01*
260020	. Beans (without pods)	0.01*
260030	. Peas (with pods)	0.01*
260040	. Peas (without pods)	0.01*
260050	. Lentils	0.01*
260990	. Others (2)	0.01*
270000	. Stem vegetables	0.01*
270010	. Asparagus	0.01*
270020	. Cardoons	0.01*
270030	. Celeries	0.01*
270040	. Florence fennels	0.01*
270050	. Globe artichokes	0.01*
270060	. Leeks	0.01*
270070	. Rhubarbs	0.01*
270080	. Bamboo shoots	0.01*
270090	. Palm hearts	0.01*
270990	. Others (2)	0.01*
280000	. Fungi, mosses and lichens	0.01*
280010	. Cultivated fungi	0.01*
280020	. Wild fungi	0.01*
280990	. Mosses and lichens	0.01*
290000	. Algae and prokaryotes organisms	0.01*
300000	. PULSES	0.01*
300010	. Beans	0.01*
300020	. Lentils	0.01*
300030	. Peas	0.01*
300040	. Lupins/lupini beans	0.01*
300990	. Others (2)	0.01*

Code number	Groups and examples of individual products to which the MRLs apply (a)	Triticonazole (mg/kg)
400000	. OILSEEDS AND OIL FRUITS	
401000	. Oilseeds	0.02*
401010	. Linseeds	0.02*
401020	. Peanuts/groundnuts	0.02*
401030	. Poppy seeds	0.02*
401040	. Sesame seeds	0.02*
401050	. Sunflower seeds	0.02*
401060	. Rapeseeds/canola seeds	0.02*
401070	. Soyabeans	0.02*
401080	. Mustard seeds	0.02*
401090	. Cotton seeds	0.02*
401100	. Pumpkin seeds	0.02*
401110	. Safflower seeds	0.02*
401120	. Borage seeds	0.02*
401130	. Gold of pleasure seeds	0.02*
401140	. Hemp seeds	0.02*
401150	. Castor beans	0.02*
401990	. Others (2)	0.02*
402000	. Oil fruits	
402010	. Olives for oil production	0.01*
402020	. Oil palms kernels	0.02*
402030	. Oil palms fruits	0.02*
402040	. Kapok	0.02*
402990	. Others (2)	0.02*
500000	. CEREALS	0.01*
500010	. Barley	0.01*
500020	. Buckwheat and other pseudo-cereals	0.01*
500030	. Maize/corn	0.01*
500040	. Common millet/proso millet	0.01*
500050	. Oat	0.01*
500060	. Rice	0.01*
500070	. Rye	0.01*
500080	. Sorghum	0.01*
500090	. Wheat	0.01*
500990	. Others (2)	0.01*
600000	. TEAS, COFFEE, HERBAL INFUSIONS, COCOA AND CAROBS	0.02*
610000	. Teas	0.02*
620000	. Coffee beans	0.02*
630000	. Herbal infusions from	0.02*
631000	. (a) flowers	0.02*
631010	. Chamomile	0.02*
631020	. Hibiscus/roselle	0.02*
631030	. Rose	0.02*
631040	. Jasmine	0.02*
631050	. Lime/linden	0.02*
631990	. Others (2)	0.02*
632000	. (b) leaves and herbs	0.02*
632010	. Strawberry	0.02*
632020	. Rooibos	0.02*
632030	. Mate/maté	0.02*
632990	. Others (2)	0.02*

Code number	Groups and examples of individual products to which the MRLs apply (a)	Triticonazole (mg/kg)
633000	. (c) roots	0.02*
633010	. Valerian	0.02*
633020	. Ginseng	0.02*
633990	. Others (2)	0.02*
639000	. (d) any other parts of the plant	0.02*
640000	. Cocoa beans	0.02*
650000	. Carobs/Saint John's breads	0.02*
700000	. HOPS	0.02*
800000	. SPICES	0.02*
810000	. Seed spices	0.02*
810010	. Anise/aniseed	0.02*
810020	. Black caraway/black cumin	0.02*
810030	. Celery	0.02*
810040	. Coriander	0.02*
810050	. Cumin	0.02*
810060	. Dill	0.02*
810070	. Fennel	0.02*
810080	. Fenugreek	0.02*
810090	. Nutmeg	0.02*
810990	. Others (2)	0.02*
820000	. Fruit spices	0.02*
820010	. Allspice/pimento	0.02*
820020	. Sichuan pepper	0.02*
820030	. Caraway	0.02*
820040	. Cardamom	0.02*
820050	. Juniper berry	0.02*
820060	. Peppercorn (black, green and white)	0.02*
820070	. Vanilla	0.02*
820080	. Tamarind	0.02*
820990	. Others (2)	0.02*
830000	. Bark spices	0.02*
830010	. Cinnamon	0.02*
830990	. Others (2)	0.02*
840000	. Root and rhizome spices	0.02*
840010	. Liquorice	0.02*
840020	. Ginger	0.02*
840030	. Turmeric/curcuma	0.02*
840040	. Horseradish	0.02*
840990	. Others (2)	0.02*
850000	. Bud spices	0.02*
850010	. Cloves	0.02*
850020	. Capers	0.02*
850990	. Others (2)	0.02*
860000	. Flower pistil spices	0.02*
860010	. Saffron	0.02*
860990	. Others (2)	0.02*
870000	. Aril spices	0.02*
870010	. Mace	0.02*
870990	. Others (2)	0.02*

Code number	Groups and examples of individual products to which the MRLs apply (a)	Triticonazole (mg/kg)
900000	. SUGAR PLANTS	0.01*
900010	. Sugar beet roots	0.01*
900020	. Sugar canes	0.01*
900030	. Chicory roots	0.01*
900990	. Others (2)	0.01*
1000000	. PRODUCTS OF ANIMAL ORIGIN -TERRESTRIAL ANIMALS	0.01*
1010000	. Tissues from	0.01*
1011000	. (a) swine	0.01*
1011010	. Muscle	0.01*
1011020	. Fat tissue	0.01*
1011030	. Liver	0.01*
1011040	. Kidney	0.01*
1011050	. Edible offals (other than liver and kidney)	0.01*
1011990	. Others (2)	0.01*
1012000	. (b) bovine	0.01*
1012010	. Muscle	0.01*
1012020	. Fat tissue	0.01*
1012030	. Liver	0.01*
1012040	. Kidney	0.01*
1012050	. Edible offals (other than liver and kidney)	0.01*
1012990	. Others (2)	0.01*
1013000	. (c) sheep	0.01*
1013010	. Muscle	0.01*
1013020	. Fat tissue	0.01*
1013030	. Liver	0.01*
1013040	. Kidney	0.01*
1013050	. Edible offals (other than liver and kidney)	0.01*
1013990	. Others (2)	0.01*
1014000	. d) goat	0.01*
1014010	. Muscle	0.01*
1014020	. Fat tissue	0.01*
1014030	. Liver	0.01*
1014040	. Kidney	0.01*
1014050	. Edible offals (other than liver and kidney)	0.01*
1014990	. Others (2)	0.01*
1015000	. (e) equine	0.01*
1015010	. Muscle	0.01*
1015020	. Fat tissue	0.01*
1015030	. Liver	0.01*
1015040	. Kidney	0.01*
1015050	. Edible offals (other than liver and kidney)	0.01*
1015990	. Others (2)	0.01*
1016000	. (f) poultry	0.01*
1016010	. Muscle	0.01*
1016020	. Fat tissue	0.01*
1016030	. Liver	0.01*
1016040	. Kidney	0.01*
1016050	. Edible offals (other than liver and kidney)	0.01*
1016990	. Others (2)	0.01*

Code number	Groups and examples of individual products to which the MRLs apply (a)	Triticonazole (mg/kg)
1017000	. (g) other farmed terrestrial animals	0.01*
1017010	. Muscle	0.01*
1017020	. Fat tissue	0.01*
1017030	. Liver	0.01*
1017040	. Kidney	0.01*
1017050	. Edible offals (other than liver and kidney)	0.01*
1017990	. Others (2)	0.01*
1020000	. Milk	0.01*
1020010	. Cattle	0.01*
1020020	. Sheep	0.01*
1020030	. Goat	0.01*
1020040	. Horse	0.01*
1020990	. Others (2)	0.01*
1030000	. Birds eggs	0.01*
1030010	. Chicken	0.01*
1030020	. Duck	0.01*
1030030	. Geese	0.01*
1030040	. Quail	0.01*
1030990	. Others (2)	0.01*
1040000	. Honey and other apiculture products	0.01*
1050000	. Amphibians and Reptiles	0.01*
1060000	. Terrestrial invertebrate animals	0.01*
1070000	. Wild terrestrial vertebrate animals	0.01*

(*) Indicates lower limit of analytical determination

Plant Matrices

Wheat

In the growing seasons 2012/2013 and 2013/2014, in total 16 field trials comprising 16 plots were performed in Northern Europe (Germany, The Netherlands, Northern France and The United Kingdom) and Southern Europe (Italy, Spain, Greece and Southern France). The trials were conducted with formulations BAS 595 01 F according to the critical GAP of formulation BAS 595 01 F (± 25%).

No residues of parent triticonazole above the LOQ (0.01 mg/kg) were found in wheat grain samples harvested at crop maturity (BBCH 89).

This is in line with the 23 field studies on wheat, barley and rye, which were submitted for Annex I inclusion of triticonazole and which also match the critical GAP of BAS 595 01 F. At harvest, no residues at or above the LOQ (0.01 mg/kg) were detected in any of the grain samples.

Based on residue results and under consideration of the extrapolation rules of the EU guidance document "Guidelines on comparability, extrapolation, group tolerances and data requirements for setting MRLs" (SANCO 7525/VI/95 rev. 9, March 2011, Table 4), the default MRL of 0.01 mg/kg should be maintained for:

Code number 0500010 (barley)
Code number 0500050 (oat)
Code number 0500070 (rye)
Code number 0500090 (wheat)

Wheat straw (feed item)

Despite MRLs are not yet set for animal feed, the residue data summarized in M-CA 6.3 have been used for deriving a *"pseudo MRL"*. The relevant STMR and HR values can be used for deriving a feed burden and subsequently, a MRL proposal for food of animal origin.

In the growing seasons 2012/2013 and 2013/2014, in total 16 field trials comprising 16 plots were performed in Northern Europe (Germany, The Netherlands, Northern France and The United Kingdom) and Southern Europe (Italy, Spain, Greece and Southern France). The trials were conducted with formulations BAS 595 01 F according to the critical GAP of formulation BAS 595 01 F (± 25%).

No residues of parent triticonazole above the LOQ (0.01 mg/kg) were found in wheat "whole plant without root samples" (which can be used as fodder or forage) harvested either at BBCH 49 (8 trials) or at BBCH 59 (8 trials).

In the following, the dietary burden calculations are conducted using the EU-agreed methodology and the OECD-agreed methodology.

The following input values are used for the calculations using the EU model:

Table 6.7.2-2: Input values for the dietary burden calculation (EU model)

Commodity	Median dietary burden		Maximum dietary burden	
	Input value (mg/kg)	Comment	Input value (mg/kg)	Comment
Residue definition for risk assessment: triticonazole				
Wheat grain	0.01	STMR	0.01	STMR
Barley grain	0.01	STMR	0.01	STMR
Rye grain	0.01	STMR	0.01	STMR
Oat grain	0.01	STMR	0.01	STMR
Wheat bran	0.08	STMR x 8[a]	0.08	STMR x 8[a]
Rye bran	0.08	STMR x 8[a]	0.08	STMR x 8[a]
Wheat straw	0.01	STMR	0.08	HR[b]
Barley straw	0.01	STMR	0.08	HR[b]
Rye straw	0.01	STMR	0.08	HR[b]
Oat straw	0.01	STMR	0.08	HR[b]

[a] processing factor see EFSA Scientific Report (2009) 277, 1-23
[b] HR see EFSA Scientific Report (2009) 277, 1-23; as those trials still meet the current critical GAP (±25%) they are considered here do depict a worst case scenario (the HR of the new trials reported in CA 6.3 is <0.01 mg/kg)

Applying those values leads to the following results:

Table 6.7.2-3: Results of the dietary burden calculation (EU model)

	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?
Residue definition for risk assessment: triticonazole					
Dairy ruminants	0.00133	0.00074	Wheat straw	0.04	No
Meat ruminants	0.00276	0.00102	Wheat straw	0.06	No
Poultry	0.00085	0.00085	Wheat bran	0.01	No
Pigs	0.00072	0.00072	Wheat bran	0.02	No

The following input values are used for the EU in context of the calculations using the OECD model:

Table 6.7.2-4: Input values for the dietary burden calculation (OECD model)

Feedstuff	Residue value for EU (mg/kg)	Comment
Forages		
Barley forage	0.01	HR
Barley straw	0.08	HR[b]
Oat forage	0.01	HR
Oat straw	0.08	HR[b]
Rye forage	0.01	HR
Rye straw	0.08	HR[b]
Triticale forage	0.01	HR
Triticale straw	0.08	HR[b]
Wheat forage	0.01	HR
Wheat straw	0.08	HR[b]
Cereal Grains/Crops Seeds		
Barley grain	0.01	STMR
Oat grain	0.01	STMR
Rye grain	0.01	STMR
Triticale grain	0.01	STMR
Wheat grain	0.01	STMR
By-products		
Barley, bran fractions	0.08	STMR x 8[a]
Wheat, milled byproducts	0.08	STMR x 8[a]

[a] processing factor see EFSA Scientific Report (2009) 277, 1-23

[b] HR see EFSA Scientific Report (2009) 277, 1-23; as those trials still meet the current critical GAP (±25%) they are considered here do depict a worst case scenario (the HR of the new trials reported in CA 6.3 is <0.01 mg/kg)

Applying those values leads to the following results:

Table 6.7.2-5: Results of the dietary burden calculation (RWCFL for EU, OECD model)

	Dietary burden (mg/kg bw/d)	Feed burden (mg/kg DM)	Trigger exceeded?
Residue definition for risk assessment: triticonazole			
Cattle - Beef	0.001	0.042	No
Cattle - Dairy	0.002	0.042	No
Sheep - Ram/Ewe	0.002	0.054	No
Sheep - Lamb	0.003	0.062	No
Swine - Breeding	0.001	0.051	No
Swine - Finishing	0.002	0.051	No
Poultry - Broiler	0.002	0.026	No
Poultry - Layer	0.002	0.027	No
Poultry - Turkey	0.002	0.025	No

When applying the EU model and the OECD model and the input values as specified above, the trigger value of 0.1 mg/kg DM is not exceeded. Therefore, no feeding studies are required (7031/VI/95 rev. 4, 22/7/96, Appendix G).

Animal matrices

In 2005 EFSA concluded in context of the Conclusion on the peer review of triticonazole (EFSA Scientific Report (2005) 33, 1-69), that after one seed treatment application in cereals at 5 g a.i. per 100 kg seed "no residues of triticonazole were quantified in any of the cereal grain or straw samples from field trials conducted according critical good agricultural practice (GAP)".

In context of this AIR3 submission, additional trials are reported (see CA 6.3) being conducted within ± 25% according to the critical GAP already evaluated by EFSA in 2005. Residues in cereal grain and straw were again always below the respective LOQs (grain: 0.01 mg/kg, straw: 0.05 mg/kg).

EFSA also concluded in 2005: "No quantifiable triticonazole residues were found in cereal grains and straw at the time of harvest and triticonazole and/or its metabolites are not deemed to accumulate in animal tissue. Therefore, metabolism studies in livestock are not necessary as long as cereal green forage is not used in animal diet and a definition of residues in food of animal origin has not to be proposed."

It can be concluded, that furthermore no residues in animal tissues can be expected and a definition of residues in food of animal origin is not needed.

CA 6.7.3 Proposed maximum residue levels (MRLs) and justification of the acceptability of the levels proposed for imported products (import tolerance)

Referring to the MRL derivations in chapter CA 6.7.2 it is proposed to keep the established EU MRLs of:

0.01* mg/kg for barley grain [group 0500010]
0.01* mg/kg for oat grain [group 0500050]
0.01* mg/kg for rye grain [group 0500070]
0.01* mg/kg for wheat (spelt, triticale) grain [group 0500090]
0.01* mg/kg for products of animal origin – terrestrial animals [group 1000000]

CA 6.8 Proposed safety intervals

Pre-harvest interval
Triticonazole is to be used for seed treatment in cereals only. The pre-harvest interval is covered by the application conditions and the growing period between sowing and harvest. Therefore, it is not necessary to lay down a pre-harvest interval in days.
The proposed pre harvest interval is **F** (ref. working doc. 7039/VI/95 EN, 1997).

Re-entry period for livestock to areas to be grazed
Because triticonazole 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
Triticonazole is used as seed treatment only. Therefore, no re-entry period can be set. However, a possible contact to treated seed is possible during sowing and a risk assessment on using of treated cereals grain for sowing by operators has been made in the supplemental product dossiers (M-CP 7.2).

Withholding period for animal feed stuffs
Treated cereals (grain and straw) may be used as fodder for livestock Due to the favorable residue situation in the cereal matrices of concern with residues consistently below LOQ no withholding period is needed.

Waiting period between application and crop sowing or planting the crop to be protected
No waiting period is necessary since triticonazole is not intended in a pre-emergence use.

Waiting period between application and handling treated produce
Treated seed has to be handled during sowing using the recommended PPE (gloves). No waiting period has to be set. A risk assessment on using of treated cereals grain for sowing has been made in the supplemental product dossiers (M-CP 7.2).

Waiting period between last application and sowing or planting succeeding crops
Neither the results from confined rotation crops study nor the results from field rotational crop studies give reason to assume the uptake of triticonazole after sowing of treated grain from soil. Therefore no waiting period for replanting is necessary.

CA 6.9 Estimation of the potential and actual exposure through diet and other sources

An assessment of the potential chronic and acute dietary consumer risk due to exposure to residues of triticonazole was performed using the EFSA model for chronic and acute risk assessment - rev. 2_0 (Model PRIMo). The EFSA model was used since it considers all the different diets in the EU and all consumer groups.
The ADI and ARfD for the active substance triticonazole are summarized in the table below.

Table 6.9-1: Toxicological endpoints – triticonazole

Endpoint	Value (mg/kg bw/d)	Study	Safety factor	Source
Acceptable Daily Intake (ADI)	0.025	1-year dog study	100	Dir 06/39
Acute Reference Dose (ARfD)	0.05	developmental rabbit study	100	Dir 06/39

Acceptable Daily Intake (ADI) and Dietary Exposure Calculation

A chronic exposure assessment was performed, for which all crops and maximum residue levels used are summarized in Table 6.9-2. For the representative use of triticonazole in cereals, no new MRLs were proposed.
The summary of the calculation using the EFSA model rev 2.0 is presented in Table 6.9-3. For the assessment, an ADI of 0.025 mg/kg bw/day was used. According to the EFSA model the TMDI has been simultaneously calculated for adults, children, toddlers and infants (different age groups), vegetarian and elderly in different EU countries.

Table 6.9-2: EU MRLs and proposed MRLs for the uses of triticonazole

Code number	Groups and examples of individual products to which the MRLs apply (a)	Triticonazole	
		established MRL (mg/kg)	proposed MRL (mg/kg)
100000	. FRUITS, FRESH or FROZEN; TREE NUTS	0.01*	
110000	. Citrus fruits	0.01*	
110010	. Grapefruits	0.01*	
110020	. Oranges	0.01*	
110030	. Lemons	0.01*	
110040	. Limes	0.01*	
110050	. Mandarins	0.01*	
110990	. Others (2)	0.01*	
120000	. Tree nuts	0.01*	
120010	. Almonds	0.01*	
120020	. Brazil nuts	0.01*	
120030	. Cashew nuts	0.01*	
120040	. Chestnuts	0.01*	
120050	. Coconuts	0.01*	
120060	. Hazelnuts/cobnuts	0.01*	
120070	. Macadamias	0.01*	
120080	. Pecans	0.01*	

Code number	Groups and examples of individual products to which the MRLs apply (a)	Triticonazole	
		established MRL (mg/kg)	proposed MRL (mg/kg)
120090	. Pine nut kernels	0.01*	
120100	. Pistachios	0.01*	
120110	. Walnuts	0.01*	
120990	. Others (2)	0.01*	
130000	. Pome fruits	0.01*	
130010	. Apples	0.01*	
130020	. Pears	0.01*	
130030	. Quinces	0.01*	
130040	. Medlars	0.01*	
130050	. Loquats/Japanese medlars	0.01*	
130990	. Others (2)	0.01*	
140000	. Stone fruits	0.01*	
140010	. Apricots	0.01*	
140020	. Cherries (sweet)	0.01*	
140030	. Peaches	0.01*	
140040	. Plums	0.01*	
140990	. Others (2)	0.01*	
150000	. Berries and small fruits	0.01*	
151000	. (a) grapes	0.01*	
151010	. Table grapes	0.01*	
151020	. Wine grapes	0.01*	
152000	. (b) strawberries	0.01*	
153000	. (c) cane fruits	0.01*	
153010	. Blackberries	0.01*	
153020	. Dewberries	0.01*	
153030	. Raspberries (red and yellow)	0.01*	
153990	. Others (2)	0.01*	
154000	. (d) other small fruits and berries	0.01*	
154010	. Blueberries	0.01*	
154020	. Cranberries	0.01*	
154030	. Currants (black, red and white)	0.01*	
154040	. Gooseberries (green, red and yellow)	0.01*	
154050	. Rose hips	0.01*	
154060	. Mulberries (black and white)	0.01*	
154070	. Azaroles/Mediterranean medlars	0.01*	
154080	. Elderberries	0.01*	
154990	. Others (2)	0.01*	
160000	. Miscellaneous fruits with	0.01*	
161000	. (a) edible peel	0.01*	
161010	. Dates	0.01*	
161020	. Figs	0.01*	
161030	. Table olives	0.01*	
161040	. Kumquats	0.01*	
161050	. Carambolas	0.01*	
161060	. Kaki/Japanese persimmons	0.01*	
161070	. Jambuls/jambolans	0.01*	
161990	. Others (2)	0.01*	

Code number	Groups and examples of individual products to which the MRLs apply (a)	Triticonazole	
		established MRL (mg/kg)	proposed MRL (mg/kg)
162000	. (b) inedible peel, small	0.01*	
162010	. Kiwi fruits (green, red, yellow)	0.01*	
162020	. Litchis/lychees	0.01*	
162030	. Passionfruits/maracujas	0.01*	
162040	. Prickly pears/cactus fruits	0.01*	
162050	. Star apples/cainitos	0.01*	
162060	. American persimmons/Virginia kaki	0.01*	
162990	. Others (2)	0.01*	
163000	. (c) inedible peel, large	0.01*	
163010	. Avocados	0.01*	
163020	. Bananas	0.01*	
163030	. Mangoes	0.01*	
163040	. Papayas	0.01*	
163050	. Granate apples/pomegranates	0.01*	
163060	. Cherimoyas	0.01*	
163070	. Guavas	0.01*	
163080	. Pineapples	0.01*	
163090	. Breadfruits	0.01*	
163100	. Durians	0.01*	
163110	. Soursops/guanabanas	0.01*	
163990	. Others (2)	0.01*	
200000	. VEGETABLES, FRESH or FROZEN	0.01*	
210000	. Root and tuber vegetables	0.01*	
211000	. (a) potatoes	0.01*	
212000	. (b) tropical root and tuber vegetables	0.01*	
212010	. Cassava roots/manioc	0.01*	
212020	. Sweet potatoes	0.01*	
212030	. Yams	0.01*	
212040	. Arrowroots	0.01*	
212990	. Others (2)	0.01*	
213000	. (c) other root and tuber vegetables except sugar beets	0.01*	
213010	. Beetroots	0.01*	
213020	. Carrots	0.01*	
213030	. Celeriacs/turnip rooted celeries	0.01*	
213040	. Horseradishes	0.01*	
213050	. Jerusalem artichokes	0.01*	
213060	. Parsnips	0.01*	
213070	. Parsley roots/Hamburg roots parsley	0.01*	
213080	. Radishes	0.01*	
213090	. Salsifies	0.01*	
213100	. Swedes/rutabagas	0.01*	
213110	. Turnips	0.01*	
213990	. Others (2)	0.01*	
220000	. Bulb vegetables	0.01*	
220010	. Garlic	0.01*	
220020	. Onions	0.01*	
220030	. Shallots	0.01*	
220040	. Spring onions/green onions and Welsh onions	0.01*	
220990	. Others (2)	0.01*	

Code number	Groups and examples of individual products to which the MRLs apply (a)	Triticonazole	
		established MRL (mg/kg)	proposed MRL (mg/kg)
230000	. Fruiting vegetables	0.01*	
231000	. (a) solanacea	0.01*	
231010	. Tomatoes	0.01*	
231020	. Sweet peppers/bell peppers	0.01*	
231030	. Aubergines/eggplants	0.01*	
231040	. Okra/lady's fingers	0.01*	
231990	. Others (2)	0.01*	
232000	. (b) cucurbits with edible peel	0.01*	
232010	. Cucumbers	0.01*	
232020	. Gherkins	0.01*	
232030	. Courgettes	0.01*	
232990	. Others (2)	0.01*	
233000	. (c) cucurbits with inedible peel	0.01*	
233010	. Melons	0.01*	
233020	. Pumpkins	0.01*	
233030	. Watermelons	0.01*	
233990	. Others (2)	0.01*	
234000	. (d) sweet corn	0.01*	
239000	. (e) other fruiting vegetables	0.01*	
240000	. Brassica vegetables (excluding brassica roots and brassica baby leaf crops)	0.01*	
241000	. (a) flowering brassica	0.01*	
241010	. Broccoli	0.01*	
241020	. Cauliflowers	0.01*	
241990	. Others (2)	0.01*	
242000	. (b) head brassica	0.01*	
242010	. Brussels sprouts	0.01*	
242020	. Head cabbages	0.01*	
242990	. Others (2)	0.01*	
243000	. (c) leafy brassica	0.01*	
243010	. Chinese cabbages/pe-tsai	0.01*	
243020	. Kales	0.01*	
243990	. Others (2)	0.01*	
244000	. (d) kohlrabies	0.01*	
250000	. Leaf vegetables, herbs and edible flowers	0.01*	
251000	. (a) lettuces and salad plants	0.01*	
251010	. Lamb's lettuces/corn salads	0.01*	
251020	. Lettuces	0.01*	
251030	. Escaroles/broad-leaved endives	0.01*	
251040	. Cresses and other sprouts and shoots	0.01*	
251050	. Land cresses	0.01*	
251060	. Roman rocket/rucola	0.01*	
251070	. Red mustards	0.01*	
251080	. Baby leaf crops (including brassica species)	0.01*	
251990	. Others (2)	0.01*	
252000	. (b) spinaches and similar leaves	0.01*	
252010	. Spinaches	0.01*	
252020	. Purslanes	0.01*	
252030	. Chards/beet leaves	0.01*	
252990	. Others (2)	0.01*	

Code number	Groups and examples of individual products to which the MRLs apply (a)	Triticonazole	
		established MRL (mg/kg)	proposed MRL (mg/kg)
253000	. (c) grape leaves and similar species	0.01*	
254000	. (d) watercresses	0.01*	
255000	. (e) witloofs/Belgian endives	0.01*	
256000	. (f) herbs and edible flowers	0.01*	
256010	. Chervil	0.01*	
256020	. Chives	0.01*	
256030	. Celery leaves	0.01*	
256040	. Parsley	0.01*	
256050	. Sage	0.01*	
256060	. Rosemary	0.01*	
256070	. Thyme	0.01*	
256080	. Basil and edible flowers	0.01*	
256090	. Laurel/bay leave	0.01*	
256100	. Tarragon	0.01*	
256990	. Others (2)	0.01*	
260000	. Legume vegetables	0.01*	
260010	. Beans (with pods)	0.01*	
260020	. Beans (without pods)	0.01*	
260030	. Peas (with pods)	0.01*	
260040	. Peas (without pods)	0.01*	
260050	. Lentils	0.01*	
260990	. Others (2)	0.01*	
270000	. Stem vegetables	0.01*	
270010	. Asparagus	0.01*	
270020	. Cardoons	0.01*	
270030	. Celeries	0.01*	
270040	. Florence fennels	0.01*	
270050	. Globe artichokes	0.01*	
270060	. Leeks	0.01*	
270070	. Rhubarbs	0.01*	
270080	. Bamboo shoots	0.01*	
270090	. Palm hearts	0.01*	
270990	. Others (2)	0.01*	
280000	. Fungi, mosses and lichens	0.01*	
280010	. Cultivated fungi	0.01*	
280020	. Wild fungi	0.01*	
280990	. Mosses and lichens	0.01*	
290000	. Algae and prokaryotes organisms	0.01*	
300000	. PULSES	0.01*	
300010	. Beans	0.01*	
300020	. Lentils	0.01*	
300030	. Peas	0.01*	
300040	. Lupins/lupini beans	0.01*	
300990	. Others (2)	0.01*	

Code number	Groups and examples of individual products to which the MRLs apply (a)	Triticonazole	
		established MRL (mg/kg)	proposed MRL (mg/kg)
400000	. OILSEEDS AND OIL FRUITS		
401000	. Oilseeds	0.02*	
401010	. Linseeds	0.02*	
401020	. Peanuts/groundnuts	0.02*	
401030	. Poppy seeds	0.02*	
401040	. Sesame seeds	0.02*	
401050	. Sunflower seeds	0.02*	
401060	. Rapeseeds/canola seeds	0.02*	
401070	. Soyabeans	0.02*	
401080	. Mustard seeds	0.02*	
401090	. Cotton seeds	0.02*	
401100	. Pumpkin seeds	0.02*	
401110	. Safflower seeds	0.02*	
401120	. Borage seeds	0.02*	
401130	. Gold of pleasure seeds	0.02*	
401140	. Hemp seeds	0.02*	
401150	. Castor beans	0.02*	
401990	. Others (2)	0.02*	
402000	. Oil fruits		
402010	. Olives for oil production	0.01*	
402020	. Oil palms kernels	0.02*	
402030	. Oil palms fruits	0.02*	
402040	. Kapok	0.02*	
402990	. Others (2)	0.02*	
500000	. CEREALS	0.01*	
500010	. Barley	0.01*	0.01*
500020	. Buckwheat and other pseudo-cereals	0.01*	
500030	. Maize/corn	0.01*	
500040	. Common millet/proso millet	0.01*	
500050	. Oat	0.01*	0.01*
500060	. Rice	0.01*	
500070	. Rye	0.01*	0.01*
500080	. Sorghum	0.01*	
500090	. Wheat	0.01*	0.01*
500990	. Others (2)	0.01*	
600000	. TEAS, COFFEE, HERBAL INFUSIONS, COCOA AND CAROBS	0.02*	
610000	. Teas	0.02*	
620000	. Coffee beans	0.02*	
630000	. Herbal infusions from	0.02*	
631000	. (a) flowers	0.02*	
631010	. Chamomile	0.02*	
631020	. Hibiscus/roselle	0.02*	
631030	. Rose	0.02*	
631040	. Jasmine	0.02*	
631050	. Lime/linden	0.02*	
631990	. Others (2)	0.02*	

Code number	Groups and examples of individual products to which the MRLs apply (a)	Triticonazole	
		established MRL (mg/kg)	proposed MRL (mg/kg)
632000	. (b) leaves and herbs	0.02*	
632010	. Strawberry	0.02*	
632020	. Rooibos	0.02*	
632030	. Mate/maté	0.02*	
632990	. Others (2)	0.02*	
633000	. (c) roots	0.02*	
633010	. Valerian	0.02*	
633020	. Ginseng	0.02*	
633990	. Others (2)	0.02*	
639000	. (d) any other parts of the plant	0.02*	
640000	. Cocoa beans	0.02*	
650000	. Carobs/Saint John's breads	0.02*	
700000	. HOPS	0.02*	
800000	. SPICES	0.02*	
810000	. Seed spices	0.02*	
810010	. Anise/aniseed	0.02*	
810020	. Black caraway/black cumin	0.02*	
810030	. Celery	0.02*	
810040	. Coriander	0.02*	
810050	. Cumin	0.02*	
810060	. Dill	0.02*	
810070	. Fennel	0.02*	
810080	. Fenugreek	0.02*	
810090	. Nutmeg	0.02*	
810990	. Others (2)	0.02*	
820000	. Fruit spices	0.02*	
820010	. Allspice/pimento	0.02*	
820020	. Sichuan pepper	0.02*	
820030	. Caraway	0.02*	
820040	. Cardamom	0.02*	
820050	. Juniper berry	0.02*	
820060	. Peppercorn (black, green and white)	0.02*	
820070	. Vanilla	0.02*	
820080	. Tamarind	0.02*	
820990	. Others (2)	0.02*	
830000	. Bark spices	0.02*	
830010	. Cinnamon	0.02*	
830990	. Others (2)	0.02*	
840000	. Root and rhizome spices	0.02*	
840010	. Liquorice	0.02*	
840020	. Ginger	0.02*	
840030	. Turmeric/curcuma	0.02*	
840040	. Horseradish	0.02*	
840990	. Others (2)	0.02*	
850000	. Bud spices	0.02*	
850010	. Cloves	0.02*	
850020	. Capers	0.02*	
850990	. Others (2)	0.02*	

Code number	Groups and examples of individual products to which the MRLs apply (a)	Triticonazole	
		established MRL (mg/kg)	proposed MRL (mg/kg)
860000	. Flower pistil spices	0.02*	
860010	. Saffron	0.02*	
860990	. Others (2)	0.02*	
870000	. Aril spices	0.02*	
870010	. Mace	0.02*	
870990	. Others (2)	0.02*	
900000	. SUGAR PLANTS	0.01*	
900010	. Sugar beet roots	0.01*	
900020	. Sugar canes	0.01*	
900030	. Chicory roots	0.01*	
900990	. Others (2)	0.01*	
1000000	. PRODUCTS OF ANIMAL ORIGIN -TERRESTRIAL ANIMALS	0.01*	0.01*
1010000	. Tissues from	0.01*	
1011000	. (a) swine	0.01*	
1011010	. Muscle	0.01*	
1011020	. Fat tissue	0.01*	
1011030	. Liver	0.01*	
1011040	. Kidney	0.01*	
1011050	. Edible offals (other than liver and kidney)	0.01*	
1011990	. Others (2)	0.01*	
1012000	. (b) bovine	0.01*	
1012010	. Muscle	0.01*	
1012020	. Fat tissue	0.01*	
1012030	. Liver	0.01*	
1012040	. Kidney	0.01*	
1012050	. Edible offals (other than liver and kidney)	0.01*	
1012990	. Others (2)	0.01*	
1013000	. (c) sheep	0.01*	
1013010	. Muscle	0.01*	
1013020	. Fat tissue	0.01*	
1013030	. Liver	0.01*	
1013040	. Kidney	0.01*	
1013050	. Edible offals (other than liver and kidney)	0.01*	
1013990	. Others (2)	0.01*	
1014000	. d) goat	0.01*	
1014010	. Muscle	0.01*	
1014020	. Fat tissue	0.01*	
1014030	. Liver	0.01*	
1014040	. Kidney	0.01*	
1014050	. Edible offals (other than liver and kidney)	0.01*	
1014990	. Others (2)	0.01*	
1015000	. (e) equine	0.01*	
1015010	. Muscle	0.01*	
1015020	. Fat tissue	0.01*	
1015030	. Liver	0.01*	
1015040	. Kidney	0.01*	
1015050	. Edible offals (other than liver and kidney)	0.01*	
1015990	. Others (2)	0.01*	

Code number	Groups and examples of individual products to which the MRLs apply (a)	Triticonazole	
		established MRL (mg/kg)	proposed MRL (mg/kg)
1016000	. (f) poultry	0.01*	
1016010	. Muscle	0.01*	
1016020	. Fat tissue	0.01*	
1016030	. Liver	0.01*	
1016040	. Kidney	0.01*	
1016050	. Edible offals (other than liver and kidney)	0.01*	
1016990	. Others (2)	0.01*	
1017000	. (g) other farmed terrestrial animals	0.01*	
1017010	. Muscle	0.01*	
1017020	. Fat tissue	0.01*	
1017030	. Liver	0.01*	
1017040	. Kidney	0.01*	
1017050	. Edible offals (other than liver and kidney)	0.01*	
1017990	. Others (2)	0.01*	
1020000	. Milk	0.01*	
1020010	. Cattle	0.01*	
1020020	. Sheep	0.01*	
1020030	. Goat	0.01*	
1020040	. Horse	0.01*	
1020990	. Others (2)	0.01*	
1030000	. Birds eggs	0.01*	
1030010	. Chicken	0.01*	
1030020	. Duck	0.01*	
1030030	. Geese	0.01*	
1030040	. Quail	0.01*	
1030990	. Others (2)	0.01*	
1040000	. Honey and other apiculture products	0.01*	
1050000	. Amphibians and Reptiles	0.01*	
1060000	. Terrestrial invertebrate animals	0.01*	
1070000	. Wild terrestrial vertebrate animals	0.01*	

(*) Indicates lower limit of analytical determination
Pesticides - Web Version - EU MRLs (File created on 30/07/2015)
Source: DG Sanco website: http://ec.europa.eu/sanco_pesticides/public/index.cfm?event =substance.resultat&s=1

With the current EFSA model the chronic risk assessment ranges from 0 to 3.0% of ADI (see Table 6.9-3). The diet with the highest TMDI is "FR toddler" with 3.0% of ADI. For this diet, the highest contributor are products of animal origin with 1.7% of ADI (this contribution is very protective as no residues in products of animal origin are anticipated). The diet with the second highest TMDI is “UK infant” with 2.9% of ADI, in which also products of animal origin are the major contributor with 1.7% of ADI.

According to the presented TMDI calculation a long-term intake of triticonazole residues is unlikely to present a public health concern.

Table 6.9-3 TMDI calculation of triticonazole (EFSA model rev. 2)

Triticonazole

Status of the active substance:		Code no.	
LOQ (mg/kg bw):	0.01	proposed LOQ:	
Toxicological end points			
ADI (mg/kg bw/day):	**0.025**	ARfD (mg/kg bw):	**0.05**
Source of ADI:	**EFSA**	Source of ARfD:	**EFSA**
Year of evaluation:	**2005**	Year of evaluation:	**2005**

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	3
No of diets exceeding ADI	---

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)
3 0	FR toddler	1.7	PRODUCTS OF AN MAL ORIG N	0.7	VEGETABLES	0.5	FRUIT (FRESH OR FROZEN)	3.0
2 9	UK Infant	1.7	PRODUCTS OF AN MAL ORIG N	0.4	SUGAR PLANTS	0.3	VEGETABLES	2.8
2 8	UK Toddler	0.9	PRODUCTS OF AN MAL ORIG N	0.9	SUGAR PLANTS	0.3	FRUIT (FRESH OR FROZEN)	2.6
2 6	NL child	1.3	PRODUCTS OF AN MAL ORIG N	0.6	FRUIT (FRESH OR FROZEN)	0.5	VEGETABLES	2.6
2 6	FR infant	1.1	PRODUCTS OF AN MAL ORIG N	0.8	VEGETABLES	0.6	FRUIT (FRESH OR FROZEN)	2.6
2.4	DE child	0.9	PRODUCTS OF AN MAL ORIG N	0.9	FRUIT (FRESH OR FROZEN)	0.3	VEGETABLES	2.4
1 9	WHO Cluster diet B	0.6	VEGETABLES	0.5	CEREALS	0.3	FRUIT (FRESH OR FROZEN)	1.8
1 8	DK child	0.9	PRODUCTS OF AN MAL ORIG N	0.4	CEREALS	0.3	VEGETABLES	1.8
1 5	SE general population 90th percentile	0.7	PRODUCTS OF AN MAL ORIG N	0.4	VEGETABLES	0.2	FRUIT (FRESH OR FROZEN)	1.5
1.4	ES child	0.7	PRODUCTS OF AN MAL ORIG N	0.2	FRUIT (FRESH OR FROZEN)	0.2	CEREALS	1.4
1.4	E adult	0.4	FRUIT (FRESH OR FROZEN)	0.4	VEGETABLES	0.3	CEREALS	1.3
1 3	WHO cluster diet E	0.4	VEGETABLES	0.3	PRODUCTS OF AN MAL ORIG N	0.2	CEREALS	1.1
1 2	WHO cluster diet D	0.4	VEGETABLES	0.3	CEREALS	0.3	PRODUCTS OF ANIMAL ORIGIN	1.1
1.1	WHO regional European diet	0.4	PRODUCTS OF AN MAL ORIG N	0.4	VEGETABLES	0.1	CEREALS	1.0
1.1	WHO Cluster diet F	0.3	PRODUCTS OF AN MAL ORIG N	0.3	VEGETABLES	0.2	CEREALS	1.0
0 9	NL general	0.3	PRODUCTS OF AN MAL ORIG N	0.2	VEGETABLES	0.2	FRUIT (FRESH OR FROZEN)	0.9
0 8	ES adult	0.3	PRODUCTS OF AN MAL ORIG N	0.2	FRUIT (FRESH OR FROZEN)	0.2	VEGETABLES	0.8
0 8	UK Adult	0.2	PRODUCTS OF AN MAL ORIG N	0.2	SUGAR PLANTS	0.1	VEGETABLES	0.7
0 8	FR all population	0.2	FRUIT (FRESH OR FROZEN)	0.2	PRODUCTS OF AN MAL ORIG N	0.1	VEGETABLES	0.7
0 8	PT General population	0.3	FRUIT (FRESH OR FROZEN)	0.2	VEGETABLES	0.2	CEREALS	0.7
0.7	UK vegetarian	0.2	SUGAR PLANTS	0.2	VEGETABLES	0.1	PRODUCTS OF ANIMAL ORIGIN	0.7
0.7	DK adult	0.3	PRODUCTS OF AN MAL ORIG N	0.1	VEGETABLES	0.1	FRUIT (FRESH OR FROZEN)	0.7
0.7	IT kids/toddler	0.3	CEREALS	0.2	VEGETABLES	0.1	FRUIT (FRESH OR FROZEN)	0.7
0 6	LT adult	0.2	PRODUCTS OF AN MAL ORIG N	0.2	VEGETABLES	0.1	CEREALS	0.6
0 6	FI adult	0.3	PRODUCTS OF AN MAL ORIG N	0.1	VEGETABLES	0.1	FRUIT (FRESH OR FROZEN)	0.6
0 5	IT adult	0.2	CEREALS	0.2	VEGETABLES	0.1	FRUIT (FRESH OR FROZEN)	0.5
0.4	PL general population	0.2	VEGETABLES	0.1	FRUIT (FRESH OR FROZEN)	0.0	PULSES DRY	0.4

Conclusion:
The estimated Theoretical Maximum Daily Intakes (TMDI), based on pTMRLs were below the ADI.
A long-term intake of residues of Triticonazole is unlikely to present a public health concern.

Acute Reference Dose (ARfD) and Dietary Exposure Calculation

For the assessment, an ARfD of 0.05 mg/kg bw/day was used. The calculation was done with the default variability factors. The following MRLs for the representative crops of the active substance triticonazole were considered as reported in Table 6.9-2:

Barley: 0.01 mg/kg
Oat: 0.01 mg/kg
Rye: 0.01 mg/kg
Wheat: 0.01 mg/kg

No exceedances of the ARfD were identified for any unprocessed or processed commodity. No commodity was noted as making contributions above 1% of the ARfD for children. The acute dietary risk assessment clearly indicates that there is no acute risk for any subpopulation group under consideration, the highest ARfD utilization being under 1%. Therefore, according to the calculations, the intake of triticonazole residues is unlikely to present a public health concern.

The acute risk assessment is based on the ARfD.
For each commodity the calculation is based on the highest reported MS consumption per kg bw and the corresponding unit weight from the MS with the critical consumption. If no data on the unit weight was available from that MS an average European unit weight was used for the IESTI calculation
In the **IESTI 1** calculation, the variability factors were 10, 7 or 5 (according to JMPR manual 2002), for lettuce a variability factor of 5 was used.
In the **IESTI 2** calculations, the variability factors of 10 and 7 were replaced by 5. For lettuce the calculation was performed with a variability factor of 3. **Threshold MRL** is the calculated residue level which would lead to an exposure equivalent to 100% of the ARfD.

Table 6.9-4 Output for IESTI calculations of triticonazole (EFSA model rev. 2)

Results for children

Unprocessed commodities						
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**	***)**	****)**
Highest % of ARfD/ADI	Commodities	pTMRL/ threshold MRL (mg/kg)	Highest % of ARfD/ADI	Commodities	pTMRL/ threshold MRL (mg/kg)
0.3	Wheat	0.01 / -	0.3	Wheat	0.01 / -
0.1	Rye	0.01 / -	0.1	Rye	0.01 / -
0.1	Oats	0.01 / -	0.1	Oats	0.01 / -
0.0	Barley	0.01 / -	0.0	Barley	0.01 / -
No of critical MRLs (IESTI 1)			---		

Processed commodities		
No of commodities for which ARfD/ADI is exceeded:		**---**
		*****)**
Highest % of ARfD/ADI	Processed commodities	pTMRL/ threshold MRL (mg/kg)
0.2	Wheat flour	0.01 / -

Results for general population

Unprocessed commodities	**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**	***)**	**)
	Highest % of ARfD/ADI	Commodities	pTMRL/ threshold MRL (mg/kg)	Highest % of ARfD/ADI	Commodities	pTMRL/ threshold MRL (mg/kg)
	0.2	Wheat	0.01 / -	0.2	Wheat	0.01 / -
	0.1	Barley	0.01 / -	0.1	Barley	0.01 / -
	0.1	Rye	0.01 / -	0.1	Rye	0.01 / -
	0.0	Oats	0.01 / -	0.0	Oats	0.01 / -
	No of critical MRLs (IESTI 2)			---		
Processed commodities	**No of commodities for which ARfD/ADI is exceeded:**		---			
			*****)**			
	Highest % of ARfD/ADI	Processed commodities	pTMRL/ threshold MRL (mg/kg)			
	0.1	Bread/pizza	0.01 / -			

CA 6.10 Other studies

No other/special studies are deemed necessary. The studies and information provided in the previous sections are considered adequate and sufficient.

CA 6.10.1 Effect on the residue level in pollen and bee products

The objective of these studies shall be to determine the residue in pollen and bee products for human consumption resulting from residues taken up by honeybees from crops at blossom.

The representative use of this dossier is wheat. Cereal crops are regarded as having no melliferous potential, and residue studies in honey are therefore not required.

Tier 1 Summaries of the Supervised Field Residue Trials for the Representative Crops

Wheat

Northern Europe

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Triticonazole	Commercial Product (name)	-
Crop/crop group:	Wheat (Cereals)	Producer of commercial product	67056 Ludwigshafen, Germany
Responsible body for reporting (name, address)	BASF SE, 67056 Ludwigshafen	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	None
Content of active substance (g/kg or g/L)	25 g/L	(common name and content)	
Formulation (e.g. WP)	BAS 595 01 F (FS)	Residues calculated as:	Triticonazole

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 per Treatment			No of Treatm. and Last Date	Growth Stage (BBCH)[1]	Portion Analysed	Residues (mg/kg)	PHI (days)	Remarks
				kg a.s./hL	Water (L/ha)	g as/ha				Triticonazole		
407785 2014/1043281 67294 Mauchenheim Rheinhessen, Germany L120653	GR 0654 KWS CHAMSIN	1. 02.04.2013 2. 18.06. - 30.06.2013 3. 09.08.2013	Seeding of treated seeds	n.a.	n.a.	11.25	1 02.04.13	n.a.	Whole plant[2)] Whole plant[2)] Whole plant[2)] Grain Straw	0.64 0.11 < 0.01 < 0.01 < 0.01	21 30 72 129 129	BASF Method No. 562/0
407785 2014/1043281 CO11 2NF Lawford Essex, UK L120654	GR 0654 KWS CHAMSIN	1. 06.03.2013 2. 24.06. - 15.07.2013 3. 23.08.2014	Seeding of treated seeds	n.a.	n.a.	11.25	1 06.03.13	n.a.	Whole plant[2)] Whole plant[2)] Whole plant[2)] Grain Straw	0.13 < 0.01 < 0.01 < 0.01 < 0.01	51 71 100 170 170	BASF Method No. 562/0
407785 2014/1043281 49700 Meigné sous Doué, Maine-et-Loire France (N) L120655	GR 0654 KWS CHAMSIN	1. 04.03.2013 2. 30.06. - 10.07.2013 3. 15.08.2013	Seeding of treated seeds	n.a.	n.a.	11.25	1 04.03.13	n.a.	Whole plant[2)] Grain Straw	< 0.01 < 0.01 < 0.01	94 163 163	BASF Method No. 562/0

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Triticonazole	Commercial Product (name)	-
Crop/crop group:	Wheat (Cereals)	Producer of commercial product	67056 Ludwigshafen, Germany
Responsible body for reporting (name, address)	BASF SE, 67056 Ludwigshafen	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation (common name and content)	None
Content of active substance (g/kg or g/L)	25 g/L		
Formulation (e.g. WP)	BAS 595 01 F (FS)	Residues calculated as:	Triticonazole

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 per Treatment			No of Treatm. and Last Date	Growth Stage (BBCH)[1]	Portion Analysed	Residues (mg/kg)	PHI (days)	Remarks
				kg a.s./hL	Water (L/ha)	g as/ha				Triticonazole		

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Triticonazole	Commercial Product (name)	-
Crop/crop group:	Wheat (Cereals)	Producer of commercial product	67056 Ludwigshafen, Germany
Responsible body for reporting (name, address)	BASF SE, 67056 Ludwigshafen	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation (common name and content)	None
Content of active substance (g/kg or g/L)	25 g/L		
Formulation (e.g. WP)	BAS 595 01 F (FS)	Residues calculated as:	Triticonazole

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 per Treatment			No of Treatm. and Last Date	Growth Stage (BBCH)[1]	Portion Analysed	Residues (mg/kg)	PHI (days)	Remarks
				kg a.s./hL	Water (L/ha)	g as/ha				Triticonazole		
407785 2014/1043281 47574 Goch Kleve, Germany L120656	GR 0654 KWS CHAMSIN	1. 12.04.2013 2. 07.07. - 19.07.2013 3. 26.08.2013	Seeding of treated seeds	n.a.	n.a.	11.25	1 12.04.13	n.a.	Whole plant[2)] Grain Straw	< 0.01 < 0.01 < 0.01	68 136 136	BASF Method No. 562/0
721605 2014/1090813 67294 Mauchenheim Rheinhessen, Germany L130814	GR 0654 KWS CHAMSIN	1. 08.03.2014 2. 08.06. - 16.06.2014 3. 25.07.2014	Seeding of treated seeds	n.a.	n.a.	11.25	1 08.03.14	n.a.	Whole plant[2)] Whole plant[2)] Whole plant[2)] Grain Straw	0.134 0.023 < 0.01 < 0.01 < 0.01	32 46 86 142 142	BASF Method No. 562/0
721605 2014/1090813 49700 Meigné sous Doué, Maine-et-Loire France (N) L130815	GR 0654 KWS CHAMSIN	1. 18.03.2014 2. 15.06. - 25.06.2014 3. 27.07.2014	Seeding of treated seeds	n.a.	n.a.	11.25	1 18.03.14	n.a.	Whole plant[2)] Whole plant[2)] Whole plant[2)]	0.202 0.028 < 0.01	28 50 85	BASF Method No. 562/0
721605 2014/1090813 6599 AV Ven Zelderheide, Gennep Netherlands L130816	GR 0654 KWS CHAMSIN	1. 01.04.2014 2. 16.06. - 01.07.2014 3. 05.08.2014	Seeding of treated seeds	n.a.	n.a.	11.25	1 01.04.14	n.a.	Whole plant[2)] Whole plant[2)] Whole plant[2)] Grain Straw	0.462 0.020 < 0.01 < 0.01 < 0.01	15 29 72 126 126	BASF Method No. 562/0

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Triticonazole	Commercial Product (name)	-
Crop/crop group:	Wheat (Cereals)	Producer of commercial product	67056 Ludwigshafen, Germany
Responsible body for reporting (name, address)	BASF SE, 67056 Ludwigshafen	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	None
Content of active substance (g/kg or g/L)	25 g/L	(common name and content)	
Formulation (e.g. WP)	BAS 595 01 F (FS)	Residues calculated as:	Triticonazole

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 per Treatment			No of Treatm. and Last Date	Growth Stage (BBCH)[1]	Portion Analysed	Residues (mg/kg)	PHI (days)	Remarks
				kg a.s./hL	Water (L/ha)	g as/ha				Triticonazole		
721605 2014/1090813 CO11 2NF Lawford Essex, UK L130817	GR 0654 KWS CHAMSIN	1. 18.03.2014 2. 20.06. - 30.06.2014 3. 01.08. - 22.08.2014	Seeding of treated seeds	n.a.	n.a.	11.25	1 18.03.14	n.a.	Whole plant[2)] Whole plant[2)] Whole plant[2)] Grain Straw	0.259 0.028 < 0.01 < 0.01 < 0.01	30 49 83 139 139	BASF Method No. 562/0

1) at treatment
2) no roots
n.a. not applicable

Southern Europe

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Triticonazole	Commercial Product (name)	-
Crop/crop group:	Wheat (Cereals)	Producer of commercial product	67056 Ludwigshafen, Germany
Responsible body for reporting (name, address)	BASF SE, 67056 Ludwigshafen	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation (common name and content)	None
Content of active substance (g/kg or g/L)	25 g/L		
Formulation (e.g. WP)	BAS 595 01 F (FS)	Residues calculated as:	Triticonazole

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 per Treatment			No of Treatm. and Last Date	Growth Stage (BBCH)[1]	Portion Analysed	Residues (mg/kg)	PHI (days)	Remarks
				kg a.s./hL	Water (L/ha)	g as/ha				Triticonazole		
407785 2014/1043281 32600 Endoufielle Gers, France L120657	GR 0654 ESTEROS	1. 01.02.2013 2. 20.05. - 25.05.2013 3. 12.07.2013	Seeding of treated seeds	n.a.	n.a.	11.25	1 01.02.13	n.a.	Whole plant[2)] Grain Straw	< 0.01 < 0.01 < 0.01	98 161 161	BASF Method No. 562/0
407785 2014/1043281 59032 Platanos Imathia, Greece L120658	GR 0654 ESTEROS	1. 20.03.2013 2. 20.05. - 30.05.2013 3. 10.07. - 25.07.2013	Seeding of treated seeds	n.a.	n.a.	11.25	1 20.03.13	n.a.	Whole plant[2)] Grain Straw	< 0.01 < 0.01 < 0.01	59 105 105	BASF Method No. 562/0
407785 2014/1043281 40051 Altedo of Malalbergo Bologna, Italy L120659	GR 0654 ESTEROS	1. 04.03.2013 2. 28.05. - 08.06.2013 3. 05.07.2013	Seeding of treated seeds	n.a.	n.a.	11.25	1 04.03.13	n.a.	Whole plant[2)] Whole plant[2)] Whole plant[2)] Grain Straw	0.20 0.087 < 0.01 < 0.01 < 0.01	23 36 71 123 123	BASF Method No. 562/0
407785 2014/1043281 ES-41710 Utrera Sevilla, Spain L120660	GR 0654 ESTEROS	1. 09.01.2013 2. 10.04. - 16.04.2013 3. 11.06.2013	Seeding of treated seeds	n.a.	n.a.	11.25	1 09.01.13	n.a.	Whole plant[2)] Whole plant[2)] Whole plant[2)] Grain Straw	0.52 0.18 < 0.01 < 0.01 < 0.01	20 34 84 153 153	BASF Method No. 562/0

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Triticonazole	Commercial Product (name)	-
Crop/crop group:	Wheat (Cereals)	Producer of commercial product	67056 Ludwigshafen, Germany
Responsible body for reporting (name, address)	BASF SE, 67056 Ludwigshafen	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation (common name and content)	None
Content of active substance (g/kg or g/L)	25 g/L		
Formulation (e.g. WP)	BAS 595 01 F (FS)	Residues calculated as:	Triticonazole

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 per Treatment			No of Treatm. and Last Date	Growth Stage (BBCH)[1]	Portion Analysed	Residues (mg/kg)	PHI (days)	Remarks
				kg a.s./hL	Water (L/ha)	g as/ha				Triticonazole		
721605 2014/1090813 32600 Endoufielle Gers, France L130818	GR 0654 ESTEROS	1. 20.02.2014 2. 30.05. - 06.06.2014 3. 17.07.2014	Seeding of treated seeds	n.a.	n.a.	11.25	1 20.02.14	n.a.	Whole plant[2)] Whole plant[2)] Whole plant[2)] Grain Straw	0.052 < 0.01 < 0.01 < 0.01 < 0.01	47 67 97 147 147	BASF Method No. 562/0
721605 2014/1090813 59032 Platanos Imathia, Greece L130819	GR 0654 ESTEROS	1. 21.02.2014 2. 10.05. - 25.05.2014 3. 01.07. - 15.07.2014	Seeding of treated seeds	n.a.	n.a.	11.25	1 21.02.14	n.a.	Whole plant[2)] Whole plant[2)] Whole plant[2)] Grain Straw	0.097 < 0.01 < 0.01 < 0.01 < 0.01	33 49 87 124 124	BASF Method No. 562/0
721605 2014/1090813 12050 Castagnito d'Alba Cuneo, Italy L130820	GR 0654 ESTEROS	1. 25.02.2014 2. 10.06. - 17.06.2014 3. 11.07.2014	Seeding of treated seeds	n.a.	n.a.	11.25	1 25.02.14	n.a.	Whole plant[2)] Whole plant[2)] Whole plant[2)] Grain Straw	0.64 < 0.01 < 0.01 < 0.01 < 0.01	35 48 104 136 136	BASF Method No. 562/0

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Triticonazole	Commercial Product (name)	-
Crop/crop group:	Wheat (Cereals)	Producer of commercial product	67056 Ludwigshafen, Germany
Responsible body for reporting (name, address)	BASF SE, 67056 Ludwigshafen	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation (common name and content)	None
Content of active substance (g/kg or g/L)	25 g/L		
Formulation (e.g. WP)	BAS 595 01 F (FS)	Residues calculated as:	Triticonazole

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 per Treatment			No of Treatm. and Last Date	Growth Stage (BBCH)[1]	Portion Analysed	Residues (mg/kg)	PHI (days)	Remarks
				kg a.s./hL	Water (L/ha)	g as/ha				Triticonazole		
721605 2014/1090813 ES-41710 Utrera Sevilla, Spain L130821	GR 0654 ESTEROS	1. 27.01.2014 2. 20.04. - 05.05.2014 3. 10.06.2014	Seeding of treated seeds	n.a.	n.a.	11.25	1 27.01.14	n.a.	Whole plant[2)] Whole plant[2)] Whole plant[2)] Grain Straw	0.446 0.064 < 0.01 < 0.01 < 0.01	22 39 79 134 134	BASF Method No. 562/0

1) at treatment
2) no roots
n.a. not applicable

Triticonazole

Document M-CA, Section 7

FATE AND BEHAVIOUR IN THE ENVIRONMENT

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[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

Data on the fate and behaviour of triticonazole in the environment were submitted within the EU Dossier which resulted in the Annex I inclusion under Directive 2006/39/EC in 2006. In this Supplemental Dossier for renewal of approval, only those environmental fate studies are summarised which are submitted for the first time. However, the relevant results of the studies already evaluated are also presented in order to provide an overall picture of the fate and behaviour of triticonazole in the environment.

The chemical structure of triticonazole contains an exocyclic double bond which allows two relative positions of the groups attached (nomenclature E/Z) as well as one chiral centre in the cyclopentane ring which allows two configurations (R/S) for the E and Z isomers, respectively.

Triticonazole is a racemate of the two enantiomers of the E isomer: (RS)-(5E)-5-(4-chlorobenzylidene)-2,2-dimethyl-1-(1H-1,2,4-triazol-1-ylmethyl)cyclopentanol.

The triticonazole test items used in the studies were either ^{14}C-labelled in the phenyl or triazole rings or non-radiolabeled. Studies with phenyl-U-^{14}C labelled triticonazole show same metabolite pattern as studies with triazole-U-^{14}C labelled triticonazole. Accordingly, the used radiolabel positions (shown below) are sufficient to define the routes of degradation of triticonazole in the environment. Furthermore, studies with radiolabelled metabolites were performed. As all of them contain the phenyl and triazole ring, the same labelling positions as for the parent where used. All major metabolites observed or used as test items in the studies are presented below.

Special emphasis was given to the investigation of the fate of the enantiomers of triticonazole. Results obtained in individual studies will be presented in the study summaries below. Additionally, a comprehensive discussion will be provided in document N5.

Names and structures of compounds mentioned in this chapter is provided in Table 7-1. CAS-Codes and IUPAC-names are included in document N3.

Table 7-1: Substances and metabolites in this chapter; structures, codes, synonyms

Substance Code Other codes	Structure
BAS 595 F **RPA 400727** Label: phenyl-U-^{14}C M595F000 Reg. No: 4378513	HO, ^{14}C, Cl
BAS 595 F **RPA 400727** Label: triazole 3(5) ^{14}C M595F000 Reg. No: 4378513	HO, Cl
RPA 404766 Cis-diol M595F001 Reg. No.: 5079285	AND Enantiomer; HO, OH, Cl
RPA 406341 Trans-diol AE 0540093 M595F002 Reg. No. 5059144	AND Enantiomer; HO, OH, Cl
RPA 407922 M595F013 Reg. No.: 5079288	HO, OH, Cl

Table 7-1: Substances and metabolites in this chapter; structures, codes, synonyms

Substance Code Other codes	Structure
RPA 406203 Z-Isomer of triticonazole M595F014 Reg. No.: 5079359	N N N Cl OH H_3C H_3C

CA 7.1 Fate and behaviour in soil

CA 7.1.1 Route of degradation in soil

CA 7.1.1.1 Aerobic degradation

A total of six studies investigating the route of degradation of triticonazole in aerobic soil were evaluated during the Annex I inclusion and accepted by the European Commission (SANCO/10442/2005-Final rev 1, 12 March 2010). All of these previously submitted studies are still considered valid and scientifically sound, although some of them show deviations to the current OECD 307 Test Guideline (e.g. study duration exceeded 120 days, different temperature and moisture conditions). In some of those studies metabolites occur above 5% which were not identified sufficiently due to a higher trigger value for identification at the time of study performance. Therefore, to comply with the current guidance, a new study was performed to confirm the metabolic patterns observed in the studies submitted previously and to elucidate the degradation of the enantiomers.

The previously submitted studies are listed below, deviations from current guidance are presented. The summary of the new soil metabolism study is provided hereafter in the annex point CA 7.1.1.1./1. A short summary of the results and relevant parameters of all individual studies is presented in Table 7.1.1.3-1.

Report:	**C017917; Ayliffe J.M., Austin D.J. (1993), also listed in CA 7.1.2.1.1** Fungicides: RPA 400727-^{14}C: Aerobic soil metabolism in three soils (Final Report). 428635 / P91/326
GLP:	yes
Guidelines:	USEPA (= EPA) N, 162-1 (1982) (not stated in report)

Please see the full summary details presented in Volume 3, Section B.8 of the DAR for triticonazole (Austrian Federal Office for Food Safety, 2003). The study is still considered valid but has some deviations to the current Test Guideline OECD 307. Two metabolites with maximum occurrence above 5% were not identified. Furthermore, in one of the investigated soils (Speyer 2.2) a low degradation was found and the results for this soil were considered to be not reliable (refer to Addendum R012981 for results of this soil). A short summary of the results and relevant parameters of the study is presented in Table 7.1.1.3-1.

Report: **R012979; Ayliffe J.M., Godward P.J., (1993), also listed in CA 7.1.2.1.1**
Fungicides: RPA 400727-^{14}C: Rate of degradation in four soils
200234 / P91/411 / GOoD2486
GLP: yes
Guidelines: Dutch Guidelines for the Submission of Applications for Registration of Pesticides, Part G.1

Please see the full summary details presented in Volume 3, Section B.8 of the DAR for triticonazole (Austrian Federal Office for Food Safety, 2003). The study is still considered valid but has some deviations to the current Test Guideline OECD 307. The study is still considered valid but has some deviations to the current Test Guideline OECD 307. Two metabolites with maximum occurrence above 5% were not identified. A short summary of the results and relevant parameters of the study is presented in Table 7.1.1.3-1.

Report: **R012981; Ayliffe J.M., McMillan-Staff S.L. (1994), also listed in CA 7.1.2.1.1**
Addendum Report: Fungicides: RPA 400727-^{14}C: Aerobic soil metabolism in three soils.
200471 / P91/326
GLP: yes
Guidelines: USEPA (= EPA) N, 162-1 (1982) (not stated in report)

Please see the full summary details presented in Volume 3, Section B.8 of the DAR for triticonazole (Austrian Federal Office for Food Safety, 2003).The study is still considered valid but has some deviations to the current Test Guideline OECD 307. Three metabolites with maximum occurrence above 5% were not identified. A short summary of the results and relevant parameters of the study is presented in Table 7.1.1.3-1.

Report: **R012994; Doble M.L., Ferreira E.M., Hardy I.A.J. (1996), also listed in CA 7.1.2.1.1**
(^{14}C)-triazole labelled triticonazole: Rate of degradation in clay soil under aerobic conditions.
201171 / P94/158 / GOoD8999
GLP: yes
Guidelines: USEPA (= EPA) N, 162-1 (1982) and Draft European Uniform Guidelines (Lynch 1993)

Please see the full summary details presented in Volume 3, Section B.8 of the DAR for triticonazole (Austrian Federal Office for Food Safety, 2003). The study is still considered valid but has some deviations to the current Test Guideline OECD 307. One non-identified metabolite reaches its maximum occurrence of 3.7% on the last day of the study. A short summary of the results and relevant parameters of the study is presented in Table 7.1.1.3-1.

Report: **R012995; Simmonds M.B., Hardy I.J., Ferreira E.M., (1996), also listed in CA 7.1.2.1.1**
Triticonazole: Rate of degradation in one soil type under aerobic conditions with regard to varying temperature, soil moisture, treatment rate and soil viability.
201173 / P94/141 / GOoD7834
GLP: yes
Guidelines: Danish Agency of Environmental Protection Guidelines Sub-Section 21.1 Transformation and Degradation in Soil (1988) and Draft European Guidelines (Lynch 1993)

Please see the full summary details presented in Volume 3, Section B.8 of the DAR for triticonazole (Austrian Federal Office for Food Safety, 2003). The study is still considered valid but has some deviations to the current Test Guideline OECD 307. The summary of minor metabolites contains individual components which were not identified. A short summary of the results and relevant parameters of the study is presented in Table 7.1.1.3-1.

Report: **C021044; Simmonds M., Lowden P., (2002)**
[^{14}C]-Triticonazole: Generation and identification of soil metabolites.
CX/01/011 / C021044 / GOoD 28047
GLP: yes
Guidelines: EU (=EEC) 95/36/EC, Section 7.1.1.2.2, (1995)

Please see the full summary details presented in Volume 3, Section B.8 of the DAR for triticonazole (Austrian Federal Office for Food Safety, 2003). The study is still considered valid. A short summary of the results and relevant parameters of the study is presented in Table 7.1.1.3-1.

Although all previously listed studies were considered as valid, to comply with the current guidance, a new study was performed. Goals of the study were to confirm the metabolic patterns observed in the studies submitted previously and to elucidate the degradation of the enantiomers.

Report: CA 7.1.1.1/1
Ta C.,Strobush A., 2015a
Aerobic soil metabolism of 14C-BAS 595 F
2014/7000472
Guidelines: EPA 835.4100, OECD 307 (2002), SETAC
GLP: yes
(certified by United States Environmental Protection Agency)

Also listed in annex Points CA 7.1.2.1.1/1 and CA 7.1.2.1.2/1.

Executive Summary

The metabolism of BAS 595 F under aerobic conditions was investigated in one German soil (Li 10) by using two separate radiolabelled BAS 595 F compounds, [phenyl-U-^{14}C] and [triazole-3(5)-^{14}C]-BAS 595 F, respectively. For this purpose, soil samples (50 g of dry soil) were treated with the test items at a rate of 0.2 mg/kg soil, respectively, corresponding to 4-times the proposed maximum field application rate of 12.5 g a.i./ha. The treated soils samples were maintained aerobically in the dark at 20 ± 2°C for 120 days. Prior to the BAS 595 F application, the soil moisture was adjusted to approximately 50% maximum water holding capacity (MWHC) which was maintained throughout the experiment. Duplicate samples were collected at 0, 3, 8, 14, 30, 59, 91, and 120 days after treatment (DAT). The soil samples were extracted with appropriate solvents and extracts were analysed for radioactivity by LSC and then combined. To determine the metabolite pattern, samples were also analysed by HPLC. A second HPLC method (chiral) was used for analysis of extracts taken at the beginning (0 DAT), middle (59 DAT) and the end (120 DAT) of the incubation. Identification of main metabolites was performed by LC-MS/MS and/or retention time matching with standards by HPLC. The evolved $^{14}CO_2$ was trapped in sodium hydroxide solutions (1N) and analysed by LSC.

The mean material balances for the [phenyl-U-^{14}C]-BAS 595 F ranged from 98.21 to 105.32% of the total applied radioactivity (TAR). The non-extractable [^{14}C]-residues (NER) increased to 13.62% TAR at 120 DAT. The recovered volatile radioactivity reached a maximum of 2.53% TAR at 120 DAT. Considering [triazole-3(5)-^{14}C]-BAS 595 F, the mean material balances ranged from 94.74 to 98.51% TAR. At the end of the study, NER amounted to 15.18% and the $^{14}CO_2$ produced was 0.41% TAR at maximum at 120 DAT.

The study demonstrated that BAS 595 F steadily degraded in soil under aerobic conditions. The DT_{50} value for BAS 595 F in Li 10 soil was approximately 149 days. There were only two metabolites, Reg. No. 5079285 (RPA 407466) and Reg. No. 5059144 (RPA 406341), greater than 5% TAR, with maximum occurrences of 6.99 and 6.17% TAR in Li 10 soil. Some minor degradation products were also found, each representing less than 5% TAR. Furthermore, it can be concluded that both the *R* and *S* isomers of the racemic parent are comparably degradable. The same is indicated for the major metabolite Reg. No. 5059144 (RPA 406341).

I. MATERIAL AND METHODS

A. MATERIALS

1. Test material:

	[phenyl-U-^{14}C]-BAS 595 F
Reg. No.	4378513
Lot/Batch number	866-1401
Molecular Weight	317.82 g/mol (non-labelled)
Site of radiocarbon labeling:	phenyl-U-^{14}C
Radiochemical purity:	99.5%
Specific activity of ai:	357000 dpm/µg (5.95 MBq/mg)

	[triazole-3(5)-^{14}C]-BAS 595 F
Reg. No.	4378513
Lot/Batch number	867-1301
Molecular Weight	317.82 g/mol (non-labelled)
Site of radiocarbon labeling:	triazole-3(5)-^{14}C
Radiochemical purity:	99.3%
Specific activity of ai:	391200 dpm/µg (6.52 MBq/mg)

2. Soil

The soil was a loamy sand (Li 10) from Germany, representative of the intended use areas. The soil was collected from the field, 2-mm mesh-sieved and stored in the refrigerator. Soil moisture was adjusted to approximately 50% MWHC prior to the application of the test solutions and maintained throughout the incubation period. Soil characterization is presented in the table below.

Table 7.1.1.1-1: Properties of the test soil

Name:		**Li 10**
USDA Textural class:		Loamy sand
Sand [%]		80
Silt [%]		12
Clay [%]		8
Cation Exchange Capacity	[meq/ 100 g]	6.2
Max. Water Hold. Capacity	[gm/100 g dry soil]	22.2
Total Organic Matter	[%]	1.4
Total Organic Carbon	[%]	0.81[a]
pH (water)		6.7
pH ($CaCl_2$)		6.3
Microbial Biomass Carbon at 0 DAT	[µg/g dry soil]	281.1
Microbial Biomass Carbon at 153 DAT	[µg/g dry soil]	313.4
Bulk Density	[g/cc]	1.37

[a]: Total organic carbon percent = percent organic matter / 1.724; DAT = days after treatment

B. STUDY DESIGN

1. Test system

Soil aliquots (50 g of dry weight) were placed in 250-mL polypropylene bottles and treated with the test item at a rate of 0.2 mg/kg soil, corresponding to 4-times the proposed maximum field application rate of 12.5 g a.i./ha. This elevated application rate will allow for the identification and quantitation of the parent and metabolites. The treated soil samples were connected to a flow-through test system and incubated in the dark at 20 ± 2°C for 120 days. Moisturized and CO_2-free air was passed over the soil in order to maintain the aerobic conditions. The evolved $^{14}CO_2$ was trapped in NaOH solutions (1 N).

2. Sampling

Duplicate samples were collected at 0, 3, 8, 14, 30, 59, 91, and 120 DAT. The traps were replaced with fresh aqueous sodium hydroxide solutions (1N) at each sampling time. The soil samples were extracted and processed immediately after sampling.

3. Analytical procedure

Each soil sample was sequentially extracted with 100 mL of acetonitrile, acetonitrile: methanol (7:3), acetonitrile: water (7:3) and methanol: water (7:3) by shaking for 30 minutes at 300 rpm and then, centrifugation for 15 minutes at 4000 rpm. Each extract was brought to a volume of 100 mL, assayed by LSC. Then all extracts were combined. An aliquot of each pooled organic solvent extracts was concentrated, diluted with acetonitrile and analyzed by LSC and HPLC. Identification of the transformation products was performed by LC-MS/MS and/or retention time matching with standards by HPLC. Furthermore, chiral reverse phase HPLC analysis for the isomeric identification and separation of BAS 595 F and its metabolites.

The extracted soil samples were air-dried, and the amount of non-extractable residues (NER) was determined by oxidative combustion analysis. The extracted soil samples at 59 DAT and 120 DAT were further characterized for fulvic acid, humic acid and humin contents using an extraction procedure with NaOH (0.5 N) and precipitation of humic acids by HCl. The respective solvents were analyzed by LSC. Humins were determined by oxidative combustion analysis.

4. Determination of degradation kinetics

Kinetic evaluations were carried out for BAS 595 F according to FOCUS kinetic guidance to derive endpoints as triggers for further analysis (triggers endpoints). In addition, a full degradation pathway model was also investigated to determine kinetic endpoints (DT_{50}, DT_{90} and formation fractions) for the metabolites exceeding 10% TAR at any single sampling time or 5% TAR in two consecutive sampling times.

The guidance of FOCUS (2006) was used as the basis for conducting the kinetic analysis, statistical assessment, and selection of the best fit kinetic model. Optimization of model parameters, including estimation of parameter standard errors, was performed using the software KinGUII v. 2.2012.202.925.

II. RESULTS AND DISCUSSION

A. MASS BALANCE

The total recoveries of radioactivity from soils treated with [phenyl-U-^{14}C]-BAS 595 F and [triazole-3(5)-^{14}C]-BAS 595 F are presented in Table 7.1.1.1-2 and Table 7.1.1.1-3, respectively.

Table 7.1.1.1-2: Material Balance of [phenyl-U-^{14}C]-BAS 595 F in Li 10 Soil (%TAR)

DAT	Extracts				ERR	NER	Volatiles NaOH	Material Balance
	1	2	3	4				
0 rep 1	83.10	14.00	2.39	0.41	99.91	0.09	NA	100.00
0 rep 2	84.36	14.05	2.34	0.43	101.19	0.10	NA	101.28
0 mean	**83.73**	**14.03**	**2.37**	**0.42**	**100.55**	**0.09**	**NA**	**100.64**
3 rep 1	80.44	14.81	4.00	0.97	100.22	1.70	0.10	102.01
3 rep 2	78.14	14.02	3.94	0.94	97.05	1.80	0.11	98.96
3 mean	**79.29**	**14.42**	**3.97**	**0.95**	**98.63**	**1.75**	**0.11**	**100.49**
8 rep 1	75.37	14.77	3.98	1.01	95.14	2.29	0.21	97.63
8 rep 2	75.73	14.90	4.49	1.21	96.33	2.94	0.23	99.50
8 mean	**75.55**	**14.84**	**4.24**	**1.11**	**95.73**	**2.61**	**0.22**	**98.57**
14 rep 1	74.92	13.93	5.11	1.38	95.34	4.20	0.38	99.92
14 rep 2	74.83	13.85	4.95	1.31	94.93	3.98	0.41	99.32
14 mean	**74.88**	**13.89**	**5.03**	**1.34**	**95.14**	**4.09**	**0.40**	**99.62**
30 rep 1	70.80	13.71	5.45	1.68	91.65	6.69	0.83	99.16
30 rep 2	69.60	14.38	5.40	1.63	91.01	6.41	0.83	98.25
30 mean	**70.20**	**14.04**	**5.42**	**1.66**	**91.33**	**6.55**	**0.83**	**98.71**
59 rep 1	67.28	13.93	5.14	1.72	88.08	8.62	1.59	98.29
59 rep 2	66.99	14.36	5.61	1.91	88.87	10.01	1.30	100.18
59 mean	**67.13**	**14.15**	**5.38**	**1.82**	**88.47**	**9.31**	**1.45**	**99.23**
91 rep 1	66.96	14.58	6.22	2.09	89.85	12.00	2.32	104.17
91 rep 2	67.27	15.08	6.84	2.29	91.48	13.22	1.76	106.47
91 mean	**67.12**	**14.83**	**6.53**	**2.19**	**90.66**	**12.61**	**2.04**	**105.32**
120 rep 1	60.55	13.15	6.13	2.17	82.00	13.24	2.85	98.09
120 rep 2	60.33	12.96	6.41	2.42	82.12	14.00	2.22	98.34
120 mean	**60.44**	**13.06**	**6.27**	**2.29**	**82.06**	**13.62**	**2.53**	**98.21**

Extract 1 = Acetonitrile; Extract 2 = Acetonitrile:Methanol (7:3); Extract 3 = Acetonitrile:Water (7:3);
Extract 4 = Methanol:Water (7:3); ERR= Extractable Radioactive Residues; NER = Non Extractable Residues (by combustion)
NA = Not Applicable (no sample analyzed). There were no volatile traps collected for 0 DAT

Table 7.1.1.1-3: Material Balance of [triazole-3(5)-^{14}C]-BAS 595 F in Li 10 Soil (%TAR)

DAT	Extracts				ERR	NER	Volatiles NaOH	Material Balance
	1	2	3	4				
0 rep 1	83.04	13.88	2.48	0.46	99.87	0.13	NA	100.00
0 rep 2	81.15	12.85	2.45	0.44	96.89	0.14	NA	97.02
0 mean	**82.09**	**13.37**	**2.47**	**0.45**	**98.38**	**0.13**	**NA**	**98.51**
3 rep 1	79.80	13.97	4.10	0.99	98.86	1.74	0.07	100.66
3 rep 2	74.96	13.64	4.28	1.04	93.92	1.92	0.06	95.91
3 mean	**77.38**	**13.81**	**4.19**	**1.01**	**96.39**	**1.83**	**0.07**	**98.29**
8 rep 1	71.33	14.76	5.23	1.36	92.67	3.39	0.09	96.15
8 rep 2	71.45	14.87	5.36	1.40	93.08	3.61	0.08	96.77
8 mean	**71.39**	**14.82**	**5.30**	**1.38**	**92.88**	**3.50**	**0.09**	**96.47**
14 rep 1	71.35	13.94	5.62	1.55	92.46	4.63	0.11	97.19
14 rep 2	71.38	14.48	5.47	1.56	92.90	4.73	0.10	97.72
14 mean	**71.37**	**14.21**	**5.55**	**1.56**	**92.68**	**4.68**	**0.10**	**97.46**
30 rep 1	65.76	13.46	5.83	1.75	86.80	7.51	0.15	94.45
30 rep 2	70.90	14.15	5.94	1.84	92.82	7.83	0.14	100.79
30 mean	**68.33**	**13.80**	**5.88**	**1.79**	**89.81**	**7.67**	**0.15**	**97.62**
59 rep 1	61.35	13.71	6.06	1.99	83.11	10.82	0.23	94.16
59 rep 2	63.44	13.92	5.99	1.95	85.29	10.54	0.22	96.05
59 mean	**62.39**	**13.82**	**6.02**	**1.97**	**84.20**	**10.68**	**0.23**	**95.10**
91 rep 1	59.02	13.39	6.76	2.24	81.40	13.49	0.33	95.23
91 rep 2	63.04	13.73	6.40	2.14	85.32	13.88	0.34	99.54
91 mean	**61.03**	**13.56**	**6.58**	**2.19**	**83.36**	**13.69**	**0.33**	**97.38**
120 rep 1	56.69	12.24	6.73	2.46	78.12	14.97	0.42	93.51
120 rep 2	57.59	13.29	6.92	2.40	80.19	15.38	0.40	95.97
120 mean	**57.14**	**12.76**	**6.82**	**2.43**	**79.16**	**15.18**	**0.41**	**94.74**

Extract 1 = Acetonitrile; Extract 2 = Acetonitrile:Methanol (7:3); Extract 3 = Acetonitrile:Water (7:3);
Extract 4 = Methanol:Water (7:3); ERR= Extractable Radioactive Residues; NER = Non Extractable Residues (by combustion)
NA = Not Applicable (no sample analyzed). There were no volatile traps collected for 0 DAT

For the 59 DAT samples treated with [phenyl-U-^{14}C]-BAS 595 F, the NER (9.31% TAR) were further characterized for their fulvic (4.73%), humic (1.54% TAR), and humin (2.76% TAR) content. At 120 DAT, the NER content increased to 13.62% TAR, from which the fulvic, humic and humin components amounted for 6.35, 2.64 and 3.82% TAR, respectively.

Considering 59 DAT samples treated with [triazole-3(5)-^{14}C]-BAS 595 F, the radioactivity recovered in the NER (10.68% TAR) as fulvic, humic and humin components was 6.19, 1.39 and 2.82% TAR, respectively. At 120 DAT, the NER content increased to 15.18% TAR, from which the fulvic, humic and humin components amounted for 8.84, 2.17 and 3.84% TAR, respectively.

B. CHARACTERISATION AND IDENTIFICATION OF RESIDUES IN EXTRACTS

The amounts of BAS 595 F and its metabolites recovered at each time point are presented in the tables below. Results showed that only two metabolites, Reg. No. 5079285 (RPA 404766) (at t_R of ~25.3 min.) and Reg. No. 5059144 (RPA 406341) (at t_R of ~28.5 min), were greater than 5% TAR, with maximum occurrences of 6.99 and 6.17% TAR. Some minor degradation products were also found, each representing less than 5% TAR.

Table 7.1.1.1-4: HPLC Quantitation of [phenyl-U-^{14}C]-BAS 595 F Residues in Li 10 Extract

DAT	tR (min)							
	25.5-25.6 Reg. No. 5079285 (RPA 404766	**25.8**	**27.1-27.3**	**28.6-28.7 Reg. No. 5059144 (RPA 406341)**	**28.9-29.0**	**29.1-29.2**	**29.5-29.8**	**30.6-30.8**
0 rep 1	**0.00**	0.00	0.00	**0.00**	0.00	0.00	0.00	0.00
0 rep 2	**0.00**	0.00	0.00	**0.00**	0.00	0.00	0.00	0.00
0 mean	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
3 rep 1	**0.00**	0.00	0.00	**0.00**	0.00	0.00	0.00	0.00
3 rep 2	**0.00**	0.00	0.00	**0.00**	0.00	0.00	0.00	0.00
3 mean	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
8 rep 1	**1.66**	0.00	0.00	**1.71**	1.42	0.00	0.00	0.00
8 rep 2	**2.39**	0.00	0.00	**1.56**	1.65	0.00	0.00	0.00
8 mean	**2.03**	**0.00**	**0.00**	**1.64**	**1.53**	**0.00**	**0.00**	**0.00**
14 rep 1	**2.49**	0.00	0.00	**2.20**	1.41	0.00	0.00	0.00
14 rep 2	**4.06**	0.00	0.00	**2.91**	1.92	0.00	0.00	0.00
14 mean	**3.28**	**0.00**	**0.00**	**2.59**	**1.66**	**0.00**	**0.00**	**0.00**
30 rep 1	**4.05**	0.00	0.42	**2.67**	1.90	0.00	0.00	0.00
30 rep 2	**5.17**	0.00	0.49	**3.64**	2.49	0.00	0.00	0.00
30 mean	**4.61**	**0.00**	**0.46**	**3.15**	**2.20**	**0.00**	**0.00**	**0.00**
59 rep 1	**6.10**	0.00	1.57	**4.35**	3.23	0.00	0.00	0.00
59 rep 2	**5.39**	0.00	1.33	**5.51**	3.77	0.00	0.00	0.00
59 mean	**5.74**	**0.00**	**1.45**	**4.93**	**3.50**	**0.00**	**0.00**	**0.00**
91 rep 1	**6.98**	0.00	1.21	**5.56**	4.08	0.00	0.00	0.35
91 rep 2	**7.00**	0.00	1.12	**6.18**	4.87	0.00	0.55	0.63
91 mean	**6.99**	**0.00**	**1.16**	**5.87**	**4.47**	**0.00**	**0.27**	**0.49**
120 rep 1	**5.35**	0.00	1.26	**5.78**	3.78	1.07	0.23	0.57
120 rep 2	**5.57**	0.44	1.68	**5.88**	4.83	0.69	0.83	0.00
120 mean	**5.46**	**0.22**	**1.47**	**5.83**	**4.30**	**0.88**	**0.53**	**0.29**

DAT	31.6-32.1	32.1-32.3	32.5-32.7	34.3-34.6 BAS 595 F	34.9-35.1	34.9-36.2	36.9-37.2
0 rep 1	0.00	0.00	0.00	**99.91**	0.00	0.00	0.00
0 rep 2	0.00	0.00	0.00	**101.19**	0.00	0.00	0.00
0 mean	**0.00**	**0.00**	**0.00**	**100.55**	**0.00**	**0.00**	**0.00**
3 rep 1	0.00	0.00	0.00	**97.29**	1.78	1.78	1.14
3 rep 2	0.00	0.00	0.00	**95.06**	1.99	1.99	0.00
3 mean	**0.00**	**0.00**	**0.00**	**96.18**	**1.89**	**1.89**	**0.57**
8 rep 1	0.00	0.00	0.00	**90.34**	0.00	0.00	0.00
8 rep 2	0.00	0.00	0.00	**90.73**	0.00	0.00	0.00
8 mean	**0.00**	**0.00**	**0.00**	**90.54**	**0.00**	**0.00**	**0.00**
14 rep 1	0.00	0.00	0.00	**89.24**	0.00	0.00	0.00
14 rep 2	0.00	0.00	0.00	**86.04**	0.00	0.00	0.00
14 mean	**0.00**	**0.00**	**0.00**	**87.64**	**0.00**	**0.00**	**0.00**
30 rep 1	0.00	0.00	1.83	**79.83**	0.00	0.62	0.33
30 rep 2	0.64	0.00	2.07	**76.51**	0.00	0.00	0.00
30 mean	**0.32**	**0.00**	**1.95**	**78.17**	**0.00**	**0.31**	**0.16**
59 rep 1	0.92	0.00	1.82	**69.46**	0.00	0.63	0.00
59 rep 2	1.44	0.00	2.42	**68.16**	0.00	0.84	0.00
59 mean	**1.18**	**0.00**	**2.12**	**68.81**	**0.00**	**0.74**	**0.00**
91 rep 1	1.5	1.07	3.25	**64.45**	0.00	1.40	0.00
91 rep 2	1.12	0.7	3.7	**64.39**	0.00	1.21	0.00
91 mean	**1.31**	**0.89**	**3.48**	**64.42**	**0.00**	**1.3**	**0.00**
120 rep 1	1.87[a]	0	2.48	**58.28**	0.00	1.31	0.00
120 rep 2	1.21[a]	1.22	3.53	**54.98**	0.00	1.28	0.00
120 mean	**1.54**	**0.61**	**3.01**	**56.63**	**0.00**	**1.30**	**0.00**

a Two extremely close peaks were summed

Table 7.1.1.1-5: HPLC Quantitation of [triazole-3(5)-^{14}C]-BAS 595 F Residues in Li 10 Extract

DAT	tR (min)							
	5.3-5.7	25.3-25.6 Reg. No. 5079285 (RPA 404766)	27.1-27.4	28.5-28.7 Reg. No. 5059144 (RPA 406341)	28.9-29.0	29.1-29.2	29.5-29.8	30.6-30.8
0 rep 1	0.00	**0.00**	0.00	**0.00**	0.00	0.00	0.00	0.00
0 rep 2	0.00	**0.00**	0.00	**0.00**	0.00	0.00	0.00	0.00
0 mean	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
3 rep 1	0.00	**0.86**	0.00	**0.00**	0.00	2.04	0.00	0.00
3 rep 2	0.00	**1.47**	0.00	**2.15**	0.00	0.00	0.00	0.00
3 mean	**0.00**	**1.16**	**0.00**	**1.08**	**0.00**	**1.02**	**0.00**	**0.00**
8 rep 1	0.00	**2.68**	0.00	**2.11**	1.06	0.00	0.00	0.00
8 rep 2	0.00	**2.34**	0.00	**3.36**	0.00	0.00	0.00	0.00
8 mean	**0.00**	**2.51**	**0.00**	**2.74**	**0.53**	**0.00**	**0.00**	**0.00**
14 rep 1	0.00	**2.90**	0.00	**2.45**	1.51	0.00	0.00	0.00
14 rep 2	0.00	**3.18**	0.00	**2.74**	1.54	0.00	0.00	0.00
14 mean	**0.00**	**3.04**	**0.00**	**2.60**	**1.52**	**0.00**	**0.00**	**0.00**
30 rep 1	0.86	**4.96**	0.82	**3.65**	3.44	0.00	0.00	0.00
30 rep 2	0.86	**5.50**	0.66	**3.87**	2.16	0.00	0.00	0.00
30 mean	**0.86**	**5.23**	**0.74**	**3.76**	**2.80**	**0.00**	**0.00**	**0.00**
59 rep 1	1.48[a]	**5.73**	1.07	**4.67**	3.57	0.00	0.00	0.00
59 rep 2	1.10[a]	**5.70**	0.60	**5.18**	2.81	0.00	0.00	0.00
59 mean	**1.29[a]**	**5.71**	**0.83**	**4.92**	**3.19**	**0.00**	**0.00**	**0.00**
91 rep 1	1.83[a]	**5.71**	0.63	**5.19**	4.38	0.00	0.69	0.00
91 rep 2	1.16	**6.25**	1.97	**5.67**	4.49	0.00	0.00	0.00
91 mean	**1.50**	**5.98**	**1.30**	**5.43**	**4.43**	**0.00**	**0.35**	**0.00**
120 rep 1	1.58[a]	**6.16**	1.80	**5.86**	4.30	0.00	0.36	0.84
120 rep 2	1.39[a]	**6.33**	1.27	**6.48**	3.70	0.00	0.00	0.90
120 mean	**1.48**	**6.25**	**1.54**	**6.17**	**4.00**	**0.00**	**0.18**	**0.87**

DAT	31.6-32.1	32.1-32.3	32.5-32.8	34.3-34.6 BAS 595F	34.9-36.2	36.8-37.2	Sum Others
0 rep 1	0.00	0.00	0.00	**98.03**	0.00	1.83	0.00
0 rep 2	0.00	0.00	0.00	**95.55**	0.00	1.33	0.00
0 mean	**0.00**	**0.00**	**0.00**	**96.80**	**0.00**	**1.58**	**0.00**
3 rep 1	0.00	0.00	0.00	**92.68**	0.00	3.28	0.00
3 rep 2	0.00	0.00	0.00	**89.09**	0.00	1.21	0.00
3 mean	**0.00**	**0.00**	**0.00**	**90.89**	**0.00**	**2.25**	**0.00**
8 rep 1	0.00	0.00	1.12	**84.50**	0.00	1.20	0.00
8 rep 2	0.00	0.00	0.00	**85.71**	0.00	1.68	0.00
8 mean	**0.00**	**0.00**	**0.56**	**85.10**	**0.00**	**1.44**	**0.00**
14 rep 1	0.00	0.00	1.28	**82.70**	0.87	0.75	0.00
14 rep 2	0.00	0.00	1.77	**82.12**	0.72	0.83	0.00
14 mean	**0.00**	**0.00**	**1.53**	**82.41**	**0.79**	**0.79**	**0.00**
30 rep 1	0.62	0.00	1.45	**68.50**	1.65	0.86	0.00
30 rep 2	0.61	0.00	1.91	**74.41**	0.96	1.86	0.00
30 mean	**0.62**	**0.00**	**1.68**	**71.46**	**1.30**	**1.36**	**0.00**
59 rep 1	1.26	0.00	2.57	**61.50**	0.58	0.67	0.00
59 rep 2	0.87	1.09	3.12	**63.06**	1.15	0.61	0.00
59 mean	**1.07**	**0.55**	**2.84**	**62.28**	**0.87**	**0.64**	**0.00**
91 rep 1	1.95[c]	0.00	3.54	**55.55**	0.75	0.51	0.64
91 rep 2	1.59	0.00	3.27	**58.11**	1.01	1.12	0.68
91 mean	**1.77**	**0.00**	**3.40**	**56.83**	**0.88**	**0.82**	**0.66**
120 rep 1	1.52	1.00	3.10	**48.50**	1.16	0.58	1.36[a]
120 rep 2	1.10	1.25	2.51	**51.87**	1.14	0.74	1.54[b]
120 mean	**1.31**	**1.13**	**2.81**	**50.19**	**1.15**	**0.66**	**1.45**

a,b Sum of three peaks
c Two extremely close peaks were summed.

C. ISOMERIZATION

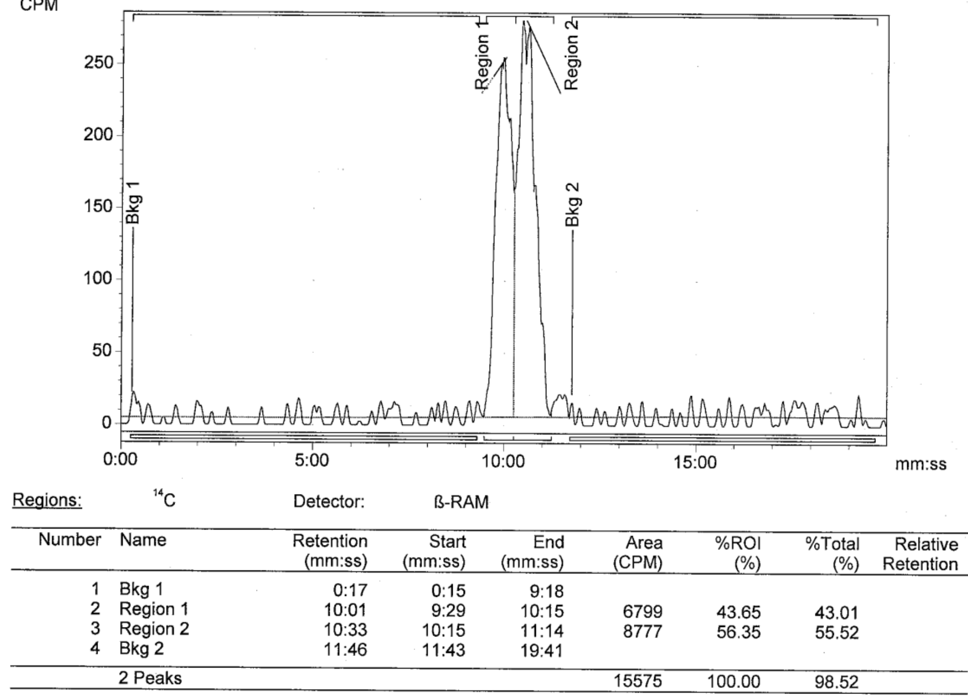
Results of the non-chiral HPLC method showed that the E isomer was the major component of BAS 595 F, while the levels of the Z isomer were not detected in all samples during the incubation, suggesting that there were no significant conversions of the E to the Z isomers.

Additionally, chiral-HPLC analysis of samples taken at the beginning (0 DAT), middle (59 DAT) and the end (120 DAT) of the incubation demonstrated that no significant metabolic preference for the R versus S stereo isomer occurred during the soil metabolism of the parent compound BAS 595 F.

Table 7.1.1.1-6: Isomer Ratios from Selected Soil Extract Samples Throughout the Study

DAT	Label	R/S Ratio
0	Phenyl	1.00
	Triazole	1.04
59	Phenyl	1.02
	Triazole	1.04
120	Phenyl	1.12
	Triazole	1.03

It can be concluded that both the R and S isomers of the racemic parent are comparably degradable. The separation of isomers of individual metabolites proved to be extremely difficult due to their low levels and other impurities co-eluted. Only metabolite of Reg. No. 5059144 (RPA 406341) exhibits an acceptable separation, which demonstrated a similar ratio with the test substance (see Figure 7.1.1.1-1). This result suggests that no significant metabolic preference for the R versus S isomer occurred during the formation of metabolites from BAS 595 F.



Regions: ^{14}C Detector: ß-RAM

Number	Name	Retention (mm:ss)	Start (mm:ss)	End (mm:ss)	Area (CPM)	%ROI (%)	%Total (%)	Relative Retention
1	Bkg 1	0:17	0:15	9:18				
2	Region 1	10:01	9:29	10:15	6799	43.65	43.01	
3	Region 2	10:33	10:15	11:14	8777	56.35	55.52	
4	Bkg 2	11:46	11:43	19:41				
	2 Peaks				15575	100.00	98.52	

Figure 7.1.1.1-1: Chiral HPLC Radiochromatogram of Isolated Metabolite 5059144

D. TRANSFORMATION OF PARENT COMPOUND

In soil and under aerobic conditions, the major degradation pathway of BAS 595 F is the hydroxylation to produce either hydroxylated derivatives or logical products from the hydroxylated derivatives of the parent compound. Ultimately, BAS 595 F and its degradation products are mineralized to CO_2 by soil microorganisms.

Trigger endpoints were derived according to FOCUS (2006) and were reported in the study summary. From the kinetic analysis, it was found that, the biphasic DFOP model produced an excellent visual and statistical fit to the parent data. A full pathway kinetic model was tested to derive metabolite trigger endpoints, which resulted in acceptable visual fits for BAS 595 F and two metabolites. Soil degradation endpoints derived from the study and from other existing studies are presented in Table 7.1.2.2.2-1 for the parent molecule triticonazole, and in Table 7.1.2.2.2-2 and in Table 7.1.2.2.2-3 for the metabolites RPAS 404766 and RPA 406341, respectively. Modelling endpoints were derived separately by Donaldson (*BASF DocID: 2015/7001309, CA 7.1.2.1.2/3, CA 7.1.2.1.1/4*) and presented in the same tables as the trigger endpoints.

Additionally, structures were proposed for the degradation products that had the highest maximum occurrences below 5% TAR. As observed amount of these compounds were decreasing from the respective maxima towards the end of the study, further assessment is not necessary. Due to missing mass specrometrical data in old studies it was not possible to assign these stuctures to one or the other non identified peaks between 5% and 10% maximum occurrences in old studies.

III. CONCLUSION

The study demonstrated that BAS 595 F steadily degraded in soil under aerobic conditions. There were only two metabolites, Reg. No. 5079285 (RPA 404766) and Reg. No. 5059144 (RPA 406341), greater than 5% TAR, with maximum occurrences of 6.99 and 6.17% TAR in Li 10 soil. Some minor degradation products were also found, each representing less than 5% TAR. Furthermore, it can be concluded that both the *R* and *S* isomers of the racemic parent are comparably degradable. The DT_{50} value for BAS 595 F was approximately 149 days in the tested soil. The DT_{50} values for metabolites Reg. No. 5079285 (RPA 404766) and Reg. No. 5059144 (RPA 406341) are 94.6 and 205 days, respectively. Unknown metabolites observed in legacy studies were not reproduced. The study is valid and the results are suitable to be used as basis for further work.

CA 7.1.1.2 Anaerobic degradation

The following study investigated the route of degradation of triticonazole in anaerobic soil. It was evaluated during the Annex I inclusion and accepted by the European Commission (SANCO/10442/2005-Final rev 1, 12 March 2010). An overview on the relevant results of the study is presented in Table 7.1.1.3-2. No new study was performed.

Report:	**R012982; Goodyear A., 1994 (+ amendment R012983 of 1998), also listed in CA 7.1.2.1.3 and CA 7.1.2.1.4** (^{14}C)-RPA 400727: Anaerobic Soil Metabolism
Report No.	200491 / 68/136-1015 / 68/136
Guidelines:	USEPA (=EPA) 162-2

Please see the full summary details presented in Volume 3, Section B.8 of the DAR for triticonazole (Austrian Federal Office for Food Safety, 2003). The study is still considered valid but has some deviations to the current Test Guideline OECD 307. An overview on the relevant results of the study is presented in Table 7.1.1.3-2.

CA 7.1.1.3 Soil photolysis

A study investigating the soil photolysis of triticonazole was evaluated during the Annex I inclusion and accepted by the European Commission (SANCO/10442/2005-Final rev 1, 12 March 2010). An overview on the relevant results of the study is presented in Table 7.1.1.3-3. No new study was performed.

Report:	**C017700; Ayliffe J.M., Jones M.K. (1998)** Fungicides: Triticonazole: Soil photolysis.
Report No.	201021 / P 95/065
Guidelines:	USEPA (=EPA) N, 161-3, (1982)

Please see the full summary details presented in Volume 3, Section B.8 of the DAR for triticonazole (Austrian Federal Office for Food Safety, 2003). The study is still considered valid. An overview on the relevant results of the study is presented in Table 7.1.1.3-3.

Route of degradation in soil - Overall Conclusion

Legacy studies on the aerobic degradation of triticonazole in soil showed that the main degradation pathway is hydroxylation to form the three major transformation products RPA 406341, RPA 404766 and RPA 407922 as well as some minor hydroxylated metabolites (*Volume 3, Section B.8 of the DAR for triticonazole, Austrian Federal Office for Food Safety, 2003*). However, in some of those studies metabolites occured above 5% which were not identified sufficiently due to a higher trigger value for identification at the time of study performance. Therefore, to comply with the current guidance, a new study was performed to confirm the metabolic patterns observed in the studies submitted previously and to elucidate the degradation of the enantiomers.

In the new study metabolites RPA 40476 and RPA 406341 were observed above the trigger of 5%. Structures were proposed for the degradation products that had the highest maximum occurrences below 5% TAR. As observed amount of these compounds were decreasing from the respective maxima towards the end of the study, further assessment is not necessary. Due to missing mass specrometrical data in legacy studies it was not possible to assign structures to any of the non-identified peaks between 5% and 10% maximum occurrences in those studies. However, all of these degradation products were observed in legacy studies beyond 120d, which would be the study duration according to the respective OECD guidance. Accordingly, considering current guidance documents, no exposure or risk assessment would be necessary for the previously not identified and not reproducible degradation product.

Thus, the degradation pathway in soil proposed for triticonazole during the data evaluation for the Annex I inclusion in 2010 is still valid. The pathway is presented in Figure 7.1.1.3-1. Outcome and main parameters of legacy (*Volume 3, Section B.8 of the DAR for triticonazole, Austrian Federal Office for Food Safety, 2003*) and new (*current dossier*) laboratory soil metabolism studies are presented in Table 7.1.1.3-1 to Table 7.1.1.3-3.

The major soil metabolites RPA 406341 and RPA 404766 are diastereomers which are hydroxylated at the same position in the cyclopentane ring. Metabolite RPA 406341 includes the two enantiomers for which the hydroxyl group has the opposite orientation as the second hydroxylgroup in the cyclopentane ring (RS, RS isomers). Metabolite RPA 404766 includes the two enantiomers for which both hydroxylgroups have the same orientation (RR, SS isomers). Metabolite RPA 407922 is hydroxylated in the phenyl ring. It is a racemate of its two isomers. Subsequent degradation processes are a further hydroxylation to minor dihydroxylated metabolites, ring cleavage as well as the formation of bound residues and carbon dioxide.

Chiral analysis of representative soil extracts shows that both the R and S isomers of the racemic parent are comparably degradable. Furthermore, the results suggest, that an enantioselective degradation of the metabolites does also not occur.

Degradation of triticonazole under anaerobic conditions was not significant and did only lead to minor metabolites as well as to the formation of bound residues.

Photolysis on soil surface can contribute to the dissipation of triticonazole in soil, resulting mainly in the formation of RPA 406203, the Z-isomer of triticonazole. However, due to the planned use as seed treatment, triticonazole is not expected to be exposed to light and, therefore, this process is not considered to be relevant in soil.

A detailed discussion of the fate of the metabolites of triticonazole is presented in Document N5.

Table 7.1.1.3-1: Overview of the laboratory aerobic route of degradation studies

Reference			Soil characteristics				Incubation conditions		Major metabolites (max. occurrence in %TAR)			Further residues	
			Soil origin	Soil type	pH	OC (%) [a]	Moisture	Temp. (°C)	RPA 404766	RPA 406341	RPA 407922	CO_2 (% AR)	BR (% AR)
CA 7.1.1.1	Studies previously evaluated during Annex I inclusion	Ayliffe &Austin, 1993	Not reported	Sandy loam	6.42 [b]	0.72	75% of 33 kPa	22	6.59	8.62	4.26	8.41	14.66
			Not reported	Clay loam	6.18 [b]	5.66	75% of 33 kPa	22	6.66	9.04	**12.77**	**23.86**	**17.92**
		Ayliffe & McMillan-Staff, 1994	Speyer 2.2	Loamy sand	6.8 [b]	2.35	75% of 33 kPa	22	9.86	15.27	2.3	2.13	6.36
		Simmonds & Lowden, 2002	Not reported	Clay loam	8.3 [d]	4.3	45% MWHC	10/20	Supplemental study for identification of metabolite assigned with MW349				
		Doble et al., 1996	Not reported	Clay [e]	5.7 [c]	1.2	75% of 33 kPa	25	9.47	10.67	-	0.81	9.14
		Ayliffe & Godward, 1993	Not reported	Loamy sand	6.24 [b]	18.70	75% of 33 kPa	22	8.39	14.76	6.20	11.03	12.68
			Not reported	Sandy loam	6.30 [b]	0.83	75% of 33 kPa	10	7.39	10.53	6.93	3.14	7.74
			Not reported	Clay loam	6.08 [b]	3.28	75% of 33 kPa	10	**13.94**	16.13	5.28	4.06	8.86
			Not reported	Loamy sand	6.24 [b]	18.70	75% of 33 kPa	10	8.2	9.15	2.33	1.67	5.97
		Simmonds et al., 1996	Manningtree	Sandy loam [e]	6.1 [c]	0.8	50% FC	25	8.72	16.72	-	17.61	15.84
			Manningtree	Sandy loam [e]			50% FC, red. application rate	25	8.20	**20.24**	-	12.65	12.86
			Manningtree	Sandy loam [e]			20% FC	25	7.21	15.78	-	10.59	15.98
			Manningtree	Sandy loam [e]			50% FC	10	6.47	14.64	-	1.46	7.42
	New Study	Ta & Strobush, 2015	Li10	Loamy Sand [e]	6.3 [c]	0.81	50% MWHC	20	6.99	6.17	-	2.53	15.18

a If not explicitly mentioned in the study report, OC was calculated: OC (%) = OM (%) / 1.724.
b Buffer solution unknown, c Buffer solution = $CaCl_2$, d Buffer solution = H_2O.
e Soil type according to USDA, for the other soils the classification is unknown

Bold: Maximum values

Table 7.1.1.3-2: Overview on the results of the anaerobic soil degradation study

Reference		Soil characteristics				Incubation conditions		Major metabolites (% max AR)	Further residues	
		Soil type		pH	OC (%)	Moisture	Temp. (°C)		Volatiles (%AR)	BR (%AR)
CA 7.1.1.2	Goodyear A., 1994 (+ amendment R012983 of 1998)	Manningtree	Sandy loam	7.6 (KCl)	0.3	75% of 33 kPa for 30 days before flooding	25°C	None	< 1% during anaerobic phase	25.2

Table 7.1.1.3-3: Overview on the results of the soil photolysis study

Reference		Soil characteristics				Incubation conditions		Major metabolite (% max AR)	Further residues	
		Soil type		pH	OC (%)	Moisture	Temp. (°C)	RPA 406203	CO_2 (%AR)	BR (%AR)
KCA 7.1.1.3	Ayliffe J.M., Jones M.K. 1998	Manningtree	Sandy loam	4.9 (KCl)	2.2	75% of 33 kPa	20°C	10.95	1.31	4.11

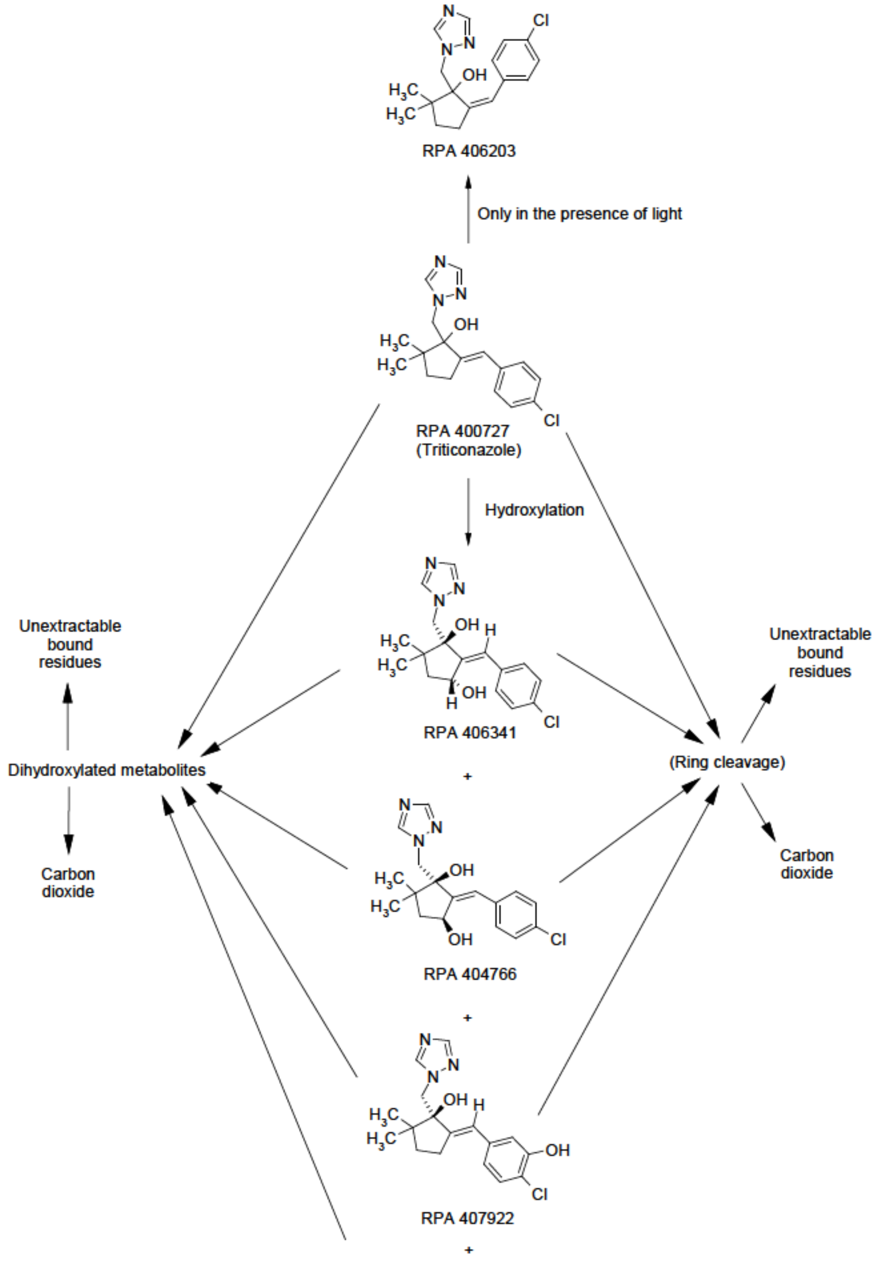


Figure 7.1.1.3-1: Proposed metabolic pathway of triticonazole: Triticonazole consists of a mixture of two possible enantiomers. For sake of simplicity, for all the metabolite structures shown above only one stereoisomer is shown. Studies indicate no shift in enantiomer ratio.

CA 7.1.2 Rate of degradation in soil

Experimental studies which were conducted to derive laboratory and field half lives of triticonazole and its metabolites are summarized in this chapter. Summaries of experimental studies are followed by respective kinetic evaluations. All derived lab and field endpoints are summarized in a tabular form at the end of the chapter (see Table 7.1.2.2.2-1 to Table 7.1.2.2.2-7).

To facilitate an easy reading of this chapter, it is shortly summarized below which endpoint is found in which kinetic evaluation report.

Table 7.1.2-1: Overview of reports containing kinetic evaluations to derive endpoint according to FOCUS (2006)

Compound, compartment	Relevant report		
	Experimental studies	**Trigger DT_{50}**	**Modelling DT_{50}**
Triticonazole And metabolites	Ayliffe & Austin (1993) Ayliffe & al (1994) Doble et al. (1996) Ayliffe&Godward (1993) Simmonds et al. (1996).	Kreschnak (2015) CA 7.1.2.1.2/4	Jarvis & Montesano (2014) CA 7.1.2.1.2/2
	Ta & Strobush (2015) CA 7.1.1.1/1	Ta & Strobush (2015) CA 7.1.1.1/1	Donaldson, 2015 CA 7.1.2.1.2/3
	Grella et al. (2014) CA 7.1.2.1.1/2	Grella et al. (2014) (parent only)	Kreschnak (2015) CA 7.1.2.1.2/4
Triticonazole field	Wicks 1996	Huber 2008 CA 7.1.2.2.1/4	Schwarz and Jarvis 2014 CA 7.1.2.2.1/3
	Duncan et al 2003	Huber 2008 CA 7.1.2.2.1/4	Schwarz and Jarvis 2014 CA 7.1.2.2.1/3
RPA 406 341 field	Richter 2009 CA 7.1.2.2.1/1	Huber 2008 CA 7.1.2.2.1/4	Schwarz N.,Jarvis T., 2014b CA 7.1.2.2.1/5

Studies without an annex point noted were summarized in Volume 3, Section B.8 of the DAR for triticonazole (Austrian Federal Office for Food Safety, 2003)

CA 7.1.2.1 Laboratory studies

CA 7.1.2.1.1 Aerobic degradation of the active substance

Five studies investigating the aerobic soil degradation rate of the active substance triticonazole were evaluated during the Annex I inclusion and accepted by the European Commission (SANCO/10442/2005-Final rev 1, 12 March 2010). Those previously submitted studies are still considered valid and scientifically sound, although some of them have slight deviations to the design of the current OECD 307 Test Guideline (e.g. study duration exceeded 120 days, different temperature and moisture conditions). In order to fully comply with current guidance and to investigate also the degradation behaviour of the enantiomers, two new laboratory studies were performed. A new laboratory study performed in 2015 to comply with current guidance documents is listed below as well (see CA 7.1.2.1.1/1), a detailed summary is presented within Section CA 7.1.1.1. The second new laboratory study is summarized thereafter in annex point CA 7.1.2.1.1/2.

The kinetic evaluation previously submitted (*Report C019871*) was not performed in compliance with current FOCUS kinetics guidance [*FOCUS (2006): Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration. Report of the Work Group on Degradation Kinetics of FOCUS. EC Document Reference SANCO/10058/2005 version 2.0, June 2006; FOCUS (2014): Generic Guidance for Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration, version 1.1*]. Therefore, a new kinetic evaluation was conducted in order to obtain revised rates of degradation which are suitable for environmental risk assessment and modelling purposes. The new evaluation was conducted according to FOCUS (2006) but is also in accordance with the current FOCUS guidance (2014) although this is not explicitly stated in the report. A summary of the evaluation is presented in annex point CA 7.1.2.1.1/3. Only the modelling endpoints are explicitly mentioned in this summary, although the applied kinetic models could also be used to derive the trigger endpoints. Therefore, the trigger endpoints were summarized in a statement, (2015/1186987) which is listed below in annex point CA 7.1.2.1.1/5. The summary includes also trigger and modelling endpoints of the new laboratory studies which were either included in the original study report or determined during dossier preparation.

A non-GLP investigation of the effects of triticonazole on soil microorganisms is available in the public literature (*Niewiadomska et al. 2011*). The study shows that formulated triticonazole applied as seed dressing did have an in part statistically significant effect on the count soil microorganisms. However, the direction (stimulating or inhibiting) of the effect on the number of selected microorganisms was ambiguous. Changes in enzymatic activity in the soil induced by seed dressings with triticonazole were not statistically significant. Thus an effect on soil degradation rates in not expected.

All previously submitted studies are listed below, including their deviations from current OECD guidance. A short summary of the results of all relevant studies is presented in Table 7.1.2.2.2-1.

Report: **C017917; Ayliffe J.M., Austin D.J., (1993), also listed in CA 7.1.1.1**
Fungicides: RPA 400727-^{14}C: Aerobic soil metabolism in three soils (Final Report).
428635 / P91/326
GLP: yes
Guidelines: USEPA (= EPA) N, 162-1 (1982) (not stated in report)

Please see the full summary details presented in Volume 3, Section B.8 of the DAR for triticonazole (Austrian Federal Office for Food Safety, 2003). The study is still considered valid but has some minor deviations to the current OECD Test Guideline 307 (study duration exceeded 120 days). Please refer to chapter 7.1.1.1 for further details. A short summary of the relevant parameters of the study and derived endpoints are presented in Table 7.1.2.2.2-1.

Report: **R012979; Ayliffe J.M., Godward P.J., (1993), also listed in CA 7.1.1.1**
Fungicides: RPA 400727-^{14}C: Rate of degradation in four soils
200234 / P91/411 / GOoD2486
GLP: yes
Guidelines: Dutch Guidelines for the Submission of Applications for Registration of Pesticides, Part G.1

Please see the full summary details presented in Volume 3, Section B.8 of the DAR for triticonazole (Austrian Federal Office for Food Safety, 2003). The study is still considered valid but has some minor deviations to the current OECD Test Guideline 307 (study duration exceeded 120 days, test was performed at 10°C for three of soils). Please refer to chapter 7.1.1.1 for further details. A short summary of the results of all relevant studies is presented in Table 7.1.2.2.2-1.

Report:	**R012981; Ayliffe J.M., McMillan-Staff S.L., (1994), also listed in CA 7.1.1.1** Addendum Report: Fungicides: RPA 400727-^{14}C: Aerobic soil metabolism in three soils. 200471 / P91/326
GLP:	yes
Guidelines:	USEPA (= EPA) N, 162-1 (1982) (not stated in report)

Please see the full summary details presented in Volume 3, Section B.8 of the DAR for triticonazole (Austrian Federal Office for Food Safety, 2003). The study is still considered valid but has some minor deviations to the current OECD Test Guideline 307 (study duration exceeded 120 days). Please refer to chapter 7.1.1.1 for further details. . A short summary of the relevant parameters of the study and derived endpoints are presented in Table 7.1.2.2.2-1.

Report:	**R012994; Doble M.L., Ferreira E.M., Hardy I.A.J., (1996), also listed in CA 7.1.1.1** (^{14}C)-triazole labelled triticonazole: Rate of degradation in clay soil under aerobic conditions. 201171 / P94/158 / GOoD8999
GLP:	yes
Guidelines:	USEPA (= EPA) N, 162-1 (1982) and Draft European Uniform Guidelines (Lynch 1993)

Please see the full summary details presented in Volume 3, Section B.8 of the DAR for triticonazole (Austrian Federal Office for Food Safety, 2003). The study is still considered valid but has some minor deviations to the current OECD Test Guideline 307 (study duration exceeded 120 days, test was performed at 25°C, only one soil was investigated).

Please refer to chapter 7.1.1.1 for further details. A short summary of the results of all relevant studies is presented in Table 7.1.2.2.2-1.

Report:	**R012995; Simmonds M.B., Hardy I.J., Ferreira E.M., (1996) also listed in CA 7.1.1.1** Triticonazole: Rate of degradation in one soil type under aerobic conditions with regard to varying temperature, soil moisture, treatment rate and soil viability. 201173 / P94/141 / GOoD7834
GLP:	yes
Guidelines:	Danish Agency of Environmental Protection Guidelines Sub-Section 21.1 Transformation and Degradation in Soil (1988) and Draft European Guidelines (Lynch 1993)

Please see the full summary details presented in Volume 3, Section B.8 of the DAR for triticonazole (Austrian Federal Office for Food Safety, 2003). The study is still considered valid but has some minor deviations to the current OECD Test Guideline 307 (study duration exceeded 120 days). Please refer to chapter 7.1.1.1 for further details. A short summary of the results of all relevant studies is presented in Table 7.1.2.2.2-1.

Although all previously listed studies were considered as valid, to comply with the current guidance, two new studies were performed, which are summarized below.

Report: CA 7.1.2.1.1/1
Ta C.,Strobush A., 2015a
Aerobic soil metabolism of 14C-BAS 595 F
2014/7000472
Guidelines: EPA 835.4100, OECD 307 (2002), SETAC
GLP: yes
(certified by United States Environmental Protection Agency)

Also listed in Annex Points CA 7.1.1.1/1 and CA 7.1.2.1.2/01Please refer to chapter 7.1.1.1.1, CA 7.1.1.1/1 for a detailed summary of the study. A short summary of the results of all relevant studies is presented in Table 7.1.2.2.2-1.

Report: CA 7.1.2.1.1/2
Grella B. et al., 2014a
Rate of degradation of BAS 595 F in soils
2014/7000471
Guidelines: EPA 835.4100, OECD 307 (2002), SETAC Procedures for assessing the environmental fate and ecotoxicity for pesticides (March 1995)
GLP: yes
(certified by United States Environmental Protection Agency)

Executive Summary

The degradation of BAS 595 F under aerobic conditions was investigated in three German soils (LUFA 2.2, LUFA 2.3, and LUFA 5M). For this purpose, soil samples (50 g of dry soil) were treated with the test item at a rate of 0.2 mg/kg soil, corresponding to 4-times the proposed maximum field application rate of 12.5 g a.i./ha. The treated soils were maintained aerobically in the dark at 20 ± 2°C for 120 days. Prior to the BAS 595 F application, the soil moisture was adjusted to approximately 50% maximum water holding capacity (MWHC) and kept and throughout the experiment. Duplicate samples were collected at 0, 3, 7, 14, 30, 59, 91, and 120 days after treatment (DAT). The soil samples were extracted with appropriates solvents and the extracts analysed by LC-MS.

The soils were still viable with microbial activity at the beginning and at the end of the study (120 DAT). The amount of BAS 595 F decreased at different rates in the three soils examined. The fastest decrease was seen for LUFA 2.3, where BAS 595 F decreased to 49.3% at day 120 with DT_{50} of 125 days. In soil LUFA 5M, the amount of BAS 595 F at day 120 (53.9%, DT_{50} of 149 days) was close to that of LUFA 2.3. A slower decrease was seen for soil LUFA 2.2 (70.7%, DT_{50} of 317 days). Different labels were treated as replicates for the kinetic evaluation.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test material: **BAS 595 F**

Common name	Triticonazole
Reg. No.	4378513
Batch number:	L76-154
CAS number:	131983-72-7
Molecular Weight	317.82 g/mol
Purity	98.6 ± 1.0%

2. Soils

The soils were representative of the intended use areas. The soil samples were collected from their respective fields (top 8-inch layer, sieved through a 2-mm mesh-sieve and stored in the refrigerator. Soil characterization is presented in the table below:

Table 7.1.2.1.1-1: Properties of the soils

Name:		LUFA 2.3	LUFA 2.2	LUFA 5M
USDA Textural class:		Sandy Loam	Loamy Sand	Sandy Loam
Sand	[%]	68	82	60
Silt	[%]	22	12	26
Clay	[%]	10	6	14
Cation Exchange Capacity	[meq/ 100 g]	9.4	8.1	10.4
Max. Water Hold. Capacity	[gm/100 g dry soil]	35.2	25.7	33.5
Moisture at 1/3 Bar	[%]	12.6	10.7	14.7
Moisture at 15 Bar	[%]	6.0	6.5	6.4
Organic Matter-Walkley Black	[%]	1.7	2.8	1.7
pH (1:1 soil: water ratio)		7.2	5.8	7.7
pH (0.01 M $CaCl_2$)		6.9	5.5	7.4
Microbial Biomass Carbon at 0 DAT	[µg/g dry soil]	311.0	430.3	353.2
Microbial Biomass Carbon at 120 DAT	[µg/g dry soil]	414.5	399.1	449.1
N total	[%]	0.09	0.14	0.09
Olsen Phosphorous	[mg/L]	80	7	48

DAT = days after treatment

B. STUDY DESIGN

1. Experimental conditions

Soil aliquots (50 g of dry weight) were placed in 250-mL polypropylene centrifuge bottles and treated with the test item at a rate of 0.2 mg/kg soil, corresponding to 4-times the proposed maximum field application rate of 12.5 g a.i./ha. The treated soils were connected to a flow-through test system and incubated in the dark at 20 ± 2°C for 120 days. Moisturized and CO_2-free air was passed over the soil in order to maintain the aerobic conditions. Soil moisture was adjusted to approximately 50% MWHC prior to the application of the test solutions and maintained throughout the incubation period.

2. Sampling

Duplicate samples were collected at 0, 3, 7, 14, 30, 59, 91, and 120 DAT.

Within every set of samples belonging to a certain sampling date, at least one sample per soil was incubated without the test item (control samples). The control samples were used for microbial biomass determination and for verification of the analytical method.

3. Description of analytical procedures

The sample analysis was performed according to the validated method No. 0051 with the exception of the LC-MS conditions. The LC-MS method is described in section 4.2.1. Each soil sample was sequentially extracted once with ammonium hydroxide (0.1 M) and twice with acetone. The solvent from the combined extracts was evaporated until 10-15 mL remained and the volume was adjusted to 20 mL by the addition of water. The samples were centrifuged (3000 rpm, 10 min.) and then further extracted by solid phase extraction (SPE), and then the samples were analyzed by LC-MS.

The analytical method was verified by using spiked samples of the soils at every sampling date. At the day of the workup, control spiked samples (instrumental recoveries) were prepared by removing 1 mL of the control extract and spiking it with 10 µL of the 10 µg/mL fortification solution. Additionally, prior to workup, fortification samples were made by treating the soil control samples with 0.1 mL of the 10 µg/mL fortification solution (LOQ) or with 0.1 mL of the 100 µg/mL fortification solution (10 x LOQ). The LOQ was set at 20 µg/kg of soil.

4. Determination of degradation Kinetics

The guidance of FOCUS (2006) was used as the basis for conducting the kinetic analysis, statistical assessment, and selection of the best fit kinetic model for each soil. Optimization of model parameters, including estimation of parameter standard errors, was performed using the software ModelMaker 4.0.

II. RESULTS AND DISCUSSION

A. DATA

Time course of the rate of degradation of BAS 595 F in the treated soils is presented in Table 7.1.2.1.1-2.

Table 7.1.2.1.1-2: Degradation of BAS 595 F in soil

DAT	Soil LUFA 2.2		Soil LUFA 2.3		Soil LUFA 5M	
	BAS 595 F [mg/kg]	BAS 595 F (% applied)	BAS 595 F [mg/kg]	BAS 595 F (% applied)	BAS 595 F [mg/kg]	BAS 595 F (% applied)
0 rep 1	0.161	84.9	0.176	99.3	0.163	86.6
0 rep 2	0.219	115.1	0.178	100.7	0.213	113.4
0 mean	**0.190**	**100.0**	**0.177**	**100.0**	**0.188**	**100.0**
3 rep 1	0.151	79.5	0.145	81.8	0.195	103.6
3 rep 2	0.155	81.8	0.145	81.9	0.207	110.4
3 mean	**0.153**	**80.7**	**0.145**	**81.8**	**0.201**	**107.0**
7 rep 1	0.142	74.6	0.155	87.5	0.151	80.3
7 rep 2	0.158	83.3	0.151	85.3	0.143	76.3
7 mean	**0.150**	**79.0**	**0.153**	**86.4**	**0.147**	**78.3**
14 rep 1	0.165	86.9	0.149	84.1	0.156	82.9
14 rep 2	0.150	78.7	0.143	81.0	0.152	80.9
14 mean	**0.157**	**82.8**	**0.146**	**82.6**	**0.154**	**81.9**
30 rep 1	0.158	82.9	0.130	73.4	0.135	71.7
30 rep 2	0.162	85.4	0.143	80.6	0.134	71.5
30 mean	**0.160**	**84.1**	**0.136**	**77.0**	**0.134**	**71.6**
59 rep 1	0.118	62.0	0.098	55.3	0.116	61.8
59 rep 2	0.127	66.6	0.099	56.1	0.121	64.2
59 mean	**0.122**	**64.3**	**0.099**	**55.7**	**0.118**	**63.0**
91 rep 1	0.138	72.4	0.098	55.2	0.104	55.5
91 rep 2	0.140	73.9	0.087	48.9	0.113	60.1
91 mean	**0.139**	**73.1**	**0.092**	**52.1**	**0.109**	**57.8**
120 rep 1	0.133	70.1	0.085	48.2	0.097	51.9
120 rep 2	0.136	71.3	0.089	50.4	0.105	56.0
120 mean	**0.134**	**70.7**	**0.087**	**49.3**	**0.101**	**53.9**

BAS 595 F (%applied) was calculated by setting 100% to the mean of the day 0 values
The data for 59-DAT and 120-DAT in soil LUFA 2.2 for both rep1 and rep2 are an average of two analyses.
The data for 91-DAT rep 1 and rep 2 in soil LUFA 2.2 are an average of three analyses each.

B. TRANSFORMATION OF PARENT COMPOUND

The amount of BAS 595 decreased at different rates in the three soils examined. The fastest decrease was seen for LUFA 2.3, where BAS 595 F decreased to 49.3% at day 120. In soil LUFA 5M, the amount of BAS 595 F at day 120 (53.9%) was close to that of LUFA 2.3. A slower decrease was seen for soil LUFA 2.2 (70.7% at day 120). Soil degradation endpoints derived from the study and from other existing studies according to FOCUS (2006) are presented in Table 7.1.2.2.2-1.

III. CONCLUSION

The rate of the degradation of BAS 595 F varied in the three soils examined under aerobic conditions. DT_{50} values of 317, 125 and 149 days were found in soils LUFA 2.2, LUFA 2.3 and LUFA 5M, respectively. The study is valid and the results are suitable to be used as basis for further work.

The kinetic evaluation previously submitted (*Report C019871*) was not performed in compliance with current FOCUS kinetics guidance. Therefore, a new kinetic evaluation was conducted, which is summarized below.

Report:	CA 7.1.2.1.1/3 Jarvis T.,Montesano V., 2014a Recalculation of Triticonazole laboratory soil degradation kinetics according to FOCUS (2006) guidance 2014/1083342
Guidelines:	FOCUS (2006): Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration Sanco/10058/2005 version 2.0 434 pp.
GLP:	no

Also listed in CA 7.1.2.1.2/2

Executive Summary

Laboratory soil degradation studies of triticonazole and its soil metabolites were re-evaluated according to the FOCUS kinetics (2006) guidance. As the evaluation showed that field dissipation studies are triggered, regardless which kinetic model was used, only modelling endpoints were derived. DT_{50} values (normalised: 20°C, pF2) for triticonazole for use in simulation modelling ranged from 61.4 to 401 days. Normalised DT_{50} values for the metabolites ranged from 102.4 to 231.7 days (RPA 406341), 0.44 to 2.0 days (RPA 407922) and 30.9 to 60.8 days (RPA 404766). The fits also included the metabolic pathways from the parent compound triticonazole to RPA 406341, RPA 404766 and RPA 407922. Arithmetic mean formation fractions were calculated as 0.561, 0.088 and 0.396 to RPA 406341, RPA 407922 and RPA 404766, respectively.

I. MATERIAL AND METHODS

Five studies were considered in which the parent triticonazole was applied (Ayliffe & Godward (*1993, Doc No. R012979),* Ayliffe & Austin (*1993, Doc No. C017917*), Ayliffe & McMillan-Staff (*1994, Doc No. R012981*), Doble et al. (*1996, Doc No. R012994*), and Simmonds et al. (*1996, Doc No. R012996*)). Additionally, three metabolite laboratory soil degradation studies (McGhee (2000, *Doc No. C010570*), Unsworth & Clarke (*2000, Doc No. R012054*) and Crowe (*2002, Doc No. C021045*)) were evaluated. Analytical results of triticonazole and its metabolites in soil (in percentage of applied) were obtained from the laboratory soil degradation studies and used as input for the kinetic evaluation. The simulation modelling was performed using KinGui Version 2.

Degradation rates of triticonazole and its metabolites and, furthermore, the formation fractions of the metabolites were derived. When necessary, the DT_{50} values were normalised to 20°C (using a Q_{10} of 2.58) and pF2.

II. RESULTS AND DISCUSSION

The evaluation of the parent degradation studies showed a noticeable deviation from SFO kinetics during the latter stages of the incubations. However, due to generally slow degradation of triticonazole in the investigated lab studies, the low formation of metabolites and long study durations (365 d), SFO was considered acceptable for modelling purposes. No separate trigger endpoints were derived as field dissipation studies are triggered regardless which kinetic model is used. Results are presented in Table 7.1.2.1.1-3. The results for the metabolites are presented in Annex Point CA 7.1.2.1.2/1.

Table 7.1.2.1.1-3: Triticonazole: Aerobic Soil Degradation Modelling Endpoints

Study	Soil	Incubation conditions		Kinetic Model	DT_{50} study cond. (d)	DT_{90} study cond. (d)	DT_{50} at 20°C, pF2 (d)
		Temp. (°C)	Moisture				
Ayliffe & Godward, 1993	Loamy sand	22	75% of 33kPa	SFO	249	828	299
	Sandy loam	10	75% of 33kPa	SFO	347	>1000	79.8
	Clay loam	10	75% of 33kPa	SFO	183	607	61.4
	Loamy sand	10	75% of 33kPa	SFO	599	>1000	234
Ayliffe & Austin, 1993 plus addendum Ayliffe & McMillan-Staff, 1994	Sandy loam	22	75% of 33kPa	SFO	253	840	176
	Clay loam	22	75% of 33kPa	SFO	136	452	145
	Loamy sand	22	75% of 33kPa	SFO	187	620	206
Doble et al.; 1996	Clay	25	75% of 33kPa	SFO	492	>1000	401
Simmonds et al., 1996	Sandy loam	25	50% FC	SFO	167	555	166
	Sandy loam (reduced rate)	25	50% FC	SFO	187	620	186
	Sandy loam	25	20% FC	SFO	215	714	344
	Sandy loam	10	50% FC	SFO	413	>1000	100

III. CONCLUSION

The rates of the degradation of triticonazole and its metabolites were re-evaluated according to FOCUS (2006). Modelling endpoints for the parent and its metabolites as well as formation fractions for the metabolites were derived and can be used in environmental fate modelling. Field dissipation studies are triggered for triticonazole.

In the study report for the soil metabolism stdy with triticonazole only trigger endpoints were presented (Ta & Strobush 2015, CA 7.1.1.1/1, 7.1.2.1.1/1 & 7.1.2.1.2/1). Corresponding modelling endpoints are presented below.

Report: CA 7.1.2.1.1/4
Donaldson F., 2015a
Kinetic evaluation of the aerobic soil metabolism of BAS 595 F (triticonazole) in a loamy sand soil
2015/7001309
Guidelines: FOCUS (2006): Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration Sanco/10058/2005 version 2.0 434 pp.
GLP: no

Also listed in CA 7.1.2.1.2/3.

Executive Summary

A laboratory soil degradation study (one soil) of triticonazole and its soil metabolites was evaluated to determine $DegT_{50}$ modeling endpoints for BAS 595 F and any major soil metabolites, taking into account the current guidance of the FOCUS workgroup on degradation kinetics (FOCUS, 2006). The study $DegT_{50}$ endpoints were further normalised to reference conditions (20°C, pF2) according to the recommendations of the FOCUS ground water workgroup (FOCUS, 2014). $DegT_{50}$ values (normalised: 20°C, pF2) for triticonazole, RPA 404766 and RPA 406341 for use in simulation modelling were 116, 20.1 and 30.3 days, respectively. Formation fractions of RPA 404766 and RPA 406341 were 0.576 and 0.386, respectively. Both metabolites are formed from the parent.

I. MATERIAL AND METHODS

One study with one soil was considered in which the parent triticonazole was applied (*Ta & Strobush, 2015, CA 7.1.1.1/1, 7.1.2.1.1/01 & 7.1.2.1.2/1*). Analytical results of triticonazole and its metabolites in soil (in percentage of applied) were obtained from the laboratory soil degradation study and used as input for the kinetic evaluation. Two radiolabels (phenyl, triazole rings) were investigated in the study, and replicate samples were taken for each sampling interval. The data for each radiolabel was combined and analysed as one data set, resulting in four replicates per sampling time. Those metabolites which exceeded 10% TAR at any single sampling time or 5% TAR in two consecutive sampling times were considered in the evaluation. Two metabolites met these criteria: Reg. No. 5079285 (RPA 404766) and Reg. No. 5059144 (RPA 406341). The simulation modelling was performed using KinGUII (v. 2.2014.224.1704). Degradation rates of triticonazole and its metabolites and, furthermore, the formation fractions of the metabolites were derived. The derived $DegT_{50}$ values were normalised to 20°C (using a Q_{10} of 2.58) and pF2.

II. RESULTS AND DISCUSSION

Parent-only kinetics were evaluated with the SFO model as a first step. As this provided an acceptable visual and statistical fit, there was no need to test any biphasic models. The $DegT_{50}$ modelling endpoint was derived from the parent-only fitting. A full degradation pathway was also investigated in order to determine metabolite kinetic endpoints ($DegT_{50}$, formation fraction) for RPA 404766 and RPA 406341. The full degradation pathway fit considered the SFO model for both parent and metabolites. Results for parent are presented in Table 7.1.2.1.1-4. The results for the metabolites are presented in Annex Point CA 7.1.2.1.2/3.

Table 7.1.2.1.1-4: Triticonazole: Aerobic Soil Degradation Modelling Endpoints

Study	Soil	Incubation conditions		Kinetic Model	$DegT_{50}$ study cond. (d)	$DegT_{50}$ at 20°C, pF2 (d)
		Temp. (°C)	Moisture			
Ta & Strobush, 2015	Loamy sand	20	50% MWHC	SFO	136	116

III. CONCLUSION

The rates of the degradation of triticonazole and its metabolites were evaluated according to FOCUS (2006). Modelling endpoints for the parent and its metabolites as well as formation fractions for the metabolites were derived and can be used in environmental fate modelling.

Only the modelling endpoints are explicitly mentioned in CA 7.1.2.1.1/3, although the applied kinetic models could also be used to derive the trigger endpoints. Therefore, the trigger endpoints were summarized in the statement below.

Report: CA 7.1.2.1.1/5
Kreschnak C., 2015a
Summary of kinetic endpoints for Triticonazole and its metabolites from laboratory soil degradation studies
2015/1186987
Guidelines: none
GLP: no

Also listed in annex point CA 7.1.2.1.2/4. The rate of degradation of triticonazole and its metabolites in aerobic laboratory soils was investigated in several studies. The kinetic evaluation of these studies was conducted either directly in the study reports or in separate kinetic reports. In some cases, not all kinetic endpoints were explicitly reported although the necessary data is included in the reports. This statement aims to summarise all kinetic endpoints of triticonazole and its metabolites that were provided in the study reports or in the kinetic reports. The relevant results of this summary are presented in Table 7.1.2.2.2-1.

The non-GLP investigation of the effects of triticonazole on soil microorganisms available in the public literature is summarized below.

Report: Niewiadomska A., Sawińska Z., Wolna-Maruwka A., 2011
Impact of Selected Seed Dressings on Soil Microbiological Activity in Spring Barley Cultivation
Fresenius Environmental Bulltein Vol 20/No 5a / 2001 pp 1252-1261
Guidelines: None reported
GLP: No

Peer rewieved public literature.

Executive Summary

The objective of this study was to examine changes in counts of total bacteria and numbers of oligotrophs, copiothrophs, actinomycetes and fungi, as well as changes in enzymatic activity of dehydrogenases and acid phosphatase following the application of different seed dressings, including triticonazole containing products. The applied seed dressings were found to exert a statistically highly significant or significant influence on total counts of bacteria, oligotrophs, copiotrophs, actinomycetes and fungi at different dates of analysis. However, the direction (stimulating or inhibiting) of the effect on the number of selected microorganisms was ambiguous. For fungi the effect was only significant on one of the five dates. Changes in enzymatic activity in the soil induced by triticonazole were not statistically significant.

Conclusions

The influence on changes in number of individual groups of microorganisms in the soil of triticonazole are, depending on the applied concentration, ambiguous. Changes in enzymatic activity in the soil induced by triticonazole were not statistically significant.

The study showed effects of triticonazole on enzymatic activity, and no clear conclusions on the the directions on the effect on count of soil bacteria. The authors performed no multifactorial analysis, although figures indicate a stronger influence of seasonal changes than the effects of different treatments. Considering the presented information, influence of the use of triticonazole in not expected to affect its degradation rate in soil.

CA 7.1.2.1.2 Aerobic degradation of metabolites, breakdown and reaction products

Three studies which investigated the aerobic soil degradation of the three major soil metabolites (RPA 404766, RPA 406341, and RPA 407922) were evaluated during the Annex I inclusion and accepted by the European Commission (SANCO/10442/2005-Final rev 1, 12 March 2010). All of the previously submitted studies are still considered valid and scientifically sound. They are listed below.

As the accepted degradation rates from the previous EU review were not determined according to current FOCUS guidance (2006 and 2014), a new kinetic evaluation of the data was performed which is summarized below in annex point CA 7.1.2.1.2/2. This evaluation includes the calculation of formation fractions based on the parent degradation studies previously submitted (refer to section CA 7.1.2.1.1).

Furthermore, degradation rates for metabolites RPA 404766 and RPA 406341 were calculated based on the new laboratory study of Ta & Strobush *(2015, BASF DocID: 2014/7000472*) performed with the parent compound triticonazole and listed below as well (CA 7.1.2.1.2/1, refer to section CA 7.1.1.1 for a detailed summary). For this study, the calculation of trigger endpoints is included in the study report. Modelling endpoints were determined by Donaldson (*BASF DocID: 2015/7001309, CA 7.1.2.1.2/3, CA 7.1.2.1.1/4*). A short summary of the results and relevant parameters of the individual studies is presented in Table 7.1.2.2.2-2 to Table 7.1.2.2.2-4.

Report: **C010570; McGhee I., (2000)**
Rate of degradation in three soils at 20 degrees Celsius (^{14}C) RPA 406341
GOoD16713 / 202643 / 16713
GLP: yes
Guidelines: EU (=EEC) 95/36/EC Section 7.1.1.2, BBA IV, 5.1, SETAC

Please see the full summary details presented in Volume 3, Section B.8 of the DAR for triticonazole (Austrian Federal Office for Food Safety, 2003). The study is still considered valid and scientifically sound. However, only single samples were analysed. A short summary of the relevant parameters of the study and derived endpoints are presented in Table 7.1.2.2.2-3.

Report: **R012054; Unsworth R. H., Clarke D. E., (2000)**
(^{14}C)-RPA 407922 Rate of aerobic soil degradation in three soil types at 20 degrees Celsius
GOoD202590
GLP: yes
Guidelines: EU (=EEC) 95/36/EC Section 7.1.1.2, BBA IV, 5.1, SETAC

Please see the full summary details presented in Volume 3, Section B.8 of the DAR for triticonazole (Austrian Federal Office for Food Safety, 2003). The study is still considered valid and scientifically sound. However, only single samples were analysed. A short summary of the relevant parameters of the study and derived endpoints are presented in Table 7.1.2.2.2-4.

Report: **C021045; Crowe A., (2002)**
(^{14}C)-RPA 404766 Rate of aerobic soil degradation in three soil types at 20 degrees Celsius
AES 065/022530 / C021045
GLP: yes
Guidelines: EU (=EEC) 95/36/EC Section 7.1.1.2, BBA IV, 5.1, SETAC

Please see the full summary details presented in Volume 3, Section B.8 of the DAR for triticonazole (Austrian Federal Office for Food Safety, 2003). The study is still considered valid and scientifically sound. Only single samples were analysed. A short summary of the relevant parameters of the study and derived endpoints are presented in Table 7.1.2.2.2-2.

Although all previously listed studies were considered as valid, to comply with the current guidance, a new studies with the parent triticonazole was performed, in which also metabolites were analyzed. The study is listed below, and summarized at CA 7.1.1.1./1.

Report: CA 7.1.2.1.2/1
Ta C.,Strobush A., 2015a
Aerobic soil metabolism of 14C-BAS 595 F
2014/7000472
Guidelines: EPA 835.4100, OECD 307 (2002), SETAC
GLP: yes
(certified by United States Environmental Protection Agency)

Also listed in Annex Points CA 7.1.1.1/1 and CA 7.1.2.1.1/1. A short summary of the relevant parameters of the study and derived endpoints are presented in Table 7.1.2.2.2-2 and in Table 7.1.2.2.2-3.
The derived trigger DegT50 of 94.6 days for RPA 404766 is not considered reliable because confidence interval of the estimated degradation rate of the metabolite extends to low negative values. This is in line with that the estimated DegT50 substantially differs from the values obtained from the metabolite study.
The occurrence of RPA 404766 was investigated in the field dissipation study of Duncan et al. (2003, refer to CA 7.1.2.2.1). Its concentration was always low (0.01 mg/kg at maximum) and it was not possible to derive reliable kinetic data from the observed residues. But it was shown that this metabolite was not formed in significant quantities in the field and that the amounts which were formed were dissipated so that there was no likelihood of accumulation.

A new kinetic evaluation of the existing studies was performed according to FOCUS (2006, 2014), which is summarized below.

Report: CA 7.1.2.1.2/2
Jarvis T.,Montesano V., 2014a
Recalculation of Triticonazole laboratory soil degradation kinetics according to FOCUS (2006) guidance
2014/1083342

Guidelines: FOCUS (2006): Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration Sanco/10058/2005 version 2.0 434 pp.

GLP: no

Also listed in Annex Point CA 7.1.2.1.1/3. A detailed summary of the kinetic evaluation is provided in Annex Point CA 7.1.2.1.1/1. The relevant results for the metabolites are presented below and in Table 7.1.2.2.2-2 to Table 7.1.2.2.2-4.

DT_{50} values for the metabolites were obtained from the studies in which the metabolites were applied. Formation fractions for the metabolites were derived from the evaluation of pathway fits using the data from the parent degradation studies. The metabolite DT_{50} values obtained from the pathway evaluations were subject to considerable error (Chi^2 error values typically »15%) and so were not considered further. However, the formation fractions were considered acceptable for regulatory use. Results are presented in Table 7.1.2.1.2-1 and Table 7.1.2.1.2-2:

Table 7.1.2.1.2-1: Metabolites of Triticonazole: Aerobic Soil Degradation Modelling Endpoints

Study	Soil	Incubation conditions		Kinetic Model	DT_{50} study cond. (d)	DT_{90} study cond. (d)	DT_{50} at 20°C, pF2 (d)
		Temp. (°C)	Moisture				
RPA 406341							
McGhee, 2000,	Clay loam	20	45% MWHC	SFO	165.2	548.8	102.4
	Sandy loam	20	45% MWHC	SFO	198.9	660.9	143.2
	Loam	20	45% MWHC	SFO	345.9	>1000	231.8
RPA 407922							
Unsworth & Clarke, 2000	Clay loam	20	45% MWHC	SFO	0.44	1.5	0.44
	Clay loam	20	45% MWHC	FOMC	2.0[a]	6.6	2.0
	Loamy sand	20	45% MWHC	SFO	1.1	3.8	1.1
RPA 404766							
Crowe, 2002	Sandy loam	20	pF2-2.5	SFO	30.9	102.8	30.9
	Silty clay loam	20	pF2-2.5	DFOP	60.8[b]	137.9	60.8
	Clay loam	20	pF2-2.5	DFOP	58.7[b]	166.2	58.7

[a] Calculated as follows: $DT_{90}/3.32$.
[b] Calculated using the slow degradation rate: $\ln(2)/k_2$.

Table 7.1.2.1.2-2: Metabolites of Triticonazole: Formation fractions from Pathway Fits

Study	Soil	Formation fraction from parent		
		RPA 406341	RPA 407922	RPA 404766
Ayliffe & Godward, 1993	Loamy sand	0.688	-[a]	0.312
	Sandy loam	0.404	-[a]	0.253
	Clay loam	0.526	-[a]	0.474
	Loamy sand	0.576	-[a]	0.424
Ayliffe & Austin, 1993 plus addendum Ayliffe & McMillan-Staff, 1994	Sandy loam	0.760	0.040	0.200
	Clay loam	0.343	0.136	0.521
	Loamy sand	0.512	-[b]	0.488
Doble et al.; 1996	Clay	0.581	nd	0.419
Simmonds et al., 1996	Sandy loam	0.563	nd	0.437
	Sandy loam (reduced rate)	0.598	nd	0.402
	Sandy loam	0.529	nd	0.471
	Sandy loam	0.655	nd	0.345

a Not evaluable (e.g. due to data scattering, insufficient amount of data points).
b Formation fraction not reliable.
nd = not determined (metabolite was not detected in these soils).

Report: CA 7.1.2.1.2/3
Donaldson F., 2015a
Kinetic evaluation of the aerobic soil metabolism of BAS 595 F (triticonazole) in a loamy sand soil
2015/7001309

Guidelines: FOCUS (2006): Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration Sanco/10058/2005 version 2.0 434 pp.

GLP: no

Also listed in Annex Point CA 7.1.2.1.1/4. A detailed summary of the kinetic evaluation is provided in Annex Point CA 7.1.2.1.1/04. The relevant results for the metabolites are presented below and in Table 7.1.2.2.2-2 to Table 7.1.2.2.2-4.

DegT50 values and formation fractions were derived for the metabolites RPA 404766 and RPA 406341 from a study in which the parent triticonazole was applied (Ta & Strobush, 2015, CA 7.1.1.1/1, 7.1.2.1.1/1 & 7.1.2.1.2/1). Results are presented in Table 7.1.2.1.2-3 below.

Table 7.1.2.1.2-3: RPA 404766 and RPA 406341: Aerobic Soil Degradation Modelling Endpoints

Compound	Soil	Incubation conditions		Kinetic Model	$DegT_{50}$ study cond. (d)	$DegT_{50}$ at 20°C, pF2 (d)	FF
		Temp. (°C)	Moisture				
RPA 404766	Loamy sand	20	50% MWHC	SFO[a]	23.6	20.1	0.576
RPA 406341				SFO[a]	35.7	30.3	0.386

a Pathway fit with parent (= SFO).

Only the modelling endpoints are explicitly mentioned in CA 7.1.2.1.2/2, although the applied kinetic models could also be used to derive the trigger endpoints. Therefore, the trigger endpoints were summarized in the statement below.

Report: CA 7.1.2.1.2/4
Kreschnak C., 2015a
Summary of kinetic endpoints for Triticonazole and its metabolites from laboratory soil degradation studies
2015/1186987
Guidelines: none
GLP: no

Also listed in annex point CA 7.1.2.1.1/05. The rate of degradation of triticonazole and its metabolites in aerobic laboratory soils was investigated in several studies. The kinetic evaluation of these studies was conducted either directly in the study reports or in separate kinetic reports. In some cases, not all kinetic endpoints were explicitly reported although the necessary data is included in the reports. This statement aims to summarise all kinetic endpoints of triticonazole and its metabolites that were provided in the study reports or in the kinetic reports. The relevant results of this summary are presented in Table 7.1.2.2.2-2 to Table 7.1.2.2.2-4.

CA 7.1.2.1.3 Anaerobic degradation of the active substance

The following study investigated the rate of degradation of triticonazole in anaerobic soil. It was evaluated during the Annex I inclusion and accepted by the European Commission (SANCO/10442/2005-Final rev 1, 12 March 2010). A short summary of the results and relevant parameters of the study is presented in Table 7.1.2.2.2-5.

Report: **R012982; Goodyear A., (1994 + amendment R012983 of 1998), also listed in chapters CA 7.1.1.2 and CA 7.1.2.1.4**
(^{14}C)-RPA 400727: Anaerobic Soil Metabolism
200491 / 68/136-1015 / 68/136
GLP: yes
Guidelines: USEPA (=EPA) 162-2

Please see the full summary details presented in Volume 3, Section B.8 of the DAR for triticonazole (Austrian Federal Office for Food Safety, 2003). The study is still considered valid. A short summary of the results and relevant parameters of the study is presented in Table 7.1.2.2.2-5.

CA 7.1.2.1.4 Anaerobic degradation of metabolites, breakdown and reaction products

Information on the anaerobic degradation of the major soil metabolites was deduced from the following parent study which is listed in Section CA 7.1.2.1.3. A short summary of the results and relevant parameters of the study is presented in Table 7.1.2.2.2-5.

Report: **R012982; Goodyear A., (1994 + amendment R012983 of 1998), also listed in chapters CA 7.1.1.2 and CA 7.1.2.1.3**
(^{14}C)-RPA 400727: Anaerobic Soil Metabolism
200491 / 68/136-1015 / 68/136
GLP: yes
Guidelines: USEPA (=EPA) 162-2

Please see the full summary details presented in Volume 3, Section B.8 of the DAR for triticonazole (Austrian Federal Office for Food Safety, 2003). The study is still considered valid. A short summary of the results and relevant parameters of the study is presented in Table 7.1.2.2.2-5.

CA 7.1.2.2 Field studies

CA 7.1.2.2.1 Soil dissipation studies

Two field dissipation studies with triticonazole were evaluated and accepted by the European Commission (SANCO/10442/2005-Final rev 1, 12 March 2010). Although the studies have some deficiencies, they are still considered valid and scientifically sound. Two further studies were submitted which are not considered valid anymore but provide supplemental information.

All previously submitted studies are listed below. Furthermore, to refine the DT_{50} of the metabolite RPA 406341 as input parameter for calculating its predicted environmental concentration in groundwater, a new field study was performed in which metabolite was directly applied. This is in line with the conclusions of EFSA during the peer review of triticonazole (*EFSA Scientific Report 2005, 33, 1-69*). A summary of this study is presented in annex point CA 7.1.2.2.1/1.

New kinetic evaluations were performed to derive reliable trigger and modelling endpoints for the parent compound triticonazole (based on the previously submitted studies) as well as for the major soil metabolite RPA 406341 (based on the new study). The evaluations are compliant with current FOCUS Kinetics guidance (2006 and 2014) and EFSA [*EFSA 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*] guidance even if this is not explicitly mentioned in the reports. They are summarized below in annex points CA 7.1.2.2.1/2 to CA 7.1.2.2.1/5.

In the following, the experimental data will be presented first, both for triticonazole and for its metabolite RPA 406203, respectively. Kinetic evaluations will be summarized subsequently.

Report:	**R012975; Wicks R.J., Guyot C.N., (1993)** RPA 400727: Field soil study in France. 200193 / P92/085 / GOoD3866
GLP:	yes
Guidelines:	Based on USEPA Guidelines

Please see the full summary details presented in Volume 3, Section B.8 of the DAR for triticonazole (Austrian Federal Office for Food Safety, 2003). The study has severe deficiencies (only 2 sampling points after the day of application, time between sampling and freezing not given) and a kinetic evaluation is not possible. Therefore, it cannot be used for the determination of modelling endpoints but only as supplemental information.

Report:	**R012980; Doble M., Parsons R. G., (1994)** Triticonazole (^{14}C-Phenyl): Soil Persistence Study Using Lysimeter Tubes. 200248
GLP:	yes
Guidelines:	Non-guideline study (Method developed for treated seeds to give both dissipation and mobility data).

Please see the full summary details presented in Volume 3, Section B.8 of the DAR for triticonazole (Austrian Federal Office for Food Safety, 2003). As the study does not investigate the degradation in field soil but in outdoor lysimeters, the results can only be used as supplemental information.

Report: **R012996; Wicks R.J., (1996)**
Triticonazole: Terrestrial field soil dissipation study in Europe (Final Report).
201284 / P92/029 / RPA/TRI/95021 / RPA/TRI/95022 / AR32-93 R&D/CRLD/AN/kd/9516604 / GOoD3865
GLP: yes
Guidelines: EU (=EEC) 95/36/EC, Section 7.1.1.2.2, (1995)

Please see the full summary details presented in Volume 3, Section B.8 of the DAR for triticonazole (Austrian Federal Office for Food Safety, 2003). The study has some deficiencies (shipment was performed at ambient temperature except for 0 and 14 DAT, duration of shipment not given, procedures applied not always described in detail). As DegT50 obtained from the study are in the range of DegT50 obtained from other studies, an influence of the shipment on degradation rate can be excluded. Accordingly, the study is still considered acceptable and scientifically sound. A new kinetic evaluation of the data was performed (refer to Annex Point CA 7.1.2.2.1/02 for trigger endpoints and to CA 7.1.2.2.1/03 for modelling endpoints). A short summary of the results and relevant parameters of the study is presented in Table 7.1.2.2.2-6.

Report: **C032147; Duncan P., Doran A., Old J., (2003)**
Triticonazole: Field Soil Dissipation Study in Europe
C032147 / Inveresk 680045
GLP: yes
Guidelines: Directive 95/36/EC Section 7.1.1.2.2 dated 14 July 1995 amending 91/414/EEC).

Please see the full summary details presented in Volume 3, Section B.8 of the DAR for triticonazole (Austrian Federal Office for Food Safety, 2003). The study has deficiencies (azoles used in previous years at 2 sites, only medium quality of analytical results (scattering data), procedures applied not always described in detail). The study is considered acceptable and scientifically sound. A new kinetic evaluation of the data was performed (refer to Annex Point CA 7.1.2.2.1/02 for trigger endpoints and to CA 7.1.2.2.1/03 for modelling endpoints). A short summary of the results and relevant parameters of the study is presented in Table 7.1.2.2.2-6.
The study contains also information on the major soil metabolites RPA 404766 and RPA 406341: The concentrations of the two metabolites always remained low. The maximum concentration of RPA 404766 detected was 0.010 mg/kg. The concentrations of RPA 406341 were generally higher than those of RPA 404766 (up to 0.023 mg/kg). At the end of the study the concentrations of both metabolites had declined to at or below the LOQ for RPA 404766 and at or below 2 x LOQ for RPA 406341. It was not possible to derive reliable kinetic data from the results but it was clear that neither metabolite was formed in significant quantities and that the amounts which were formed were dissipated.

To refine the DT_{50} of the metabolite RPA 406341 as input parameter for calculating its predicted environmental concentration in groundwater, a new field study was performed in which metabolite was directly applied. The study is summarized below.

Report:	CA 7.1.2.2.1/1 Richter T., 2009a Field soil dissipation study of RPA406341 (metabolite of BAS 595 F - Triticonazole) in the formulation EXP 5059144 F on bare soil at four different locations in Europe, 2007-2008 2009/1049703
Guidelines:	EEC 95/36, EEC 91/414, SETAC Procedures for assessing the environmental fate and behaviour and ecotoxicity of pesticides (March 1995), BBA VI 4-1 (December 1986), ECPA Guidance Document on Field Soil Dissipation Studies Aug. 1997, EPA 171-4(e), IVA-Leitlinie Rueckstandsversuche Teil II Lagerstabilitaet von Rueckstandsproben (Frankfurt/Main 1990), SANCO/825/00 rev. 7 (17 March 2004), SANCO/3029/99 rev. 4 (11 July 2000)
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Refer to Annex Point CA 7.1.2.2.1/4 for trigger endpoints and to CA 7.1.2.2.1/5 for modelling endpoints. A short summary of the results and relevant parameters of the study is presented in Table 7.1.2.2.2-7.

Executive Summary

The dissipation of metabolite RPA 406341 under field conditions was investigated at four sites in Europe representative of Northern and Southern EU conditions. The product, formulated as an SC, was broadcast applied to bare soil in a single application at a nominal rate of 100 g a.s./ha using a target water volume of 300 L/ha. Applications were conducted between late August to middle September 2007. Following application and day 0 sampling, the plots were irrigated with an amount of about 10 mm. Soil specimens were taken at intervals up to nominal 340 days and down to a soil depth of 50 cm. RPA 406341 degraded in soil under field conditions at all four European sites with no residues above the LOQ (0.01 mg/kg) left after about 340 days. Residues of the test substance RPA 406341 were found only in the upper 10 cm of the soil, indicating a very low potential of RPA 406341 residues to appear in groundwater.

I. MATERIAL AND METHODS

1. Field sites, sampling, and analysis

The dissipation of metabolite RPA 406341 was studied under bare soil conditions for up to 12 months in Germany, Belgium, South of France, and Spain. Basic soil properties for the top layer of the four sites are listed in Table 7.1.2.2.1-1.

Table 7.1.2.2.1-1: Basic soil properties of sites

Soil properties	L070921 (Germany)	L070922 (Belgium)	L070923 (France)	L070924 (Spain)
Top soil depth [cm]	0-30	0-30	0-20	0-30
Soil Type (USDA)	Silt loam	Silt loam	Loam	Clay
Sand [%]	26.6	44.2	48.9	5.8
Silt [%]	70	51.3	40.3	70.3
Clay [%]	3.5	4.4	10.8	23.9
Organic Matter [%]	2.9	2.4	2.0	2.6
pH ($CaCl_2$) [-]	4.7	5.1	5.4	7.6

The selected fields represented typical regions of agricultural practice 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 if considered necessary, but then was left fallow. Field maintenance measurement, crop and pesticide history information were not subjected to GLP.

The trial area at each site was divided into two plots, one untreated control plot (size: 30-72 m2) and one treated plot (size: 168-180 m2) which consisted of four equal sized subplots A, B, C and D that were assigned for replicates. The untreated control plot was subdivided into 2 subsubplots of the equal size. Each of the four treated subplots was subdivided into eight subsubplots of equal size and 2 buffer stripes. The width of the treated subplots was 2.5 or 3 m and adapted to the size of the spraying boom used. The buffer stripes at the beginning and at the end of each treated subplot were treated with the test item but were not sampled. The distance between the treated subplots was at least 2 m, the distance between treated and untreated plot at least 10 m.

In late summer of 2007, RPA 406341 was applied using broadcast sprayer on the bare plots at a nominal rate of 100 g ha^{-1}. To avoid any potential effect of photolysis the plots were irrigated after application to wash in the substance.

Climatic conditions were based on records of appropriate weather stations located on-site or at a distance of maximal 12 km from site. Monthly summary results on temperature and precipitation are presented in the study report.

Replicate soil specimens (20 per treated plot and 20 per control plot) were taken at intervals up to about 340 days and down to a soil depth of up to 50 cm. At day 0, immediately after application, the treated plots were sampled down to 10 cm only.

Soil samples were analysed for RPA 406341 according to the validated method No. 0051, which is presented in chapter CA 4.1.2 of this dossier, i.e by solid-liquid extraction using sonication and shaking with ammonium hydroxide and acetone, followed by sample clean-up by solid phase using C18 phase. The analyte was detected and quantified by HPLC/MS/MS.

2. Application verification and recovery rates

Procedural recovery experiments were conducted with untreated field soil as well as with untreated LUFA 2.2 soil (during analysis of storage samples) which was used for the Petri dish samples that served as application monitors. Untreated and fortified samples of field soil were analyzed along with the applied Petri dishes as well as with samples from the field. Fortification levels were at 0.01, 2.0 and 5.0 mg/kg. The fortification experiments yielded average recoveries for RPA406341 at 0.01 mg/kg level of 88 ± 10 % (n=25), at 2.0 mg/kg level of 84 t 7 % (n=10) and at 5.0 mg/kg level of 85 t 7 % (n=14). These data prove that the analytical method applied is able to accurately determine RPA406341 residues in soil down to a concentration of 0.01 mg/kg. Residues in blank samples were not detectable.

Residue levels of RPA406341 achieved on extraction and analysis of the application monitors (Petri dishes filled with soil LUFA 2.2) were corrected for mean procedural recoveries of the respective analytical set of samples and then converted into residue rates (in g/ha) taking into account the area of the Petri dishes (91.6 cm2). The obtained rates ranged from 70 to 94 g/ha (see Table 3 for individual figures) representing 70-94% of the target application rate.

3. Sample storage and Storage stability

All soil specimens (inclusive of Petri dish samples, main samples and double samples) were placed into freezer storage at about -18°C within a maximum of 6.5 hours of being taken. The specimens remained frozen at about -18°C until shipment to BASF SE, Limburgerhof, Germany. For exceptions, the temperature was higher (max. -8° C), this was only for a short period and the samples remained in any case frozen.

In order to verify that residues in soil specimens did not deteriorate during the time of storage in the freezers, the storage stability of RPA406341 was checked in frozen soil. The soil arrived in the laboratory in Brazil on September 1th, 2007. From this time the soil was stored at the cold chamber at -20° C or cooler. The shipment was conducted at frozen conditions and the samples were homogeneised before analysis. For all experiments, LUFA 2.2 soil, batch 07/736/01, was used. Untreated soil aliquots of 5 g were weighed into plastic containers.Five sets of samples were prepared at one time point consisting of 5 specimens each. Each set of samples was intended for an analytical queue covering one nominal sampling time point between 14 and 365 days. Two specimens of each set were spiked at a level of 100 Ng/kg and were intended for storage. The other 3 specimens of each set were not initially treated but two of them were intended to be freshly fortified on the day of analysis of the sample set. The fifth specimen remained untreated and served as control sample. An additional set of samples consisting of one untreated and two freshly fortified specimens was prepared and worked up immediately. This set served as the day 0 sample. All samples besides the day 0 samples were stored in the dark at about -20 °C for the intended storage period. These conditions were in agreement with those used for the storage of field residue samples.

The storage stability samples were worked up and analysed for RPA406341 according to the same method used for the analysis of field residue samples. Details are given in the analytical phase report in Attachment 7.

II. RESULTS

1. Residue data

For all sampling dates and at all sites residue of RPA 406341 was only found in the top 10 cm of the soil profile (Table 7.1.2.2.1-2). No movement below this first soil layer occurred.

Table 7.1.2.2.1-2: Residue data of RPA 406341 [mg/kg] for 0-10 cm soil depth

L070921 (Germany)		L070922 (Belgium)		L070923 (France)		L070924 (Spain)	
DAT [a]	Residue [b]	DAT	Residue [b]	DAT	Residue [b]	DAT	Residue [b]
0	0.082	0	0.065	0	0.044	0	0.087
3	0.059	4	0.072	3	0.041	2	0.047
9	0.067	11	0.033	10	0.028	12	0.051
28	0.050	32	0.047	31	0.025	30	0.020
65	0.026	67	0.032	64	0.020	62	0.018
97	0.022	103	0.022	100	0.025	96	0.017
176	0.020	175	0.019	184	0.017	182	n.d.
346	< 0.01	344	< 0.01	336	< 0.01	351	< 0.01

a DAT = Days after treatment
b Residue: units in [mg RPA 406341 / kg soil]

2. Storage stability

The results show no significant decline of RPA406341 concentrations up to 365 days. From these data it can be concluded that residues of RPA406341 are stable in soil for at least 365 days when stored frozen at about -20°C. This period covers the maximum storage time from sampling to the first analysis of samples collected during the course of the field soil dissipation study except three samples from the 20-30 cm layer. They were analysed later in order to demonstrate two residue free soil layers below the 0-10 cm horizon wich provide residues.

III. CONCLUSIONS

The study delivers appropriate data to derive endpoints for subsequent model calculations.

Kinetic evaluation of existing terrestrial field dissipation studies with triticonazole according to FOCUS Kinetics (2006, 2014) to obtain endpoints as triggers for further use is presented below. Kinetic evaluation to derive normalized $DegT_{50}$ to be used as input for groundwater and surface water models are summarized hereafter.

Report: CA 7.1.2.2.1/2
Huber S., 2008a
Best-fit analysis and normalization of the field dissipation of the Triticonazole (BAS 595 F) metabolite RPA 406341
2008/1089810
Guidelines: FOCUS Kinetics (2006)
GLP: no

Executive Summary

Two field soil dissipation studies of triticonazole were re-evaluated according to the FOCUS kinetics (2006) guidance in order to derive trigger endpoints. DT_{50} values for triticonazole were in the range of 38 to 250 days with DT_{90} values between 360 and >1000 days.

I. MATERIAL AND METHODS

Two field soil dissipation studies (Wicks, 1996 & Duncan et al., 2003) with altogether eight trials across Europe (Germany, UK, France, Italy, Spain) were considered. The parent triticonazole was applied in all trials. Residues of triticonazole in the soil layers were accumulated on a 10-cm basis and used as input for the kinetic evaluation. The simulation modelling was performed with the software package ModelMaker 3.0.4 (Family Genetix Ltd). Trigger endpoints of triticonazole were derived following the recommended procedures outlined by FOCUS (2006).

II. RESULTS AND DISCUSSION

For all but two sites the dissipation behaviour could be best described by bi-phasic kinetics. For the Italian site (Bologna), a modified hockey-stick model was fitted to the data to account for the initial lag-phase (the first degradation rate constant k_1 was fixed to zero). Results are valid for use as persistence endpoints and are presented in the table below:

Table 7.1.2.2.1-3: Triticonazole: Trigger endpoints in field soils

Study	System	Kinetic model	DT_{50} (d)	DT_{90} (d)
Wicks, 1996	Bologna (IT)	Modified HS [a]	230	360
	Goch (DE)	DFOP	85	632
	Manningtree (UK), spray treatment	DFOP	55	641
	Mereville (FR)	FOMC	133	1237
Duncan et al., 2003	Brentwood (UK)	DFOP	250	831
	Saint Trivier sur Moigans (FR)	SFO	109	360
	Vallfogona de Balaquer (ES)	DFOP	54	398
	Goch (DE)	DFOP	38	500

[a] First degradation rate k_1 fixed to zero to account for the initial lag-phase.

III. CONCLUSION

Field soil dissipation of triticonazole was re-evaluated according to FOCUS (2006). Trigger endpoints for the parent were derived.

Kinetic evaluation of existing terrestrial field dissipation studies with triticonazole to derive normalized $DegT_{50}$ according to the new EFSA (2010) guidance are summarized below.

Report: CA 7.1.2.2.1/3
Schwarz N.,Jarvis T., 2014c
Determination of normalised rates of decline for Triticonazole from two field dissipation studies
2014/1083344

Guidelines: FOCUS (2006): Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration Sanco/10058/2005 version 2.0 434 pp., FOCUS Kinetics Generic Guidance v1.0 (2011)

GLP: no

Executive Summary

Field soil dissipation studies of triticonazole were re-evaluated according to EFSA (2010) and the FOCUS kinetics [*FOCUS (2006), FOCUS (2011): Generic Guidance for Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration, version 1.0*] guidance. Degradation rates for eight sites in different regions of Europe were derived that can be used in environmental fate modelling (modelling endpoints). $DegT_{50}$ values (normalised: 20°C, pF2) for triticonazole for use in simulation modelling ranged from 49.5 to 252 days.

I. MATERIAL AND METHODS

Two field soil dissipation studies carried out at eight sites in different regions of Europe (Italy, Germany, UK, France, Spain) were considered (refer to CA 7.1.2.2.1: Wicks (*1996, Doc No. R012996*) and Duncan et al., (*2003, C032147)).* The parent triticonazole was applied onto bare soil at a nominal application rate of 240 g a.s./ha and then incorporated into the soil on the same day and winter wheat was then planted. The application of triticonazole was conducted as spray application at all sites. At one site (Manningtree, UK) the application was additionally carried out as seed treatment. Residue data of triticonazole in different depths (in percentage of applied) were obtained from the studies and used as input for the kinetic evaluation. Replicates from four subplots per trial site were considered.

Due to the incorporation of triticonazole into soil, the EFSA (2010) recommendation of incorporation to about 10 cm to prevent surface processes taking place was considered to be fulfilled and all data points were considered for the kinetic evaluation.

The field data were normalised to 20°C and pF2 using the time-step normalisation approach. The PERSIST model was used to calculate normalised day lengths based on temperature and moisture data which requires that daily average soil moisture and temperature data are either directly available or can be calculated from other data, e.g. daily maximum/minimum air temperatures. Weather data from all sites were provided in the reports and were sufficient for normalisation.

The simulation modelling was performed using KinGUI Version 2. Degradation rates of triticonazole were derived. FOCUS (2006, 2011) approaches were used to determine the appropriate kinetic fit in each soil.

II. RESULTS AND DISCUSSION

In general, the replicate results from the subplots showed high variability and hence in all cases the χ^2 error values were considerably high. Please see the full summary details presented in Volume 3, Section B.8 of the DAR for triticonazole (Austrian Federal Office for Food Safety, 2003).

The SFO model did not always provide acceptable fits. Biphasic degradation kinetics (FOMC or DFOP) were considered more appropriate at Manningtree (UK, spray treatment), Vallfogona (ES), Goch (DE) and Mereville (FR), both by statistical and visual measure. There was no significant difference in the $DegT_{50}$ values for spray application and seed treatment at the Manningtree site, hence indicating that the differing methods of application did not affect the degradation rate. The derived modelling endpoints are shown in Table 7.1.2.2.1-4.

Table 7.1.2.2.1-4: Triticonazole - Soil Degradation Modelling Endpoints from Field Studies

Study	Site	Application method	Kinetic Model	χ^2 (%)	$DegT_{50}$ at 20°C, pF2 (d)	$DegT_{90}$ at 20°C, pF2 (d)
Wicks, 1996	Bologna (IT)	Spray	SFO	22.8	75.5	251
	Goch (DE)	Spray	DFOP	21.6	81.4[a]	211
	Manningtree (UK)	Spray	FOMC	15.7	92.2[b]	306
	Manningtree (UK)	Seed treatment	SFO	36.5	88.8	295
	Mereville (FR)	Spray	DFOP	14.1	252[a]	467
Duncan, 2003	Brentwood (UK)	Spray	SFO	32.9	100	333
	Saint Trivier sur Moigans (FR)	Spray	SFO	19.3	49.5	165
	Vallfogona de Balaquer (ES)	Spray	FOMC	33.4	57.2[b]	190
	Goch (DE)	Spray	FOMC	12.6	52.4[b]	174

[a] Calculated using the slow degradation rate: $\ln(2)/k_2$.
[b] Calculated as follows: $DT_{90}/3.32$.

III. CONCLUSION

Field soil dissipation of triticonazole was re-evaluated according to FOCUS (2006, 2001) and EFSA (2010). Modelling endpoints for the parent were derived.

Kinetic evaluation of existing terrestrial field dissipation studies with the metabolite RPA 406341 according to FOCUS Kinetics (2006, 2014) to obtain endpoints as triggers for further use is presented below. Kinetic evaluation to derive normalized $DegT_{50}$ to be used as input for groundwater and surface water models are summarized hereafter.

Report: CA 7.1.2.2.1/2
Huber S., 2008a
Best-fit analysis and normalization of the field dissipation of the Triticonazole (BAS 595 F) metabolite RPA 406341
2008/1089810
Guidelines: FOCUS Kinetics (2006)
GLP: no

I. MATERIAL AND METHODS

The study by Richter [see KCA 7.1.2.2.1/3 2009/1049703] was evaluated according to guidance outlined by FOCUS Kinetics (2006). The goal was to derive best-fit dissipation DT_{50} values, as well as to reference conditions (20°C; Q_{10}=2.58; pF 2) normalized single-first-order (SFO) DT_{50}.

The kinetic evaluation was based on the interim report for the field study. Thus, report for the kinetic evaluation is dated earlier than the report for the study.

Best-fit models were derived within the software package KinGUI 1.1 according to FOCUS Kinetics guidance on determination of trigger endpoints. Normalization to reference conditions was conducted by linking the pesticide fate model FOCUS Pearl 3.3.3 to the optimization software PEST. This approach consists of repeatedly running the model and automatically adjusting the SFO-reference DT_{50} until the fit between simulated and measured soil residues is acceptable.

II. RESULTS

For all but one site the dissipation behaviour could be best described by bi-phasic FOMC models (Table 7.1.2.2.1-5). For those three sites with bi-phasic dissipation kinetics DFOP did not improve the fits.

Table 7.1.2.2.1-5: Best-fit models for field dissipation of RPA 406341

Site	Best-fit DT_{50} [d]	Best-fit DT_{90} [d]	Best fit model	Chi^2-error [%]
L070921 (Germany)	36.1	> 1000	FOMC	12.7
L070922 (Belgium)	78.2	259.8	SFO	20.7
L070923 (France)	46.9	> 1000	FOMC	13.6
L070924 (Spain)	4.6	302.7	FOMC	24.5

Additionally, normalized endpoints were derived in the study report. However, they do not fulfil latest guidance documents. Thus, a new evaluation was performed by Schwarz and Jarvis (BASF DocID 2014/1083343), which is summarized at the Annex Point CA 7.1.2.2.1/5.

III. CONCLUSIONS

Best-fit dissipation endpoints (trigger endpoints) were derived within this study.

Kinetic evaluation of existing terrestrial field dissipation studies with the metabolite to derive normalized $DegT_{50}$ according to the new EFSA (2010) guidance are summarized below.

Report:	CA 7.1.2.2.1/4 Schwarz N.,Jarvis T., 2014b Determination of normalised rates of decline for Triticonazole metabolite RPA406341 from a field dissipation study 2014/1083343
Guidelines:	FOCUS (2006): Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration Sanco/10058/2005 version 2.0 434 pp., FOCUS Kinetics (2011) Generic Guidance v 1.0
GLP:	no

Executive Summary

Field soil dissipation data for the metabolite RPA 406341 (refer to CA 7.1.2.2.1/1) were normalised to 20°C and a moisture content of pF 2 using the time step normalisation approach. The data were evaluated according to FOCUS (2006 and 2011) and the requirements of EFSA (2010 and 2014) using SFO, FOMC and DFOP kinetic models. The resulting $DegT_{50}$ values for RPA 406341 for use in simulation modelling ranged from 33.6 to 70.0 days.

I. MATERIAL AND METHODS

The degradation of the triticonazole metabolite RPA 406341 on bare soil was investigated under field conditions at four sites situated in different regions of Europe (Germany, Belgium, France and Spain). The nominal application rate was 100 g a.s./ha to bare soil. Following the day 0 sampling, the untreated and treated plots were irrigated with about 10 mm water. Thus, according to EFSA (2014) the influence of surface processes on the degradation of triticonazole in the study can be excluded. No tillage was performed during the course of the study and no crops were grown throughout any of the trials. Residue data of RPA 406341 (in mg/kg) were obtained from the studies and used as input for the kinetic evaluation. Replicates from four subplots per trial site were considered. Residues of RPA 406341 were only present in the upper 0-10 cm soil layer and degraded to concentrations below the LOQ of 0.01 mg/kg at the last sampling point at all 4 sites (ca. 336 - 346 days after application).

The field data were normalised to 20°C and pF2 using the time-step normalisation approach. The PERSIST model was used to calculate normalised day lengths based on temperature and moisture data which requires that daily average soil moisture and temperature data are either directly available or can be calculated from other data, e.g. daily maximum/minimum air temperatures. Overall, weather data from all sites were provided in the reports and were sufficient for normalisation. However, some data gaps are reported which were filled by adding the average values of the measurements before and/or afterwards.

The modelling was performed using KinGUI Version 2. FOCUS (2006 and 2011) approaches were used to determine the appropriate kinetic fit in each soil for use in determining simulation endpoints.

II. RESULTS AND DISCUSSION

In general, the results showed some variability as would be expected for field data. However, in all cases the χ^2 error values were < 24%. For all for sites, the SFO model provided an acceptable fit and the SFO $DegT_{50}$ was the preferred endpoint. The derived modelling endpoints are summarized in Table 7.1.2.2.1-4.

Table 7.1.2.2.1-6: RPA 406341 - Modelling Endpoints from Field Studies

Study	Site	Kinetic Model	χ^2 (%)	$DegT_{50}$ at 20°C, pF2 (d)	$DegT_{90}$ at 20°C, pF2 (d)
Richter, 2009	Goch, Germany	SFO	12.24	35.8	118.9
	Rummen, Belgium	SFO	23.19	33.6	111.5
	Meauzac , France	SFO	14.19	70.0	229.1
	Valencia, Spain	SFO	16.76	37.4	124.2

III. CONCLUSIONS

Modelling endpoints for the field dissipation of RPA 406341 were derived within this study.

CA 7.1.2.2.2 Soil accumulation studies

The following soil accumulation study was evaluated and accepted by the European Commission (SANCO/10442/2005-Final rev 1, 12 March 2010). The study was performed for 4 years in Germany and the UK with an elevated dose rate of yearly 112.5 g/ha. The plateau concentration for triticonazole was in the range of 0.001 mg/kg with peak concentrations ranging from 0.0022 to 0.0044 mg/kg. Concentrations of the major soil metabolite RPA 406341 were below LOQ (0.002 mg/kg), except for six times in Germany (0.002 – 0.005 ppm). Furthermore, the potential for soil accumulation was assessed in the M-CP document, also for the metabolite RPA 406341.

Furthermore, the potential for soil accumulation was assessed for triticonazole and its metabolite RPA 406341. For details, please refer to M-CP, Section 9, point 9.1.3.

Report:	**Davis, H. (2004)** Triticonazole - Long term soil dissipation study with repeated applications BASF DocID 2004/5000553 Original report number: 1849/034-D2149
GLP:	yes
Guidelines:	EEC 95/36 7.1.1.2.2; EEC 91/414

Please see the full summary details presented in Volume 3, Section B.8 of the DAR for triticonazole (Austrian Federal Office for Food Safety, 2003). The study is still considered acceptable but it has some minor deficiencies (some procedures are not described in detail or are not state of the art, no analysis of enantiomers). Furthermore, ploughing and sampling were both performed to a depth of 30 cm and deeper soil layers were not analyzed.

Rate of degradation in soil - Overall Conclusions

Aerobic soil degradation of the active substance triticonazole and its metabolites RPA 404766, RPA 406341 and RPA 407922 in was investigated in the laboratory and reliable trigger and modelling endpoints were calculated. The values are presented in Table 7.1.2.2.2-1 to Table 7.1.2.2.2-4.

As the trigger $DegT_{50}$ values for the parent compound triticonazole and the metabolite RPA 406341 exceed 60 days, field soil dissipation studies were performed for these compounds. The corresponding trigger and modelling endpoints are presented in Table 7.1.2.2.2-6 and Table 7.1.2.2.2-7. For both, triticonazole and RPA 406341, $DisT_{90}$ vales were greater than one year in more than one soil. Therefore, the accumulation behaviour of these substances was addressed.

For triticonazole, a field accumulation study revealed that after correction for the application rate, the plateau concentration for triticonazole was in the range of 0.001 mg/kg with peak concentrations ranging from 0.0022 to 0.0044 mg/kg for a ~9-times elevated use rate if triticonazole. Concentrations of the major soil metabolite RPA 406341 were below LOQ (0.002 mg/kg), except for six times in Germany (0.002 – 0.005 ppm). Furthermore, the potential for soil accumulation was assessed in the M-CP document, also for the metabolite RPA 406341.

During the anaerobic soil degradation study, no significant degradation of triticonazole occurs. Therefore, anaerobic degradation rates for triticonazole and its major soil metabolites were not calculated.

Table 7.1.2.2.2-1: Overview of the laboratory aerobic degradation rate studies – active substance triticonazole

Reference			Soil characteristics				Incubation conditions		Trigger Endpoints			Modelling Endpoints		
			Soil origin	Soil type	pH	OC (%) [d]	Moisture	Temp. (°C)	Kinetic Model	DT_{50} (days)	DT_{90} (days)	Kinetic Model	DT_{50} (d) study cond.	DT_{50} (d) at 20°C and pF2
CA 7.1.2.1.1	Studies previously evaluated during Annex I inclusion	Ayliffe &Austin, 1993	Not reported	Sandy loam	6.42 [b]	0.72	75% of 33 kPa	22	SFO	253	840	SFO	253	176
			Not reported	Clay loam	6.18 [b]	5.66		22	SFO	136	452	SFO	136	145
		Ayliffe & McMillan-Staff, 1994	Speyer 2.2	Loamy sand	6.8 [b]	2.35		22	SFO	187	620	SFO	187	206
		Doble et al., 1996	Not reported	Clay [a]	5.7 [c]	1.2		25	SFO	492	>1000	SFO	492	401
		Ayliffe & Godward, 1993	Not reported	Loamy sand	6.24 [b]	18.70		22	SFO	249	828	SFO	249	299 [e]
			Not reported	Loamy sand	6.24 [b]	18.70		10	SFO	599	>1000	SFO	599	234 [e]
			Not reported	Sandy loam	6.30 [b]	0.83		10	SFO	347	>1000	SFO	347	79.8
			Not reported	Clay loam	6.08 [b]	3.28		10	SFO	183	607	SFO	183	61.4
		Simmonds et al., 1996	Manningtree	Sandy loam [a]	6.1 [c]	0.8	50% FC	25	SFO	167	555	SFO	167	166 [e]
			Manningtree (reduced rate)				50% FC	25	SFO	187	620	SFO	187	186 [e]
			Manningtree				20% FC	25	SFO	215	714	SFO	215	344 [e]
			Manningtree				50% FC	10	SFO	413	>1000	SFO	413	100 [e]
	New Studies	Ta & Strobush, 2015	Li10	Loamy Sand [a]	6.3 [c]	0.81	50% MWHC	20	DFOP	149	640	SFO	136	116.0
		Grella et al. 2014	LUFA 2.2	Loamy sand [a]	5.5 [c]	2.8		20	SFO	317	>1000	SFO	317	299
			LUFA 2.3	Sandy loam [a]	6.9 [c]	1.7		20	FOMC	125	>1000	SFO	115	100
			LUFA 5M	Sandy loam [a]	7.4 [c]	1.7		20	FOMC	149	>1000	- [f]	-	-
											Geometric mean:			161

a Soil type according to USDA, for the other soils the classification is unknown.
b Buffer solution unknown.
c Buffer solution = 0.01 M $CaCl_2$
d If not explicitly mentioned in the study report, OC was calculated: OC (%) = OM (%) / 1.724.
e The geometric mean value determined from single values for the same soil (values *in italic*) was used for the calculation of the overall geometric mean value.
f Modelling endpoints could not be determined since the SFO model did not provide a reliable fit and the slow degradation rate obtained from DFOP model was not significant.

Studies/ Values shaded in grey have been reviewed as part of the first EU review of triticonazole.

Table 7.1.2.2.2-2: Overview of the laboratory aerobic degradation rates – major soil metabolite RPA 404766

Reference			Soil characteristics				Incubation conditions		Trigger Endpoints			Modelling Endpoints			
			Soil origin	Soil type	pH	OC (%) [d]	Moisture	Temp. (°C)	Kinetic Model	DT50 (d)	DT90 (d)	Kinetic Model	DT50 (d) study cond.	DT50 (d) at 20°C and pF2	FF
CA 7.1.2.1.2	Studies previously evaluated during Annex I inclusion	Ayliffe &Austin, 1993	Not reported	Sandy loam	6.42 [b]	0.72	75% of 33 kPa	22	Degradation rates from pathway fits are not reliable			Degradation rates from pathway fits are not reliable.			0.200
			Not reported	Clay loam	6.18 [b]	5.66		22							0.521
		Ayliffe & McMillan-Staff, 1994	Speyer 2.2	Loamy sand	6.8 [b]	2.35		22							0.488
		Doble et al., 1996	Not reported	Clay [a]	6.5 [c]	1.2		25							0.419
		Ayliffe & Godward, 1993	Not reported	Loamy sand	6.24 [b]	18.70		22							*0.312* [e]
			Not reported	Loamy sand	6.24 [b]	18.70		10							*0.424* [e]
			Not reported	Sandy loam	6.30 [b]	0.83		10							0.253
			Not reported	Clay loam	6.08 [b]	3.28		10							0.474
		Simmonds et al., 1996	Manningtree	Sandy loam [a]	6.1 [c]	0.8	50% FC	25							*0.437* [e]
			Manningtree (reduced rate)				50% FC	25							*0.402* [e]
			Manningtree				20% FC	25							*0.471* [e]
			Manningtree				50% FC	10							*0.345* [e]
		Crowe, 2002	Baylham	Sandy loam	4.5 [c]	1.2	pF2-2.5	20	SFO	30.9	102.8	SFO	30.9	30.9	-
			Royston	Silty clay loam	7.2 [c]	2.1		20	DFOP	11.0	137.9	DFOP	60.8	60.8	-
			Ongar	Clay loam	6.9 [c]	2.6		20	DFOP	30.2	166.2	DFOP	58.7	58.7	-
	New Study	Ta & Strobush, 2015	Li10	Loamy Sand[a]	6.3 [c]	0.81	50% MWHC	20	DFOP-SFO	- [f]		SFO-SFO	23.6	20.1	0.576
Geometric mean:														**38.6**	
Arithmetic mean:															**0.413**

a Soil type according to USDA, for the other soils the classification is unknown.
b Buffer solution unknown , c Buffer solution = 0.01 M $CaCl_2$
d If not explicitly mentioned in the study report, OC was calculated: OC (%) = OM (%) / 1.724.
e The arithmetic mean value determined from single values for the same soil (values in italic) was used for the calculation of the overall arithmetic mean value.
f The derived trigger DegT50 of 94.6 days is not considered reliable because no degradation of the metabolite was observed and the value differs distinctly from the values obtained from the metabolite study
- not calculated since the metabolite was directly applied to soil

Studies/values shaded in grey have been reviewed as part of the first EU review of triticonazole.

Table 7.1.2.2.2-3: Overview of the laboratory aerobic degradation rates – major soil metabolite RPA 406341

Reference			Soil characteristics				Incubation conditions		Trigger Endpoints			Modelling Endpoints			
			Soil origin	Soil type	pH	OC (%) [d]	Moisture	Temp. (°C)	Kinetic Model	DT_{50} (d)	DT_{90} (d)	Kinetic Model	DT_{50} (d) tudy cond	DT_{50} (d) at 20°C and pF2	FF [e]
CA 7.1.2.1.2	Studies previously evaluated during Annex I inclusion	Ayliffe &Austin, 1993	Not reported	Sandy loam	6.42 [b]	0.72	75% of 33 kPa	22	Degradation rates from pathway fits are not significan			Degradation rates from pathway fits are not significant			0.760
			Not reported	Clay loam	6.18 [b]	5.66		22							0.343
		Ayliffe & McMillan-Staff, 1994	Speyer 2.2	Loamy sand	6.8 [b]	2.35		22							0.512
		Doble et al., 1996	Not reported	Clay [a]	6.5 [c]	1.2		25							0.581
		Ayliffe & Godward, 1993	Not reported	Loamy sand	6.24 [b]	18.70		22							*0.688* [f]
			Not reported	Loamy sand	6.24 [b]	18.70		10							*0.576* [f]
			Not reported	Sandy loam	6.30 [b]	0.83		10							0.404
			Not reported	Clay loam	6.08 [b]	3.28		10							0.526
		Simmonds et al., 1996	Manningtree	Sandy loam [a]	6.1 [c]	0.8	50% FC	25							*0.563* [f]
			Manningtree (reduced rate)				50% FC	25							*0.598* [f]
			Manningtree				20% FC	25							*0.529* [f]
			Manningtree				50% FC	10							*0.655* [f]
		McGhee, 2000	Royston	Clay Loam [a]	7.0 [c]	1.2	45% MWHC	20	SFO	165.2	548.8	SFO	165.2	102.4	N/A
			Ipswich	Sandy Loam [a]	5.3 [c]	2.1		20	SFO	198.9	660.9	SFO	198.9	143.2	N/A
			Ongar	Loam [a]	6.2 [c]	2.6		20	SFO	345.9	>1000	SFO	345.9	231.7	N/A
	New Study	Ta & Strobush, 2015	Li10	Loamy Sand[a]	6.3 [c]	0.81	50% MWHC	20	DFOP-SFO	205	682	SFO -SFO	35.7	30.3	0.386
											Geometric mean:			**100.7**	
											Arithmetic mean:				**0.526**

a Soil type according to USDA, for the other soils the classification is unknown.
b Buffer solution unknown
c = 0.01 M $CaCl_2$
d If not explicitly mentioned in the study report, OC was calculated: OC (%) = OM (%) / 1.724.
e FF is the formation fraction from parent triticonazole
f The arithmetic mean value determined from single values for the same soil (values *in italic*) was used for the calculation of the overall arithmetic mean value.

Studies/ Values shaded in grey have been reviewed as part of the first EU review of triticonazole.

Table 7.1.2.2.2-4: Overview of the laboratory aerobic degradation rates – major soil metabolite RPA 407922

Reference			Soil characteristics				Incubation conditions		Trigger Endpoints			Modelling Endpoints			
			Soil origin	Soil type	pH	OC (%) [d]	Moisture	Temp. (°C)	Kinetic Model	DT_{50} (d)	DT_{90} (d)	Kinetic Model	DT_{50} (d) study cond.	DT_{50} (d) at 20°C and pF2	FF [e]
CA 7.1.2.1.2	Studies previously evaluated during Annex I inclusion	Ayliffe &Austin, 1993	Not reported	Sandy loam	6.42 [b]	0.72	75% of 33 kPa	22	Degradation rates from pathway fits are not significant			Degradation rates from pathway fits are not significant			0.040
			Not reported	Clay loam	6.18 [b]	5.66		22							0.136
		Ayliffe & McMillan-	Speyer 2.2	Loamy sand	6.8 [b]	2.35		22							-
		Doble et al., 1996	Not reported	Clay [a]	6.5 [c]	1.2		25							-
		Ayliffe & Godward, 1993	Not reported	Loamy sand	6.24 [b]	18.70		22							-
			Not reported	Loamy sand	6.24 [b]	18.70		10							
			Not reported	Sandy loam	6.30 [b]	0.83		10							-
			Not reported	Clay loam	6.08 [b]	3.28		10							-
		Simmonds et al., 1996	Manningtree	Sandy loam [a]	6.1 [c]	0.8	50% FC	25							-
			Manningtree (reduced rate)				50% FC	25							-
			Manningtree				20% FC	25							-
			Manningtree				50% FC	10							-
		Unsworth & Clark, 2000	Not reported	Clay loam [a]	7.4 [c]	4.1	45% MWHC	20	SFO	0.44	1.5	SFO	0.44	0.44	-
			Not reported	Clay loam [a]	7.3 [c]	2.3		20	FOMC	0.7	6.6	FOMC	2.0	2.0	-
			Not reported	Loamy sand [a]	6.2 [c]	1.3		20	SFO	1.1	3.8	SFO	1.1	1.1	-
Geometric mean:														**0.99**	
Maximum:															**0.136**

a Soil type according to USDA, for the other soils the classification is unknown.
b Buffer solution unknown.
c = 0.01 M $CaCl_2$.
d If not explicitly mentioned in the study report, OC was calculated: OC (%) = OM (%) / 1.724.
e FF is the formation fraction from parent triticonazole
\- no reliable formation fraction available or not calculated due to application of metabolite
Studies/ Values shaded in grey have been reviewed as part of the first EU review of triticonazole.

Table 7.1.2.2.2-5: Overview of the results of the anaerobic soil degradation study

Reference			Soil characteristics				Incubation conditions		Information on degradation rates
			Soil type		pH	OC (%)	Moisture	Temp. (°C)	
CA 7.1.2.1.3 and CA 7.1.2.1.4	Studies previously evaluated during Annex I inclusion	Goodyear A., 1994 (+ amendment R012983 of 1998)	Manningtree	Sandy loam	7.6 (KCl)	0.3	75% of 33 kPa for 30 days before flooding	25°C	Triticonazole and its major soil metabolites RPA 406341, RPA 404766 and RPA 405826 were not degraded under anaerobic conditions. Therefore, no anaerobic degradation rates were calculated

Studies/ Values shaded in grey have been reviewed as part of the first EU review of triticonazole.

Table 7.1.2.2.2-6: Overview of the field degradation rates of triticonazole

Reference			Soil characteristics (0 – 10 cm or 0 – 30 cm)				Application Method	Trigger Endpoints			Modelling Endpoints		
			Soil origin	Soil type [a]	pH [b]	OC (%) [c]		Kinetic Model	DT_{50} (d)	DT_{90} (d)	Kinetic Model	$DegT_{50}$ (d) at 20°C and pF2	$DegT_{90}$ (d) at 20°C and pF2
CA 7.1.2.2.1	Studies previously submitted	Wicks, 1996	Bologna, Italy	Loam	8.4	0.9	Spray	Modified HS	230	360	SFO	75.5	251
			Goch, Germany	Sandy loam	6.6	1.2	Spray	DFOP	85	632	DFOP	81.4	211
			Manningtree, UK	Sandy loam	5.3	0.9	Spray	DFOP	55	641	FOMC	92.2 [d]	306
			Manningtree, UK	Sandy loam	5.3	0.9	Seed treatment	Not determined	-	-	SFO	*88.8* [d]	295
			Mereville, France	Silty clay loam	7.8	1.4	Spray	FOMC	133	1237	DFOP	252	467
		Duncan et al., 2003	Brentwood, UK	Sandy silt loam	7.9 / 7.3	1.9	Spray	DFOP	250	831	SFO	100	333
			St Trivier, France	Sandy silt loam	8.1 / 7.1	1.1	Spray	SFO	109	360	SFO	49.5	165
			Balaquer, Spain	Clay loam	8.0 / 7.4	1.2	Spray	DFOP	54	398	FOMC	57.2	190
			Goch, Germany	Sandy silt loam	7.2 / 6.7	0.9	Spray	DFOP	38	500	FOMC	52.4	174
											Geometric mean:	**82.2**	

a Soil type is reported according to USDA (Wicks, 1996) and ADAS (Duncan et al., 1996)
b Buffer solution = H_2O (Wicks, 1996) and H_2O / $CaCl_2$ (Duncan et al., 1996)
c If not explicitly mentioned in the study report, OC was calculated according to: OC (%) = OM (%) / 1.724.
d The geometric mean of the singles values of the Manning tree soil was used for the calculation of the overall geometric mean value.

Studies shaded in grey have been reviewed as part of the first EU review of triticonazole.

Table 7.1.2.2.2-7: Overview of the field degradation rates of RPA 406341

Reference			Soil characteristics (0 – 20 or 30 cm)				Application Method	Trigger Endpoints			Modelling Endpoints		
			Soil origin	Soil type (USDA)	pH [a]	OC (%)		Kinetic Model	DT_{50} (d)	DT_{90} (d)	Kinetic Model	DT_{50} (d) at 20°C and pF2	DT_{90} (d) at 20°C and pF2
	New study	Richter, 2009	Goch, Germany	Silt loam	4.7	1.7	Spray	FOMC	36.1	> 1000	SFO	35.8	118.9
	New study	Richter, 2009	Rummen, Belgium	Silt loam	5.1	1.4	Spray	SFO	78.2	259.8	SFO	33.6	111.5
	New study	Richter, 2009	Meauzac, France	Loam	5.4	1.2	Spray	FOMC	46.9	> 1000	SFO	70.0	229.1
	New study	Richter, 2009	Valencia, Spain	Clay	7.6	1.5	Spray	FOMC	4.6	302.7	SFO	37.4	124.2
										Geometric mean:		**42.1**	

a Buffer solution = $CaCl_2$

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

Two studies investigating the adsorption and desorption behaviour of triticonazole in a total of 9 soils were evaluated during the Annex I inclusion and accepted by the European Commission (SANCO/10442/2005-Final rev 1, 12 March 2010). Those studies are still considered valid and scientifically sound, although they have some minor deviations to the current OECD Test Guideline 106 (e.g. only four concentrations tested, short desorption cycle). To fully comply with the current guidance and to confirm the results of the previously submitted studies, a new study was performed.

The previously submitted studies are listed below, deviations from current guidance are presented. The summary of the new adsorption/desorption study is provided thereafter in annex point CA 7.1.3.1.1/1. A short summary of the results and relevant parameters of all individual studies is presented in Table 7.1.3.2-1.

Report:	**R013051; Burr C.M., Austin D.J., (1992)** RPA 400727-^{14}C: Adsorption / desorption on five soils. 428632 / P91/325
GLP:	yes
Guidelines:	USEPA (=EPA) 163-1 / OECD 106

Please see the full summary details presented in Volume 3, Section B.8 of the DAR for triticonazole (Austrian Federal Office for Food Safety, 2003). The study is still considered acceptable but it has some minor deviations to the current OECD Test Guideline 106 (soil classification system unknown, buffer for determination of pH unknown). Results of the study were confirmed by the new study by Vasques (CA 7.1.3.1.1/01). Results of both studies and their main parameters are given in Table 7.1.3.2-1.

Report: **R000496; Burr C.M., (1998)**
[14C]-Triticonazole: Adsorption/Desorption to and from four soils and a sediment
201670 / 12966
GLP: yes
Guidelines: 95/36/EC, July 1995 referencing OECD method 106
USEPA (=EPA) Subdivision N, 163-1

Please see the full summary details presented in Volume 3, Section B.8 of the DAR for triticonazole (Austrian Federal Office for Food Safety, 2003). The study is still considered acceptable but it has some minor deviations to the current OECD Test Guideline 106 (only four test concentrations used, desorption cycle was only performed for one hour). Results of the study were confirmed by the new study by Vasques (CA 7.1.3.1.1/01). Results of both studies and their main parameters are given in Table 7.1.3.2-1.

Report: CA 7.1.3.1.1/1
Vasques A.C., 2015a
Adsorption / desorption behavior of 14C-BAS 595 F on different European soils
2014/3001242
Guidelines: OECD 106 (2000), EPA 835.1230
GLP: yes
(certified by Instituto Nacional de Metrologia, Normalizacao e Qualidade Industrial - INMETRO, Rio de Janeiro, Brazil)

Executive Summary

The adsorption/desorption behaviour of radiolabelled ^{14}C-BAS 595 F was investigated on five different European soils which covered a pH range of from 5.6 to 7.4 (in $CaCl_2$), a range of organic carbon content from 0.60% to 1.85%, and five different USDA textural classes (sandy clay loam, loamy sand, sand, sandy loam and silt loam).

For the determination of the adsorption/desorption isotherms, five different concentrations from nominal 0.01 to 1.00 µg/mL of the test item in 0.01 M $CaCl_2$ solutions were used. Soil/ test solution ratio was 1/5 and the adsorption equilibrium time was 24 hours for all five soils. Desorption was determined in two steps starting after the adsorption isotherm determination test, by adding 0.01 mol/L $CaCl_2$ solution without test item onto the sample from the previous test, consisting of soil and remaining liquid from the supernatant decanted. The concentrations of the test item in the test solutions and soils extracts were determined by LSC. The stability of the test substance in the test system was confirmed by Radio-HPLC analysis.

The mass balance of ^{14}C-BAS 595 F for the test soils during determination of adsorption/desorption isotherms ranged from 95.1 to 97.3% of the total of test item applied.

Freundlich adsorption coefficients K_F ranged from 3.7 to 11.8 mL/g in the five soils, which corresponded to K_{FOC} values ranging from 325 to 871 mL/g. Freundlich exponents 1/n varied between 0.89 and 0.94. The desorption coefficients K_{Fdes1} and K_{Fdes2} ranged from 5.1 to 18.0 mL/g in the five soils, with K_{FOCdes} values between 444 mL/g and 1437 mL/g.

I. MATERIAL AND METHODS

A. MATERIALS

1. Radiolabeled test material: **^{14}C-BAS 595 F**

Batch No.:	866-1401
Label position:	phenyl-U-^{14}C
Specific Activity:	5.95 MBq/mg
Radiochemical purity:	99.5%
Molecular weight:	317.82 g/mol (non-radiolabelled)

2. Soils

The study was conducted with five different soils from Europe. The characterisation of the soils is presented in Table 7.1.3.1.1-1. Soil samples were < 2 mm-mesh sieved and air dried at room temperature. The actual water content of the soils, determined using a halogen moisture analyser, was taken into account for the calculations.

Table 7.1.3.1.1-1: Characteristics of the soils used to investigate the adsorption and desorption of ^{14}C-BAS 595 F

Soil designation Origin	La Gironda (Spain)	Li 10 (Germany)	LUFA 2.1 (Germany)	LUFA 2.3 (Germany)	Nierswalde Wildacker (Germany)
USDA textural class:	Sandy clay loam	Loamy sand	Sand	Sandy loam	Silt Loam
Sand [%]	49.2	83.5	90.8	68.6	17.7
Silt [%]	23.0	12.2	6.9	23.1	73.5
Clay [%]	27.7	4.3	2.3	8.3	8.8
Organic carbon [%]	1.22	0.95	0.60	0.99	1.85
CEC [$cmol^+/kg$]	26.3	5.5	-0.7	7.5	3.1
pH ($CaCl_2$)	7.4	6.2	5.6	6.7	5.7

B. STUDY DESIGN

1. Experimental conditions

All tests were carried out in duplicate at room temperature. Test samples were prepared by applying on soil a treatment solution containing test item in a $CaCl_2$ 0.01 mol/L solution. Controls were prepared with only the treatment solution (no soil) in the tube and soil blanks were prepared by weighing soil in the tube and applying $CaCl_2$ 0.01 mol/L solution (no test item). For all tests carried out, the tubes, containing soil or not, after application of the proper solution, were closed and then shaken horizontally on a mechanical shaker at 150 rpm at temperature controlled room (20 ± 2°C) and dark conditions for the indicated test period.

After test period is reached, the soil / solution suspensions were centrifuged at 3000 rpm for 5 minutes and the supernatants were isolated by pouring to storage flasks.

Both, supernatants and initial solution applied were analysed in order to determine the concentration of the test item in the aqueous solution after adsorption, as well as the initial concentration. The amount of test item adsorbed is indirectly calculated by the depletion of the total applied to the amount determined in the aqueous phase at the end of the test period.

Aliquots of samples were analyzed in triplicates by liquid scintillation counter (LSC) for quantification and by radio-HPLC for formation of any degradation products and determination of the nature of the radioactivity.

Extraction procedure for ^{14}C-BAS 595 F consists of a single extraction of 2 g soil (after removal of supernatant) with 20 mL of acetonitrile/water 4/1 (v/v). For each extraction step, the tubes were closed and then shaken horizontally on a mechanical shaker at 250 rpm for 30 minutes, then centrifuged at 3000 rpm for 5 minutes and the extracts were isolated.

The extraction of the soil samples was performed to provide the extraction efficiency, evaluate the nature of items adsorbed to soil and to determine the stability of test item on soil. The total aqueous phase to be considered for the calculations includes the supernatant decanted from soil after centrifugation and the remaining volume of this solution in the soil. In order to determine the volume of the remaining solution on soil right before extraction, soil samples are weighed throughout the experiments at several steps: Before and after treatment and after removal of supernatant. The soils dried weights were used for the calculations.

Preliminary tests

Preliminary experiments revealed that the optimal soil / solution ratio and adsorption equilibrium time for the adsorption/desorption tests were 1/5 and 24 h, respectively. Further preliminary experiments indicated that glass centrifuge tubes are suitable for conducting the adsorption/desorption studies as no significant adsorption on the test vessel surface occurs.

2. Adsorption-Desorption Isotherm Determination

To determine adsorption isotherms, standard solutions of the test item in 0.01 M $CaCl_2$ were prepared at five concentrations levels (nominal concentrations: from 0.01, 0.05, 0.1, 0.5 and 1.00 µg/mL) and applied directly on the soils (duplicates per concentration level dosed, 2 g soil and 10 mL solution). The experiment was performed by the indirect method. Samples were shaken for 24 hours. Thereafter, the soil/water specimens were centrifuged, decanted, and aliquots of the supernatants were assayed by LSC. Samples with the highest concentration were additionally analysed by radio-HPLC. After adsorption, two desorption steps were performed (24 hours each) by replacing the removed supernatants with an equal volume of 0.01 M $CaCl_2$ solution without test item. After the desorption experiments, the samples from the isotherms determination with the highest concentration were extracted to show the extractability and stability of the test item. A mass balance was calculated for these samples.

3. Description of analytical procedures

The amounts of radioactivity were determined by radioactivity measurements. Therefore, aliquots of the decanted supernatants were added to scintillation cocktail and radioassayed in a liquid scintillation counter. Radio-HPLC was used to show the purity and stability of the test item during the study.

II. RESULTS AND DISCUSSION

A. MASS BALANCE

During the main test, acceptable mass balances of the test item for the highest concentration in each soil were achieved with mean mass balances of 97.3, 97.1, 96.5, 95.3 and 95.1% in soils La Gironda, Li 10, LUFA 2.2, LUFA 2.3 and Nierswalde Wildacker, respectively.

C. FINDINGS

The stability of the test item in the adsorption/desorption experiments was proven by radio-HPLC analysis of the adsorption, desorption and extraction supernatants from the high dose samples and test item treatment solution fresh and after 48 hours shaking.

Detailed results from the adsorption and desorption tests for ^{14}C-BAS 595 F in all five soils are presented in Table 7.1.3.1.1-2 and Table 7.1.3.1.1-3.

Table 7.1.3.1.1-2: Adsorption Isotherms of ^{14}C-BAS 595 F in five soils

Soil	Soil Type (USDA)	K_F [mL/g]	1/n	K_{FOC} [mL/g] [a]	R^2
GI	Sandy clay loam	3.97	0.94	325.02	0.9998
Li 10	Loamy sand	4.79	0.91	504.13	0.9992
Lufa 2.1	Sand	5.23	0.93	871.37	0.9991
Lufa 2.3	Sandy loam	3.67	0.89	370.45	0.9966
NW	Silt loam	11.77	0.92	636.10	0.9994

GI = La Gironda; NW = Nierswalde Wildacker;
[a] Based on organic-carbon content

Table 7.1.3.1.1-3: Desorption Isotherms of ^{14}C-BAS 595 F in five soils

Soil	Desorption 1				Desorption 2			
	K_{Fdes1} [mL/g]	1/n	$K_{FOCdes1}$ [mL/g] [a]	R^2	K_{Fdes2} [mL/g]	1/n	$K_{FOCdes2}$ [mL/g] [a]	R^2
GI	5.41	0.92	443.50	0.9995	6.87	0.94	563.17	0.9994
Li 10	6.15	0.92	647.47	0.9993	8.25	0.93	868.43	0.9994
Lufa 2.1	6.20	0.94	1032.63	0.9994	8.62	0.93	1437.30	0.9984
Lufa 2.3	5.13	0.90	518.06	0.9950	8.40	0.92	848.04	0.9950
NW	14.91	0.93	806.07	0.9996	17.99	0.92	972.59	0.9995

GI = La Gironda; NW = Nierswalde Wildacker
[a] Based on organic-carbon content

III. CONCLUSION

Freundlich adsorption coefficients K_F in the five soils investigated range from 3.7 to 11.8 mL/g, corresponding to K_{FOC} values ranging from 325 to 871 mL/g. Freundlich exponents 1/n varied between 0.89 and 0.94. The study is valid and the results are suitable to be used as basis for further work.

CA 7.1.3.1.2 Adsorption and desorption of metabolites, breakdown and reaction products

Three studies investigating the adsorption and desorption behaviour of the major soil metabolites RPA 404766, RPA 406341 and RPA 407922, respectively, were evaluated during the Annex I inclusion and accepted by the European Commission (SANCO/10442/2005-Final rev 1, 12 March 2010). Those studies are still considered valid and scientifically sound, although they have some minor deviations to the current OECD 106 Test Guideline. To fully comply with the current guidance and to confirm the results of the previously submitted studies, a new study was performed.

The previously submitted studies are listed below, deviations from current guidance are presented. A summary of the new adsorption/desorption study, including experiments on all three metabolites, is provided thereafter in annex point CA 7.1.3.1.2/1. A summary of the results and relevant parameters of all individual studies is presented Table 7.1.3.2-2 to Table 7.1.3.2-4.

Report:	**C010431; Simmonds M., Lowden P., (2000a)** Adsorption to and desorption from four soils and a sediment (^{14}C) RPA 406341. GOoD16714 / 202662 / 16714
GLP:	yes
Guidelines:	EU (=EEC) 95/36/EC, Section 7.1.2, USEPA (=EPA) N, 163-1, (1982), SETAC

Please see the full summary details presented in Volume 3, Section B.8 of the DAR for triticonazole (Austrian Federal Office for Food Safety, 2003). The study is still considered acceptable but it has some minor deviations the current OECD Test Guideline 106 (only four concentrations tested, very short equilibration time for desorption cycle). Results of the study were confirmed by the new study by Vasques (CA 7.1.3.1.2/01). Results of both studies and their main parameters are given in Table 7.1.3.2-3.

Report: **C012304; Simmonds M., Lowden P., (2001)**
Adsorption to and desorption from four soils and a sediment (^{14}C) RPA 404766
C012304 / GOoD16953
GLP: yes
Guidelines: EU (=EEC) 95/36/EC, USEPA (=EPA) PAG Subdiv.N, Par 163-1 (1982), SETAC

Please see the full summary details presented in Volume 3, Section B.8 of the DAR for triticonazole (Austrian Federal Office for Food Safety, 2003). The study is still considered acceptable but it has some minor deviations the current OECD Test Guideline 106 (only four concentrations tested, very short equilibration time for desorption cycle). Results of the study were confirmed by the new study by Vasques (CA 7.1.3.1.2/01). Results of both studies and their main parameters are given in Table 7.1.3.2-2.

Report: **C010432; Simmonds M., Lowden P., (2000b)**
Adsorption to and desorption from four soils and a sediment (^{14}C)-RPA 407922.
GOoD16952 / 202633
GLP: yes
Guidelines: EU (=EEC) 95/36/EC, USEPA (=EPA) PAG Subdiv.N, Par 163-1 (1982), SETAC

Please see the full summary details presented in Volume 3, Section B.8 of the DAR for triticonazole (Austrian Federal Office for Food Safety, 2003). The study is still considered acceptable but it has some minor deviations the current OECD Test Guideline 106 (only four concentrations tested, very short equilibration time for desorption cycle). Results of the study were confirmed by the new study by Vasques (CA 7.1.3.1.2/01). Results of both studies and their main parameters are given in Table 7.1.3.2-4.

Report: CA 7.1.3.1.2/1
Vasques A.C., 2015b
Adsorption / desorption behavior of 14C-RPA404766 / M595F001 (Reg. 5079295), 14C-RPA406341 / M595F002 (Reg. 5059144) and 14C-RPA407922 (Reg. 5079288) (metabolites of 14C-BAS 595 F) on different European soils
2015/3000503
Guidelines: OECD 106 (2000)
GLP: yes
(certified by Instituto Nacional de Metrologia, Normalizacao e Qualidade Industrial - INMETRO, Rio de Janeiro, Brazil)

Executive Summary

The adsorption behaviour of radiolabelled ^{14}C-RPA 404766 (Reg. No. 5079285) and ^{14}C-RPA 406341 (Reg. No. 5059144) as well as the adsorption and desorption behaviour of ^{14}C-RPA 407922 (Reg. No. 5079288) were investigated in five different European soils which covered a pH range from 5.5 to 7.7 (in $CaCl_2$), a range of organic carbon content from 0.6% to 1.97% and five different DIN textural classes: sandy clay loam, silty sand, sand, loamy sand and clay silt.

For the determination of the adsorption isotherm, five different concentrations (nominal 1.0, 0.50, 0.10, 0.050 and 0.010 µg/mL) of each test item in 0.01 mol/L $CaCl_2$ solutions were used. The ratio of soil mass and test solution volume was 1/1 for ^{14}C-RPA 404766 and ^{14}C-RPA 406341 and 1/10 for ^{14}C-RPA 407922. The test periods used were 24 hours for ^{14}C-RPA 404766 and 48 hours for ^{14}C-RPA 406341. For ^{14}C-RPA 407922, 48 hours was the time chosen for conduction of isotherms test with one soil (La Gironda) and 24 hours for the others. Two desorption steps were conducted

for ^{14}C-RPA407922, starting after adsorption isotherm determination test by adding 0.01 mol/L $CaCl_2$ solution without test item onto the sample from the previous test, consisting of soil and remaining liquid from the supernatant decanted.

The tests with ^{14}C-RPA 404766 and ^{14}C-RPA 406341 were conducted with the direct method, due to low purity of stock solution and formation of unknown peaks in the $CaCl_2$ supernatant of preliminary tests, respectively. The test with ^{14}C-RPA 407922 was conducted with the indirect method. The amounts of radioactivity in the test solutions and sample supernatants or extracts were determined by liquid scintillation counting (LSC). The stability of the test items was investigated by radio-HPLC analysis.

The mass balance of ^{14}C-RPA 404766 for the test soils at determination of adsorption isotherms ranged from 86.3 to 98.7%, with radioactivity recoveries ranging from 91.2 to 98.7%. Mass balance of ^{14}C-RPA 406341 ranged from 95.1 to 102.7%, with radioactivity recoveries from 95.6 to 104.0%. Mass balance of ^{14}C-RPA 407922 ranged from 90.4 to 97.2%, with radioactivity recovery from 94.8 to 98.5% of the total of test item applied.

Freundlich adsorption coefficients K_F for ^{14}C-RPA 404766 ranged from 0.3 to 3.2 mL/g in the five soils with corresponding K_{FOC} values ranging from 46 to 161 mL/g. Freundlich exponents 1/n varied between 0.90 and 0.99.

Freundlich adsorption coefficients K_F for ^{14}C-RPA 406341 ranged from 0.7 to 2.6 mL/g in the five soils with corresponding K_{FOC} values ranging from 106 to 324 mL/g. Freundlich exponents 1/n varied between 0.94 and 1.00.

Freundlich adsorption coefficients K_F for ^{14}C-RPA 407922 ranged from 1.6 to 8.7 mL/g in the five soils with corresponding K_{FOC} values ranging from 228 to 743 mL/g. Freundlich exponents 1/n varied between 0.89 and 0.98. The desorption coefficients K_{Fdes1} and K_{Fdes2} ranged from 4.9 to 36.9 mL/g in the five soils, corresponding to K_{FOCdes} values between 611 and 3702 mL/g.

I. MATERIAL AND METHODS

A. MATERIALS

1. Radiolabeled test item: **^{14}C-RPA 406341**

Batch No.:	1102-1002
Label position:	Triazole-3(5)-^{14}C
Specific Activity:	6.33 MBq/mg
Radiochemical purity:	98.5%
Molecular weight:	333.82 g/mol (non-radiolabelled)

2. Radiolabeled test item: **^{14}C-RPA 404766**

Batch No.:	1104-1056
Label position:	Triazole-3(5)-14C
Specific Activity:	6.65 MBq/mg
Radiochemical purity:	98.5%
Molecular weight:	333.82 g/mol (non-radiolabelled)

3. Radiolabeled test item: **^{14}C-RPA 407922**

Batch No.:	1103-1028
Label position:	Triazole-3(5)-14C
Specific Activity:	5.66 MBq/mg
Radiochemical purity:	97.4%
Molecular weight:	333.82 g/mol (non-radiolabelled)

4. Soils

The study was conducted with five different European soils. The characterisation of the soils is presented in Table 7.1.3.1.2-1. Soil samples used were < 2 mm-mesh sieved and air dried at room temperature. The actual water content of the soils, determined using a halogen moisture analyser, was taken into account for the calculations.

Table 7.1.3.1.2-1: Characterisation of the soils used to investigate the adsorption and desorption of ^{14}C-RPA404766, ^{14}C-RPA406341 and ^{14}C-RPA407922

Soil designation Origin	La Gironda (Spain)	Li 10 (Germany)	LUFA 2.1 (Germany)	LUFA 2.3 (Germany)	Nierswalde Wildacker (Germany)
DIN textural class:	Sandy clay loam	Silty sand	Sand	Loamy sand	Clay silt
Sand [%]	48.0	82.2	89.5	66.9	17.7
Silt [%]	24.3	13.5	8.2	24.8	72.9
Clay [%]	27.7	4.3	2.3	8.3	9.4
Organic carbon [%]	1.3	0.6	0.6	0.7	1.97
CEC [mmolc/kg][a] or [$cmol^+$/kg][b]	452 [a]	67 [a]	28 [a]	72 [a]	7.6 [b]
pH ($CaCl_2$)	7.7	5.5	6.0	7.1	5.8

B. STUDY DESIGN

1. Experimental conditions

All tests were carried out in duplicate at room temperature. Test samples were prepared by applying a treatment solution containing the test item in a 0.01 mol/L $CaCl_2$ solution on soil.

Controls were prepared with only the treatment solution (no soil) in the tube and soil blanks were prepared by weighing soil in the tube and applying 0.01 mol/L $CaCl_2$ solution (no test item). For all tests carried out, the tubes, containing soil or not, after application of the proper solution, were closed and then shaken horizontally on a mechanical shaker at 150 rpm at temperature controlled room (20 ± 2°C) and at dark conditions for the indicated test period. After the test period is reached, suspensions were centrifuged at 3000 rpm for 5 minutes and the supernatants were isolated by pouring to storage flasks.

Aliquots of the supernatant are analysed in triplicates by liquid scintillation counter (LSC) for quantification and by radio-HPLC, for formation of any degradation products and determination of the nature of the radioactivity. At adsorption equilibrium test, at least the sampling of interest (equilibrium time) or longer test period samples were analysed by radio-HPLC. At isotherms determination test, only supernatants from samples treated with the treatment solution of the highest dose were analysed by radio-HPLC.

The extraction procedure for ^{14}C-RPA 404766 consisted of two consecutive extractions conducted with a 5 g soil sample (after removal of the supernatant), one with 20 mL acetone, and one with 20 mL acetonitrile/water 1/1 (v/v). At each extraction step, the tubes were closed and then shaken horizontally on a mechanical shaker at 250 rpm for 30 minutes, then centrifuged at 3000 rpm for 5 minutes. The extracts were then combined and made up to a volume of 50 mL with acetone.

The extraction procedure for ^{14}C-RPA 406341 consisted of four consecutive extraction step conducted with a 5 g soil sample (after removal of supernatant): two extractions steps with methanol and two extraction steps with acetonitrile/water 1/1 (v/v) solution. Each extraction step was conducted by adding 20 mL of the respective solution. At each extraction step, the tubes were closed and then shaken horizontally on a mechanical shaker at 250 rpm for 30 minutes, then centrifuged at 3000 rpm for 5 minutes. The extracts were then combined and brought to a volume of 100 mL with methanol. 15 mL aliquots of extracts were concentrated at turbovap down to almost dryness and brought to 1 mL with methanol.

The extraction procedure for ^{14}C-RPA 407922 consisted of two consecutive extractions conducted with a 5 g soil sample (after removal of supernatant) with 20 mL of methanol each. At each step, the tubes were closed and then shaken horizontally on a mechanical shaker at 250 rpm for 30 minutes, then centrifuged at 3000 rpm for 5 minutes. The extracts were then combined and made up to a volume of 50 mL with methanol. 15 mL aliquots of the extracts were concentrated at turbovap down to almost dryness and brought to 1 mL with methanol.

For the indirect method, both supernatants and initial solution applied were analysed in order to determine the concentration of the test item in the aqueous solution after adsorption, as well as the initial concentration. The amount of test item adsorbed is indirectly calculated by the depletion of the total applied amount to the amount determined in the aqueous phase at the end of the test period. The extraction of the soil samples provides the extraction efficiency, enables to evaluate the nature of the items adsorbed to soil and to determine the stability of the test item in soil. For the adsorption equilibrium test, at least the sampling of interest (equilibrium time) or longer test period samplings were extracted, for determination of the extraction method. For the determination of isotherms, only the supernatants from samples originally treated with the treatment solution of the highest dose, were extracted. The total aqueous phase considered for the calculations included the supernatant decanted from soil after centrifugation and the remaining volume of this solution in the soil. In order to determine the volume of the remaining solution in soil right before extraction, soil samples were weighed throughout the experiments at several steps: before and after treatment as well as after removal of the supernatant.

For the direct method, the amount adsorbed onto soil is given by the amount of test item extracted, considering the volume of the supernatant remaining in the soil after decantation. Therefore, the extraction of the soil samples enables the direct determination of the amount adsorbed onto soil and the determination of the nature of items adsorbed to soil. Besides the information of concentration on both, aqueous phase and extracts, soil sample weights throughout the experiments at several steps are needed: before and after treatment as well as after removal of the supernatant, for determination of the volume of the solution remaining in the soil right before extraction. The extraction efficiency may be demonstrated by the mass balance. Aliquots of the extracts were analysed in triplicates by liquid scintillation for quantification and by radio-HPLC, for formation of any degradation products and determination of the nature of the radioactivity. The soil dry weights were used for the calculation.

Preliminary tests

Preliminary experiments revealed that the optimal soil / solution ratio for metabolites RPA 404766 and RPA 406341 in the main test is 1/1 with adsorption in the range of 27.5 to 80.8% in the two soils tested. For the metabolite RPA 407922, a ratio of 1/10 was chosen. The adsorption equilibrium test revealed that the appropriate equilibrium times for metabolites RPA 404766 and RPA 406341 were 24 and 48 hours, respectively. For metabolite RPA 407922 an equilibration time of 48 hours was chosen for the La Gironda soil and an equilibration time of 24 hours was chosen for the other four soils.

2. Adsorption-Desorption Isotherm Determination

The adsorption isotherm determination was performed with all five concentration levels nominal 1.0, 0.50, 0.10, 0.050 and 0.010 µg/mL) and all five soils. For ^{14}C-RPA404766 and ^{14}C-RPA406341, centrifuge Teflon tubes were used. For ^{14}C-RPA 407922, centrifuge glass tubes were used.

Appropriate volumes of application solutions were applied directly to the soils. The test with ^{14}C-RPA 404766 was conducted with the direct method, due to presence of unknown peaks in the treatment solution with the highest concentration, summering around 8% (radio purity checked before tests 92.0%). The test with ^{14}C-RPA 406341 was also conducted with the direct method, due to formation of unknown peaks in the $CaCl_2$ supernatant of preliminary tests. Subsequent extractions with 0.01 mol/aqueous $CaCl_2$ L, inherent from indirect method, could lead to higher degradation of test item.

The test with ^{14}C-RPA 407922 was conducted with the indirect method. The method was chosen due to formation of unknown peaks on solvent extract chromatograms of preliminary tests samples. The indirect method was expected to reduce the amount available for solvent extraction due to the subsequent aqueous $CaCl_2$ 0.01 mol/L and, consequently, reach a higher mass balance at Freundlich isotherm determination tests.

Desorption experiments were not conducted for test items ^{14}C-RPA 404766 and ^{14}C-RPA 406341. The soil was extracted right after the adsorption phase with organic solvent. For ^{14}C-RPA 407922, the Freundlich desorption isotherms test was conducted with the same soil samples used for the determination of adsorption isotherms after removal of the supernatant. The test was performed with all the five soils and at five concentration levels. Desorption step 1 was performed as follows: The removed supernatant was replaced by an equal volume of 0.01 mol/L $CaCl_2$ solution without test item. The new mixture was gently agitated for the same test period and conditions. The volumes of the supernatants were measured gravimetrically. Desorption step 2 was performed with the soil samples left from desorption step 1 as described above.

3. Description of analytical procedures

The concentrations of the ^{14}C-test items were determined by radioactivity measurements. Therefore, aliquots of the decanted supernatants were added to scintillation cocktail and radioassayed in a liquid scintillation counter.

Radio-HPLC was used to demonstrate the purity and stability of the test item during the study.

When recovery of radioactivity fell below 90%, the extracted soil was combusted. Therefore, the extracted soil was air dried and homogenized. Aliquots were weighed in triplicates and submitted to combustion in an oxidizer. The gases formed in the combustion were trapped in a scintillation solution and analysed with LSC.

II. RESULTS AND DISCUSSION

A. MASS BALANCE

The mass balance determination was carried out for all samples at the end of the isotherm experiments. The mass balance of ^{14}C-RPA 404766 ranged from 86.3% to 98.7%, with radioactivity recoveries ranging from 91.2 to 98.7%.The mass balance of ^{14}C-RPA 406341 ranged from 95.1 to 102.7%, with radioactivity recovery from 95.6 to 104.0% and the mass balance of ^{14}C-RPA 407922 ranged from 90.4 to 97.2%, with radioactivity recovery from 94.8% to 98.5%. Unknown compounds observed during the tests in both phases, accounted only for a maximum of 1.2% of total applied radioactivity (average of duplicates) for all soils.

B. TRANSFORMATION OF PARENT COMPOUND

The purity of test substance in the treatment solution at the beginning of the study was 92.0% for ^{14}C-RPA 404766 and 100.0% for ^{14}C-RPA 406341 and ^{14}C-RPA 407922.

From the control run in parallel to tests, it was inferred that the test item was stable in 0.01 mol/L $CaCl_2$ solution in absence of soil during 48 h, compared to original solution applied. Also, the test solution of the highest concentration samples and of the samples which were analysed for formation of degradation products or test item, was considered stable during the test period, by recovery of test item higher or equal to 90%, except for ^{14}C-RPA 404766 recoveries from one soil (Nierswalde Wildacker), due to formation of bound residues.

C. FINDINGS

Detailed results from the adsorption and desorption tests for the three metabolites in all five soils are presented in to Table 7.1.3.1.2-5.

Table 7.1.3.1.2-2: Adsorption Isotherms of ^{14}C-RPA 404766 in five soils

Soil	Soil Type (DIN)	K_F [mL/g]	1/n	K_{FOC} [a] [mL/g]	R^2
GI	Sandy clay loam	0.68	0.99	52.60	0.9999
Li 10	Silty sand	0.83	0.98	138.94	0.9995
Lufa 2.1	Sand	0.28	0.97	46.06	0.9987
Lufa 2.3	Loamy sand	0.34	0.90	49.01	0.9987
NW	Clay silt	3.17	0.95	161.11	0.9987

GI = La Gironda; NW = Nierswalde Wildacker
[a] Based on organic-carbon content

Table 7.1.3.1.2-3: Adsorption Isotherms of ^{14}C-RPA 406341 in five soils

Soil	Soil Type (DIN)	K_F [mL/g]	1/n	K_{FOC} [a] [mL/g]	R^2
GI	Sandy clay loam	1.38	0.94	106.27	0.9989
Li 10	Silty sand	1.94	1.00	323.66	0.9952
Lufa 2.1	Sand	0.68	0.98	113.67	0.9997
Lufa 2.3	Loamy sand	0.80	0.96	114.19	0.9972
NW	Clay silt	2.59	0.95	131.53	0.9971

GI = La Gironda; NW = Nierswalde Wildacker
[a] Based on organic-carbon content

Table 7.1.3.1.2-4: Adsorption Isotherms of ^{14}C-RPA 407922 in five soils

Soil	Soil Type (DIN)	K_F [mL/g]	1/n	K_{FOC} [a] [mL/g]	R^2
GI	Sandy clay loam	3.57	0.93	274.74	0.9997
Li 10	Silty sand	4.46	0.94	742.71	0.9994
Lufa 2.1	Sand	1.89	0.98	314.70	0.9994
Lufa 2.3	Loamy sand	1.59	0.92	227.51	0.9995
NW	Clay silt	8.70	0.89	441.83	0.9951

GI = La Gironda; NW = Nierswalde Wildacker
[a] Based on organic-carbon content

Table 7.1.3.1.2-5: Desorption Isotherms of ^{14}C-RPA 407922 F in five soils

Soil	Desorption 1				Desorption 2			
	K_{Fdes1} [mL/g]	1/n	$K_{FOCdes1}$ [a] [mL/g]	R^2	K_{Fdes2} [mL/g]	1/n	$K_{FOCdes2}$ [a] [mL/g]	R^2
GI	7.94	0.92	611.03	0.9967	16.29	0.95	1252.99	0.9963
Li 10	8.82	0.96	1470.58	0.9988	14.96	0.96	2493.34	0.9969
Lufa 2.1	4.89	0.96	814.69	0.9990	11.27	0.94	1877.87	0.9917
Lufa 2.3	4.88	0.94	697.40	0.9946	25.92	1.02	3702.28	0.9945
NW	18.73	0.92	950.96	0.9987	36.92	0.97	1874.07	0.9961

GI = La Gironda; NW = Nierswalde Wildacker
[a] Based on organic-carbon content

III. CONCLUSION

The Freundlich adsorption coefficients K_F for ^{14}C-RPA 404766 ranged from 0.3 to 3.2 mL/g. The K_{FOC} values ranged from 46 to 161 mL/g and 1/n values ranged from 0.90 to 0.99.

The Freundlich adsorption coefficients K_F for ^{14}C-RPA 406341 ranged from 0.7 to 2.6 mL/g. The K_{FOC} values ranged from 106 to 324 mL/g and 1/n values ranged from 0.94 to 1.00.

The Freundlich adsorption coefficients K_F for ^{14}C-RPA 407922 ranged from 1.6 to 8.7 mL/g. The K_{FOC} values ranged from 228 to 743 mL/g and 1/n values ranged from 0.89 to 0.98.

Freundlich desorption isotherms were established for ^{14}C-RPA407922 and K_{Fdes} values ranged from 4.9 to 18.7 mL/g for desorption step 1 and from 11.3 to 36.9 mL/g for desorption step 2. The corresponding K_{FOCdes} values ranged from 611 to 1471 mL/g for desorption step 1 and from 1253 to 3702 mL/g for desorption step 2.

The study is valid and the results are suitable to be used as basis for further work.

CA 7.1.3.2 Aged sorption

Studies on aged sorption were not performed for the active substance triticonazole since they are not required under Commission Regulation (EU) No 283/2013 in accordance with Regulation (EC) No 1107/2009.

Adsorption and desorption in soil - Overall Conclusions

In the batch equilibrium experiments triticonazole has K_{FOC} values ranging from 324 to 871 mL/g. The adsorption values for triticonazole are summarized in Table 7.1.3.2-1.

RPA 404766 and RPA 406341 showed K_{FOC} values ranging from 35 to 161 mL/g and from 61 to 324 mL/g, respectively. RPA 407922 showed K_{FOC} values ranging from 275 to 1305 mL/g. The adsorption values for the metabolites are summarized in Table 7.1.3.2-2 to Table 7.1.3.2-4.

Existing studies showed minor deviations from current guidance documents. The studies are still considered as scientifically sound, and the derived sorption parameters are in the same range as sorption parameters derived following latest guidance documents. Thus it is reasonable to pool existing and newly derived sorption parameters.

Table 7.1.3.2-1: Overview on the adsorption parameters for Triticonazole

Reference			Soil characteristics						Adsorption Parameters		
			Soil origin	Soil type	Clay [%]	pH	OC[d] [%]	CEC [meq/100 g]	K_F [mL/g]	K_{FOC} [mL/g]	1/n [-]
CA 7.1.2.3	Studies previously evaluated during Annex I inclusion	Burr, 1998	Leland, USA	Silt loam[a]	8.23	5.1[c]	0.5	5.7	4.060	812	0.857
			Iola, USA	Sandy loam[a]	6.72	5.9[c]	1.2	6.5	5.621	468	0.813
			Essex, UK	Loam[a]	23.87	6.5[c]	2.2	15.0	8.665	394	0.864
			Suffolk, UK	Sand[a]	5.13	6.2[c]	2.4	13.2	14.383	599	0.857
			Ongar, sediment[e]	*Clay sediment[a]*	*43.49*	*7.5[c]*	*3.4*	*62.3*	*11.701[e]*	*344[e]*	*0.890[e]*
		Burr & Austin, 1992	Manningtree, UK	Sandy loam	13.5	6.30[b]	0.8	5.99	4.103	485.43	0.925
			Ongar, UK	Clay loam	32	6.08[b]	3.3	28.50	12.891	386.06	0.893
			Bury-St-Edmunds, UK[e]	*Loamy sand*	*9.5*	*6.24[b]*	*17.0*	*51.12*	*31.729[e]*	*183.61[e]*	*0.917[e]*
			Mildenhall, UK	Sand	2.5	6.23[b]	0.5	2.30	1.745	324.38	0.964
			Speyer, Germany	Sand	6.0	6.12[b]	0.8	2.95	4.389	562.60	0.862
	New study	Vasques, 2015a	La Gironda, Spain	Sandy clay loam[a]	27.7	7.4[c]	1.22	26.30[g]	3.97	325.02	0.94
			Li10, Germany	Loamy sand[a]	4.3	6.2[c]	0.95	5.50[g]	4.79	504.13	0.91
			Lufa 2.1, Germany	Sand[a]	2.3	5.6[c]	0.6	*-0.70[f,g]*	5.23	871.37	0.93
			Lufa 2.3, Germany	Sandy loam[a]	8.3	6.7[c]	0.99	7.50[g]	3.67	370.45	0.89
			Nierswalde Wildacker, Germany	Silt loam[a]	8.8	5.7[c]	1.85	3.10[g]	11.77	636.10	0.92
								Arithmetic mean	**6.56**	**518**	**0.89**
								Geometric mean	**5.57**	**494**	**0.89**

a Soil type according to USDA, for the other soils the classification is unknown.
b Buffer solution unknown , c = 0.01 M $CaCl_2$
d If not explicitly mentioned in the study report, OC was calculated: OC (%) = OM (%) / 1.724.
e Data from Ongar sediment and high organic soil Bury-St-Edmunds, were not included in the calculation of mean values as they were considered not relevant to the proposed European application scenarios.
f Value is considered to be an outlier.
g unit is $cmol^+/kg$

Studies/ Values shaded in grey have been reviewed as part of the first EU review of triticonazole.

Table 7.1.3.2-2: Overview on the adsorption parameters for RPA 404766

Reference			Soil characteristics						Adsorption Parameters		
			Soil origin	Soil type	Clay [%]	pH ($CaCl_2$)	OC [b] [%]	CEC	K_F [mL/g]	K_{FOC} [mL/g]	1/n [-]
CA 7.1.2.3	Study previously evaluated	Simmonds & Lowden, 2001	Leland, USA	Silt loam [a]	5.48	6.0	0.5	6.3 [c]	0.67	133	0.825
			Iola, USA	Sandy loam [a]	6.55	5.2	1.3	5.0 [c]	1.09	84	0.850
			Ongar, UK	Loam [a]	25.12	6.4	1.9	10.0 [c]	1.51	79	0.844
			Royston, UK	Clay loam [a]	35.97	7.4	4.1	51.9 [c]	1.43	35	0.854
			Ongar sediment [f]	*Sandy clay loam [a]*	*25.98*	*7.7*	*2.6*	*43.8 [c]*	*1.62 [f]*	*62 [f]*	*0.877 [f]*
	New study	Vasques, 2015b	La Gironda	Sandy clay loam [b]	27.7	7.7	1.3	452.0 [d]	0.68	52.6	0.99
			Li10	Silty sand [b]	4.3	5.5	0.6	67.0 [d]	0.83	138.94	0.98
			Lufa 2.1	Sand [b]	2.3	6.0	0.6	28.0 [d]	0.28	46.06	0.97
			Lufa 2.3	Loamy sand [b]	8.3	7.1	0.7	72.0 [d]	0.34	49.01	0.90
			Nierswalde Wildacker	Clay silt [b]	9.4	5.8	1.97	7.6 [e]	3.17	161.11	0.95
								Arithmetic mean	**1.11**	**87**	**0.91**
								Geometric mean	**0.86**	**76**	**0.90**

a Soil type according to USDA, b soil type according to DIN.
b If not explicitly mentioned in the study report, OC was calculated: OC (%) = OM (%) / 1.724.
c unit is meq/100 g
d unit is mmolc/kg
e unit is cmol+/kg
f Data from sediment (Ongar, UK) were not included in the calculation of mean values as they were considered not relevant to the proposed European application scenarios

Studies/ Values shaded in grey have been reviewed as part of the first EU review of triticonazole.

Table 7.1.3.2-3: Overview on the adsorption parameters for RPA 406341

Reference			Soil characteristics						Adsorption Parameters		
			Soil origin	**Soil type**	**Clay [%]**	**pH ($CaCl_2$)**	**OC [c] [%]**	**CEC**	**K_F [mL/g]**	**K_{FOC} [mL/g]**	**1/n [-]**
CA 7.1.2.3	Study previously evaluated	Simmonds & Lowden, 2000a	Leland, USA	Silt loam [a]	5.48	6.0	0.5	6.3 [d]	0.82	163	0.849
			Iola, USA	Sandy loam [a]	6.55	5.2	1.3	5.0 [d]	1.64	126	0.840
			Ongar, UK	Loam [a]	25.12	6.4	1.9	10.0 [d]	2.65	140	0.868
			Royston, UK	Clay loam [a]	35.97	7.4	4.1	51.9 [d]	2.50	61	0.861
			Ongar sediment [f]	*Sandy clay loam [a]*	*25.98*	*7.7*	*2.6*	*43.8 [d]*	*3.31 [h]*	*127 [h]*	*0.877 [h]*
	New study	Vasques, 2015b	La Gironda	Sandy clay loam [b]	27.7	7.7	1.3	452.0 [e]	1.38	106.27	0.94
			Li10	Silty sand [b]	4.3	5.5	0.6	67.0 [e]	1.94	323.66	1.00
			Lufa 2.1	Sand [b]	2.3	6.0	0.6	28.0 [e]	0.68	113.67	0.98
			Lufa 2.3	Loamy sand [b]	8.3	7.1	0.7	72.0 [e]	0.80	114.19	0.96
			Nierswalde Wildacker	Clay silt [b]	9.4	5.8	1.97	7.6 [f]	2.59	131.53	0.95
								Arithmetic mean	**1.67**	**142**	**0.92**
								Geometric mean	**1.48**	**130**	**0.91**

a Soil type according to USDA
b soil type according to DIN
c If not explicitly mentioned in the study report, OC was calculated: OC (%) = OM (%) / 1.724.
d unit is meq/100 g
e unit is mmolc/kg,
f unit is cmol+/kg
h Data from sediment (Ongar, UK) were not included in calculation of mean values as they were considered not relevant to the proposed European application scenarios

Studies/ Values shaded in grey have been reviewed as part of the first EU review of triticonazole.

Table 7.1.3.2-4: Overview on the adsorption parameters for RPA 407922

Reference			Soil characteristics						Adsorption Parameters		
			Soil origin	Soil type	Clay [%]	pH ($CaCl_2$)	OC [c] [%]	CEC	K_F [mL/g]	K_{FOC} [mL/g]	1/n [-]
CA 7.1.2.3	Study previously evaluated	Simmonds & Lowden, 2000b	Leland, USA	Silt loam [a]	5.48	6.0	0.5	6.3 [d]	3.88	775	0.755
			Iola, USA	Sandy loam [a]	6.55	5.2	1.3	5.0 [d]	16.96	1305	0.825
			Ongar, UK	Loam [a]	25.12	6.4	1.9	10.0 [d]	9.44	497	0.825
			Royston, UK	Clay loam [a]	35.97	7.4	4.1	51.9 [d]	19.13	467	0.708
			Ongar sediment [g]	*Sandy clay loam* [a]	*25.98*	*7.7*	*2.6*	*43.8* [d]	*10.57* [g]	*407* [g]	*0.865* [g]
	New study	Vasques, 2015b	La Gironda	Sandy clay loam [b]	27.7	7.7	1.3	452.0 [e]	3.57	274.74	0.93
			Li10	Silty sand [b]	4.3	5.5	0.6	67.0 [e]	4.46	742.71	0.94
			Lufa 2.1	Sand [b]	2.3	6.0	0.6	28.0 [e]	1.89	314.7	0.98
			Lufa 2.3	Loamy sand [b]	8.3	7.1	0.7	72.0 [e]	1.59	227.51	0.92
			Nierswalde Wildacker	Clay silt [b]	9.4	5.8	1.97	7.6 [f]	8.70	441.83	0.89
								Arithmetic mean	**7.74**	**561**	**0.86**
								Geometric mean	**5.54**	**486**	**0.86**

a Soil type according to USDA
b soil type according to DIN
c If not explicitly mentioned in the study report, OC was calculated: OC (%) = OM (%) / 1.724.
d unit is mew/100 g]
e unit is mmolc/kg,
f unit is cmol+/kg
g Data from sediment (Ongar, UK) were not included in calculation of mean values as they were considered not relevant to the proposed European application scenarios

Studies/ Values shaded in grey have been reviewed as part of the first EU review of triticonazole.

CA 7.1.4 Mobility in soil

Studies on the mobility of triticonazole and its metabolites in soil are not required since reliable K_{FOC} values for triticonazole and its major soil metabolites were determined within the batch equilibrium experiments presented in section CA 7.1.3.

However, several studies on the mobility were evaluated during the Annex I inclusion and accepted by the European Commission (SANCO/10442/2005-Final rev 1, 12 March 2010). They are listed in the sections below. The soil column leaching studies are considered not to be valid due to severe deviation from current guidance documents. The lysimeter studies show minor deviations from the current guidelines and can be used as additional information. The results are presented in Table 7.1.4.3-1.

CA 7.1.4.1 Column leaching studies

These kind of studies on the mobility of triticonazole and its metabolites in soil are not required since reliable K_{FOC} values for triticonazole and its major soil metabolites are available. Moreover, both studies show severe deviations from current guidance documents.

Accordingly, the applicant considers both studies as not necessary for the current evaluation and not valid as well. Mobility of triticonazole and its metabolites can be evaluated based on the information presented in section CA 7.1.3. Risk of leaching of triticonazole and its metabolites can be assessed based on the information provided in chapter CP 9.1.4.

CA 7.1.4.1.1 Column leaching of the active substance

Report:	**R012972; John A.E., Lowden P., Austin D.J. (1993) (+ amendment R012973 of 1998)** Fungicides: RPA 400727-^{14}C (phenyl label): Fresh and aged leaching study with five soils (final report). 200173 / P91/357 / GOoD735
GLP:	yes
Guidelines:	USEPA (=EPA) 163-1, BBA IV, 4-2

Please see the full summary details presented in Volume 3, Section B.8 of the DAR for triticonazole (Austrian Federal Office for Food Safety, 2003). The study has severe deviations to the current OECD Test Guideline 312: 500mm 0.005 M $CaCl_2$ was used for irrigation, at the rate close to the hydraulic conductivity of the columns. Collection time of leachate exceeded the time stated in OECD 312. Selected soils do not fulfil the selection criteria given in the OECD Guidance 312. Moreover, the application rate of 360g/ha strongly exceeds the application rate of 12.5g/ha of triticonazole as seed treatment. Thus, the study is not considered as valid. However, a short summary of the results of the study is presented in Table 7.1.4.3-1 for information purposes.

CA 7.1.4.1.2 Column leaching of metabolites, breakdown and reaction products

Report:	**R012040; Völkel W., (1995) (+ amendment R012041 of 1995)** Leaching characteristics of three metabolites of Triticonazole (RPA 406780, RPA 406341, RPA 407922) in three soils 201160 / 394942 / 8535
GLP:	yes
Guidelines:	BBA IV, 4-2

Please see the full summary details presented in Volume 3, Section B.8 of the DAR for triticonazole (Austrian Federal Office for Food Safety, 2003). The study has severe deviations to the current OECD Test Guideline 312 (e.g. deionized water was used for irrigation; too high limit of detection.). The study is not considered as valid. However, a short summary of the results of the study is presented in Table 7.1.4.3-1 for information purposes.

CA 7.1.4.2 Lysimeter studies

Mobility of triticonazole and its metabolites can be evaluated based on the information presented in section CA 7.1.3. Risk of leaching of triticonazole and its metabolites can be assessed based on the information provided in chapter CP 9.1.4. Accordingly, the applicant considers both studies as not necessary for the current evaluation. However, the lysimeter studies can be used as additional information.

Report:	**C032148; Schnoeder F., (2003)** Triticonazole[benzene ring-U-^{14}C]: Outdoor Lysimeter Study C032148 / 1756-1849-009
GLP:	yes
Guidelines:	Federal Biological Office of Agriculture and Forestry (BBA) Guideline IV, 4-3, 1990

Please see the full summary details presented in Volume 3, Section B.8 of the DAR for triticonazole (Austrian Federal Office for Food Safety, 2003). The study is considered as supplemental information. As a deviation to the BBA Guideline, the second sowing of winter barley was performed with treated seeds. A short summary of the results of the study is presented below.

Two square lysimeters (1 m^2 surface, 1.1 m depth) of undisturbed soil were used. [Benzene ring-U-14C]-Triticonazole was applied as a seed dressing formulation on winter wheat seeds at an actual application rate of 13.1 g/ha (nominal 12.5 g/ha).

The leachate was monitored for radioactivity at bi-weekly intervals, except when no leachate had been produced, for two years. At the end of the study, two years after application, soil samples were analysed in 10°cm segments. Total radioactive residues in the crops were also determined.

Annual average concentrations in the leachates (ai equivalents) were below 0.10 µg/L. Radioactivity in the sample with the highest concentration consisted mainly of components of medium polarity that did not match with known reference compounds.

Report: **Schnoeder F. (2004a)**
(+ Amendment 1, BASF DocID: 2004/5000555, from 2004b and Amendment 2, BASF DocID: 2004/5000556 from 2004c)
Triticonazole [triazole-3,5-^{14}C]: Outdoor lysimeter study
BASF DocID: 2004/5000399
GLP: Yes
Guidelines: Federal Biological Office of Agriculture and Forestry (BBA) Guideline IV, 4-3, 1990

Please see the full summary details presented in Volume 3, Section B.8 of the DAR for triticonazole (Austrian Federal Office for Food Safety, 2003). The study is considered as supplemental information. The study is not valid without Amendment 1 (BASF DocID: 2004/5000555) and Amendment 2 (BASF DocID: 2004/5000556). A short summary of the results of the study is presented below.

Two square lysimeters (1 m^2 surface, 1.1 m depth) of undisturbed soil were used. [Triazole-3,5-14C]-Triticonazole was applied as a seed treatment formulation. The actual application rate was 12.4 g/ha (nominal 12.5 g/ha). In one of the lysimeters a second application was performed about one year after the first application with an actual application rate of 13.1 g/ha.

The leachate was monitored for radioactivity in bi-weekly intervals for three years. At the end of the study, two years after the last application, soil samples were analysed in 10°cm segments. Total radioactive residues in the crops were also determined.

Annual mean concentrations in the leachates (ai equivalents) exceeded 0.1 µg/L in the third year and were close to 0.1 µg/L in the second year. HPLC revealed that the radioactivity consists of polar material with no single component expected to exceed 0.1 µg/l.

Triticonazole and its metabolites RPA 406341 and RPA 404766 have not been detected in the leachates during the course of study.

CA 7.1.4.3 Field leaching studies

A field leaching study for the active substance triticonazole was not performed since it is not required under Commission Regulation (EU) No 283/2013 in accordance with Regulation (EC) No 1107/2009.

Mobility in soil - Overall Conclusions

The results of column leaching studies show that the mobility of triticonazole was dependent on the soil type, having a medium to low mobility in all but a sand soil where up to 75% of the applied radioactivity was found in the leachates.

A column leaching study performed with the two major soil metabolites RPA 407922 and RPA 406341, as well as with the minor metabolite RPA 406780 (peak mixed up in soil metabolism studies with the peak for RPA 404766 in a first step) indicates that these metabolites of triticonazole do not constitute a leaching risk.

Within the lysimeters studies, no leaching risk was identified for triticonazole and its metabolites. In samples for which the leached amount, expressed in a.i. equivalents, exceeded 0.1 µg/L, radioactivity mainly consisted of very polar components with no single component expected to exceed 0.1 µg/L. Triticonazole and its soil metabolites were not detected in the leachates.

Table 7.1.4.3-1: Overview on the mobility of triticonazole and its metabolites in soil columns

Reference			Test conditions	Results
CA 7.1.4.1.1	Study previously evaluated	John et al., 1993, with amendment	The leaching of fresh and aged ^{14}C-phenyl-**triticonazole** residues through soil columns was investigated using 5 different soils. The columns were leached with calcium chloride solution (0.005 M) at a rate that did not exceed the infiltration capacity of the soil. Leachate collected was equivalent to 500 mm of precipitation with leaching times varying from 3 to 12 days. Application rate was equivalent o 360g/ha. The study has severe deviations to the current OECD Test Guideline 312 (e.g. 0.005 M CaCl2 was used for irrigation, different times and volumes of leaching, strongly increased irrigation rate.). **Thus, the study is not considered as valid.**	For non-aged triticonazole, < 1% applied radioactivity was detected in leachates, except for the sand soil for which up to 75% emerged. HPLC analysis revealed the presence of predominantly triticonazole in the extracts of the soil segments with small amounts of RPA 404766 and RPA 406341. For aged triticonazole (30 days), < 1% applied radioactivity was detected in leachates, except for the sand soil for which up to 32% emerged. This leached amount is lower than for the non-aged triticonazole which was suggested to be caused by the increased binding to soil.

Reference			Test conditions	Results
CA 7.1.4.1.2		Völkel, W., 1995, with amendment	The leaching of **RPA 406341, RPA 407922,** and the minor metabolite RPA 406780, was investigated in a column leaching study using three different standard German soil types. The non-radiolabelled test substances were applied as a mixture. The soil columns were leached with deionised water equivalent to 200 mm for a two-day period. The study has severe deviations to the current OECD Test Guideline 312 (e.g. 0.005 M $CaCl_2$ was used for irrigation, different times and volumes of leaching, strongly increased irrigation rate.). **Thus, the study is not considered as valid.**	All residues in the leachate were below the 0.125 µg/L limit of detection.

Studies shaded in grey have been reviewed as part of the first EU review of triticonazole.

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

One study investigating the hydrolysis of triticonazole was evaluated during the Annex I inclusion and accepted by the European Commission (SANCO/10442/2005-Final rev 1, 12 March 2010). The previously submitted study is still considered valid and scientifically sound. However, a new study was performed which included a chiral analysis to confirm the behaviour of the individual stereoisomers. As triticonazole was hydrolytically stable, no studies on metabolites were performed.

The previously submitted study is listed below. The summary of the new hydrolysis study is provided hereafter in the annex point CA 7.2.1.1/1.

Report:	**R013023; Corgier M. M. C., Robin, J. M., (1991)** ^{14}C-RPA 400727: Hydrolysis at 25ºC. 426940
GLP:	yes
Guidelines:	USEPA (=EPA) N, 161-1

Please see the full summary details presented in Volume 3, Section B.8 of the DAR for triticonazole (Austrian Federal Office for Food Safety, 2003). The study is still considered valid with some minor deviations to the current OECD Test Guideline 111 (detection limits were not reported). Results of the study were confirmed in a new study performed following latest guidance documents (Hassink 2013, CA 7.2.1.1/01).

Report: CA 7.2.1.1/1
Hassink J., 2013a
Triticonazole: Aqueous hydrolysis at four different pH values
2012/1300793

Guidelines: EEC 94/37, EEC 95/36, EEC 91/414, SETAC Procedures for assessing the environmental fate and ecotoxicity for pesticides (March 1995), JMAFF 2-6-1, OECD 111, EPA 835.2120

GLP: yes
(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

A hydrolysis study with ^{14}C-labelled triticonazole was conducted at 25°C for 30 days at pH 4, 5, 7 and 9 under sterile conditions. During the testing period the material balance was in the range of 96.0% TAR to 102.7% of total applied radioactivity (TAR).

At all pH values no degradation of the test item occurred within the study period of 30 days. The Z-isomer of triticonazole (RPA 406203, Reg. No. 5079359) was already present at the start of the incubations (1-2% TAR) and was observed with a maximum of 2.6% TAR in the course of the study period. Besides this, some minor peaks with ≤ 2% TAR were detected.

All samples were analysed using a chiral HPLC column to obtain a separation of the S- and R-enantiomers triticonazole. However, there was no indication that a change in the S-/R-ratio (1:1) occurred in the course of the study period. It was concluded that triticonazole was stable in aqueous solution at pH 4, 5, 7 and 9 (25°C) and that no change in the isomer ratio occurred. Due to the stability of triticonazole, no estimation of half-lives was performed.

I. MATERIAL AND METHODS

A. MATERIALS

1. Radiolabeled test material:	**[triazole-3(5)-^{14}C]triticonazole**
Reg. No.:	4378513
Batch No.:	867-1201
Specific Activity:	5.07 MBq/mg
Radiochemical purity:	> 99%
2. Reference items:	**Triticonazole**
Reg. No.:	4378513
Batch No.:	L76-154
Purity	99.8%
	Z-isomer (RPA 406203)
Reg. No.:	Reg. No. 5079359
Batch No.:	BESS0578
Purity	99.9%
	S-Triticonazole
Reg. No.:	5079361
Batch No.:	L76-160
Purity	99.0%
	R-Triticonazole
Reg. No.:	5079385
Batch No.:	HUT918
Purity	99.9 %

3. Test solutions:

The buffer solutions were prepared from Titrisol-solutions (Merck) by 10-fold dilution:

pH 4:	Titrisol 1.09884 (Citrate- HCI)
pH 5:	Titrisol 1.09885 (Citrate - NaOH)
pH 7:	Titrisol 1.09887 (phosphate)
pH 9:	Titrisol 1.09889 (boric acid/ KCI - NaOH)

B. STUDY DESIGN

Test solutions were prepared by evaporating a volume of 3.2 mL of stock solution (1.27 mg/mL, purity 97.5%) to dryness and reconstituting the residue with 5 mL acetonitrile and bringing up to 1000 mL with the respective diluted buffer. The sterile samples (100 mL subsets) were stored in a climatic chamber at 25 ± 1°C for up to 30 days in the dark. The sampling times were 0 d, 2 d, 5 d, 8 d, 12 d, 15 d and 30 d after treatment. At each sampling time, samples were checked for pH and sterility. All samples of the test solutions were analysed directly without work-up. They were analysed by LSC for radioactivity determination and by HPLC to determine the residue pattern. Furthermore, all samples were analysed using a chiral HPLC column to obtain a separation of S- and R-triticonazole.

II. RESULTS AND DISCUSSION

Results from the hydrolysis test, including the material balances and the results obtained with both HPLC methods are presented in the tables below (Table 7.2.1.1-1 to Table 7.2.1.1-4).

Table 7.2.1.1-1: Hydrolysis of triticonazole at pH 4, 25°C

DAT	Material Balance	HPLC – non-chiral column [% TAR]			HPLC – chiral column [% TAR]		
		Triticonazole (sum of R and S isomers)	**Z-isomer** (Reg. No. 5079359, RPA 406203)	**Sum** [a]	**S-isomer** (Reg. No. 5079361)	**R-isomer** (Reg. No. 5079385)	**Z-isomer** (Reg. No. 5079359, (RPA 406203)
0	100.0	95.4	2.0	97.4	50.9	47.9	1.1
2	99.0	95.6	1.6	97.2	50.7	48.3	n.d.
5	99.5	95.4	1.4	96.8	49.3	50.1	n.d.
8	99.5	95.1	2.5	97.6	49.6	49.0	0.9
12	99.4	94.5	1.8	96.3	49.8	48.5	1.0
15	98.6	94.5	2.1	96.6	49.3	49.3	n.d.
30	97.5	92.6	2.1	94.7	49.7	47.8	n.d.

[a] some minor peaks ≤ 2% TAR not included

Table 7.2.1.1-2: Hydrolysis of triticonazole at pH 5, 25°C

DAT	Material Balance	HPLC – non-chiral column [% TAR]			HPLC – chiral column [% TAR]		
		Triticonazole (sum of R and S isomers)	**Z-isomer** (Reg. No. 5079359, RPA 406203)	**Sum** [a]	**S-isomer** (Reg. No. 5079361)	**R-isomer** (Reg. No. 5079385)	**Z-isomer** (Reg. No. 5079359, RPA 406203)
0	100.0	96.8	1.3	98.1	50.0	48.8	1.3
2	101.3	97.0	1.8	98.8	49.6	51.6	n.d.
5	102.7	99.7	1.7	101.4	52.0	50.7	n.d.
8	100.9	96.5	2.3	98.8	50.8	50.1	n.d.
12	99.7	96.0	2.1	98.1	50.2	48.6	0.9
15	100.8	95.6	2.1	97.7	51.2	49.6	n.d.
30	97.4	92.1	1.6	93.7	48.8	48.6	n.d.

[a] some minor peaks ≤ 2% TAR not included

Table 7.2.1.1-3: Hydrolysis of triticonazole at pH 7, 25°C

DAT	Material Balance	HPLC – non-chiral column [% TAR]			HPLC – chiral column [% TAR]		
		Triticonazole (sum of R and S isomers)	**Z-isomer** (Reg. No. 5079359, RPA 406203)	**Sum** [a]	**S-isomer** (Reg. No. 5079361)	**R-isomer** (Reg. No. 5079385)	**Z-isomer** (Reg. No. 5079359, RPA 406203)
0	100.0	95.9	2.3	98.2	49.7	50.3	n.d.
2	90.7	86.4	1.3	97.7	46.1	43.4	1.2
5	99.8	95.4	1.7	97.1	49.5	49.4	0.8
8	97.1	94.1	2.0	96.1	48.4	47.6	1.1
12	100.5	97.3	1.8	99.1	51.3	49.3	n.d.
15	99.5	95.7	2.2	97.9	50.2	48.3	0.9
30	100.4	95.6	1.7	97.3	50.4	49.9	n.d.

[a] some minor peaks ≤ 2% TAR not included

Table 7.2.1.1-4: Hydrolysis of triticonazole at pH 9, 25°C

DAT	Material Balance	HPLC – non-chiral column [% TAR]			HPLC – chiral column [% TAR]		
		Triticonazole (sum of R and S isomers)	**Z-isomer** (Reg. No. 5079359, RPA 406203)	**Sum** [a]	**S-isomer** (Reg. No. 5079361)	**R-isomer** (Reg. No. 5079385)	**Z-isomer** (Reg. No. 5079359, RPA 406203)
0	100.0	94.7	2.3	97.0	49.2	49.8	1.1
2	98.3	94.9	1.7	96.6	49.2	47.8	1.3
5	98.4	93.5	1.9	95.4	49.9	48.4	n.d.
8	98.5	93.3	2.2	95.5	50.4	48.1	n.d.
12	98.9	95.1	2.6	97.7	49.2	49.6	n.d.
15	97.7	92.4	1.9	94.3	50.2	46.5	1.0
30	96.0	91.5	1.8	93.3	47.7	48.3	n.d.

[a] some minor peaks ≤ 2 % TAR not included

III. CONCLUSION

Triticonazole is stable in aqueous solution at pH 4, 5, 7 and 9 (25°C). There are no hints that a change to the Z-isomer or a change in the ratio of S- and R-enantiomers of triticonazole will occur in aqueous solutions in the range of pH 4 to pH 9 and at 25 °C. The study is valid.

CA 7.2.1.2 Direct photochemical degradation

Two studies investigating the direct photodegradation and the quantum yield of triticonazole were evaluated during the Annex I inclusion and accepted by the European Commission (SANCO/10442/2005-Final rev 1, 12 March 2010). The previously submitted studies are still considered valid and scientifically sound and they are listed below. However, in order to confirm and supplement the results of the previous studies, a new study was performed. The new study includes information on the photolytic degradation behaviour of the major photolysis metabolite RPA 406203 (Z-isomer of triticonazole).

The previously submitted studies are listed below. The summary of the new photolysis study is provided hereafter in the annex point CA 7.2.1.2/1.

Report: **R013068; Corgier M. M. C, Robin J.M., (1992)**
^{14}C-RPA 400727: Aqueous photolysis.
429307 / 91-50 / AG/CRLD/AN/9216236
GLP: yes
Guidelines: USEPA (=EPA) N, 161-2

Please see the full summary details presented in Volume 3, Section B.8 of the DAR for triticonazole (Austrian Federal Office for Food Safety, 2003). The study is still considered valid but it has some deficiencies: The irradiation period for the irradiated samples was not long enough to investigate the decline of the major photolysis metabolite RPA 406203 (Z-isomer) and to demonstrate that equilibrium between triticonazole and RPA 406203 is established. Furthermore, the half-life of triticonazole could only be graphically determined.

Report: **R012072; Corgier M. M. C., Turier G.P., (1995)**
Triticonazole - Quantum yield and environmental half-life in water
439237 / 95-90 / R&D/CRLD/AN/9516639 / 8533
GLP: yes
Guidelines: BBA Part IV, 6-1, UBA Phototransformation of Chemicals, Part A

Please see the full summary details presented in Volume 3, Section B.8 of the DAR for triticonazole (Austrian Federal Office for Food Safety, 2003). The study is still considered valid.

Report: CA 7.2.1.2/1
Singh M., 2007a
Aqueous photolysis of 14C-BAS 595 F (Triticonazole)
2007/7001058
Guidelines: EPA 161-2
GLP: yes
(certified by United States Environmental Protection Agency)

Executive Summary

The aqueous photolysis study with ^{14}C-BAS 595 F (triazole and phenyl radiolabelled triticonazole) was performed in in aqueous buffer of pH 5 at 22 ± 1°C under sterile conditions. The buffer test solutions (200 mL), with initial triticonazole concentrations of 3.6 (triazole label) and 5.5 mg/L (phenyl label), were continuously exposed to a Xenon arc lamp (wavelength > 290 nm) for up to 408 hours in quartz glass reaction vessels. The vessels were continuously flushed with sterile moistened CO_2-free air, and volatiles were collected in a series of trapping solutions (ethylene glycol and NaOH). Dark control samples were incubated under the same conditions except for irradiation.

Treated pH 5 buffer samples were analysed by LSC and HPLC after 0, 8/19, 24, 48, 72,168, 240, 360/336 and 408 hours of irradiation (phenyl label/triazole label). The dark control samples were analysed by LSC and HPLC concurrently with the irradiated samples.

During the course of the study the mean material balance for the irradiated samples ranged between 100.1 and 102.5% of total applied radioactivity (TAR) (triazole label) and between 94.3 and 103.6% TAR (phenyl label). The mean material balance for the dark control samples ranged between 100.2 and 106.5% TAR (triazole label) and 99.8 - 104.4% TAR (phenyl label). Less than 1% TAR was found as volatile radioactivity for both labels, all of which was identified as $^{14}CO_2$.

The major photo-degradation pathway involved isomerization of triticonazole (E-isomer) to its Z-isomer (RPA 406203). The minor degradation pathway involved hydroxylation at various positions of triticonazole and the Z-isomer and furthermore, oxidation of these hydroxylated derivatives to corresponding carbonyl compounds (all < 5% TAR).

Triticonazole degraded photolytically with DT_{50}, DT_{75} and DT_{90} values of 9.3, 18.7 and 31.0 hours, respectively. ^{14}C-BAS 595 F is being reformed from the Z-isomer concurrently with its loss. Therefore, the DT_{50} value is short compared to the graphical time at which 50% of the initial amount remains (about 250 hours). The DT_{50} and DT_{90} values for the degradation of the Z-isomer (RPA 406203) were 5.9 and 19.7 hours, respectively.
BAS 595 F is stable in pH 5 buffer solution under darkness.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test material:

	^{14}C-triazole-BAS 595 F
Reg. No.	4378513
Molecular Weight	317.82 g/mol (unlabelled)
Site of radiocarbon labeling:	triazole-4(5)-^{14}C
Batch No.:	867-1103
Specific Activity:	391780.8 dpm/µg
Radiochemical purity:	> 99.3%

	^{14}C-phenyl-BAS 595 F
Reg. No.	4378513
Molecular Weight	317.82 g/mol (unlabelled)
Site of radiocarbon labeling:	phenyl-U-^{14}C
Batch No.:	866-1103
Specific Activity:	400915 dpm/µg
Radiochemical purity:	> 99.4%

2. Test System

The pH 5 buffer solutions were prepared by dissolving sodium acetate (1.36 g) in HPLC grade water (1000 mL), and then adding 235 µL of acetic acid to obtain a 0.01 M pH 5 buffer solution. The buffer solution was filtered using a sterilized filtration unit (pore size 0.2 µm) prior to the treatment to make it sterile.

B. STUDY DESIGN

1. Experimental conditions

The photolysis setup consisted of a rectangular metallic hollow block equipped with coolant inlet and outlet. The thermostated block was provided with 6 wells to house 6 photolysis glass vessels with a quartz glass disc at the top. Each test vessel was filled with 200 mL of a treated buffer solution which was prepared by adding appropriated amounts of ^{14}C-labelled triticonazole stock solution to sterile pH 5 buffer. Initial concentrations of ^{14}C-labeled triticonazole in the test solutions (irradiated samples and dark controls) were 3.6 and 5.5 mg/L for the triazole and phenyl label, respectively.

Samples were continuously exposed to a Xenon arc lamp in an Atlas Suntest CPS Plus apparatus. Wavelengths < 290 nm were filtered out. The measured intensity and spectrum of the irradiation was comparable to natural sunlight at 40° N latitude.

The irradiation temperature was maintained at 22 ± 1°C. The test vessels were continuously flushed with sterile moistened CO_2-free air, and volatiles were collected in a series of trapping solutions (ethylene glycol and NaOH). Possible losses of water during irradiation were fixed by weighing the irradiated test vessel and adding appropriate amounts of untreated sterile buffer solutions

In addition, aliquots (~1 mL each) of the treated solution were transferred to a number of HPLC vials. Vials were capped and stored in the dark inside an incubator maintained at 22 ± 1°C. These samples were used as dark controls.

2. Sampling

The sampling intervals for the triazole label experiment were 0, 19, 24, 48, 72, 168, 240, 336 and 408 hours. The sampling intervals for the phenyl label experiment were 0, 8, 24, 48, 72, 168, 240, 360 and 408 hours. Irradiated samples and dark controls were removed at the same time. Volatile trapping solutions were removed at every sampling interval.

3. Description of analytical procedures

Volatile trapping solutions from irradiated samples were analysed by LSC to quantify the amount of volatile radioactivity. For a selected time interval, the trapped carbon dioxide was characterized by reacting a measured aliquot of the trapping solution with sulfuric acid, trapping the carbon dioxide generated into Harvey cocktail, and finally counting the Harvey sample by LSC.

To determine the material balances in irradiated and dark samples, aliquots of the solutions were analysed by LSC. HPLC was used to obtain the quantitative distribution profile of radioactive residues in the sample.

At the end of the study, the remaining buffer solutions in the irradiated triazole-labelled samples were pooled and partitioned with ethyl acetate. The resultant fractions were assayed by LSC, concentrated to dryness, reconstituted with an acetonitrile water mixture and fractionated using a HPLC method. Each relevant fraction was assayed by LSC to generate a histogram. Fractions of relevance were pooled assayed by LSC and HPLC. Then, they were concentrated further and analysed by HPLC co-chromatography with reference standard (if available) and mass spectrometry.

A similar procedure was used for the irradiated phenyl-labelled samples, but lyophilisation was applied in the first step instead of partitioning and NMR was used as an additional identification method.

4. Determination of degradation kinetics

Estimation of the half-life of BAS 595 F was done only for the irradiated samples. The half-life estimation was not done for the dark control samples because BAS 595 F was stable under this condition for at least 17 days after the treatment. The guidance of FOCUS (2006) was used as the basis for conducting the kinetic analysis, statistical assessment, and selection of the best fit kinetic model. Optimization of model parameters, including estimation of parameter standard errors, was performed using the software ModelMaker 4.0. Decline of ^{14}C-BAS 595 F and rise and decline of the Z-isomer photoproduct were evaluated using a single first-order (SFO) reversible model and three biphasic models (first-order multi compartment or FOMC, double first-order in series or DFOS, and double first-order in parallel or DFOP).

II. RESULTS AND DISCUSSION

A. MASS BALANCE

During the course of the study the mean material balance for the irradiated samples ranged between 100.1 - 102.5% of the total applied radioactivity (TAR) (triazole label) and 94.3 - 103.6% TAR (phenyl label). The mean material balance for the dark control samples ranged between 100.2 - 106.5% TAR (triazole label) and 99.8 - 104.4% TAR (phenyl label). Less than 1% TAR was found as volatile radioactivity for both labels, all of which was identified as $^{14}CO_2$.

B. FINDINGS

BAS 595 F degraded photolytically with the major photo-degradation pathway involving isomerization of triticonazole to its Z-isomer (RPA 406203). The minor degradation pathway involved hydroxylation at various positions of triticonazole (parent) and the Z-isomer and further oxidation of these hydroxylated derivatives to corresponding carbonyl compounds.

The distribution of radioactivity in the test solutions is summarized in Table 7.2.1.2-1 and Table 7.2.1.2-2. In the irradiation experiment using both labels, a large number of radioactive residues was observed but BAS 595 F and the Z-isomer were the only components > 5% TAR. The test item BAS 595 F decreased with time while the Z-isomer first increased and started declining after about 48 hours of irradiation. All other degradation products were minor (none > 5% TAR).

Table 7.2.1.2-1: Distribution of radioactivity in irradiated and dark samples of ^{14}C-phenyl-labelled-BAS 595 F in aqueous pH 5 buffer solution; mean of two replicates [%TAR]

Peak name TR (min.)	Sampling time [h]								
	0	**8**	**24**	**48**	**72**	**168**	**240**	**360**	**408**
Phenyl label – irradiated samples									
Peak 1	n.d.	n.d.	n.d.	n.d.	n.d.	0.7	0.9	1.9	1.7
Peak 2	n.d.	n.d.	n.d.	n.d.	n.d.	1.4	2.0	2.9	2.9
Peak 3	n.d.	n.d.	n.d.	n.d.	n.d.	1.0	1.4	2.1	2.2
Peak 4	n.d.	n.d.	n.d.	n.d.	n.d.	0.7	1.0	2.7	2.7
Peak 5	n.d.	n.d.	n.d.	n.d.	n.d.	1.1	1.1	1.5	1.5
BAS 595 F	**100.0**	**70.7**	**62.9**	**61.2**	**61.0**	**56.0**	**54.7**	**51.1**	**47.0**
Z-isomer	**1.1**	**30.5**	**40.7**	**40.2**	**40.1**	**36.2**	**34.2**	**30.9**	**27.8**
Others [a]	n.d.	n.d.	n.d.	0.9	n.d.	3.7	3.8	8.1	8.6
Phenyl label – dark samples									
BAS 595 F	100.0	102.8	101.0	100.2	101.3	98.2	98.9	98.4	96.0
Z-isomer	1.1	1.6	1.2	0.8	1.5	1.6	1.4	1.5	1.7
Others						0.8			2.1

n.d. = not detected;
[a] Sum of multiple components, none of them more than 2% TAR

Table 7.2.1.2-2: Distribution of radioactivity in irradiated and dark samples of ^{14}C-triazole labelled-BAS 595 F in aqueous pH 5 buffer solution; mean of two replicates [%TAR]

Peak name T_R (min.)	Sampling time [h]								
	0	19	24	48	72	168	240	336	408
Triazole label - irradiated samples									
Peak 1 [a]	n.d.	n.d.	n.d.	n.d.	1.9	5.0	7.9	11.9	16.3
Peak 2	n.d.	n.d.	n.d.	n.d.	n.d.	1.3	2.2	3.6	4.3
Peak 3	n.d.	n.d.	n.d.	n.d.	n.d.	0.8	1.5	2.1	2.5
Peak 4	n.d.	n.d.	n.d.	n.d.	n.d.	1.5	2.1	2.6	3.4
Peak 5	n.d.	n.d.	n.d.	n.d.	n.d.	0.8	0.6	1.9	1.9
BAS 595 F	**99.2**	**61.1**	**60.9**	**61.2**	**60.0**	**53.7**	**49.6**	**43.9**	**41.2**
Z-isomer	**1.0**	**39.0**	**39.5**	**40.0**	**38.9**	**33.9**	**32.1**	**27.6**	**26.4**
Others [b]					0.8	4.2	4.7	7.1	6.7
Triazole label – dark samples									
BAS 595 F	99.2	105.4	100.0	101.0	100.9	100.5	100.7	99.8	100.1
Z-isomer	1.0	1.1	1.2	1.1	1.2	1.3	1.2	1.3	1.2

n.d. = not detected;
[a] Peak 1 is a mixture of several products (> 6)
[b] Sum of multiple components, none of them more than 2% TAR

Considering that photolytic conversion of ^{14}C-BAS 595 F (E-isomer) to the Z-isomer is reversible and that pseudo equilibrium is established, the aqueous photolysis of ^{14}C-BAS 595 F was well described by a SFO kinetic model. The photochemical degradation trigger endpoints in aqueous pH 5 buffer solution are presented in Table 7.2.1.2-3.

Table 7.2.1.2-3: BAS 595 F Photochemical Degradation Trigger Endpoints in aqueous pH 5 buffer solution

Soil	Parameter estimates ± standard error [a]	χ^2 error	r^2	DT_{50} [h]	DT_{75} [h]	DT_{90} [h]
BAS 595 F	M_o = 102.23 ± 0.54751 k = 0.074232 ± 0.004914	2.80	0.973	9.34	18.7	31.0
Z-isomer	C_o = .98197 ±0.0012318 k = 0.11662 ± 0.008152	3.47	0.981	5.94	11.9	19.7

[a] Units: Mo [% TAR]; k [d-1]

Given the reversible process, ^{14}C-BAS 595 F is being reformed concurrently with its loss.

III. CONCLUSION

The major photo-degradation pathway for BAS 595 F in irradiated aqueous buffer solutions involved reversible isomerization of the E-isomer (parent triticonazole) to its Z-isomer. All other degradation products were minor (none > 5% TAR).

The DT_{50} and DT_{90} values for the degradation of BAS 595 F were 9.3 and 31.0 hours, respectively, considering that photolytic conversion is reversible. Therefore, the DT_{50} value is short compared to the graphical time at which 50% of the initial amount remains (about 250 hours). The DT_{50} and DT_{90} values for the degradation of the Z-isomer (RPA 406203) were 5.9 and 19.7 hours, respectively.

CA 7.2.1.3 Indirect photochemical degradation

The indirect photochemical degradation of triticonazole was not investigated.

Route and rate of degradation in aquatic systems (chemical and photochemical degradation) - Overall conclusions

Triticonazole is hydrolytically stable at environmentally relevant pH values (pH 4 - 9) at 25°C.

Under irradiated conditions, triticonazole is converted to RPA 406203 (Z-isomer) and furthermore to numerous minor metabolites, including carbon dioxide. The photolytic conversion to the Z-isomer is considered to be reversible since equilibrium with the parent compound triticonazole was established. The DT_{50} and DT_{90} values for the degradation of BAS 595 F were 9.3 and 31.0 hours, respectively. The DT_{50} and DT_{90} values for the degradation of the Z-isomer (RPA 406203) were 5.9 and 19.7 hours, respectively.

A detailed discussion of the fate of the isomers of triticonazole is presented in Document N5.

CA 7.2.2 Route and rate of biological degradation in aquatic systems

CA 7.2.2.1 "Ready biodegradability"

One study investigating the ready biodegradability of triticonazole was evaluated during the Annex I inclusion and accepted by the European Commission (SANCO/10442/2005-Final rev 1, 12 March 2010). The previously submitted study is still considered valid and scientifically sound and it is listed below.

According to this study, triticonazole is not readily biodegradable.

Report: **R013073; Handley J.W., Horton M.R., (1992)**
Assessment of the ready biodegradability (modified Sturm Test) of RPA 400727
430264 / 282/321
GLP: yes
Guidelines: OECD 301B, (1981)

Please see the full summary details presented in Volume 3, Section B.8 of the DAR for triticonazole (Austrian Federal Office for Food Safety, 2003). The study is still considered valid.

CA 7.2.2.2 Aerobic mineralisation in surface water

This type of study is a new data requirement according to Commission Regulation (EU) No. 283/2013 and was, therefore, not addressed within the former Annex I inclusion dossier. A new study was performed which is summarized below.

According to this study, triticonazole is not significantly degraded in natural surface water.

Report:	CA 7.2.2.2/1 Adam D., 2014a 14C-Triticonazole (BAS 595 F): Aerobic mineralisation in surface water - Simulation biodegradation test 2014/1083345
Guidelines:	OECD 309 (April 2004), SANCO 11802/2010/ rev.7 amending EC 1107/2009
GLP:	yes (certified by Swiss Federal Office of Public Health, Berne, Switzerland)

Executive Summary

Aerobic mineralization of ^{14}C-BAS 595 F (triticonazole) in surface water was investigated under laboratory conditions using two separately labelled test items: [phenyl-U-^{14}C]-labelled BAS 595 F and [triazole-3(5)-^{14}C]-labelled BAS 595 F. Natural pond water was treated with two concentrations (high dose and a low dose) which represented the expected range of concentrations in the environment. Additionally, single test vessels of the high dose experiment of the phenyl-label were incubated under sterile conditions in order to gain information about abiotic degradability of the test item. The water samples were incubated in the dark for 59 days at 21.2 ± 0.2°C. The test system was continuously flushed with moistened CO_2-free air, and volatiles were collected in a series of trapping solutions (ethylene glycol and NaOH). Microbial activity of the test system was assessed through the degradation of [^{14}C(U)]-benzoic acid.

Duplicate samples per label and test concentration were taken for analysis 0, 1, 3, 8, 14, 31 and 59 days after treatment (DAT). At each sampling time, the water phase was analysed by LSC and later on submitted to HPLC analysis. For confirmatory purposes, selected samples were additionally analysed by TLC. To check the stability of the enantiomeric ratio of triticonazole, a second HPLC method (chiral) was used for sample analysis at 0 and 59 DAT. Radioactivity in the volatile trapping solutions was monitored by LSC and solutions were exchanged after 36 days.

For the [phenyl-U-^{14}C]-labelled BAS 595 F, the total mean recoveries were 100.7 ± 2.0, 102.4 ± 2.8, 100.4 ± 1.8% of the total applied radioactivity (TAR) for the high dose, high dose sterile and for the low dose experiments, respectively. Considering [triazole-3(5)-^{14}C]-labelled BAS 595 F, the total mean recoveries were 96.1 ± 2.3% TAR for the high dose and 101.4 ± 3.7% TAR for the low dose experiments. The evolved $^{14}CO_2$ and other ^{14}C-volatiles did not exceed mean amounts of 3.1% TAR in either of the treated systems, irrespective of dosage level.

Triticonazole remained stable throughout the study and all metabolites detected in the test systems are classified as minor (each ≤ 4.3% TAR). The enantiomeric ratio in the radiolabelled BAS 595 F remained stable throughout the entire incubation time.

Because of the stable nature of triticonazole, kinetic analysis and subsequent determination of trigger endpoints was not possible.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test material:

	[phenyl-U-^{14}C]-BAS 595 F
Reg. No.	4378513
Lot/Batch number	866-1501
Molecular Weight	317.82 g/mol (non-labelled)
Site of radiocarbon labelling:	phenyl-U-^{14}C
Radiochemical purity:	99.4% (96.6% determined before use)
Specific activity of ai:	5.86 MBq/mg

2. Test material:

	[triazole-3(5)-^{14}C]-BAS 595 F
Reg. No.	4378513
Lot/Batch number	867-1401
Molecular Weight	317.82 g/mol (non-labelled)
Site of radiocarbon labelling:	triazole-3(5)-^{14}C
Radiochemical purity:	98.8% (97.5% determined before use)
Specific activity of ai:	6.44 MBq/mg

3. Reference material:

	[phenyl-U-^{14}C]Benzoic acid
Lot/Batch number	121214
Molecular Weight	122.12 g/mol
Site of radiocarbon labelling:	phenyl-U-^{14}C
Radiochemical purity:	> 99%
Specific activity of ai:	37.87 MBq/mg

4. Water

Water was freshly sampled from a pond in Rheinfelden, Switzerland. The sampling location was in an area not subject to effluent discharges and removed from human activity. The water was sampled from the surface and filtered with a 0.1 mm sieve and then transported to IES Ltd in sealed containers. The water was stored at about 4 °C in an open container under aerated conditions in the dark for one day until use. Physicochemical characteristics of the test water are presented in Table 7.2.2.2-1.

Table 7.2.2.2-1: Pond water characteristics

Origin:		**Fröschweiher, Rheinfelden, Switzerland N°47°543.495' E07°817899'**
Sampling date:		22 May 2014
Water parameters measured at field sampling		
Temperature	[°C]	19.2
pH		8.02
Oxygen concentration	[mg/L]	8.83
Redox potential (E_h) *	[mV]	236
Sampling depth	[cm]	On the surface (5 – 10 cm)
Water parameters measured post-handling		
DOC	[mg/L]	8.18
Nitrate	[mg/L]	1.57
Nitrite	[mg/L]	<0.82
Ammonium	[mg/L]	0.45
N total	[mg/L]	2.65
P total	[mg/L]	0.39

B. STUDY DESIGN

1. Test system

Each test system consisted of an open gas-flow-system with 350 mL Erlenmeyer glass flasks, containing 100 mL of natural pond water. For the high dose experiment, concentrations of 0.093 and 0.084 mg/L were assayed in [phenyl-U-^{14}C] and [triazole-3(5)-^{14}C]BAS 595 F treated systems, respectively. Additionally, single test systems of the high dose experiments of the phenyl label were incubated under sterile conditions to get information about abiotic degradability of the test item. For the low dose experiment, concentrations of 0.008 and 0.009 mg/L were assayed in [phenyl-U-^{14}C] and [triazole-3(5)-^{14}C]BAS 595 F treated systems, respectively.

Treated and untreated test systems (two non-sterile and one sterile) were incubated in the dark for up to 59 days at 21.2 ± 0.2 °C. The test systems were continuously flushed with moistened CO_2-free air, and volatiles were collected in a series of trapping solutions (ethylene glycol and NaOH). The samples were continuously and gently stirred to maintain particles and microorganisms in suspension.

The microbial activity of the test system was assessed by using the same experimental set-up and monitoring the degradation of [^{14}C(U)]-benzoic acid in duplicate samples.

2. Sampling

Duplicate water samples per label and test concentration were taken for analysis 0, 1, 3, 8, 14, 31 and 59 days after treatment (DAT). Radioactivity in the trapping solutions was also monitored by LSC and solutions were exchanged after 36 days.

Single samples were taken for the sterile experiment of [phenyl-U-^{14}C]BAS 595 F at DAT 0, 1, 3, 8, 14, 31, 59.

Duplicate water samples of [^{14}C(U)]-benzoic acid were sampled at 0, 7 and 14 DAT.

3. Analytical procedures

At each sampling time, pH and oxygen concentration were measured, the water phase volume recorded and the radioactivity present analysed by LSC. Subsequently, an aliquot of the water phase of each sample was concentrated by rotary evaporation, re-analysed by LSC and later on submitted to HPLC analysis. Selected samples were additionally analysed by TLC in order to confirm the HPLC results. Furthermore, the stability of the enantiomeric ratio of triticonazole was checked in selected samples collected at DAT 0 and 59 using a chiral HPLC method.

Radioactivity in the volatile trapping solutions was monitored by LSC, and solutions were exchanged after 36 days. Prior to measuring the radioactivity, the volume of liquid in each ethylene glycol and sodium hydroxide trap was recorded.

Aliquots (1 mL) of the samples treated with [^{14}C(U)]-benzoic acid were directly analysed by LSC and submitted for HPLC analysis in order to obtain the remaining concentration of benzoic acid in the test system. Additionally, to determine the associated amounts of dissolved radioactive carbon dioxide and volatile radioactivity, duplicate aliquots were removed from the trapping solutions.

For the LSC analysis of the water samples, the limit of detection (LOD) was 0.093 and 1.063 % AR for the high and low dosed samples, respectively. The limit of quantification (LOQ) was 0.139 and 1.594 % TAR in the high and low dosed samples, respectively.

For the HPLC analysis of the water samples, LOD was 0.690 and 0.780 % TAR for the high and low dosed samples, respectively. LOQ was 1.380 and 1.560% TAR in the high and low dosed samples, respectively.

4. Calculation of DT_{50} and DT_{90} values

As the test item was stable under all conditions in all test systems, the calculation of meaningful DT_{50} and DT_{90} values was not possible. Therefore, there was no kinetics component to this study.

II. RESULTS AND DISCUSSION

The degradation of the reference item [phenyl-U-^{14}C] benzoic acid confirmed that the test systems are microbially active.

A. MASS BALANCE

In the tested systems, the total mean recoveries ranged from 96.1 ± 2.3 to 102.4 ± 2.8% TAR. Volatiles represented less than 3.1% TAR in either of the treated systems, regardless of dosage level. The material balance and the distribution of radioactivity in the systems treated with [phenyl-U-^{14}C]-BAS 595 F and [triazole-3(5)-^{14}C]-BAS 595 F are presented in Table 7.2.2.2-2 and Table 7.2.2.2-3, respectively.

Table 7.2.2.2-2: Material balance and distribution of radioactivity after application of [phenyl-U-^{14}C]-BAS 595 F [%TAR]

DAT	Aqueous phase (mean) [% TAR]	Volatiles (mean) [% TAR]		Recovery (mean) [% TAR]
		$^{14}CO_2$	Other ^{14}C-volatiles	
Test systems – low dose				
0	102.0	n. a.	n. a.	102.0
1	98.8	<1.0	<LOQ	98.9
3	98.4	<1.0	<LOQ	98.5
8	101.2	<1.0	<LOQ	101.3
14	99.6	<1.0	<LOQ	99.6
31	100.7	<1.0	<LOQ	100.8
59	97.0	3.1	1.3	101.4
			Mean ± SD	**100.4 ± 1.8**
Test systems – high dose				
0	102.0	n. a.	n. a.	102.0
1	99.5	<0.1	<0.1	99.5
3	101.0	<0.1	<0.1	101.1
8	102.1	<0.1	<0.1	102.2
14	101.8	0.3	<0.1	102.2
31	100.7	0.1	<0.1	100.9
59	96.0	1.0	<0.1	97.0
			Mean ± SD	**100.7 ± 2.0**
Sterile samples – high dose (single values)				
0	108.2	n. a.	n. a.	108.2
1	99.5	<0.1	<0.1	99.5
3	100.8	<0.1	<0.1	100.9
8	102.9	<0.1	<0.1	102.9
14	101.0	<0.1	0.2	101.3
31	101.0	<0.1	0.2	101.3
59	102.5	<0.1	<0.1	102.6
			Mean ± SD	**102.4 ± 2.8**

n.a. - not applicable; SD = Standard deviation; LOQ = Limit of quantification

Table 7.2.2.2-3: Material balance and distribution of radioactivity (LSC) after application of [triazole-3(5)-^{14}C]-BAS 595 F [%TAR]

DAT	Aqueous phase (mean) [% TAR]	Volatiles (mean) [% TAR]		Recovery (mean) [% TAR]
		$^{14}CO_2$	Other ^{14}C-volatiles	
Test systems – low dose				
0	102.5	n. a.	n. a.	102.5
1	99.2	<0.1	<0.1	99.3
3	97.9	<0.1	<0.1	98.3
8	100.8	<0.1	<0.1	100.8
14	105.1	<0.1	<0.1	105.6
31	99.9	<0.1	<0.1	100.4
59	102.3	<0.1	<0.1	102.7
			Mean ± SD	**101.4 ± 3.7**
Test systems – high dose				
0	99.1	n. a.	n. a.	99.1
1	98.5	<0.1	<0.1	98.5
3	93.6	<0.1	<0.1	93.6
8	96.6	<0.1	<0.1	96.6
14	95.6	<0.1	<0.1	95.7
31	95.4	0.2	<0.1	95.6
59	93.5	0.3	<0.1	93.8
			Mean ± SD	**96.1 ± 2.3**

n.a. - not applicable; SD = Standard deviation

B. TRANSFORMATION OF PARENT COMPOUND

The amounts of BAS 595 F and its metabolites recovered at each sampling time in the viable test systems are presented in Table 7.2.2.2-4 and Table 7.2.2.2-5. Results showed that triticonazole remained stable throughout the study and that all metabolites are classified as minor (each ≤ 4.3% TAR). In the high dose sterile system, the parent was also stable (102.5% at DAT 59).

MI, M2 and M3 had the same elution time on HPLC as the standards 5079359 (RPA 406203) Z-isomer), 5079285 (RPA 404766) and 5059144 (RPA 406341), respectively, while the others remained unknown. As all fractions were classified as minor metabolites, none of them were identified or characterised further. There were no metabolites detected in the high dose sterile system.

Table 7.2.2.2-4: Pattern of degradation and formation of metabolites after application of [phenyl-U-^{14}C]-BAS 595 F [%TAR]

Compound	Incubation time (days)						
	0	1	3	8	14	31	59
Test systems – low dose							
Triticonazole	97.9	98.8	96.0	100.2	96.9	79.1	94.4
M1 (RPA 406203)	4.1	n.d.	2.4	1.0	2.7	1.9	2.6
M2 (RPA 404766)	n.d.	n.d.	n.d.	n.d.	n.d.	1.3	n.d.
M3 (RPA 406341)	n.d.	n.d.	n.d.	n.d.	n.d.	1.8	n.d.
M4	n.d.	n.d.	n.d.	n.d.	n.d.	2.2	n.d.
M6	n.d.	n.d.	n.d.	n.d.	n.d.	1.1	n.d.
M7	n.d.	n.d.	n.d.	n.d.	n.d.	1.5	n.d.
M8	n.d.	n.d.	n.d.	n.d.	n.d.	1.6	n.d.
M9	n.d.	n.d.	n.d.	n.d.	n.d.	2.6	n.d.
M10	n.d.	n.d.	n.d.	n.d.	n.d.	3.9	n.d.
M11	n.d.	n.d.	n.d.	n.d.	n.d.	3.7	n.d.
Test systems – high dose							
Triticonazole	102.0	99.5	101.0	99.5	99.4	98.2	94.4
M1 (RPA 406203)	n.d.	n.d.	n.d.	2.6	n.d.	n.d.	1.6
M4	n.d.	n.d.	n.d.	n.d.	2.3	1.1	n.d.
M5	n.d.	n.d.	n.d.	n.d.	n.d.	1.4	n.d.

n.d. not detected

Table 7.2.2.2-5: Pattern of degradation and formation of metabolites after application of [triazole-3(5)-^{14}C]-BAS 595 F [%TAR]

Compound	Incubation time (days)						
	0 d	1d	3d	8 d	14 d	31 d	59 d
Test systems – low dose							
Triticonazole	98.2	98.3	96.1	100.8	103.0	95.3	99.9
M1 (RPA 406203)	4.3	0.9	1.8	n.d.	2.1	n.d.	2.4
M4	n.d.	n.d.	n.d.	n.d.	n.d.	2.6	n.d.
M8	n.d.	n.d.	n.d.	n.d.	n.d.	2.0	n.d.
Test systems – high dose							
Triticonazole	99.1	98.5	93.6	95.5	95.6	92.8	92.4
M1 M1 (RPA 406203)	n.d.	n.d.	n.d.	1.1	n.d.	n.d.	1.1
M4	n.d.	n.d.	n.d.	n.d.	n.d.	2.5	n.d.

n.d. not detected

C. ISOMERIZATION

Results of chiral-HPLC analysis of treated systems taken at 0 DAT and 59 DAT showed that the ratio between the R and S enantiomers of [phenyl-U-^{14}C]BAS 595 F remained stable at approximately 1:1 throughout the study.

III. CONCLUSION

In conclusion, [^{14}C]-BAS 595 F, regardless of its concentration and ^{14}C-label position, remained stable and degraded in neither biotic nor abiotic surface water systems. Additionally, the enantiomeric ratio of [^{14}C]-BAS 595 F remained stable throughout the entire incubation period. The stable nature of the test item precluded the use of kinetics and thus the determination of meaningful DT_{50} and DT_{90} values was not possible.

CA 7.2.2.3 Water/sediment studies

A study investigating the degradation of triticonazole in water/sediment systems was evaluated during the Annex I inclusion and accepted by the European Commission (SANCO/10442/2005-Final rev 1, 12 March 2010). The previously submitted study is still considered valid and scientifically sound and it is listed below.

A new kinetic evaluation of the study was performed which is summarized hereafter in Annex point CA 7.2.2.3/1. The new evaluation is in accordance with FOCUS guidance (2014) [*FOCUS (2014): Generic Guidance for Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration, version 1.1*] although this is not explicitly stated in the report.

A short summary of the results and relevant parameters is presented Table 7.2.3-1.

Report:	**R012987; Wyss-Benz M., (1995)** ^{14}C-RPA 400727: Degradation and metabolism in aerobic aquatic systems. 200901 / 374850
GLP:	yes
Guidelines:	BBA Part IV, 5-1, (1990)

Please see the full summary details presented in Volume 3, Section B.8 of the DAR for triticonazole (Austrian Federal Office for Food Safety, 2003). The study is still considered valid and has only slight deviations) to the current OECD Test Guideline 308 (pre-incubation phase exceeded four weeks, oxygen concentration for river water < 7 mg/L.

Report: CA 7.2.2.3/1
Jarvis T.J.,Montesano V., 2014d
Recalculation of Triticonazole water sediment study kinetics according to FOCUS (2006) Guidance
2014/1083346
Guidelines: FOCUS Kinetics (2006)
GLP: no

Executive Summary

A water/sediment study of triticonazole with two systems (river and pond) was re-evaluated according to the FOCUS kinetics (2006) guidance. Level P-I dissipation and degradation rates were derived. No P-II level was calculated. $DisT_{50}$ values for triticonazole in the water phase were 5.3 and 9.5 days, $DegT_{50}$ values in the whole system were 399 and 225 days. No degradation rates could be derived for the sediment phase.

I. MATERIAL AND METHODS

One water/sediment study with two systems (river and pond) was considered in which the parent triticonazole was applied (Wyss-Benz, *1995, Doc No. R012987*). Residues of triticonazole in water and sediment (in percentage of applied) were obtained from the water/sediment study and used as input for the kinetic evaluation. Please see the full summary details on the experimental study the summary in Volume 3, Section B.8 of the DAR for triticonazole (Austrian Federal Office for Food Safety, 2003). Analytical data used for the kinetic evaluation are presented in the table below.

Table 7.2.2.3-1: Analytical results for triticonazole degradation (percentage of applied) in water sediment systems (Wyss-Benz, 1995)

	River system			Pond system		
Time (day)	Water phase	Sediment phase	Whole system	Water phase	Sediment phase	Whole system
0	95.0	2.0	97.0	97.1	1.4	98.5
0	93.8	4.3	98.1	100.4	1.1	101.5
0.25	92.9	4.7	97.6	95.4	1.8	97.2
0.25	96.2	2.1	98.3	96.3	2.7	99.0
1	85.4	12.2	97.6	90.2	7.4	97.6
1	86.8	11.6	98.4	90.2	9.3	99.5
2	58.9	34.6	93.5	75.6	22.0	97.6
2	61.0	34.1	95.1	74.8	22.5	97.3
7	48.4	47.7	96.1	57.0	37.8	94.8
7	43.7	52.7	96.4	55.8	40.0	95.8
14	35.6	55.7	91.3	42.7	52.5	95.2
14	27.5	63.1	90.6	45.5	51.2	96.7
28	24.8	69.1	93.9	24.0	62.1	86.2
28	14.1	74.8	88.9	27.3	58.0	85.3
63	14.0	73.2	87.3	15.8	65.2	81.0
63	11.6	78.7	90.3	16.1	68.0	84.1
105	9.1	70.2	79.3	10.0	62.4	72.4
105	8.7	71.7	80.3	10.4	59.9	70.3

The simulation modelling was performed using KinGui Version 2. Dissipation and degradation rates of triticonazole at level P-I were derived. P-II approaches were not considered. Comparing the rapid dissipation rate of triticonazole from the water phase to its slower degradation in the whole system it can be expected that the P-II level fit would not result in significant degradation rates.

II. RESULTS AND DISCUSSION

The evaluation of the water/sediment study showed that the FOMC model provided the best fit for dissipation in the water phase while SFO was chosen for degradation in the whole system. No endpoints could be derived for the sediment phase. Results are valid as persistence and modelling endpoints and are presented in Table 7.2.2.3-2:

Table 7.2.2.3-2: Degradation and Dissipation of Triticonazole in Water and Sediment

Study	System	Water phase			Total system		
		Kinetic model	$DisT_{50}$ (d)	$DisT_{90}$ (d)	Kinetic model	$DegT_{50}$ (d)	$DegT_{90}$ (d)
Wyss-Benz, 1995	River	FOMC	5.3	97.8	SFO	399	1325
	Pond	FOMC	9.5	125	SFO	225	748

III. CONCLUSION

Degradation and dissipation of triticonazole in water and sediment was re-evaluated according to FOCUS (2006). Modelling endpoints for the parent were derived and can be used in environmental fate modelling. The same endpoints are also valid for use as persistence endpoints.

CA 7.2.2.4 Irradiated water/sediment study

This type of study was not performed as it is not needed as a higher tier option.

CA 7.2.3 Degradation in the saturated zone

As neither triticonazole, nor any of its soil metabolites are expected to constitute a leaching risk, no investigations on the fate of these compounds in the saturated zone are required.

Route and rate of biological degradation in aquatic systems - Overall Conclusions

Triticonazole is not readily biodegradable.

Furthermore, it is not significantly degraded in natural surface water since no major metabolites were detected in the course of the study and the ratio of R/S enantiomers remained stable throughout the incubation period.

Also in the available water/sediment study no major metabolites were detected. The geometric mean $DegT_{50}$ value for the degradation in total water/sediment systems is 300 days, the geometric mean for dissipation in the water phase is 7.1 days. No degradation/dissipation rates for sediment could be determined.

Table 7.2.3-1: Degradation/dissipation of triticonazole in water sediment systems

Reference			System	Test Conditions	Metabolites	Kinetic Evaluation	
						Water $DisT_{50}$ (days)	Whole System $DegT_{50}$ (days)
CA 7.2.2.3	Study previously evaluated	Wyss-Benz, 1995	River	The test was conducted at 20°C in the dark for up to 105 days using ^{14}C-phenyl-triticonazole	No major metabolites	5.3 (FOMC)	399 (SFO)
			Pond			9.5 (FOMC)	225 (SFO)
Geometric mean						7.1	300

Study/Values shaded in grey have been reviewed as part of the first EU review of triticonazole

CA 7.2.4 Water treatment

Potential effects of water treatment procedures on triticonazole and on its metabolites

Currently there is neither a guideline for testing the effect of water treatment on pesticides (or other chemicals) nor is there a risk assessment procedure. Since conditions of water treatment are extremely variable across Europe (different treatment methods and intensities used in different sequences on different types of raw waters) it is currently not possible to comprehensively assess the potential formation of harmful by-products during drinking water production. An experimental guideline is essential because the effect of ozonation or chlorination strongly depends on treatment conditions (e.g. duration, applied concentration, properties of the raw water) which should be representative for real water treatment plants.

In the absence of such guidance documents an evaluation was made based on knowledge on the chemistry of triticonazole and its degradation products and applying chemical principles.

Experimental data on the aerobic degradation of triticonazole in soil demonstrate that the main degradation pathway is hydroxylation to form the three major transformation products RPA 406341, RPA 404766 and RPA 407922 as well as some minor hydroxylated metabolites. Subsequent degradation processes are a further hydroxylation to minor dihydroxylated metabolites, ring cleavage as well as the formation of bound residues and carbon dioxide. Triticonazole is hydrolytically stable at environmentally relevant pH values (pH 4 - 9) at 25°C.

Neither triticonazole nor its metabolites contain any comparable aliphatic side chains as present in the chemical structure of tolylfluanid, which caused the problem of nitrosamine formation during water treatment for drinking water production. No N-nitrosamine formation is expected for triticonazole and its metabolites, since no secondary amine function is present. The only nitrogen-containing moiety is the electron-deficient heteroaromatic triazine ring, which is little prone to electrophilic attack of either ozone or NO^+.

With chlorine-based treatments, chlorination or hydroxylation and the possible loss of substituted chlorinated and hydroxylated structures is conceivable. However, when chlorine enters water it reacts chemically with any organic matter found in the water. There is always some organic matter in natural waters and by-products of this reaction include e.g. trihalomethanes. Any potential harmful degradation products resulting from any organic matter in the water treatment process will be eliminated in subsequent clean-up steps by using e.g. activated carbon filtration or sand filter beds.

It is therefore highly unlikely that water treatment processes such as ozonation or chlorination will result in the formation of by-products that would require a detailed health risk assessment. Consequently, further information on the effect of water treatment processes on the nature of residues present in surface water and groundwater is not considered necessar

CA 7.3 Fate and behaviour in air

CA 7.3.1 Route and rate of degradation in air

Studies on the vapour pressure and the half-life of triticonazole in air were evaluated during the Annex I inclusion and accepted by the European Commission (SANCO/10442/2005-Final rev 1, 12 March 2010). The vapour pressure of triticonazole was determined as <0.1 x 10^{-5} Pa at 50°C. The DT_{50} in air was calculated to be 0.113 days (for a 12 hrs day). Latter study is listed below.

To fulfil current guidelines, a new vapour pressure study was conducted and the vapour pressure of triticonazole was determined as 9 x 10^{-8} Pa at 25°C (CA 7.3.1/1).Therefore, concentrations in air are considered to be negligible.

Furthermore, the atmospheric half-life was re-calculated according to Atkinson using the current version of AOPWIN in EPI Suite v4.11 and the same parameters as volkel [*EPI Suite v4.11: U.S. Environmental Protection Agency, Nov. 2012.*]. A value of 1.4 hrs (corresponding to 0.114 days) was determined (for a 12 hrs day), thus confirming the previously obtained results. Thus, long range transport of triticonazole can be disclosed.

Report:	**R012052; Völkel W., (1999)** Estimation of the degradation of triticonazole by photo-oxidation in Air R012052 / 202492 / 751983
GLP:	yes
Guidelines:	94/37/EC (of 22 July 1994) Point 2.10

The study is still considered valid. Results (DT50 = 0.113d) are confirmed by repeating the Atkinson calculation using the current version of AOPWIN in EPI Suite v4.11 [*EPI Suite v4.11: U.S. Environmental Protection Agency, Nov. 2012. http://www.epa.gov/opptintr/exposure/pubs/episuitedl.htm (7th July 2015)*]. A value of 1.4 hrs (corresponding to 0.114 days) was determined (for a 12 hrs day).

Report: CA 7.3.1/1
Cowlyn N., 2014a
Triticonazole - Surface tension and vapour pressure
2014/1001862
Guidelines: EU Regulation 1107/2009 with Regulation 283/2013, UK Health and Safety Good Laboratory Practice Regulations 1999 (No. 3106), 2004/10/EC of 11 February 2004, OECD-DOC ENV/MC/CHEM(98)17 Paris 1998
GLP: yes
(certified by Department of Health of the Government of the United Kingdom, United Kingdom)

The study is summarized in annex point KCA 2.2 of this dossier. The obtained vapour pressure is 9 x 10-8 Pa at 25°C

CA 7.3.2 Transport via air

The transport of triticonazole via air was not studied since its vapour pressure is below the trigger values of 10^{-5} Pa (plants) and 10^{-4} Pa (soil).

CA 7.3.3 Local and global effects

As triticonazole is not applied in high volumes, local and global effects are not expected. The long range transport of triticonazole can be disclosed due to the short atmospheric half-life of the substance.

CA 7.4 Definition of the residue

The residue definition accepted by the European Commission (SANCO/10442/2005-Final rev 1, 12 March 2010) is amended by including all major soil metabolites in the residue definition for soil, groundwater and surface water. With this, the possibility of soil metabolites reaching water via drainage or run-off routes is considered, although it is not envisaged that either triticonazole or its metabolites will reach water bodies.

The photolysis metabolite RPA 406203 (Z-isomer of triticonazole) is not included in the residue definition for soil since the parent compound will be used as seed treatment only. It is, however, included in the residue definition for surface water due to its possible formation by aqueous photolysis.

CA 7.4.1 Definition of the residue for risk assessment

The proposed residue definitions relevant for risk assessment for each compartment are listed in the table below:

Compartment	Residue Definition
Soil	Triticonazole (parent) RPA 406341 (max. 20.2% TAR) RPA 404766 (max. 13.9% TAR) RPA 407922 (max. 12.8% TAR)
Groundwater	Triticonazole RPA 406341 RPA 404766 RPA 407922
Surface water and Sediment	Triticonazole RPA 406341 RPA 404766 RPA 407922 RPA 406203
Air	Triticonazole

CA 7.4.2 Definition of the residue for monitoring

The residue definition accepted by the European Commission (SANCO/10442/2005-Final rev 1, 12 March 2010) is still considered valid and includes the parent triticonazole only.

CA 7.5 Monitoring data

Publically available ground and surface water monitoring data as well as peer reviewed scientific literature were checked for analyses and findings of triticonazole. No data were found for the metabolites in either ground or surface water. Additional monitoring studies were not conducted.

Publically available groundwater monitoring data for triticonazole shows that, following application according to the label, the leaching of unacceptable amounts of triticonazole is highly unlikely. Additionally entry of unacceptable amounts of triticonazole into surface water is highly unlikely.

1. Publically available ground and surface water monitoring data

Data for triticonazole in ground water were found for France. Data for triticonazole in surface water were found for France, for the Czech Republic and for Italy. These data are summarized in Table 7.5-1 to Table 7.5-3 for groundwater and in Table 7.5-4 for surface water.

All detections in groundwater were below 0.1 µg/L. Thus, the leaching of unacceptable amounts of triticonazole is highly unlikely.

Out of several thousand samples, concentration in surface water exceeded on two times 0.1 µg/L. Due to the low number of findings a risk to aquatic organisms is not expected.

Table 7.5-1: Monitoring data for triticonazole in ground water available for France

Country	FR				
Data Source	ADES Database				
Data for years	2007 - 2011				
Year	Number of wells sampled	Number of analyses	Number of detections >LOQ	Number of detections >0.1 µg/l	Number of wells with detections >0.1 µg/l
2007	52	75	0	0	0
2008	284	1001	0	0	0
2009	1301	2186	0	0	0
2010	1503	4116	2	0	0
2011	1655	4448	0	0	0

Table 7.5-2: Monitoring data for triticonazole in ground water available for Czech Republic

Country	CZ				
Data Source	CHMI				
Data for years	2009 - 2012				
Year	Number of wells sampled	Number of analyses	Number of detections >LOQ	Number of detections >0.1 µg/l	Number of wells with detections >0.1 µg/l
2009	652	1265	1	0	0
2010	653	1264	1	0	0
2011	653	653	5	0	0
2012	651	1260	0	0	0

Table 7.5-3: Monitoring data for triticonazole in ground water available for Italy

Country	IT				
Data Source	ISPRA				
Data for years	2009 - 2010				
Year	Number of wells sampled	Number of analyses	Number of detections >LOQ	Number of detections >0.1 µg/l	Number of wells with detections >0.1 µg/l
2009	12	25	0	0	0
2010	1	2	0	0	0

Table 7.5-4: Monitoring data for triticonazole in surface water available for France

Country	**FR**				
Data Source	**IFEN Database**				
Data for years	**2008 - 2012**				
Year	**Number of wells sampled**	**Number of analyses**	**Number of detections >LOQ**	**Number of detections >0.1 µg/l**	**Number of wells with detections >0.1 µg/l**
2008	311	1567	23	0	0
2009	578	5446	52	1	1
2010	1091	6898	27	0	0
2011	1136	8428	23	2	2
2012	1471	9815	15	1	1

2. Peer reviewed scientific literature

Additionally, some information on its occurrence in the environment is available in parts of the study of Reilly *et al.* (2012) who investigated the occurrence of fungicides in surface water, groundwater, bed sediments and suspended solids. Triticonazole was detected in one surface water sample out of a total of 60 samples with a concentration of 66.8 ng/L. The study of Reilly *et al.* (2012) can be regarded as supplemental information but does not affect the risk assessment. It is summarized below.

Report: Reilly T.J., Smalling K.L., Orlando J.L., Kuivila K.M., (2012)
Occurrence of boscalid and other selected fungicides in surface water and groundwater in three targeted use areas in the United States
Chemosphere 89 (2012) 228–234
Guidelines: None
GLP: No
Peer reviewed public literature.

To provide an assessment of the occurrence of fungicides in water resources, the US Geological Survey used a newly developed analytical method to measure 33 fungicides and an additional 57 current-use pesticides in water samples from streams, ponds, and shallow groundwater in areas of intense fungicide use within three geographic areas across the United States. Sampling sites were selected near or within farms using prophylactic fungicides at rates and types typical of their geographic location. Triticonazole was detected in 2 of 72 surface water samples with a maximum concentration of 66.8 ng/L.

3. Conclusions

Publically available groundwater monitoring data for triticonazole shows that, following application according to the label, the leaching of unacceptable amounts of triticonazole is highly unlikely. Entry of unacceptable amounts of triticonazole into surface water is highly unlikely.

Triticonazole

Document M-CA, Section 8

ECOTOXICOLOGICAL STUDIES ON THE ACTIVE SUBSTANCE

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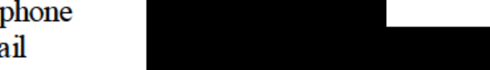

Version history[1]

Date	Data points containing amendments or additions and brief description	Document identifier and version number
23-Oct-2015		BASF DocID 2014/1092383 (version 1)
04-Jan-2016	The following chapter was updated in course of the completeness check: CA 8	BASF DocID 2015/1275301 (version 2)

[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 CA 8 ECOTOXICOLOGICAL STUDIES ON THE ACTIVE SUBSTANCE

The ecotoxicological relevant studies conducted with the active substance triticonazole, were conducted with certified triticonazole technical grade active ingredient (TGAI). The TGAI is a stable racemic mixture, as shown via storage testing (see Doc J).

CA 8.1 Effects on birds and other terrestrial vertebrates

Since Annex I inclusion of triticonazole (BAS 595 F), a new one-generation bird toxicity study on the active substance has been performed.

Details on the EU agreed studies which have been already evaluated within the Annex I inclusion of triticonazole are provided in the EU Review documents of triticonazole (*i.e.* Draft Assessment Report (DAR), Volume 3, Annex B.9., 2003; Addendum to the DAR May, 2005; EFSA Scientific Report (2005) 33, 1 - 69, "Addendum with respect to confirmatory data submitted after Annex I inclusion" from zRMS Austria, July 2009).

For better transparency and traceability of the active substance history, the results of all studies are summarized in Table 8.1-1.

Table 8.1-1: Summary of EU-reviewed and agreed, as well as additional toxicity studies relevant for AIR3 for the active substance triticonazole (BAS 595 F) for assessing the risk to birds and mammals [1)]

Test system	Test species	Reference BASF DocID	EU-agreed
BIRDS			
Acute oral toxicity	*Colinus virginianus*	R013025	EFSA Scientific Report (2005) & DAR (2003) (still valid for AIR 3 according to EFSA/2009/1438)
	Perdix perdix	R013069	EFSA Scientific Report (2005) & DAR (2003) (still valid for AIR 3 according to EFSA/2009/1438)
	Phasianus colchicus	R013004	EFSA Scientific Report (2005) & DAR (2003) (still valid for AIR 3 according to EFSA/2009/1438)
	Alectoris rufa	R013065	EFSA Scientific Report (2005) & DAR (2003) (still valid for AIR 3 according to EFSA/2009/1438)
	Columba livia [5)]	R013005	EFSA Scientific Report (2005) & DAR (2003) (still valid for AIR 3 according to EFSA/2009/1438)
	Anas platyrhynchos	R013024	EFSA Scientific Report (2005) & DAR (2003) (still valid for AIR 3 according to EFSA/2009/1438)
Short-term dietary toxicity	*Colinus virginianus*	R013037	EFSA Scientific Report (2005) & DAR (2003) (no longer part of core data package according to EFSA/2009/1438)
	Anas platyrhynchos	R013038	DAR (2003) (no longer part of core data package according to EFSA/2009/1438)
Sub-chronic toxicity and reproduction	*Colinus virginianus*	R013161	EFSA Scientific Report (2005) & DAR (2003)
	Colinus virginianus	2006/1026908	Confirmatory Data Addendum (July 2009) (still valid for AIR 3 according to EFSA/2009/1438)
	Colinus virginianus (Modified 1-generation bird study for higher tier)	2008/1023059	Confirmatory Data Addendum (July 2009) (still valid for AIR 3 according to EFSA/2009/1438)
	Anas platyrhynchos	R000098	DAR (2003) (still valid for AIR 3 according to EFSA/2009/1438)
	Colinus virginianus	2011/1269059	No (new study, cp. 8.1.1.3)

Test system	Test species	Reference BASF DocID	EU-agreed
MAMMALS			
Acute oral toxicity	Rat	R013003	EFSA Scientific Report (2005) & DAR (2003) (still valid for AIR 3 according to EFSA/2009/1438)
2-Generation reproductive toxicity	Rat	R013085	EFSA Scientific Report (2005) & DAR (2003) (still valid for AIR 3 according to EFSA/2009/1438)
Prenatal Development toxicity	Rat	C018955	DAR (2003) (still valid for AIR 3 according to EFSA/2009/1438)
	Rabbit	C018959	EFSA Scientific Report (2005) & DAR (2003) (still valid for AIR 3 according to EFSA/2009/1438)

DAR 2003: Draft Assessment Report - Triticonazole, September 2003.

[1] EU agreed means assessed during the previous EU evaluation process, but not necessarily listed in the list of endpoint as in the Conclusion on the peer review of triticonazole, *EFSA Scientific Report* (2005) 33, 1-69.

Table 8.1-2: Summary of EU-reviewed and agreed studies relevant for AIR3 for the active substance triticonazole (BAS 595 F) for assessing the risk to birds and mammals [1)]

Test system	Test species	Reference BASF DocID	EU-agreed
Rate of degradation of triticonazole on wheat seeds	n.a.	2007/1016397	Confirmatory Data Addendum (July 2009) (still valid for AIR 3 according to EFSA/2009/1438)
Rate of degradation of triticonazole (and prochloraz) on wheat seeds	n.a.	2006/1015760	Confirmatory Data Addendum (July 2009) (still valid for AIR 3 according to EFSA/2009/1438)

DAR 2003: Draft Assessment Report - Triticonazole, September 2003.

CA 8.1.1 Effect on birds

CA 8.1.1.1 Acute oral toxicity to birds

Six studies with six different bird species performed with triticonazole have already been evaluated during the previous Annex I inclusion process of triticonazole. No additional studies are required and no (new) study has been conducted.

CA 8.1.1.2 Short-term dietary toxicity to birds

Two studies with *Colinus virginianus* and *Anas platyrhynchos* performed with triticonazole have already been evaluated during the previous Annex I inclusion process of triticonazole. Please note that short-term dietary studies are no longer part of core data package according to EFSA/2009/1438. Therefore, no (new) study has been conducted.

CA 8.1.1.3 Sub-chronic and reproductive toxicity to birds

Overall, four reproductive toxicity studies in birds are available. Two standard reproductive toxicity studies with bobwhite quail and mallard duck which have already been evaluated during the Annex I inclusion process, and a modified 1-generation bird study with bobwhite quail evaluated by RMS Austria in the confirmatory data addendum. In addition, a new 1-generation study on bobwhite quail was conducted. The study has already been used in previous EU end-use product registrations, but has not been evaluated previously on EU level and is provided in support of the bird risk assessment.

Report: CA 8.1.1.3/1
[redacted]
BAS 595 F (Triticonazole) - 1-Generation reproduction study on the bobwhite quail (Colinus virginianus) by administration in the diet
2011/1269059
Guidelines: OECD 206, EPA 540/9-82-024, EPA 540/9-86-139, EPA 71-4, EPA 850.2300, EPA 712-C-96-141
GLP: yes
(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

The study was designed to determine the effect of administration of BAS 595 F (triticonazole) via food on the reproduction in the bobwhite quail (*Colinus virginianus*). After an acclimation period of two weeks, the birds were offered the test substance in the diet at a dose rate of 0 (control), 50, 150, or 450 mg a.s./kg diet (nominal), for a period of 22 weeks (10 weeks pre-egg-production, 12 weeks egg-production period). Eggs were collected, incubated and hatched and the offspring (F1-generation) were raised for 2 weeks.

Analysis of the test concentrations in the diet was carried out and recoveries of mean values ranged between 89.1% and 95.7% of the nominal concentrations during the test. Therefore, the biological results were based on the nominal test concentrations.

Parent generation:

No test substance-related effects in the parent generation on mortality and birds´ health could be detected in any of the treatment groups. Avoidance of feed was not observed. Therefore a No Observed Effect Level for the non-reproductive effects in the parental generation is 450 mg a.s./kg diet.

Effects on eggs and chicks:

In the 50 mg a.s./kg diet group (daily dose 3.63 mg a.s./kg bw d), reproductive values in general were similar or exceeded those of the control group. Therefore, treatment related effects can be excluded.

In the 150 mg a.s./kg diet group (daily dose 10.98 mg a.s./kg bw d) no biologically relevant test substance-related effects were observed.

In the 450 mg a.s./kg diet group (daily dose 32.76 mg a.s./kg bw d) the number of eggs laid per female and the survival rate of chicks for the first two weeks after hatch was clearly lower. These two effects resulted in a 50% reduction of the number of 14-day surviving chicks per hen in this diet group. However, the body weight of hatched chicks and surviving chicks 14 days after hatch was not impaired by the test substance.

In a bobwhite quail avian reproduction test with triticonazole the NOEL was determined at 10.98 mg a.s./kg b.w./day (NOEC = 150 mg a.s./kg).

I. MATERIAL AND METHODS

Test substance: BAS 595 F (triticonazole, Reg. no. 4378513); batch no. COD-001440, purity: 91.3% (analysed).

Test species: Bobwhite quail (*Colinus virginianus*), both male and females about 6 months of age, approaching their first breeding season, the birds were indistinguishable from wild birds; supplier: [redacted] [redacted].

Test design: Bobwhite quails approaching their first breeding season were kept in groups of 1 male and 1 female per replicate (16). They were acclimated for a period of 2 weeks under laboratory test conditions. After acclimation, there was a pre-egg laying period of 10 weeks and an egg-laying period of 12 weeks during which the birds were offered feed with test substance *ad libitum* at levels of 0 (control), 50, 150 and 450 mg a.s./kg diet (see diagram of test layout below).

Endpoints: Adult birds: mortality, clinical signs, feed consumption, development of body weight.
Reproduction parameters e.g. egg production, egg weight, egg quality, fertility rate, egg shell thickness, early and late embryonic deaths, hatching rate and 14-day survival of the chicks.

Test concentrations: 0 (control), 50, 150, or 450 mg a.s./kg diet (nominal).

Test conditions: Adult bobwhite room: temperature 18.1-27.4°C; relative humidity: 36% - 100%; photoperiod: week -2 to 7: 7 hours light, 17 hours darkness, week 8 to 9: 14 hours light, 10 hours darkness, week 10 to 22: 17 hours light, 7 hours darkness: intensity: 20-80 lux.
Eggs collected during the 12 week egg-laying period were placed in a special refrigerator (temperature15-18°C, relative humidity 51% - 83%). Eggs were generally turned daily. Eggs were set into the incubator weekly. They were acclimated to room temperature for about 1 day before incubation. Incubator temperature: 37.5 ± 0.5°C, relative humidity 61% - 84%. Hatcher temperature was adjusted at 37.1-38.0 and 78-97% relative humidity.

Analytics: The test substance concentrations and homogeneity in the diet were analyzed using HPLC.
Before the study was started, the stability of the test substance in the diet was verified in a separate "stability study" at a concentration of 100 mg/kg diet for a time period of 40 days.

Statistics: Descriptive statistics; Dunnett-test for body weight and food consumption of parent quails, for the egg weight, egg shell thickness and chicks' body weight, and for comparing each dose group with the control group. Wilcoxon-test for count data and proportions ($\alpha = 0.05$, $\alpha = 0.01$).

II. RESULTS AND DISCUSSION

Dietary concentration verification:

The results of the analytical verification of the test substance concentration in the diet were within a range of 89.1% and 95.7% of the nominal concentrations. The biological results are based on the nominal values.

Biological results

Parent generation:
There were no mortalities of adult quails observed in any concentration group during the whole exposure period, nor over the whole pre-egg-laying and the egg-laying period. Generally, the birds were in good health throughout the experimental period except isolated findings, mostly moderate lesions from fighting and consequential injuries (e.g. foot and eye lesions were noted during the macroscopic pathological examination). Clinical signs attributable to the test substance were not observed.

In comparison to the control, the food consumption was statistically significantly increased in test group 1 in week 5 and in test group 2 in weeks 1 and 2. In test group 3 a statistically significant decrease was observed in week 19. All these deviations were considered to be incidental. Over the whole exposure period no trend towards an increase or decrease of the food consumption was observed in any of the tested concentration groups.

The body weights of the exposed birds were not markedly increased or decreased in comparison to the control group. The statistical analyses revealed no evidence of any treatment-related effect for all measurements and both sexes.

Reproduction:

In the groups receiving **up to 150 mg a.s./kg diet** showed no test substance-related effects, except for:

- The proportion of chicks dead-in-shell of fertile eggs which was statistically significantly increased in comparison to the control group in weeks 9 – 12 and for the whole egg-laying period (Wilcoxon-test). The difference was statistically significant only on the 5% level and no effect was seen at the higher treatment level.

- The proportion of hatched chicks of eggs set at day 18, which reflects the proportion of chicks dead-in-shell, was statistically significantly decreased in weeks 9 – 12, but not over the whole egg-laying period (Wilcoxon-test). An analysis of the individual data shows that there was no clear trend over the whole egg-laying period and that the high proportion of chicks that died in the last week of the egg-laying period (28 of 66 viable 18-day embryos = 42.4%) contributed markedly to the deviation. For both parameters the highest concentration group (the 450 mg a.s./kg diet) was clearly not affected. No concentration-related trend could be seen. In conclusion, the statistically significant increased proportion of chicks dead-in-shell can be regarded as incidental and may have been caused by specific unfavourable conditions for the eggs in that test group during the last hatch.

- Survival of chicks after hatch was slightly lower than in the control group, but not statistically significantly reduced over the whole egg-laying period. Only during the second part of the egg-laying period the reduction was statistically significant. It was concluded, that the survival after hatch was affected by the test substance in the highest concentration group only since in the 150 mg a.s./kg diet group the effect was not significant over the whole egg-laying period.

- Effect over the whole embryonic development in 150 mg a.s./kg diet group was considered to not be treatment-related.

In the group receiving **450 mg a.s./kg diet** only a few of the reproduction parameters were affected:

- Egg production was statistically significantly reduced (Wilcoxon-test) during weeks 1-4, weeks 5-8, weeks 9-12 and for the whole egg-laying period.

- The number of hatched chicks per female quail was statistically significantly lower in comparison to the control group. Since no effects on fertility, embryonic survival and death-in-shell were observed for this group, the decrease reflects mainly the decreased egg-laying, which was a clear substance-related effect.

- The proportion of chicks that survived the first 14 days after hatch was clearly and statistically significantly decreased in comparison to the control group in weeks 5 – 8, weeks 9 – 12 and of the whole egg-laying period.

- The overall reproductive performance was statistically significantly decreased in comparison to the control group (50% of the control group) and was a consequence of a decreased egg-laying rate (65% of the control group) and a decreased survival rate after hatch (70% of the control group).

-

Overall, the other parameters such as proportion of damaged eggs, egg weight, and egg shell thickness resulted in no statistically significant differences compared to the control in any of the treatment groups.

Fertility was also considered not to deviate from the control. In general, the fertility was comparably low in the first phase of the egg-laying period but the overall fertile eggs throughout the egg-laying period did not indicate any abnormalities in the treatment groups compared to the control group.

The proportion of early and late embryonic mortalities of fertile eggs was not statistically significantly decreased in comparison to the control group in any of the treatment groups. The early embryonic survival plus the fertility rate are reflected in the rate of viable 14-day embryos of eggs initially set and the proportion of late embryonic mortality (proportion of eggs set at day 18 of eggs set at day 11). At both growth stages, the statistical evaluation revealed no substance-related effect. The proportion of hatched chicks of eggs initially set and the hatchability as proportion of chicks hatched of fertile eggs did not indicate any treatment-related effect.

For the F1-generation, no toxic signs and/or significant malformations exceeding the normal proportion were seen in the chicks. The statistical evaluation of the chicks´ body weights at hatch and 14 days after hatch was performed with the Dunnett's test. The body weights of the chicks 14 days after hatch were similar in all test groups and no statistically significant deviations were observed.

In summary, the most sensitive endpoints were the egg production and the survival after hatch as shown in the 450 mg a.s./kg diet group. Statistically significant differences from the control group in 150 mg a.s./kg diet were driven by the incidentally increased proportion of chicks “dead-in-shell” and were not considered to be treatment related.

Table 8.1.1.3-1: Summary of effects of triticonazole on the reproduction of the bobwhite quail (*Colinus virginianus*)

	Experimental group [mg a.s./kg diet]			
	Control	**50**	**150**	**450**
Number of replicates	16	16	16	16
No. treatment-related mortality of adult birds	0	0	0	0
Adult body weight [g] (male/female) end of egg laying period	216.1/226.1	219.1/234.7	211.3/238.8	213.5/225.2
No. of eggs laid / group	892	913	883	569
No. of cracked and broken eggs / group	63	56	79	22
Mean egg weight (g) [a]	9.9	10.1	10.1	9.8
Mean egg shell thickness (mm) [a]	0.20	0.19	0.20	0.19
No. of eggs set [b]/ group	750	771	723	476
No. of fertile eggs / group	706	736	662	455
No. of infertile eggs / group	44	35	61	21
No. of early embryonic mortalities / group	28	27	24	14
No. of viable 11-day old embryos / group	678	709	638	441
No. of late embryonic mortalities / group	13	14	8	12
No. of viable 18-day old embryos / group	665	695	630	429
No. of total embryonic deaths / group	41	41	32	26
No. of "dead-in-shell" / group	146	116	152	86
No. of chicks hatched / group	519	579	478	343
No. of 14-day surviving chicks / group	424	466	356	206
No. of eggs laid / female bird / week [a]	4.6	4.8	4.6	3.0**
No. of chicks hatched / female bird / week [a]	2.7	3.0	2.5	1.8**
No. of 14-day surviving chicks / female bird / week [a]	2.2	2.4	1.9	1.1**
Mean body weight of chicks at hatching (g) [a]	6.2	6.3	6.1	6.0
Mean body weight of chicks 14 days after hatching (g) [a]	23.7	23.9	23.3	22.4
% fertile eggs of eggs set [b]	92.8	95.3	92.6	93.5
% viable 11 day old embryos of eggs set [b]	89.4	91.7	89.2	90.5
% viable eggs at day 18 of eggs set [b] at day 11	98.3	98.2	98.7	97.4
% hatched chicks of eggs set at day 18	78.9	83.3	74.3	78.9
% 14-day survivors of chicks hatched	79.4	79.6	73.1	55.3**
% cracked and broken eggs of eggs laid	6.3	6.0	8.5	4.1
% early embryonic mortalities of fertile eggs	3.6	3.9	3.7	3.2
% late embryonic mortalities of fertile eggs	1.6	1.7	1.2	2.4
% "dead-in-shell" of fertile eggs	19.7	15.6	24.3*[c]	19.5
Hatchability (% chicks hatched of total eggs set)	69.3	75.1	65.8	70.1
Hatchability (% chicks hatched of fertile eggs)	75.1	78.9	70.8	74.9

[a] mean values calculated as means from mean values of each replicate
[b] incubated
[c] this deviation was considered to be not test substance related
* statistically significant differences compared to the control ($p \leq 0.05$)
** statistically significant differences compared to the control ($p \leq 0.01$)

III. CONCLUSION

In a bobwhite quail avian reproduction test with triticonazole the NOEL was determined at 10.98 mg a.s./kg b.w./day (NOEC = 150 mg a.s./kg).

CA 8.1.2 Effects on terrestrial vertebrates other than birds

CA 8.1.2.1 Acute oral toxicity to mammals

Acute oral toxicity to mammals is addressed in M-CA 5.2.1. The studies previously evaluated during the Annex I inclusion process are considered to be still valid and sufficient. Therefore, no new studies were conducted with the active substance.

CA 8.1.2.2 Long-term and reproductive toxicity to mammals

Long-term and reproductive toxicity to mammals is addressed in M-CA 5.6. Studies and endpoints relevant for the reproductive risk assessment of wild mammals are discussed in M-CP 10.1.2. No new studies were conducted with the active substance.

CA 8.1.3 Effects of active substance bioconcentration in prey of birds and mammals

The potential effects of active substance bioconcentration in prey of birds and mammals are addressed according to the guidance document EFSA/2009/1438 in the documents M-CP 10.1.1 and M-CP 10.1.2.

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.

An extensive literature search for triticonazole has not revealed any relevant information with respect to the toxicity of triticonazole to reptiles and amphibians.

However, the risk to reptiles and amphibians from triticonazole is considered to be very low due to the fact that triticonazole is used as a seed treatment and therefore no contact exposure in terrestrial ecosystems for reptiles and amphibians is expected to occur.

CA 8.1.5 Endocrine disrupting properties

Mammals

For mammals, there is no concern for endocrine disrupting properties of triticonazole as outlined in detail in chapter CA 5.8.3.

In the public literature search one paper was obtained. The results from the paper from De Jong et al. (2011) were classified as not relevant under regulatory aspects and is therefore not further discussed.

Birds

For birds, there is no indication from the reproductive toxicity studies for an endocrine disrupting potential of triticonazole. Please refer to chapter CP 10.1.1.

References

De Jong, E., Marta Barenys, M., Hermsen, S.A.B., Verhoef, A., Ossendorp, B.C., Bessems, J.G.M., Piersma, A.H. 2011. Comparison of the mouse Embryonic Stem cell Test, the rat Whole Embryo Culture and the Zebrafish Embryotoxicity Test as alternative methods for developmental toxicity testing of six 1,2,4-triazoles. Toxicology and Applied Pharmacology (2011), 253(2), 103-111.

CA 8.2 Effects on aquatic organisms

Since Annex I inclusion of triticonazole (BAS 595 F), new toxicity studies on the active substance triticonazole and metabolites/intermediates of triticonazole 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 sake, 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, summaries are provided for a peer-reviewed scientific literature study in which acute test with fish and daphnids were conducted with triticonazole. This literature study was considered to be of relevance for the aquatic risk assessment of triticonazole and is provided as additional information.

Details on the EU agreed studies which have been already evaluated within the Annex I inclusion of triticonazole are provided in the EU Review documents of triticonazole (*i.e.* Draft Assessment Report (DAR), Volume 3, Annex B.9., 2003; Addendum to the DAR May, 2005; EFSA Scientific Report (2005) 33, 1 - 69).

For better transparency and traceability of the active substance history, the results of all studies are summarized in Table 8.2-1 and Table 8.2-2. Studies from the former EU review are given in grey letters.

Full references to cited literature used within the following chapters are given at the end MCA 8.2. Document N3 contains structures and synonyms for all metabolites.

Table 8.2-1: List of studies and endpoints for aquatic organisms exposed to the active substance triticonazole (BAS 595 F)

Organism	Endpoint	Value [mg/L] (except BCF)	Reference (BASF Name / DocID)	EU agreed * (Justification for submission of new data)
Fish				
Oncorhynchus mykiss	96 h LC_{50}	> 3.6	C017670 / 1998/1003136	yes *EFSA Scientific Report* (2005) 33, 1-69
Oncorhynchus mykiss	96 h LC_{50}	> 10.0	R013008 / 1990/1001356	yes DAR Volume 3, Annex B.9. (2003)
Oncorhynchus mykiss [1)]	96 h LC_{50}	> 12.4	2006/1015993	no (new study; conducted after Annex I inclusion)
Cyprinodon variegatus [1), 2)]	96 h LC_{50}	> 9.1	R000095 / 1998/1005064	no (new data; generated to address US EPA requirements)

Organism	Endpoint	Value [mg/L] (except BCF)	Reference (BASF Name / DocID)	EU agreed * (Justification for submission of new data)
Cyprinus carpio 1)	96 h LC_{50}	> 18.0	2014/1095638	no (new data; generated for AIR 3 submission)
Lepomis macrochirus	96 h LC_{50}	> 8.9	R012019 / 1998/1003128	yes DAR Volume 3, Annex B.9. (2003)
Lepomis macrochirus 1)	96 h LC_{50}	> 10.1	2006/1018146	no (new data; study conducted after Annex I inclusion)
Oncorhynchus mykiss	28 d NOEC	**0.010**	R013166 / 1996/1001985	yes *EFSA Scientific Report* (2005) 33, 1-69
Cyprinodon variegatus (ELS study) 1), 2)	34 d NOEC	≥ 0.120	2006/7007245	no (new data; generated to address US EPA requirements)
Pimephales promelas (ELS study) 1)	34 d NOEC	≤ 0.024	B003479 / 1998/1005099	no (new data; generated to address US EPA requirements; study not valid as no clear endpoint could be derived)
Pimephales promelas (ELS study) 1)	34 d NOEC	0.021	C044319	no (new data; generated to address US EPA requirements)
Pimephales promelas (FLC study) 1)	6 months NOEC	0.0114	2008/1028361	no (new data; generated to address US EPA requirements)
Pimephales promelas (FLC study) 1)	257 d NOEC	0.0473	2012/1079000	no (new data; generated to address US EPA requirements)

Organism	Endpoint	Value [mg/L] (except BCF)	Reference (BASF Name / DocID)	EU agreed * (Justification for submission of new data)
Aquatic invertebrates				
Daphnia magna	48 h EC_{50}	**9.0**	R013007 / 1990/7002515	yes *EFSA Scientific Report* (2005) 33, 1-69
Americamysis bahia [1), 2)]	48 h LC_{50} [3)]	**> 7.5**	B004421	no (new data; generated to address US EPA requirements)
Crassostrea virginica [1), 2)]	96 h EC_{50}	8.9	C019777 / 1998/1005118	no (new data; generated to address US EPA requirements)
Daphnia magna [#]	21 d NOEC	0.092	R013032 / 1992/7003213	yes *EFSA Scientific Report (2005) 33, 1-69* (but not valid according to current standard; replaced by new studies)[#]
Daphnia magna [1)]	21 d NOEC	1.5	2006/7007209	no (new data; generated to address US EPA requirements)
Daphnia magna [1)]	21 d NOEC	**0.430**	2012/7003660	no (new data; generated to address US EPA requirements)
Daphnia magna [1)]	21 d NOEC	1.3	R013169 / 1998/1005098	no (new data; generated to address US EPA requirements)
Americamysis bahia [1), 2)]	28 d NOEC	**0.041**	2006/7007246	no (new data; generated to address US EPA requirements)

Organism	Endpoint	Value [mg/L] (except BCF)	Reference (BASF Name / DocID)	EU agreed * (Justification for submission of new data)
Sediment dwelling aquatic invertebrates				
Chironomus riparius	26 d NOEC	≥ 0.0777	R005755 / 1998/7002186	yes *EFSA Scientific Report* (2005) 33, 1-69
Algae [4]				
Pseudokirchneriella subcapitata (Syn. *Selenastrum capricornutum*) [1]	72 h E_rC_{50} / E_yC_{50}	**> 10**	2014/1083347	no (new study; generated for AIR 3 submission; replaces EU agreed study on green alga)
Selenastrum capricornutum [5]	24 h E_rC_{50} / 96 h E_bC_{50}	> 1.0	R013056 / 1992/1001384	yes *EFSA Scientific Report* (2005) 33, 1-69 (but not valid according to current standard; replaced by a new study on *P. subcapitata*)
Selenastrum capricornutum [1), 5)]	72 h E_bC_{50} [6]	> 2.5	R012017 / 1998/1005080	no (new data; generated to address US EPA requirements; not valid according to current standard)
Anabaena flos-aquae [5]	72 h E_bC_{50} [6]	> 2.6	R012015 / 1998/1003127	yes *EFSA Scientific Report* (2005) 33, 1-69 (but not valid according to current standard)
Navicula pelliculosa [1), 5)]	72 h E_bC_{50} [6]	1.5	R012969 / 1998/1005094	no (new data; generated to address US EPA requirements; not valid according to current standard)
Skeletonema costatum [1), 2), 5)]	72 h E_bC_{50} [6]	0.29	B004429	no (new data; generated to address US EPA requirements; not valid according to current standard)

Organism	Endpoint	Value [mg/L] (except BCF)	Reference (BASF Name / DocID)	EU agreed * (Justification for submission of new data)
Aquatic macrophytes [4]				
Lemna gibba	14 d E_bC_{50}	1.1	R012023 / 1998/1003129	yes *EFSA Scientific Report* (2005) 33, 1-69
Bioconcentration				
Lepomis macrochirus (28 d exposure, 14 d depuration)	BCF_k BCF_{SS}	72.6 94.0	C017724 / 1996/1001987 + Amendment R005940 / 1999/1007239	yes *EFSA Scientific Report* (2005) 33, 1-69
Oncorhynchus mykiss (28 d exposure, 14 d depuration)	BCF	11.7 - 12.8	R013103 / 1994/7002603	no (included in DAR 2003, but study is not valid)
Peer-reviewed literature studies				
Dani rerio [1],	72 h LC_{50}	≥ 31.80	2015/1177633	no (peer-reviewed scientific study; provided as additional information) [7]
Daphnia magna [1],	48 h LC_{50}	9.56		

Bold figures: Where several endpoints are available for the same group or where several endpoints are available for one study based on different effect parameters (*e.g.* for algae and/or macrophytes), only the relevant endpoint(s) is used in the risk assessment presented in chapter 10.2 of the MCP dossier part for Annex I renewal.

Abbreviations: ELS = early life stage; FLC = full life cycle; BCF= bioconcentration factor

* EU agreed denotes studies assessed during the previous EU evaluation process that are not necessarily listed in the list of endpoint as in the Conclusion on the peer review of triticonazole, *EFSA Scientific Report* (2005) 33, 1-69.

[#] This chronic study on *Daphnia magna* is considered to be not valid according to current standard; it is replaced by new chronic studies on *D. magna*. For details please see the justification provided below.

[1] Study has not been submitted during the Annex I inclusion process of triticonazole. A study summary is provided below.

[2] Marine / saltwater species

[3] In accordance with the new regulation 283/2013 the 48 h endpoint obtained in the 96 h study is considered as relevant endpoint and is presented here.

[4] In accordance with the EFSA Aquatic Guidance Document (EFSA, 2013) only the EC_{50} values determined for the endpoint 'growth rate' (E_rC_{50}) are considered for the risk assessment for aquatic primary producers if both "growth rate" and "yield / biomass" endpoints are available.

[5] The study on this alga species is considered to be not valid. For details please see the justifications and study summaries provided below.

[6] In accordance to the EFSA Aquatic Guidance Document (EFSA, 2013) and OECD guideline 201 (2011) the 72 h endpoints obtained in the 120 h alga studies are considered as relevant endpoints and are presented here.

[7] Data derived from relevant peer-reviewed scientific study (for details see summary provided under CA 8.2.1 below).

Table 8.2-2: List of studies and endpoints for aquatic organisms exposed to metabolites / intermediates of triticonazole (BAS 595 F)

Organism	Endpoint	Value [mg/L]	Reference (BASF Name / DocID)	EU agreed * (Justification for submission of new data)
Metabolite: RPA 406203 (Reg. No. 5079359)				
Aquatic invertebrates				
Daphnia magna [1)]	48 h EC_{50}	**3.4**	C044320	no (new data; generated to address US EPA requirements)
Daphnia magna [1)]	48 h EC_{50}	> 10	2009/1075083	no (new data; study conducted after Annex I inclusion)
Algae [2)]				
Pseudokirchneriella subcapitata [1)]	72 h E_rC_{50} 72 h E_yC_{50}	**73.32** 9.29	2009/1050280	no (new data; study conducted after Annex I inclusion)
Metabolite: RPA 406341 (= AE 0540093; Reg. No. 5059144)				
Aquatic invertebrates				
Daphnia magna	48 h EC_{50}	50	C020498 / 2002/1016123	Yes *EFSA Scientific Report* (2005) 33, 1-69
Metabolite: RPA 404766 (Reg. No. 5079285)				
Aquatic invertebrates				
Daphnia magna	48 h EC_{50}	> 100	C017902 / 2001/1023409	Yes *EFSA Scientific Report* (2005) 33, 1-69
Metabolite: RPA 407922 (Reg. No. 5079288)				
Aquatic invertebrates				
Daphnia magna	48 h EC_{50}	> 100	C017901 / 2001/1023408	Yes *EFSA Scientific Report* (2005) 33, 1-69
Intermediate: RPA 405217 (Dimethylbencylidenecyclopentanone; DMBCP)				
Algae [2)]				
Scenedesmus subspicatus [1), 3)]	72 h E_rC_{50} [4)] 72 h E_bC_{50} [4)]	35.41 8.08	C039712	no (study is not valid; included for completeness)

Bold figures: Where several endpoints are available for the same group or where several endpoints are available for one study based on different effect parameters (*e.g.* for algae), the relevant endpoint is used in the risk assessment presented in chapter 10.2 of the MCP dossier part for Annex I renewal.

* EU agreed denotes studies assessed during the previous EU evaluation process that are not necessarily listed in the list of endpoint as in the Conclusion on the peer review of triticonazole, *EFSA Scientific Report* (2005) 33, 1-69.

1) Study has not been submitted during the Annex I inclusion process of triticonazole. A study summary is provided below.

2) In accordance with the EFSA Aquatic Guidance Document (EFSA, 2013) only the EC_{50} values determined for the endpoint 'growth rate' (E_rC_{50}) are considered for the risk assessment for aquatic primary producers if both "growth rate" and "yield / biomass" endpoints are available.

3) No analytical verification of the test item concentrations was conducted in this study; study is invalid according to the current guideline for testing of algae (OECD 201). Intermediate is not relevant for risk assessment.

4) In accordance to the EFSA Aquatic Guidance Document (EFSA, 2013) and OECD guideline 201 (2011) the 72 h endpoints obtained in the 96 h alga study are considered as relevant endpoints and are presented here.

CA 8.2.1 Acute toxicity to fish

In addition to the two standard acute studies on *Oncorhynchus mykiss* which have already been evaluated during the Annex I inclusion process, a further acute study on this fish species was conducted. The study has already been used in previous EU end-use product registrations, but has not been evaluated previously on EU level and is provided in support of the aquatic risk assessment.

Report: CA 8.2.1/1
[redacted]
BAS 595 F - Acute toxicity study on the rainbow trout (Oncorhynchus mykiss) in a static system over 96 hours
2006/1015993
Guidelines: OECD 203, EEC 92/69 A V C 1, 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, rainbow trout were exposed to 0, 5, 10, 22, 50 and 100% saturated solution of triticonazole (corresponding to 0, 0.58, 1.18, 2.62, 6.25 and 12.4 mg/L based on mean measured concentrations of the technical test substance) in groups of 10 animals in glass aquaria containing 50 L water. Fish were observed for survival and symptoms of toxicity 1, 4, 24, 48, 72 and 96 hours after start of exposure.

The biological results are based on the mean measured concentrations. After 96 hours of exposure, no mortality was observed except for one fish in the 50% (6.35 mg a.s./L) and one fish in the 100% (12.6 mg a.s./L) saturated groups after 72 hours of exposure. At the two highest test item concentrations of 6.35 and 12.6 mg a.s./L, the fish were observed swimming at the bottom of the aquarium.

In a static acute toxicity study with rainbow trout, the LC_{50} (96 h) of triticonazole was greater than 12.4 mg a.s./L and the NOEC (96 h) was determined to be 2.62 mg a.s./L based on the mean of analytically determined concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Triticonazole (BAS 595 F; Reg. no.: 4378513); batch no. COD-000601, purity: 90.3% (analyzed).

B. STUDY DESIGN

Test species: Rainbow trout (*Oncorhynchus mykiss*), approx. 8 months old; body length 7.1 cm (5.7 – 8.3 cm); body weight 3.39 g (1.55 – 4.88 g); obtained from [redacted].

Test design: Static system (96 hours); 10 fish per aquarium (loading about 0.7 g fish/L): 1 aquarium per concentration; assessment of mortality and symptoms of toxicity 1, 4, 24, 48, 72 and 96 hours after start of exposure.

Endpoints: LC_{50}, NOEC, mortality and sub-lethal effects.

Test concentrations: 0 (control), 5, 10, 22, 50 and 100% saturated solution; corresponding to mean measured concentrations of 0 (control), 0.58, 1.18, 2.62, 6.25 and 12.4 mg triticonazole/L.

Test conditions: Glass aquaria with stainless steel frame (60 x 35 x 40 cm), test volume: 50 L, non-chlorinated, charcoal filtered tap water mixed with deionized water, aerated; temperature: 12 - 13°C; pH 7.6 – 8.3; oxygen content: 6.6 - 10.3 mg/L; total hardness: 100 mg $CaCO_3$/L; photoperiod 16 h light : 8 h dark (36-191 lux); no feeding during the exposure phase.

Analytics: Analytical verification of test item concentrations 30 minutes before insertion of the fish (start of exposure), after 48 hours and at the end of the exposure after approx. 96 hours was conducted using a HPLC-method with MS detection.

Statistics: Descriptive statistics; no statistical analysis was carried out since only one fish in the 50% and 100% test solution groups died.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of the test substance was conducted in each concentration at the beginning, after 48 hours and at the end of the test. Analytically determined concentration at the end of the test ranged from 98.1% to 102% of initial measured concentration. Although the mean measured concentrations confirmed correct application of the test substance, the following biological results are based on mean of analytically determined concentrations of the test item (mean measured).

Biological results: After 96 hours of exposure, no mortality was observed except for one fish in the 50% (6.25 mg a.s./L) and one fish in the 100% (12.4 mg a.s./L) saturated groups after 72 hours of exposure. At the two highest test item concentrations of 6.25 and 12.4 mg a.s./L, the fish were observed swimming at the bottom of the aquarium. The results are summarized in Table 8.2.1-1.

Table 8.2.1-1: Acute toxicity (96 hours) of triticonazole to rainbow trout (*Oncorhynchus mykiss*)

Saturated solution %	**Control**	**5**	**10**	**22**	**50**	**100**
Mean measured concentrations [mg a.s./L]	**0**	**0.58**	**1.18**	**2.62**	**6.25**	**12.4**
Mortality [%]	0	0	0	0	10	10
Symptoms *	none	none	none	none	D[a]	D[b]
Endpoints [mg a.s./L] (mean measured)						
LC_{50} (96 h)	> 12.4					
NOEC (96 h)	2.62					

Symptoms: D = swimming near the bottom.
[a] from 72 hours on
[b] from 24 hours on

III. CONCLUSION

In a static acute toxicity study with rainbow trout, the LC_{50} (96 h) of triticonazole was greater than 12.4 mg a.s./L and the NOEC (96 h) was determined to be 2.62 mg a.s./L based on the mean of analytically determined concentrations.

The following acute fish toxicity study on bluegill sunfish (*Lepomis macrochirus*) conducted with technical triticonazole has not been evaluated previously on EU level and is provided in support of the aquatic risk assessment.

Report:	CA 8.2.1/2 [redacted] BAS 595 F - Acute toxicity study on the bluegill sunfish (Lepomis macrochirus) in a static system over 96 hours 2006/1018146
Guidelines:	OECD 203, EEC 92/69 A V C 1, 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 were exposed to 0, 5, 10, 22, 50 and 100% saturated solution of triticonazole (corresponding to 0, 0.52, 1.01, 2.23, 5.10 and 10.1 mg/L based on mean measured concentrations of the technical test substance) in groups of 10 animals in glass aquaria (2 replicates: total of 20 fish / test concentration) containing 50 L water. Fish were observed for survival and symptoms of toxicity 1, 4, 24, 48, 72 and 96 hours after start of exposure.

The biological results are based on the mean measured concentrations. After 96 hours of exposure, no mortality or abnormal behavior was observed at any of the test concentrations.

In a static acute toxicity study with bluegill sunfish, the LC_{50} (96 h) of triticonazole was greater than 10.1 mg a.s./L and the NOEC (96 h) was determined to be ≥ 10.1 mg a.s./L based on the mean of analytically determined concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Triticonazole (BAS 595 F, Reg. no.: 4378513); batch no. COD-000601, purity: 90.3% (analyzed).

B. STUDY DESIGN

Test species: Bluegill sunfish (*Lepomis macrochirus*), approx. 12 months old; body length 4.5 cm (4.2 – 5.1 cm); body weight 1.20 g (0.94 – 2.01 g); obtained from [redacted].

Test design: Static system (96 hours); 10 fish per aquarium (loading about 0.2 g fish/L): 2 aquarium per concentration (total 20 fish per test concentration); assessment of mortality and symptoms of toxicity 1, 4, 24, 48, 72 and 96 hours after start of exposure.

Endpoints: LC_{50}, NOEC, mortality and sub-lethal effects.

Test concentrations: 0 (control), 5, 10, 22, 50 and 100% saturated solution; corresponding to mean measured concentrations of 0 (control), 0.52, 1.01, 2.23, 5.10 and 10.1 mg triticonazole/L.

Test conditions: Glass aquaria with stainless steel frame (60 x 35 x 40 cm), test volume: 50 L, non-chlorinated, filtered tap water; temperature: 22°C; pH 7.7 – 8.3; oxygen content: 6.0 - 8.9 mg/L; total hardness: 100 mg $CaCO_3$/L; photoperiod 16 h light : 8 h dark (36-191 lux); no aeration, no feeding during the exposure phase.

Analytics: Analytical verification of test item concentrations 30 minutes before insertion of the fish (start of exposure), after 48 hours and at the end of the exposure after approx. 96 hours was conducted using a HPLC-method with MS detection.

Statistics: Descriptive statistics; no statistical analysis was carried out since no lethality was observed up to the highest tested concentration.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of the test substance was conducted in each concentration at the beginning, after 48 hours and at the end of the test. Analytically determined concentration at the end of the test ranged from 95.2% to 108% of initial measured concentration. Although the mean measured concentrations confirmed correct application of the test substance, the following biological results are based on mean of analytically determined concentrations of the test item (mean measured).

Biological results: After 96 hours of exposure, no mortality was observed at any of the test concentrations. In addition, all organisms appeared normal throughout the exposure period in all of the test groups. The results are summarized in Table 8.2.1-2.

Table 8.2.1-2: Acute toxicity (96 h) of triticonazole on bluegill sunfish (*Lepomis macrochirus*)

Saturated solution %	**Control**	**5**	**10**	**22**	**50**	**100**
Mean measured concentrations [mg a.s./L]	**0**	**0.52**	**1.01**	**2.23**	**5.10**	**10.1**
Mortality [%]	0	0	0	0	0	0
Symptoms	none	none	none	none	none	none
Endpoints [mg a.s./L] (mean measured)						
LC_{50} (96 h)	> 10.1					
NOEC (96 h)	≥ 10.1					

III. CONCLUSION

In a static acute toxicity study with bluegill sunfish, the LC_{50} (96 h) of triticonazole was greater than 10.1 mg a.s./L and the NOEC (96 h) was determined to be ≥ 10.1 mg a.s./L based on the mean of analytically determined concentrations.

The following acute fish toxicity study on sheepshead minnow performed with the active substance triticonazole is not required for registration in the EU. The study was conducted due to U.S. data requirements and has not been evaluated previously on EU level. It is now included for completeness.

Report:	CA 8.2.1/3 [redacted] Triticonazole technical - Acute toxicity to sheepshead minnow (Cyprinodon variegatus) under flow-through conditions R000095
Guidelines:	EPA 72-3
GLP:	yes

Executive Summary

In a 96-hour flow-through acute toxicity laboratory study, sheepshead minnow were exposed to a dilution water control, a solvent control (acetone) and to nominal concentrations of 1.3, 2.1, 3.6, 5.9 and 9.9 mg triticonazole/L (corresponding to mean measured concentrations of 1.2, 2.0, 3.4, 5.7 and 9.1 mg a.s./L) in groups of 10 animals in glass aquaria containing 11 L water. Fish were observed for survival and symptoms of toxicity directly after start of exposure and 24, 48, 72 and 96 hours after start of exposure.

The biological results are based on mean measured concentrations of the test item. After 96 hours of exposure, no mortality was observed in the dilution water control, the solvent control and at up to and including the highest test item concentration. Sub-lethal effects (*i.e.* lethargy) were found at the highest test item concentration after 96 hours.

In a flow-through acute toxicity study with sheepshead minnow the LC_{50} (96 h) of triticonazole was > 9.1 mg a.s./L based on mean measured concentrations. The NOEC (96 h) was determined to be 5.7 mg a.s./L (mean measured).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Triticonazole technical (BAS 595 F; Reg. no.: 4378513), batch no. OP9750057, purity: 90.52%.

B. STUDY DESIGN

Test species: Sheepshead minnow (*Cyprinodon variegatus*), mean body length: 35 mm (range: 30 - 40 mm); mean wet weight: 0.74 g (range: 0.48 - 0.98 g); obtained from [redacted].

Test design: Flow through system (96 h); 5 test item concentrations plus a dilution water control and a solvent control, 2 replicates per treatment; 10 fish per aquarium (max. loading 0.10 g fish/L); assessment of mortality and sub-lethal effects at 0, 24, 48, 72 and 96 hours after start of exposure.

Endpoints: LC_{50}, NOEC, mortality and sub-lethal effects.

Test concentrations: Control (dilution water), solvent control (0.37 mL acetone/L), 1.3, 2.1, 3.6, 5.9 and 9.9 mg triticonazole/L (nominal), corresponding to mean measured concentrations of 1.2, 2.0, 3.4, 5.7 and 9.1 mg a.s./L.

Test conditions: Glass aquaria (39 x 20 x 25 cm), test volume: 11 L; dilution water: natural filtered seawater; flow rate: 0.50 L per minute; approx. 6.4 volume replacements per day in each aquarium; temperature: 22 - 23°C; pH 7.7 - 7.8; oxygen content: 5.4 mg/L - 7.6 mg/L; salinity: 32‰; photoperiod 16 h light : 8 h dark; light intensity: 20 - 100 foot candles (220 - 1100 lux); no aeration; no feeding.

Analytics: Analytical verification of test item concentrations was conducted using a HPLC-method with UV detection.

Statistics: Descriptive statistics.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of triticonazole concentrations was conducted in each concentration at the beginning and at the end of the test. The analyzed contents of triticonazole ranged from 97.0% to 101.7% of nominal at test initiation and from 88.9% to 95.2% of nominal at test termination. The following biological results are based on mean measured concentrations.

Biological results: After 96 hours of exposure, no mortality was observed in the dilution water control, the solvent control and at up to and including the highest test item concentration. Sub-lethal effects (*i.e.* lethargy) were found at the highest test item concentration after 96 hours. The results are summarized in Table 8.2.1-3.

Table 8.2.1-3: Acute toxicity (96 h) of triticonazole to sheepshead minnow (*Cyprinodon variegatus*)

Concentration [mg a.s./L] (nominal)	**Control**	**Solvent control**	**1.3**	**2.1**	**3.6**	**5.9**	**9.9**
Concentration [mg a.s./L] (mean measured)	**--**	**--**	**1.2**	**2.0**	**3.4**	**5.7**	**9.1**
Mortality [%] (96 h)	0	0	0	0	0	0	0 [a)]
Symptoms (after 96 h) *	none	none	none	none	none	none	L
Endpoints [mg triticonazole/L] (mean measured)							
LC_{50} (96 h)	> 9.1 (95% confidence limits: n.d.)						
NOEC (96 h)	5.7						

n.d. = not determined
* Symptoms after 96 h: L = lethargy.
[a)] Undissolved test substance was observed in the test vessels.

III. CONCLUSION

In a flow-through acute toxicity study with sheepshead minnow the LC_{50} (96 h) of triticonazole was > 9.1 mg a.s./L based on mean measured concentrations. The NOEC (96 h) was determined to be 5.7 mg a.s./L (mean measured).

The following acute fish toxicity study on common carp performed with the active substance triticonazole was (wrongly) not listed in the "Application" document submitted for the triticonazole AIR 3 renewal process. The study has not been evaluated previously on EU level and is provided in support of the aquatic risk assessment.

Report:	CA 8.2.1/4 [redacted] BAS 595 F (Triticonazole) - Acute toxicity study in the common carp (Cyprinus carpio) 2014/1095638
Guidelines:	EC 440/2008 C.1 Acute Toxicity for Fish, OECD 203 (1992), EPA 540/9-82-024, EPA 72-1, EPA 850.1075
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

In a 96-hour static acute toxicity laboratory study, common carp were exposed to a dilution water control and to 6.25%, 12.5%, 25%, 50% and 100% of a saturated solution of the test item (corresponding to mean measured concentrations of 1.1, 2.2, 4.4, 9.1 and 18 mg triticonazole/L) in groups of 10 animals in glass aquaria containing approx. 50 L water. Fish were observed for survival and symptoms of toxicity 1, 6, 24, 48, 72 and 96 hours after start of exposure.

The biological results are based on mean measured concentrations of the test item. After 96 hours of exposure, no mortality was observed in the control and at test item concentrations of up to and including the second highest concentration of 9.1 mg a.s./L. At the highest test item concentration of 18 mg a.s./L, 5% mortality was observed. Sub-lethal effects (*i.e.* tottering, swimming at the bottom) were found at the highest test item concentration after 96 hours of exposure.

In a static acute toxicity study with common carp the LC_{50} (96 h) of triticonazole was > 18 mg a.s./L based on mean measured concentrations. The NOEC (96 h) was determined to be 9.1 mg a.s./L (mean measured).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Triticonazole (BAS 595 F; Reg. no.: 4378513), batch no. COD-001440, purity: 91.3% (tolerance ± 1.0%).

B. STUDY DESIGN

Test species: Common carp (*Cyprinus carpio*), age: 3 months; fish length: 4.1 cm (3.7 cm - 4.5 cm); fish weight: 0.82 g (range: 0.54 - 1.09 g); obtained from [redacted].

Test design: Static system (96 h); 5 test item concentrations plus a dilution water control, 2 replicates per treatment; 10 fish per test vessel (max. loading 0.21 g fish/L); assessment of mortality and sub-lethal effects at 1, 6, 24, 48, 72 and 96 hours after start of exposure.

Endpoints: LC_{50}, NOEC, mortality and sub-lethal effects.

Test concentrations: Control (dilution water), 6.25%, 12.5%, 25%, 50% and 100% of saturated solution (nominal), corresponding to mean measured concentrations of 0, 1.1, 2.2, 4.4, 9.1 and 18 mg a.s./L.

Test conditions: Stainless steel aquaria (approx. 50 L, 60 x 35 x 40 cm), test volume: 40 L; dilution water: charcoal-filtered drinking water mixed with deionized water; temperature: 22 - 23°C; pH 8.1 - 8.4; oxygen content: 6.1 mg/L - 8.4 mg/L; hardness: 1.23 mmol/L; conductivity: 296 µS/cm; photoperiod 16 h light : 8 h dark; light intensity: 60 - 585 lux; no aeration; no feeding.

Analytics: Analytical verification of test item concentrations was conducted using a HPLC-method with MS detection.

Statistics: Descriptive statistics.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of triticonazole concentrations was conducted in each concentration at the beginning of the test, 48 h after start of exposure and at the end of the test. The mean measured concentrations of the saturated solutions were 1.1, 2.2, 4.4, 9.1 and 18 mg a.s./L. The analyzed contents of triticonazole ranged 93% to 103% of the nominal concentrations, thus the overall mean measured concentrations are an accurate representation of exposure levels maintained throughout the test period. The following biological results are based on mean measured concentrations.

Biological results: After 96 hours of exposure, no mortality was observed in the control and at test item concentrations of up to and including the second highest concentration of 9.1 mg a.s./L. At the highest test item concentration of 18 mg a.s./L, 5% mortality was observed. Sub-lethal effects (*i.e.* tottering, swimming at the bottom) were found at the highest test item concentration after 96 hours of exposure. The results are summarized in Table 8.2.1-4.

Table 8.2.1-4: Acute toxicity (96 h) of triticonazole to common carp (*Cyprinus carpio*)

Nominal concentration [% of saturated solution]	**Control**	**6.25%**	**12.5%**	**25%**	**50%**	**100%**
Concentration [mg a.s./L] (mean measured)	**--**	**1.1**	**2.2**	**4.4**	**9.1**	**18**
Mortality [%] (96 h)	0	0	0	0	0	5
Symptoms (after 96 h) *	none	none	none	none	none	T, D
Endpoints [mg triticonazole/L] (mean measured)						
LC_{50} (96 h)	> 18					
NOEC (96 h)	9.1					

* Symptoms after 96 h: T = tottering, D = swimming at the bottom

III. CONCLUSION

In a static acute toxicity study with common carp the LC_{50} (96 h) of triticonazole was > 18 mg a.s./L based on mean measured concentrations. The NOEC (96 h) was determined to be 9.1 mg a.s./L (mean measured).

From the literature search the following peer-reviewed scientific study was considered relevant and reliable although with restrictions (RI 2; as this a non-GLP study). For details please see the literature search and evaluation files also provided within the submission for Annex I Renewal. In this study acute toxicity tests were performed on *Danio rerio* and *Daphnia magna* with triazoles and benzotriazoles. In the following summary, the experimental data and results for *D. rerio* and triticonazole are presented. The results for *D. magna* and triticonazole are presented under point CA 8.2.4.1.

Report: CA 8.2.1/5
Experimental assessment of the environmental fate and effects of Triazoles and Benzotriazole
2015/1177633
Guidelines: none
GLP: no

Executive Summary

In a 72-hour static acute toxicity study, zebrafish embryos were exposed to a range of 6 triticonazole test concentrations. Additionally a negative- and positive control were set up. The test was repeated three times. Viable eggs were incubated individually in well flat-bottomed plates; 4 wells per plate were used for control and 10 wells were used for each test concentration. Microscopic observations were conducted 24, 48 and 72 hours after start of the experiments.

The biological results are based on nominal concentrations of the test item. LC_{50} values for *D. rerio* were determined after 72 hours of exposure.

In a static acute toxicity study with zebrafish (test repeated three times), the LC_{50} values (72 h) of triticonazole were ≥ 31.80 mg a.s./L (test 1) and ≥ 95.30 mg a.s./L (test 2 and 3) based on nominal concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Triticonazole (BAS 595 F), CAS no.: 131983-72-7; purchased from Fluka® Analytical (Sigma-Aldrich).

B. STUDY DESIGN

Test species: Zebrafish (*D. rerio*), embryos (viable eggs).

Test design: Static system (72 h); each test was repeated three times; if necessary, the concentration range was adjusted between different experiments; viable eggs were incubated individually in 2 mL of medium in well flat-bottomed plates; 4 wells per plate were used for control, 10 wells for each test concentration; stock solutions were prepared in DMSO, with a final test concentration of 0.2% carrier (2 mL/L) in the dilution series; microscopic observations 24, 48 and 72 hours after start of the experiments.

Endpoints: LC_{50}, mortality, no heartbeat, no somite formation, no detachment of tail.

Test concentrations: 6 triticonazole test concentrations (range was adjusted between different experiments if necessary), solvent: DMSO (2 ml/L), negative control: potassium dichromate (1 mg/L); positive control: 3,4-dichloroaniline (8 mg/L).

Test conditions: Well flat-bottomed plates; 2 mL of medium per well; dilution water: Dutch Standard Water (DSW; demineralized water supplemented with 100 mg/L $NHCO_3$, 20 mg/L $KHCO_3$, 200 mg/L $CaCl_2 \cdot 2H_2O$ and 180 mg/L $MgSO_4 \cdot 7H_2O$, aerated for 24 hours at 27°C); temperature: 26.5 ± 0.5°C; pH of dilution water: 8.1 (7.4 - 8.3); oxygen content: ≥ 6.6 mg/L; water hardness: 214 mg $CaCO_3$/L.

Analytics: Chemical measurements of test item concentrations was conducted using a GC-method with MS detection.

Statistics: Descriptive statistics; trimmed Spearman-Karber method for calculation of LC_{50} values.

II. RESULTS AND DISCUSSION

Analytical measurements: Chemical measurements of test item concentrations in the test cultures was conducted at the beginning and at the end of the test. The following biological results are based on nominal concentrations.

Biological results: LC_{50} values for *D. rerio* were determined after 72 hours of exposure; the results are summarized in Table 8.2.1-5.

Table 8.2.1-5: Acute toxicity (72 h) of triticonazole to zebrafish (*D. rerio*)

		Endpoints [mg triticonazole/L] (nominal)
LC_{50} (72 h)	test 1	≥ 31.80 (95% confidence limits: n.a.)
	test 2	≥ 95.30 (95% confidence limits: n.a.)
	test 3	≥ 95.30 (95% confidence limits: n.a.)

n.a. = not applicable

III. CONCLUSION

In a static acute toxicity study with zebrafish (test repeated three times), the LC_{50} values (72 h) of triticonazole were ≥ 31.80 mg a.s./L (test 1) and ≥ 95.30 mg a.s./L (test 2 and 3) based on nominal concentrations.

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 (ELS) test on fathead minnow performed with technical triticonazole according to U.S. specific data requirements was not submitted for Annex I inclusion because no clear endpoint could be derived from this study (*i.e.* effects were measured at all tested concentrations). Thus, a second ELS study has been conducted in which a lower concentration range was tested (*i.e.* DocID C044319; see summary below). Nevertheless a summary of the first (invalid) study is provided below for completeness.

Report: CA 8.2.2.1/1
Triticonazole technical - Early life-stage toxicity test with fathead minnow (Pimephales promelas)
B003479
Guidelines: FIFRA 72-4
GLP: yes

Executive Summary

The chronic toxicity of triticonazole to fathead minnows (*Pimephales promelas*) was evaluated in a 34-day early life-stage test under flow-through conditions. Embryos were exposed to a dilution water control, a solvent control and to nominal concentrations of 0.020, 0.050, 0.130, 0.320 and 0.790 mg triticonazole/L (corresponding to mean measured concentrations of 0.024, 0.056, 0.120, 0.310 and 0.780 mg a.s./L). Hatchability of embryos and survival and growth of larvae at test termination were assessed in this study.

The results are based on mean measured concentrations. Day of hatch was considered to be exposure day 4, when no more than 10% unhatched viable embryos remained in any control or treatment level egg incubation cup. At the completion of the hatching period (day 5), embryo survival in the control and solvent control averaged 96% and 91%, respectively. No statistically significant differences were determined between the control and the solvent control data. Thus, the data from both control groups were pooled and the pooled data set was used for statistical evaluation of treatment related effects. After the 30 days post-hatch exposure period, larval survival was statistically significantly reduced at the highest test item concentration compared to the pooled control. Length of the test organisms was statistically significantly reduced at the test item concentrations of 0.056, 0.120, 0.310 and 0.780 mg a.s./L compared to the solvent control. Wet weight and dry weight was statistically significantly different compared to the pooled control at 0.024, 0.056, 0.120, 0.310 and 0.780 mg a.s./L.

In an early life stage study with fathead minnows (*Pimephales promelas*) the overall NOEC (34 d) for triticonazole was determined to be < 0.024 mg a.s./L based on mean measured concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Triticonazole technical (BAS 595 F; Reg. no.: 4378513), batch no. OP9750057, purity: 90.52%.

B. STUDY DESIGN

Test species: Fathead minnow (*Pimephales promelas*); eggs less than 24 hours post fertilization at test initiation, eggs obtained from in-house culture, brood stock originally obtained from [redacted] [redacted] [redacted] [redacted].

Test design: Flow-through system (34 d); 5 test item concentrations plus a dilution water control and a solvent control; 2 replicate test chambers per treatment; 1 incubation cup per replicate test vessel with 60 fertilized eggs in each; reduction of surviving larvae to 40 organisms per replicate test vessel on day 5; daily assessment of survival until end of hatch; daily recording of sublethal effects (behavior and appearance) and daily removal of dead larvae during 30 days post-hatch exposure; estimation of post-hatch survival at least twice weekly; determination of wet weight, dry weight and total length at test end.

Endpoints: NOEC values based on embryo survival at hatch, survival and growth (wet weight, dry weight and total length) at test termination.

Test concentrations: Control (dilution water), solvent control (0.00164 mL acetone/L), 0.020, 0.050, 0.130, 0.320 and 0.790 mg triticonazole/L (nominal); corresponding to mean measured concentrations of 0.024, 0.056, 0.120, 0.310 and 0.780 mg a.s./L.

Test conditions: Test vessels: glass aquaria (39 x 20 x 25 cm); test volume: approx. 15 L; embryo incubation cups: glass jars (5 cm diameter, 8 cm high) with 40-mesh Nitex® screen bottoms; dilution water: aerated well water from two natural sources; temperature: 25°C - 26°C; pH 6.8 - 7.6; oxygen content: 6.1 mg/L - 9.5 mg/L; total hardness: 36 - 40 mg $CaCO_3$; total alkalinity: 24 - 28 $CaCO_3$; specific conductance: 150 - 170 µmhos/cm; light intensity: 30 - 100 foot candles (320 - 1100 lux); photoperiod: 16 hours light : 8 hours dark; flow rate: approx. 6.8 volume additions per 24 hours per vessel (90% replacement time of approx. 8.0 h); feeding (from day 4 on): live brine shrimp nauplii (*Artemia salina*) *ad libitum* three times daily until 24 h before study termination; no aeration; gentle oscillation of embryo incubation cups in test solutions.

Analytics: Analytical verification of test item concentrations was conducted using an HPLC-method with variable wavelength detection.

Statistics: Descriptive statistics; t-test for comparison of the dilution water control and solvent data ($\alpha = 0.05$); William's test to determine NOEC values for survival and growth ($\alpha = 0.05$).

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of the test item concentrations was conducted in all concentrations at test initiation, at regular intervals during the study and at test end. Mean recoveries of triticonazole ranged from 81% to 135% of nominal concentrations during the test period. The following biological results are based on mean measured concentrations.

Biological results: Day of hatch was considered to be exposure day 4, when no more than 10% unhatched viable embryos remained in any control or treatment level egg incubation cup. At the completion of the hatching period (day 5), embryo survival in the control and solvent control averaged 96% and 91%, respectively. No statistically significant differences were determined between the control and the solvent control data (t-test; $\alpha = 0.05$). Thus, the data from both control groups were pooled and the pooled data set was used for statistical evaluation of treatment related effects. After the 30 days post-hatch exposure period, larval survival was statistically significantly reduced at the highest test item concentration compared to the pooled control (Williams' test, $\alpha = 0.05$). Length of the test organisms was statistically significantly reduced at the test item concentrations of 0.056, 0.120, 0.310 and 0.780 mg a.s./L compared to the solvent control (Williams' test, $\alpha = 0.05$). Wet weight and dry weight was statistically significantly different compared to the pooled control at 0.024, 0.056, 0.120, 0.310 and 0.780 mg a.s./L (Williams' test, $\alpha = 0.05$). The results are summarized in Table 8.2.2.1-1.

Table 8.2.2.1-1: Chronic toxicity of triticonazole to fathead minnow (*Pimephales promelas*) in a fish early life stage test (34 d)

Concentration [mg a.s./L] (nominal)	**Control**	**Solvent control**	**0.020**	**0.050**	**0.130**	**0.320**	**0.790**
Concentration [mg a.s./L] (mean measured)	**--**	**--**	**0.024**	**0.056**	**0.120**	**0.310**	**0.780**
Mean embryo survival at hatch [%]	96	91	92	94	93	93	97
Mean larval survival (day 34) [%]	90	93	98	98	98	96	36 *
Mean total length (day 34) [mm]	30.7 ± 1.6	30.1 ± 1.6	29.8 ± 1.6	29.1 ± 1.7 +	28.4 ± 1.8 +	23.2 ± 2.5 +	15.1 ± 3.4 a)
Mean wet weight (day 34) [g]	0.28 ± 0.04	0.27 ± 0.05	0.26 ± 0.04 *	0.25 ± 0.05 *	0.22 ± 0.04 *	0.12 ± 0.04 *	0.037 ± 0.02 a)
Mean dry weight (day 34) [g]	0.075 ± 0.01	0.071 ± 0.01	0.067 ± 0.01 *	0.061 ± 0.01 *	0.054 ± 0.01 *	0.028 ± 0.01 *	0.0078 ± 0.005 a)
	Endpoint [mg triticonazole/L] (mean measured)						
$NOEC_{overall}$ (34 d)	< 0.024						

* Statistically significantly reduced compared to the pooled control (Williams' test, $\alpha = 0.05$).
+ Statistically significantly reduced compared to the solvent control (Williams' test, $\alpha = 0.05$).
a) Growth data from this test item group was excluded from statistical analysis because survival was statistically significantly affected.

III. CONCLUSION

In an early life stage study with fathead minnows (*Pimephales promelas*) the overall NOEC (34 d) for triticonazole was determined to be < 0.024 mg a.s./L based on mean measured concentrations.

The following early life-stage test on sheepshead minnow performed with the active substance triticonazole is not required for registration in the EU. The study was conducted due to U.S. data requirements and has not been evaluated previously on EU level. It is now included for completeness.

Report:	CA 8.2.2.1/2 [redacted] 2006a BAS 595 F (Triticonazole) - Early life-stage toxicity test with the Sheepshead minnow (Cyprinodon variegatus) 2006/7007245
Guidelines:	EPA 850.1400
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary

The toxicity of triticonazole to sheepshead minnow (*Cyprinodon variegatus*) embryos and larvae was evaluated in a 34-days (28-days post hatch) early life-stage (ELS) toxicity test under flow-through conditions. The test organisms were exposed to 5 test concentrations plus controls. To initiate the study, incubation cups containing 40 eggs were suspended in respective exposure aquaria (one cup per replicate vessel). Beginning on day 6 (day 0 post-hatch) the surviving larvae present in each incubation cup were thinned to 20 organisms per replicate per 80 organisms per treatment level or control and placed into their respective exposure aquaria. Viability of embryos, survival of fry, appearance and behavior, and growth were observed.

Analysis of the test solutions resulted in recoveries ranging from 100% to 110% of the nominal concentration; nevertheless biological results were based on mean measured concentrations. Based on the evaluation of organism hatching success, percent of embryos that produced normal fry at hatch and at test termination, and larval growth (total length and dry weight), no concentration tested resulted in any statistically significant effects.

In an early-life stage (ELS) toxicity test conducted under flow through conditions, sheepshead minnow were exposed to triticonazole over a period of 28-days. The overall NOEC identified in this study was ≥ 0.120 mg a.s./L based on the highest mean measured concentration tested.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Triticonazole (BAS 595 F; Reg. No. 4378513), batch no. not reported, purity: 90.3%.

B. STUDY DESIGN

Test species: Sheepshead minnow embryos (Cyprinodon variegatus), < 30 hours old, source: [redacted].

Test design: Flow-through system (34-days, 28-days post hatch); 5 test concentrations plus control, 40 embryos per aquarium, 28 aquariums. On test day 6 (day of hatch), the surviving larvae present in each incubation cup were thinned to 20 organisms per replicate/80 organisms per treatment level or control and placed into their respective exposure aquaria. Viability of embryos, survival of the fry, appearance and behavior, and growth were observed.

Endpoints: NOEC, based on survival, appearance and behavior, growth and reproduction.

Test concentrations: Control, solvent control, 0.0075, 0.015, 0.030, 0.060 and 0.120 mg a.s./L (nominal); corresponding to mean measured concentrations of 0, 0, 0.0083, 0.016, 0.033, 0.064 and 0.120 mg a.s./L (mean measured).

Test conditions: Test vessels: test aquaria were impartially positioned in a heated water bath to maintain a stable test solution temperature. Each test aquarium measured 30 x 14.5 x 20 cm with a 15-cm high side drain that maintained a constant exposure solution volume of 6.5 L. Embryo incubation cups were round glass jars (5 cm) diameter, 8 cm high with 475-µm nylon screen bottoms. A rocker arm apparatus was used to gently oscillate the incubation cups in the test solutions during incubation.
Dilution water: natural filtered seawater pumped from the Cape Cod Canal, Bourne, Massachusetts (salinity adjusted with laboratory well water); temperature 24-26°C; pH 7.5-8.1; salinity 19-21‰; oxygen content 3.1-8.0 mg/L.
Flow rate: 50 L/aquarium/day.
Photoperiod: 16 hours light: 8 hours darkness; 570 to 1000 lux.
Feeding: live brine shrimp nauplii (*Artemia salina*) three times daily.
Aeration: on test day 29 in the solvent control, 0.015, 0.030 and 0.120 mg a.s./L treatment groups since dissolved O_2 levels dropped below 60% saturation for less than 24 hours.

Analytics: The test item concentrations were analyzed using a HPLC/UV method.

Statistics: Descriptive statistics; Student's t-Test for performance of controls; pooled control data (dilution water and solvent) used to determine treatment effects for survival, length and weight. For normality, the Shapiro-Wilks' Test was conducted and the Bartlett's Test to check on the assumption of homogeneity of variance. All endpoints met the assumptions for normality and homogeneity of variance, therefore, the Williams' Test was used to determine the performance of the organisms in each treatment level.

II. RESULTS AND DISCUSSION

Analytical measurements: The analysis of the exposure solutions during the in-life portion of the definitive study resulted in mean measured concentrations that ranged from 100% to 110% of the nominal levels. Throughout the study period, all exposure solutions were observed to be clear and colorless.

The following biological results are based on mean measured concentrations and are summarized in Table 8.2.2.1-2.

At the completion of the hatching period (day 6), embryos exposed to the control and solvent control averaged 98% and 96% hatching success, respectively (pooled control = 97%). The mean hatching success values in treatment levels 0.0083, 0.016, 0.033, 0.064 and 0.120 mg a.s./L ranged from 96% to 100% and were not statistically different from the survival of the pooled control organisms.

Following 28-days post-hatch exposure (day 34), larval survival in the control and solvent control both averaged 96% (pooled control = 96%). Mean larval survival in the 0.0083, 0.016, 0.033, 0.064 and 0.120 mg a.s./L treatment levels ranged from 90 to 99% and was not statistically different from the survival of the pooled control organisms.

At test termination, total length of larvae exposed to the control and solvent control averaged 22.2 and 22.7 mm, respectively (pooled control = 22.5 mm). Mean total length of larvae exposed to the 0.0083, 0.016, 0.033, 0.064 and 0.120 mg a.s./L test concentrations were 22.3, 22.0, 22.1, 22.1 and 22.0 mm, respectively, and was not statistically different from the survival of the pooled control organisms.

Dry weight of the test organisms in the control and solvent control averaged 0.0402 and 0.0428 g, respectively (pooled control = 0.0415 g). Mean dry weight of larvae exposed to the 0.0083, 0.016, 0.033, 0.064 and 0.120 mg a.s./L test concentrations was 0.0410, 0.0386, 0.0398, 0.0402 and 0.0396 g, respectively, and was not statistically different from the survival of the pooled control organisms.

Table 8.2.2.1-2: Mean values of biological results determined in the early life-stage toxicity test of triticonazole on sheepshead minnow

Concentration [mg a.s./L] (mean measured)	Embryo hatching success [a] [%]	28-Days post-hatch		
		Larval survival [%]	Total length (SD) [mm]	Dry weight (SD) [g]
Control	98	96	22.2 (1.7)	0.0402 (0.0084)
Solvent control	96	96	22.7 (0.85)	0.0428 (0.0068)
Pooled control	97	96	22.5 (1.4)	0.0415 (0.0077)
0.0083	97	95	22.3 (1.4)	0.0410 (0.0081)
0.016	100	99	22.0 (1.8)	0.0386 (0.0097)
0.030	96	93	22.1 (1.6)	0.0398 (0.0083)
0.060	97	90	22.1 (1.9)	0.0402 (0.0099)
0.120	99	94	22.0 (1.3)	0.0396 (0.0079)
Endpoints [mg triticonazole/L] (mean measured)				
$NOEC_{overall}$	≥ 0.120			

[a] Adjusted for the percent of viable eggs at test initiation (i.e., 96%). No deformed fry were observed.
SD = Standard Deviation.

III. CONCLUSION

In an early-life stage (ELS) toxicity test conducted under flow through conditions, sheepshead minnow were exposed to triticonazole over a period of 28-days. The overall NOEC identified in this study was ≥ 0.120 mg a.s./L based on the highest mean measured concentration tested.

The following early life-stage test on fathead minnow performed with the active substance triticonazole is not required for registration in the EU. The study was conducted due to U.S. data requirements and has not been evaluated previously on EU level. It is now included for completeness.

Report: CA 8.2.2.1/3
[redacted] 1998c
Triticonazole technical - Early life-stage toxicity test with fathead minnow (Pimephales promelas)
C044319
Guidelines: FIFRA 72-4, EPA 850.1400
GLP: yes

Executive Summary

The chronic toxicity of triticonazole to fathead minnows (*Pimephales promelas*) was evaluated in a 34-day early life-stage test under flow-through conditions. Embryos were exposed to a dilution water control, a solvent control and to nominal concentrations of 0.0014, 0.0036, 0.009, 0.022 and 0.056 mg triticonazole/L (corresponding to mean measured concentrations of 0.017, 0.037, 0.087, 0.021 and 0.051 mg a.s./L). Hatchability of embryos and survival and growth of larvae at test termination were assessed in this study.

The results are based on mean measured concentrations. Day of hatch was considered to be exposure day 4, when no more than 10% unhatched viable embryos remained in any control or treatment level egg incubation cup. At the completion of the hatching period (day 5), embryo survival in the control and solvent control averaged 88% and 86%, respectively. No statistically significant differences were determined between the control and the solvent control data. Thus, the data from both control groups were pooled and the pooled data set was used for statistical evaluation of treatment related effects. After the 30 days post-hatch exposure period, total length, wet weight and dry weight of the test organisms was statistically significantly reduced at the highest test item concentration compared to the pooled control.

In an early life stage study with fathead minnows (*Pimephales promelas*) the overall NOEC (34 d) for triticonazole was determined to be 0.021 mg a.s./L based on mean measured concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Triticonazole technical (BAS 595 F; Reg. no.: 4378513), batch no. OP9750057, purity: 90.52%.

B. STUDY DESIGN

Test species: Fathead minnow (*Pimephales promelas*); eggs less than 24 hours post fertilization at test initiation, eggs obtained from in-house culture, brood stock originally obtained from

Test design: Flow-through system (34 d); 5 test item concentrations plus a dilution water control and a solvent control; 2 replicate test chambers per treatment; 1 incubation cup per replicate test vessel with 60 fertilized eggs in each; reduction of surviving larvae to 40 organisms per replicate test vessel on day 5; daily assessment of survival until end of hatch; daily recording of sublethal effects (behavior and appearance) and daily removal of dead larvae during 30 days post-hatch exposure; estimation of post-hatch survival at least twice weekly; determination of wet weight, dry weight and total length at test end.

Endpoints: NOEC values based on embryo survival at hatch, survival and growth (wet weight, dry weight and total length) at test termination.

Test concentrations: Control (dilution water), solvent control (0.0129µL acetone/L), 0.0014, 0.0036, 0.009, 0.022 and 0.056 mg triticonazole/L (nominal); corresponding to mean measured concentrations of 0.017, 0.037, 0.087, 0.021 and 0.051 mg a.s./L.

Test conditions: Test vessels: glass aquaria (39 x 20 x 25 cm); test volume: approx. 15 L; embryo incubation cups: glass jars (5 cm diameter, 8 cm high) with 40-mesh Nitex® screen bottoms; dilution water: aerated well water from two natural sources; temperature: 25°C - 26°C; pH 6.7 - 7.5; oxygen content: 5.2 mg/L - 8.7 mg/L; total hardness: 36 - 40 mg $CaCO_3$; total alkalinity: 28 - 30 $CaCO_3$; specific conductance: 160 - 170 µmhos/cm; light intensity: 20 - 100 foot candles (220 - 1100 lux); photoperiod: 16 hours light : 8 hours dark; flow rate: approx. 6.9 volume additions per 24 hours per vessel (90% replacement time of approx. 7.0 h); feeding (from day 4 on): live brine shrimp nauplii (*Artemia salina*) *ad libitum* three times daily until 24 h before study termination; no aeration; gentle oscillation of embryo incubation cups in test solutions.

Analytics: Analytical verification of test item concentrations was conducted using an HPLC-method with UV-detection.

Statistics: Descriptive statistics; t-test for comparison of the dilution water control and solvent data ($\alpha = 0.05$); William's test to determine NOEC values for survival and growth ($\alpha = 0.05$).

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of the test item concentrations was conducted in all concentrations at test initiation, at regular intervals during the study and at test end. Mean recoveries of triticonazole ranged from 68% to 136% of nominal concentrations during the test period. The following biological results are based on mean measured concentrations.

Biological results: Day of hatch was considered to be exposure day 4, when no more than 10% unhatched viable embryos remained in any control or treatment level egg incubation cup. At the completion of the hatching period (day 5), embryo survival in the control and solvent control averaged between 88% and 86%, respectively. No statistically significant differences were determined between the control and the solvent control data (t-test; $\alpha = 0.05$). Thus, the data from both control groups were pooled and the pooled data set was used for statistical evaluation of treatment related effects. After the 30 days post-hatch exposure period, total length, wet weight and dry weight of the test organisms was statistically significantly reduced at the highest test item concentration compared to the pooled control (Williams' test, $\alpha = 0.05$). The results are summarized in Table 8.2.2.1-3.

Table 8.2.2.1-3: Chronic toxicity of triticonazole to fathead minnow (*Pimephales promelas*) in a fish early life stage test (34 d)

Concentration [mg a.s./L] (nominal)	**Control**	**Solvent control**	**0.0014**	**0.0036**	**0.009**	**0.022**	**0.056**
Concentration [mg a.s./L] (mean measured)	--	--	**0.017**	**0.037**	**0.087**	**0.021**	**0.051**
Mean embryo survival at hatch [%]	88	86	87	84	86	86	88
Mean larval survival (day 34) [%]	90	88	94	91	91	98	90
Mean total length (day 34) [mm]	31.6 ± 2.1	32.2 ± 1.8	32.5 ± 2.0	32.0 ± 2.0	31.7 ± 1.9	31.5 ± 2.3	30.5 ± 2.0 *
Mean wet weight (day 34) [g]	0.29 ± 0.05	0.31 ± 0.06	0.32 ± 0.06	0.31 ± 0.06	0.31 ± 0.07	0.29 ± 0.06	0.27 ± 0.06 *
Mean dry weight (day 34) [g]	0.076 ± 0.02	0.083 ± 0.02	0.085 ± 0.02	0.083 ± 0.02	0.079 ± 0.02	0.076 ± 002	0.067 ± 0.02 *
	Endpoint [mg triticonazole/L] (mean measured)						
$NOEC_{overall}$ (34 d)	0.021						

* Statistically significantly reduced compared to the pooled control (Williams' test, $\alpha = 0.05$).

III. CONCLUSION

In an early life stage study with fathead minnows (*Pimephales promelas*) the overall NOEC (34 d) for triticonazole was determined to be 0.021 mg a.s./L based on mean measured concentrations.

CA 8.2.2.2 Fish full life cycle test

The following fish full life cycle test on fathead minnow performed with the active substance triticonazole is not required for registration in the EU. The study was conducted due to U.S. data requirements and has not been evaluated previously on EU level. It is now included for completeness.

Report:	CA 8.2.2.2/1 [redacted] 2008a BAS 595 F (Triticonazol) - Life cycle test of the fathead minnow (Pimephales promelas) in a flow through system 2008/1028361
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

A fish full life cycle (FFLC) study on fathead minnow (*Pimephales promelas*) with triticonazole was performed under flow-through aerated conditions for 6 months with 5 concentrations of the test substance and a dilution water control. The test was started with the exposure of freshly fertilized eggs derived from 14 egg clutches. The test organisms were exposed in 4 replicates per concentration group 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 68 and a pairwise exposure was started on day 95. The reproduction of 8 pairs per test group was monitored exactly over 14 days. The F2-generation was started on days 116 - 117 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 61 days after insertion of eggs. Nominal concentrations of triticonazole were: 0 (control), 0.003, 0.006, 0.012, 0.024 and 0.048 mg a.s./L; mean measured concentrations were 0, 0.029, 0.006, 0.0114, 0.0229 and 0.0462 mg a.s./L. In this study effects on survival, appearance and behavior, and growth of fathead minnow were observed over two generations. The reproduction of the F1-generation was monitored by recording egg-laying and fertility.

The following results are based on nominal and mean measured concentrations. In the nominal test concentrations up to and including 0.012 mg a.s./L (0.0114 mg a.s./L mean measured) no treatment related effects on the test organisms were observed. In the tests groups exposed to a nominal concentration of 0.024 mg a.s./L (0.0229 mg a.s./L mean measured concentration) the body length and weight of the fish of the F2-generation were statistically significantly decreased. In the test groups exposed to a nominal concentration of 0.048 mg a.s./L (0.0462 mg a.s./L mean measured concentration) a statistically significantly decreased body length of the fish of the F2-generation was observed. However, the decrease was less pronounced than in the lower test concentration of 0.024 mg a.s./L and within the normal variability. Nevertheless, the effects on growth in both test groups (0.024 and 0.048 mg a.s./L) were considered for the evaluation following a very conservative approach. In the 0.048 mg a.s/L concentration group a slight delay of the time to maturity in the F1-generation could not be excluded. No test substance-related effects on survival of both generations, on the growth of the F1-generation or on the reproduction of the F1-generation (fertility and fecundity) were observed in any of the test groups.

In a full life cycle test conducted under flow through conditions, fathead minnow were exposed to triticonazole over a period of 6 months. The overall NOEC identified in this study was 0.012 mg a.s./L (nominal concentration) and 0.0114 mg a.s./L (mean measured concentration). The LOEC was 0.024 mg a.s./L (nominal concentration) and 0.0229 mg a.s./L (mean analytically determined concentration). The effects observed in this concentration group were a statistically significantly reduced growth of the F2-generation.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Triticonazole (BAS 595 F, Reg. No. 4378513), batch no. COD-000601, purity: 93.6%.

B. STUDY DESIGN

Test species: Fathead minnow (*Pimephales promelas*), freshly fertilized eggs (< 6 hours), source: [redacted]

Test design: Flow-through system (6 months); 5 test concentrations plus control, 4 replicates per treatment with 25 fertilized eggs in each. On day 36 the number of individuals/replicate was reduced to 15. Pairwise exposure was started on day 95. F2-generation was started on days 116 - 117 with 4 replicates/test group, with 25 eggs in each. F2-generation was exposed for 61 days after insertion of eggs. Survival, appearance and behavior, and growth were observed over two generations. Reproduction of the F1-generation was monitored by recording egg-laying and fertility.

Endpoints:	NOEC values based on survival, appearance and behavior, growth and reproduction.
Test concentrations:	0 (control), 0.003, 0.006, 0.012, 0.024 and 0.048 mg a.s./L (nominal). 0, 0.0029, 0.006, 0.0114, 0.0229 and 0.0462 mg a.s./L (mean measured of the three study parts).
Test conditions:	Test vessels: cylindrical glass vessels for eggs, larvae and juveniles; 30 L glass aquaria from day 18 on; stainless steel aquaria (29 cm x 21 cm x 22 cm) for spawning pairs. Dilution water: aerated, non-chlorinated, filtered tap water (mixed with deionized water); temperature 25 ± 1°C. F1: pH 7.5 - 7.8; oxygen content 6.0 - 8.5 mg/L; total hardness: 100 - 102 mg; conductivity 257 - 269 µSi; acid capacity 2.12 - 2.18 mmol/L; F1 pair groups: pH 7.6 - 8.0; oxygen content 6.1 - 8.4 mg/L; total hardness: 98 - 104 mg; conductivity 258 µSi; acid capacity 2.16 mmol/L; F2: pH 7.2 - 7.9; oxygen content 6.5 - 10.1 mg/L; total hardness: 102 - 104 mg; conductivity 264 - 270 µSi; acid capacity 2.22 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. Light intensity: F1: 1.7 L test vessels: 255 - 515 lux, glass aquaria (24 L): 102 - 230 Lux; F1 pair groups: stainless steel aquaria (9 L): 87 - 257 lux; F2: 1.7 L vessels: 264 - 492 lux, glass aquaria (24 L): 162 - 419 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, two times daily. Approximately 24 hours before sacrifice no feeding of animals was performed. Aeration: from day 22 (F1), from day 106 (F1 pair groups) or from day 145 (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 growth data; one-sided Wilcoxon-Test for number of eggs/day and fertility and two-sided for number of clutches/day, respectively.

II. RESULTS AND DISCUSSION

Analytical measurements: The control groups were free of test substance contaminations over the whole exposure period. The mean analytically determined concentrations of the test substance in the test water were generally in the range of ± 20% of the nominal concentration, but some deviations occurred during the 6 months exposure period.

Values below 80% of the nominal concentration were observed on one occasion at the test item concentration of 0.012 mg a.s./L in the F1-generation until reproduction and on one occasion during exposure of the F1-generation pair groups at the test concentrations of 0.003 and 0.048 mg a.s./L. In the F2-generation values below 80% were determined during the first week of exposure and on one additional occasion in the test groups 0.012 and 0.048 mg a.s./L and on two occasions in the test concentration of 0.024 mg a.s./L. The lowest value determined in the concentration of 0.012 mg a.s./L (= NOEC) was 65% of the nominal concentration over less than one day at the start of the F1-generation and 74% of nominal during the exposure of the F2-generation. Therefore, the analytical results for this concentration confirm that it is not likely that test substance effects were underestimated because of low actual concentrations.

Deviations from the nominal concentration > 120% of the nominal concentration (up to 140%) were observed on 2 - 3 occasions during the exposure of the F1-generation until formation of pair groups. During exposure of the pair groups a concentration of 0.276 mg a.s./L (574% of nominal) was determined in a sample relevant for 4 of the 8 pair groups of the 0.048 mg a.s./L group and 147% of nominal was determined for the other 4 replicates at the same time. It was concluded that these samples were contaminated and that the values were not actually that high, however, this assumption could not be proved by additional measurements. Since the concentration of 0.048 mg a.s./L was above the LOEC, a possible peak exposure would not change the interpretation of the overall results of the study.

During exposure of the F2-generation the measured concentrations exceeded 120% of the nominal concentration on one occasion in the test concentrations 0.003, 0.006, 0.012 and 0.048 mg a.s./L. The highest measured concentrations in 0.024 mg a.s./L group were 139% of nominal during exposure of the F1-generation and 120% of nominal during exposure of the F2-generation. In conclusion, it is not likely that deviations from the nominal concentration had an influence on the determination of the LOEC.

The mean analytical values are presented in the following table.

Table 8.2.2.2-1: Mean analytically determined concentration for triticonazole during the 6 month FFLC study

mg/L (nominal)	F1-generation day 0-95	F1-generation day 95-131, pair groups	F2-generation day 0-61 after egg insertion
0	Not detectable	Not detectable	Not detectable
3	0.0031 ± 0.0004 mg/L (103%)	0.0027 ± 0.00018 mg/L (91%)	0.003 ± 0.00065 mg/L (100%)
6	0.0061 ± 0.00102 mg/L (101%)	0.0058 ± 0.00025 mg/L (96%)	0.006 ± 0.00146 mg/L (100%)
12	0.0117 ± .00225 mg/L (97%)	0.011 ± 0.00054 mg/L (92%)	0.0116 ± 0.00203 mg/L (97%)
24	0.0246 ± 0.00372 mg/L (103%)	0.0216 ± 0.00151 mg/L (90%)	0.0225 ± 0.00382 mg/L (94%)
48	0.0485 ± 0.00594 mg/L (101%)	0.0425 ± 0.00373 mg/L (89%)	0.0477 ± 0.00771 mg/L (99%)

The following biological results are based on nominal and mean measured concentrations.

In the nominal test concentrations up to and including 0.012 mg a.s./L (0.0114 mg a.s./L mean measured) no treatment related effects on the test organisms were observed. In the tests group exposed to a nominal concentration of 0.024 mg a.s./L (0.0229 mg a.s./L mean measured concentration) the body length and weight of the fish of the F2-generation was statistically significantly decreased. In the test group exposed to a nominal concentration of 0.048 mg a.s./L (0.0462 mg a.s./L mean measured concentration) a statistically significantly decreased body length of the fish of the F2-generation was observed. However, the decrease was less pronounced than in the lower test concentration 0.024 mg/L and within the normal variability. Nevertheless, the effects on growth in both test groups (0.024 and 0.048 mg a.s./L) were considered for the evaluation following a very conservative approach. In the 0.048 mg a.s/L group a slight delay of the time to maturity in the F1-generation could not be excluded. No test substance-related effects on survival of both generations, on the growth of the F1-generation or on the reproduction of the F1-generation (fertility and fecundity) were observed in any of the test groups. The results are summarized in Table 8.2.2.2-2.

Table 8.2.2.2-2: Chronic toxicity (FFLC, 6 months) of triticonazole on fathead minnow (*Pimephales promelas*)

Concentration [mg a.s./L] nominal			**Control**	**0.003**	**0.006**	**0.012**	**0.024**	**0.048**
Concentration [mg a.s./L] mean measured over all study parts			**Control**	**0.0029**	**0.006**	**0.0114**	**0.0229**	**0.0462**
Survival	F1	start - hatch [%]	84	86	87	91	89	89
		hatch - swim up [%]	92	94	87	90	90	90
		swim up - reduction [%]	97	95	99	96	98	98
		reduction - reproduction [%]	100	100	98	95	98	100
		start reproduction - sacrifice [%]	93	100	97	95	98	100
	F2	start - hatch [%]	94	93	94	95	97	96
		hatch - swim up [%]	91	95	93	95	93	95
		swim up - end [%]	90	94	98	90	93	92
Growth	F1	length on day 36 [cm]	2.77	2.77	2.68	2.60**	2.68	2.73
		deviation [%]	--	-0.1	-3.3	-6.2	-3.5	-1.6
		length on day 68 [cm]	4.43	4.82**	4.71*	4.61	4.63	4.66
		deviation [%]	--	+8.9	+6.4	+4.2	+4.6	+5.3
		male length at sacrifice [cm] pairs	6.70	6.86	6.61	6.93	6.73	6.69
		female length at sacrifice [cm] pairs	5.39	5.73	5.71	5.67	5.61	5.54
		male weight at sacrifice [g] pairs	4.46	4.65	4.44	4.37	4.22	4.43
		female weight at sacrifice [g] pairs	2.10	2.33	2.24	2.32	2.10	2.02
	F2	length at day 35 [cm]	2.47	2.62**	2.55	2.53	2.48	2.66**
		deviation [%]	--	+6.3	+3.2	+2.5	+0.5	+7.7
		length at end [cm]	4.52	4.44	4.42	4.43	**4.25****	**4.29****
		deviation [%]	--	-1.8	-2.3	-2.1	-6.1	-5.2
		weight at end [g]	0.94	0.92	0.86	0.87	**0.76****	0.89
		deviation [%]	--	-2.4	-8.6	-7.7	-18.8	-5.8
Reproduction	F1	% fertility	98.6	98.4	98.3	99.0	99.4	98.3
		eggs/female/day	17.9	17.0	16.4	17.3	17.4	14.3
		clutches/female/day	0.225	0.171	0.175	0.175	0.133	0.217
Endpoints [mg a.s./L]								
Overall NOEC (nominal)			0.012					
Overall NOEC (mean measured)			0.0114					

* 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**

III. CONCLUSION

In a full life cycle test conducted under flow through conditions, fathead minnow were exposed to triticonazole over a period of 6 months. The overall NOEC identified in this study was 0.012 mg a.s./L (nominal concentration) and 0.0114 mg a.s./L (mean measured concentration). The LOEC was 0.024 mg a.s./L (nominal concentration) and 0.0229 mg a.s./L (mean analytically determined concentration). The effects observed in this concentration group were a statistically significantly reduced growth of the F2-generation.

The following fish full life cycle test on fathead minnow performed with the active substance triticonazole is not required for registration in the EU. The study was conducted due to U.S. data requirements and has not been evaluated previously on EU level. It is now included for completeness.

Report:	CA 8.2.2.2/2 [redacted] 2012a BAS 595 F (Triticonazole) - Life cycle toxicity test on the fathead minnow (Pimephales promelas) in a flow through system 2012/1079000
Guidelines:	EPA 72-5, EPA 850.1500
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

The chronic toxicity of triticonazole to fathead minnow (*Pimephales promelas*) was evaluated in a 257 d full life-cycle test under flow-through conditions. The test was started with the exposure of fathead minnow embryos (< 7 hours old). Each test group initially consisted of 200 eggs evenly distributed among four replicates. After hatching (day 5, end of hatch) the number of individuals per replicate was reduced to 25 and the fish were allowed to grow and mature with further periodic reductions of fish per test group so that biological loading requirements were not exceeded. Egg laying was first observed on day 81 and, on day 143, sexually mature fathead minnows were placed into spawning groups of 2 males and 3 females (four spawning groups per test group). Egg production was enumerated daily for 114 days starting on day 144. The parental (F0) exposure period lasted for a total of 257 days. Spawning groups were used to start the second generation (F1-generation) on day 153. The F1-generation consisted of 2 replicates per test group, initiated with 50 pooled eggs from the corresponding F0 test group. After hatching (day 158, end of hatch) the number of individuals per replicate was reduced to 25 and the F1 fish were allowed to grow for a total of 62 days. Effects on hatching success, time to hatch, survival, growth (body length and weight) and signs of toxicity (appearance/behavior) were observed over the two generations. Additionally the reproduction of the F0-generation was evaluated by assessing fecundity (eggs per female reproductive day) and fertility.

The following biological results are reported both based on nominal and time weighted mean measured (TWM) concentrations. There was no adverse treatment-related effect on hatching success, time to hatch, survival, signs of toxicity (appearance/behavior), or reproduction (fertility and fecundity) in the F0-generation fish. Effects on fish length were observed in the F0-generation at the highest test item concentration. The F1-generation fish were exposed from the embryonic stage to approximately 8 weeks post-hatch. There was no adverse treatment-related effect on hatching success, time to hatch, or survival. F1-generation growth was reduced compared to the control at the highest test-item concentration and 14% of fish had morphological deformations after 8 weeks. Based on the design of the fish full life cycle study, a genetic factor (unrelated to test substance) cannot be excluded as the cause of the observed F1-generation responses. The most sensitive effect observed in this study was an adverse effect on growth in the F0 and F1 generations.

In a fish full life cycle test with fathead minnow (*Pimephales promelas*) the overall NOEC (257 d) for triticonazole was determined to be 0.048 mg a.s./L based on nominal concentrations and 0.0473 mg a.s./L based on time weighted mean measured concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Triticonazole (BAS 595 F; Reg. no.: 4378513), batch no. COD-001440, purity: 91.3%.

B. STUDY DESIGN

Test species: Fathead minnow (*Pimephales promelas*), eggs less 7 h post-fertilization at test initiation; parental fish obtained from [redacted]

Test design: Flow-through system (257 d); 5 test item concentrations plus a dilution water control; max. fish loading rate ≤ 0.5 g fish/L/day.

F0-generation: F0 embryo groups: 4 replicates per treatment with 50 fertilized eggs per replicate until end of hatch; F0 larval-juvenile groups: 4 replicates per treatment with 25 larvae per replicate until 8 weeks post-hatch; F0 juvenile-adult groups: 2 replicates per treatment with 25 fish in each from day 60 on until maturity; F0 reproduction groups: 4 replicates per treatment with 2 reproduction groups of 2 male and 3 female fish per test vessel plus 1 group of reserve fish (1 male and 2 female, kept in a mesh basket without spawning tiles) per test vessel.

F1-generation: F1 embryo groups: 2 replicates per treatment with 50 embryos in each initiated on day 153; F1 larval-juvenile groups: larval fish were impartially reduced to 25 per replicate on day 5.

	Hatching success, time to hatch, survival, growth (body length and weight) and signs of toxicity were observed over two generations. Reproduction of the F0-generation was monitored by recording egg-laying and fertility.
Endpoints:	NOEC values based on survival, appearance and behavior, growth and reproduction.
Test concentrations:	Control (dilution water), 0.006, 0.012, 0.024, 0.048 and 0.096 mg a.s./L (nominal), corresponding to time weighted mean measured concentrations of 0.007, 0.0125, 0.0238, 0.0473 and 0.0937 mg a.s./L.
Test conditions:	Test vessels: 1.7 L cylindrical glass vessels for F0 and F1 embryo groups; 9 L stainless steel aquaria for F0 and F1 larval-juvenile groups; 45 L glass aquaria for F0 juvenile-adult groups; 45 L glass aquaria divided into 3 compartments using a stainless steel mesh basket, two of the compartments had spawning tiles for F0 reproduction groups. Dilution water: aerated, charcoal filtered, non-chlorinated drinking water (mixed with deionized water). F0: water temperature 24°C - 26°C; pH 7.6 - 8.3; oxygen content 6.1 - 8.2 mg/L; light intensity 96 - 230 lux (without lid), 34 - 178 lux (under transparent lid); water hardness 1.02 - 1.04 mmol/L. F1: water temperature 25°C - 26°C; pH 7.7 - 8.4; oxygen content 5.8 - 8.3 mg/L; light intensity 115 - 160 lux (without lid), 53 - 94 lux (under transparent lid); water hardness 1.00 - 1.04 mmol/L. Flow rate: 1.7 L- glass test vessels (embryo/larval exposure): > 0.5 L/hour (> 5 fold volume exchange); 9 L stainless steel aquaria (juvenile exposure): 1.9 L/hour (5 fold volume exchange); 45 L glass aquaria (juvenile/adult exposure): 7.5 L/hour (4-fold volume exchange). Water conductivity: 258 - 274 µS. Photoperiod: 16 hours light : 8 hours dark. Feeding: live brine shrimp nauplii (*Artemia* sp.) and a fine milled commercial fish diet (“TetraMin”) from day 5 on at least once daily. Aeration: generally no aeration but slight aeration through the end of exposure.
Analytics:	Analytical verification of test item concentrations was conducted using an HPLC-method with MS-detection.
Statistics:	Descriptive statistics; determination of NOEC values using Fisher’s exact test (one-sided) for survival data, Wilcoxon-Test (one-sided) for reproduction data and Dunnett’s test (two-sided) for growth data ($p < 0.01$ or $p < 0.05$).

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of the test item concentrations was conducted for all exposure phases of the F0- and F1-generation, as test organisms were transferred to new exposure vessels between exposure phases with a change in the test solution delivery system.
During the F0 embryo and larval-juvenile exposure phase time-weighted mean measured concentrations were within the range ± 20% of the nominal concentrations. Most individually measured concentrations during this exposure phase were within the range ± 20% of the TWM concentrations; however, deviations greater than 20% of TWM occurred on 2 occasions out of the exposure period.
During the juvenile-adult exposure phase mean measured concentrations of the exposure period were within the range ±20% of the nominal concentrations, with the exception of the lowest test item treatment. On day 82 the measured value in one replicate was 0.0588 mg/L, approx. a factor of 10 over nominal. Most individually measured concentrations during this exposure phase were within the range ± 20% of the TWM concentrations; however deviations greater than ± 20% of TWM were measured on 3 days out of the exposure period. During the F0 reproduction exposure phase and the exposure of the F1 generation all measured concentrations were within the range ± 20% of the TWM concentrations.

Therefore, over the nearly 9 month exposure period in this study, most individually measured concentrations during the exposure period were within the range ± 20% of the TWM concentrations; however, isolated deviations exceeding ± 20% of the TWM concentration were recorded in all treatment groups. However, at no time did mean measured test concentrations overlap between test levels and in all cases the deviations were concentration increases, consistent with a worst case scenario exposure. Taking into account the duration of the exposure period, overall variability was not excessive. The following biological results are reported both based on nominal and TWM concentrations.

Biological results: There was no adverse treatment-related effect on hatching success, time to hatch, survival, signs of toxicity (appearance/behavior), or reproduction (fertility and fecundity) in the F0-generation fish. Effects on fish length were observed in the F0-generation at the highest test item concentration. The F1-generation fish were exposed from the embryonic stage to approximately 8 weeks post-hatch. There was no adverse treatment-related effect on hatching success, time to hatch, or survival. F1-generation growth was reduced compared to the control at the highest test-item concentration and 14% of fish had morphological deformations after 8 weeks. Based on the design of the fish full life cycle study, a genetic factor (unrelated to test substance) cannot be excluded as the cause of the observed F1-generation responses. The most sensitive effect observed in this study was an adverse effect on growth in the F0 and F1 generations. The results are summarized in Table 8.2.2.2-3.

Table 8.2.2.2-3: Chronic toxicity of triticonazole to fathead minnow (*Pimephales promelas*) in an fish full life cycle test (257 d)

Concentration [mg a.s./L] (nominal)			Control	0.006	0.012	0.024	0.048	0.096
Concentration [mg a.s./L] (TWM concentration)			Control	0.007	0.0125	0.0238	0.0473	0.0937
Survival [1]	F0	hatching success [%]	92	93	93	93	94	92
		larval survival at end of hatch (day 5) [%]	99	99	99	99	98	99
		post hatch survival (day 5 (after reduction) - day 60) [%]	97	98	97	99	98	96
		juvenile-adult survival (day 60 (after reduction) - day 143) [%]	98	98	100	98	98	98
		adult survival (day 143 (after reduction) -day 257 (sacrifice)) [%]	95	95	90	95	100	95
	F1	hatching success [%]	95	95	94	95	95	95
		larval survival at end of hatch (F1 day 5) [%]	99	100	100	100	99	99
		post hatch survival (F1 day 5 (after reduction) - day F1 day 62 (sacrifice)) [%]	98	100	100	98	100	100
Growth [2]	F0	length on day 31 (cm) #	2.1	2.1	2.1	2.1	2.1	**2.0** **
		length on day 60 (cm) #	3.4	3.5 ** a)	3.5 **, a)	3.3	3.4	**3.3** *
		length on day 60 (cm) §	3.7	3.7	3.7	3.5 **, b)	3.6	3.6
		wet weight on day 60 (g) §	0. 5	0.5	0.5	0.4 *, b)	0.4	0.4
		male length, day 143 (cm) §	5.9	6.4 *, a)	6.2	6.2	6.1	5.8
		female length, day 143 (cm) §	5.4	5.7	5.7	5.1	4.6	5.0
		male wet weight, day 143 (g) §	2.9	3.5	3.2	3.2	3.1	3.1
		female wet weight, day 143 (g) §	2.1	2.3	2.2	1.6	1.2	1.6
		male length at day 257 (cm)	6.4	6.0	6.2	6.5	6.3	**5.8** *
		female length at day 257 (cm)	5.7	5.8	5.7	5.8	5.5	5.7
		male wet weight at day 257 (g)	4.1	3.5	3.4	4.3	4.0	3.3
		female wet weight at day 257(g)	2.2	2.3	2.2	2.2	2.0	2.3
	F1	length on F1 day 33 (28 days post hatch) (cm) #	2.4	2.5 ** a)	2.3	2.4	2.5	**2.2** **
		length on F1 day 62 (57 days post hatch) (cm)	4.3	4.3	4.2	4.3	4.3	**3.9** **
		wet weight on F1 day 62 (57 days post hatch) (g)	0.8	0.8	0.8	0.8	0.8	**0.7** **
Reproduction [3]	F0	% fertile eggs	99.3	99.3	99.4	99.6	99.1	99.2
		fecundity (total eggs/female reproductive day)	21	25	20	14	17	17

Sublethal effects [c)]	F0	Symptoms at day 257	0	0	0	N	0	0
	F1	Symptoms at day 215	0	0	0	0	0	D
			Endpoints [mg triticonazole/L]					
Overall NOEC (257 d) (nominal)			0.048					
Overall NOEC (257 d) (TWM)			0.0473					

TWM - time weighted mean measured
Deviations which are considered to be substance-related are printed **bold**
** $p < 0.01$
* $p < 0.05$
\# photographic measurement
§ total body length or wet weight of sacrificed fish
1) Statistically significant differences compared to the control were determined using Fisher's exact test (one-sided) for survival data.
2) Statistically significant differences compared to the control were determined using Dunnett's test (two-sided) for growth data.
3) Statistically significant differences compared to the control were determined using Wilcoxon-Test (one-sided) for reproduction data.
a) Larger than control fish, not an adverse or treatment-related effect
b) Not a treatment-related effect
c) Symptoms: N = abdominal extension, D = deformations.

III. CONCLUSION

In a fish full life cycle test with fathead minnow (*Pimephales promelas*) the overall NOEC (257 d) for triticonazole was determined to be 0.048 mg a.s./L based on nominal concentrations and 0.0473 mg a.s./L based on time weighted mean measured concentrations.

CA 8.2.2.3 Bioconcentration in fish

Bioconcentration in fish was addressed in respective studies, which were evaluated already during the previous Annex I inclusion process. No further studies are required or were conducted.

CA 8.2.3 Endocrine disrupting properties

Based on the physical, chemical and structural characteristics of the active substance triticonazole as well as based on results of available long-term fish studies (see summaries for ELS and FLC studies above) and studies on terrestrial vertebrates (see chapter MCA-8.1.5) there is no indication of endocrine disrupting properties of this active substance.
This is supported by a peer-reviewed scientific study by Hermsen et al. (2011) on relative embryotoxicity of two classes of chemicals in a modified zebrafish embryotoxicity test. Triticonazole showed minor effects only in the highest concentration tested and was the least toxic of all the triazoles tested. Thus, this paper raised no concerns with regards to endocrine disrupting (ED) effects. The literature study was considered as relevant but not reliable (RI 3); thus, reference is made to this study but no study summary is provided. For details please see the literature search and evaluation files also provided within the submission for Annex I Renewal.
Finally, no endocrine activity of triticonazole was detected by several toxicological impact assessments, *i.e.* triticonazole did not exert significant androgenic or anti-androgenic effects and no estrogenic or anti-estrogenic effects (see MCA 5.8.3).
Thus, based on available information it can be concluded that there is no ecotoxicological concern regarding the ED potential of triticonazole and no further studies are required.

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 RPA 406203 was (wrongly) not listed in the "Application" document submitted for the triticonazole AIR 3 renewal process. The study was conducted due to U.S. requirements and has not been evaluated previously on EU level. It is now included in support of the aquatic risk assessment.

Report: CA 8.2.4.1/1
Putt A.E., 1998a
RPA 406203 - Acute toxicity to daphnids (Daphnia magna) under flow-through conditions
C044320
Guidelines: EPA 72-2
GLP: yes

Executive Summary

In a 48-hour flow-through acute toxicity laboratory study water flea neonates were exposed to a dilution water control and a solvent control and to RPA 406203 at nominal concentrations of 1.3, 2.2, 3.6, 6.0 and 10 mg RPA 406203/L (corresponding to mean measured concentrations of 0.93, 1.8, 2.4, 4.0 and 6.1 mg/L) in 2 replicates per concentration containing 10 daphnids each. Daphnids were observed for immobility 24 hours and 48 hours after start of exposure.

The following biological results are based on mean measured concentrations. After 48 h of exposure, no immobility of daphnids was observed in the control and at mean measured test item concentrations of up to and including 1.8 mg/L, whereas 5%, 20%, 70% and 95% immobility were observed in the solvent control and the test item concentrations of 2.4 mg/L, 4.0 mg/L and 6.1 mg/L, respectively. Sub-lethal effects (*i.e.* lethargy) were observed at the three highest test item concentrations after 48 hours of exposure.

In a 48-hour flow-through acute toxicity study with *Daphnia magna*, the EC_{50} of RPA 406203 was determined to be 3.4 mg/L based on mean measured concentrations. The NOEC was determined to be 1.8 mg/L (mean measured).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: RPA 406203 (Reg. No. 5079359; photo-metabolite of triticonazole), batch no. OB0012; purity: 99.8%.

B. STUDY DESIGN

Test species: Water flea (*Daphnia magna* STRAUS), neonates collected from in house culture; < 24 h old at test initiation.

Test design: Flow-through system (48 hours), 5 test item concentrations plus dilution water control and solvent control, 2 replicates per treatment with 10 daphnids in each replicate; assessment of immobility after 24 and 48 hours.

Endpoints: NOEC and EC_{50} based on immobility of daphnids.

Test concentrations: Control (dilution water), solvent control (0.50 mL acetone/L), 1.3, 2.2, 3.6, 6.0 and 10 mg RPA 406203/L (nominal), corresponding to mean measured concentrations of 0.93, 1.8, 2.4, 4.0 and 6.1 mg/L.

Test conditions: 400 mL glass beakers, test volume: 325 mL, dilution water: fortified well water, filtered and aerated; flow rate: approx. 10 volume replacements per vessel and per day; pH 7.9 - 8.2; oxygen content: 6.8 - 8.5 mg/L (75% - 94%); temperature: 20 - 21°C; total hardness: 160 -170 mg $CaCO_3$/L; total alkalinity: 120 mg $CaCO_3$/L; conductivity: 500 µS/cm; photoperiod: 16 h light : 8 h dark; light intensity: 320 - 540 lux; no feeding.

Analytics: Analytical verification of test item concentrations was conducted using an HPLC-method with UV detection.

Statistics: Descriptive statistics, moving average angle analysis for determination of the EC_{50} value.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of the test item concentrations was conducted in each concentration at the beginning and at the end of the test. Measured values for RPA 406203 ranged from 70.0% to 100.0% of nominal concentrations at test initiation and from 52.0% to 63.6% of nominal at test termination. The following biological results are based on mean measured concentrations.

Biological results: After 48 h of exposure, no immobility of daphnids was observed in the control and at mean measured test item concentrations of up to and including 1.8 mg/L, whereas 5%, 20%, 70% and 95% immobility were observed in the solvent control and the test item concentrations of 2.4 mg/L, 4.0 mg/L and 6.1 mg/L, respectively. Sub-lethal effects (*i.e.* lethargy) were observed at the three highest test item concentrations after 48 hours of exposure. For results see Table 8.2.4.1-1.

Table 8.2.4.1-1: Effects of RPA 406203 on *Daphnia magna* immobility

Concentration [mg/L] (nominal)	**Control**	**Solvent control**	**1.3**	**2.2**	**3.6**	**6.0**	**10**
Concentration [mg/L] (mean measured)	**--**	**--**	**0.93**	**1.8**	**2.4**	**4.0**	**6.1**
Immobility (24 h) [%]	0	5	0	0	15	20	55
Immobility (48 h) [%]	0	5	0	0	20	70	95
	Endpoints [mg RPA 406203/L] (mean measured)						
EC_{50} (48 h)	3.4 (95% confidence limits: 3.0 - 3.9)						
NOEC (48 h)	1.8						

III. CONCLUSION

In a 48-hour flow-through acute toxicity study with *Daphnia magna*, the EC_{50} of RPA 406203 was determined to be 3.4 mg/L based on mean measured concentrations. The NOEC was determined to be 1.8 mg/L (mean measured).

The following acute toxicity study on *Daphnia magna* performed with RPA 406203 is provided in support of the aquatic risk assessment and has not been evaluated previously on EU level. The study was (wrongly) not listed in the "Application" document submitted for the triticonazole AIR 3 renewal process.

Report:	CA 8.2.4.1/2 Janson G.-M., 2009a Acute toxicity of Reg.No. 5079359 (metabolite of BAS 595 F) to Daphnia magna STRAUS in a 48 hour static test 2009/1075083
Guidelines:	OECD 202, EPA 850.1010
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

In a 48-hour static acute toxicity laboratory study water flea neonates were exposed to a dilution water control and a solvent control and to RPA 406203 at nominal concentrations of 3.5, 4.6, 5.9, 7.7 and 10 mg RPA 406203/L in 4 replicates per concentration containing 5 daphnids each. Daphnids were observed for immobility 24 hours and 48 hours after start of exposure.

The following biological results are based on nominal concentrations. After 48 h of exposure, no immobility of daphnids was observed in the control and at test item concentrations of up to and including 7.7 mg/L, whereas 15% immobility was observed at the highest test item concentration of 10 mg/L. No statistically significant effects compared to control data were observed up to and including the highest concentration tested.

In a 48-hour static acute toxicity study with *Daphnia magna*, the EC_{50} of RPA 406203 was determined to be > 10 mg/L based on nominal concentrations. The NOEC was determined to be ≥10 mg/L (nominal).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: RPA 406203 (Reg. No. 5079359; photo-metabolite of triticonazole), batch no. BESS0578; purity: 99.9% (tolerance ± 1%).

B. STUDY DESIGN

Test species: Water flea (*Daphnia magna* STRAUS), neonates collected from in house culture (originally obtained from Institute National de Recherché Chimique Appliquée, France); > 2 h < 24 h old at test initiation.

Test design: Static system (48 hours), 5 test item concentrations plus dilution water control and solvent control, 4 replicates per treatment with 5 daphnids in each; assessment of immobility after 24 and 48 hours.

Endpoints: NOEC and EC_{50} based on immobility of daphnids.

Test concentrations: Control (dilution water), solvent control (0.1 mL DMF/Cremophor (v:v, 9:1)/L), 3.5, 4.6, 5.9, 7.7 and 10 mg RPA 406203/L (nominal).

Test conditions: Glass vessels, test volume: 50 mL, dilution water: "M4" (Elendt medium); pH 7.94 - 8.04; oxygen content: 8.7 - 9.0 mg/L; temperature: 20.1 - 21.0°C; total hardness at test initiation: 2.4 mmol/L; conductivity at test initiation: 665 µS/cm; photoperiod: 16 h light : 8 h dark; light intensity: 324 - 638 lux; no feeding; no aeration.

Analytics: Analytical verification of test item concentrations was conducted using an HPLC-method with MS detection.

Statistics: Descriptive statistics; Fisher's Exact Test for determination of the NOEC ($p < 0.05$).

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of the test item concentrations was conducted in each concentration at the beginning and at the end of the test. Measured values for RPA 406203 ranged from 98.2% to 105.3% of nominal concentrations at test initiation and from 97.6% to 102.4% of nominal at test termination. As analytical data confirmed correct application of the test item, the following biological results are based on nominal concentrations.

Biological results: After 48 h of exposure, no immobility of daphnids was observed in the control and at test item concentrations of up to and including 7.7 mg/L, whereas 15% immobility was observed at the highest test item concentration of 10 mg/L. No statistically significant effects compared to control data were observed up to and including the highest concentration tested (Fisher's Exact Test, $p < 0.05$). For results see Table 8.2.4.1-2.

Table 8.2.4.1-2: Effects of RPA 406203 on *Daphnia magna* immobility

Concentration [mg/L] (nominal)	**Control**	**Solvent control**	**3.5**	**4.6**	**5.9**	**7.7**	**10**
Immobility (24 h) [%]	0	0	0	0	0	0	0
Immobility (48 h) [%]	0	0	0	0	0	0	15
	Endpoints [mg RPA 406203/L] (nominal)						
EC_{50} (48 h)	> 10						
NOEC (48 h)	≥ 10						

III. CONCLUSION

In a 48-hour static acute toxicity study with *Daphnia magna*, the EC_{50} of RPA 406203 was determined to be > 10 mg/L based on nominal concentrations. The NOEC was determined to be ≥10 mg/L (nominal).

From the literature search the following peer-reviewed scientific study was considered relevant and reliable although with restrictions (RI 2; as this a non-GLP study). For details please see the literature search and evaluation files also provided within the submission for Annex I Renewal. In this study acute toxicity tests were performed on *Danio rerio* and *Daphnia magna* with triazoles and benzotriazoles. In the following summary only the experimental data and results for *D. magna* and triticonazole are presented. The results for *D. rerio* and triticonazole are presented under point CA 8.2.1.

Report:	CA 8.2.4.1/3 Durjava M.K. et al., 2013a Experimental assessment of the environmental fate and effects of Triazoles and Benzotriazole 2015/1177633
Guidelines:	none
GLP:	no

Executive Summary

In a 48-hour static acute toxicity laboratory study, water flea neonates were exposed to triticonazole at a range of at least 5 concentrations in 4 replicates per concentration, containing 5 daphnids each. Daphnids were observed for immobility 48 hours after start of exposure.

The biological results are based on nominal concentrations. EC_{50} values for *D. magna* were determined after 48 hours of exposure.

In a 48-hour static acute toxicity study with *Daphnia magna* the EC_{50} of triticonazole was 9.56 mg a.s./L based on nominal concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Triticonazole (BAS 595 F), CAS no.: 131983-72-7; purchased from Fluka® Analytical (Sigma-Aldrich).

B. STUDY DESIGN

Test species: Water flea (*Daphnia magna* STRAUS), < 24 h old at test initiation.

Test design: Static system (48 hours), minimum of 5 test concentrations plus control, 4 replicates with 5 daphnids in each; assessment of immobility after 48 hours.

Endpoints: EC_{50} based on immobility of daphnids.

Test concentrations: Range of triticonazole concentrations (5 concentrations at least).

Test conditions: 100 mL glass beakers, test volume 20 mL; dilution water "M4-medium"; substances were dissolved in dimethyl sulphoxide (DMSO); pH, oxygen content and temperature not reported; dissolved oxygen levels and pH did not change significantly during the experiments.

Analytics: Chemical measurements of test item concentrations was conducted using a GC-method with MS detection.

Statistics: Descriptive statistics; non-linear regression curve fitting assuming a sigmoid dose–response curve for EC_{50} calculation.

II. RESULTS AND DISCUSSION

Analytical measurements: Chemical measurements of test item concentrations in the test cultures was conducted at the beginning and at the end of the test. The following biological results are based on nominal concentrations.

Biological results: The 48 h EC_{50} value for *D. magna* was determined to be 9.56 mg/L (95% confidence limits: 5.70 - 16.0 mg/L).

III. CONCLUSION

In a 48-hour static acute toxicity study with *Daphnia magna* the EC_{50} of triticonazole was 9.56 mg a.s./L based on nominal concentrations.

CA 8.2.4.2 Acute toxicity to an additional aquatic invertebrate species

The following acute toxicity study on the saltwater mysid *Americamysis bahia* performed with technical triticonazole is not required for registration in the EU and it has not been evaluated previously on EU level. However, the study was conducted due to U.S. data requirements and it is provided for completeness.
The 48-h LC_{50} obtained in the 96 h study on *A. bahia* is used as relevant endpoint for the risk assessment in accordance with the EU Regulation 283/2013 (European Commission, 2013) which describes the data requirements for active substances. Also the recent EFSA Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters (EFSA, 2013) advises to use the 48-h endpoint, which allows an easy comparison to the other standard acute invertebrate tests. Therefore only the 48-h results are shown below.

Report:	CA 8.2.4.2/1 Sousa J.V., 1998d Triticonazole technical - Acute toxicity to mysids (Mysidopsis bahia) under flow-through conditions B004421
Guidelines:	EPA 72-3
GLP:	yes

Executive Summary

In a 96-hour flow through acute toxicity laboratory study, saltwater mysids were exposed in a first exposure series to triticonazole at nominal concentrations of 0.64, 1.1, 1.8, 3.0 and 4.9 mg a.s./L (corresponding to mean measured concentrations of 0.60, 1.0, 1.7, 2.9 and 4.6 mg a.s./L) and in a second exposure series to triticonazole at nominal concentrations of 1.0, 1.7, 2.8, 4.7 and 7.9 mg a.s./L (corresponding to mean measured concentrations of 1.1, 1.8, 2.9, 4.9 and 7.5 mg a.s./L) in groups of 10 animals in glass aquaria with 2 replicates per concentration. Additionally, a dilution water control and a solvent control was set up in both exposure series. Saltwater mysids were observed for survival and symptoms of toxicity 0, 24, 48, 72 and 96 hours after start of exposure.

The biological results are based on mean measured concentrations (measured over the 96 h study period). After 48 hours in the first exposure series no mortality and toxic effects were observed in the control, the solvent control and in the tested concentrations up to and including 1.7 mg triticonazole/L. Mortality rates of 20% and 30% were observed in the 2.9 and 4.6 mg triticonazole/L test item groups after 48 hours. At concentrations of 2.9 mg triticonazole/L and above, signs of toxicity were evident among mysids, including loss of equilibrium and lethargy.
After 48 hours in the second exposure series no mortality and toxic effects were observed in the control, the solvent control and in the tested concentrations up to and including 1.1 mg triticonazole/L. Mortality rates of 55%, 20% and 40% were observed in the 2.9, 4.9 and 7.5 mg triticonazole/L test item groups after 48 hours, respectively. At the four highest test item concentrations signs of toxicity were evident among mysids, including loss of equilibrium, lethargy and erratic swimming.

In a flow-through acute toxicity study with saltwater mysids (*Americamysis bahia*) the LC_{50} (48 h) for triticonazole was determined to be > 7.5 mg a.s./L based on mean measured concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Triticonazole technical (BAS 595 F; Reg. No. 4378513), batch no. OP9750057, purity: 90.52%.

B. STUDY DESIGN

Test species: Saltwater mysid (*Mysidopsis bahia;* nowadays called *Americamysis bahia*), juveniles, age: less than 24 hours old; source: in-house cultures.

Test design: Flow-through system (96 hours); 10 mysids per replicate, 2 replicates per concentration; assessment of mortality and symptoms of toxicity 0, 24, 48, 72 and 96 hours after start of exposure.

Endpoints: LC_{50} (48 h), mortality and sub-lethal effects.

Test concentrations: Exposure 1: Control (dilution water), solvent control (0.084 mL acetone/L), 0.64, 1.1, 1.8, 3.0 and 4.9 mg a.s./L (nominal), corresponding to mean measured concentrations of 0.60, 1.0, 1.7, 2.9 and 4.6 mg a.s./L.
Exposure 2: Control (dilution water), solvent control (0.140 mL acetone/L), 1.0, 1.7, 2.8, 4.7 and 7.9 mg a.s./L corresponding to mean measured concentrations of 1.1, 1.8, 2.9, 4.9 and 7.5 mg a.s./L.

Test conditions: Glass aquaria (39 x 20 x 25 cm), each with one mysid retention chamber (petri dishes, 10 cm in diameter, 2 cm deep, to which a 21.5 cm high Nitex screen collar was attached); retention chambers were partially submerged in exposure aquaria), filtered natural seawater, salinity: 31 - 33‰; flow rate: approximately 6.5 volume exchanges/aquarium/24 h; temperature: 24 - 25°C; pH 7.8 - 7.9; oxygen content: 5.2 mg/L - 7.9 mg/L; photoperiod 16 h light : 8 h dark; light intensity: 240 - 1100 lux; no aeration.

Analytics: Analytical verification of test item concentrations was conducted using a HPLC-method with UV detection.

Statistics: Descriptive statistics.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentration was conducted in each concentration at test initiation and at test termination. Measured concentrations of triticonazole ranged from 83.7% to 120.0% of nominal at test initiation and from 92.4% to 110.0% of nominal at test termination during both exposure series. The following biological results are based on mean measured concentrations.

Biological results: After 48 hours in the first exposure series no mortality and toxic effects were observed in the control, the solvent control and in the tested concentrations up to and including 1.7 mg triticonazole/L. Mortality rates of 20% and 30% were observed in the 2.9 and 4.6 mg triticonazole/L test item groups after 48 hours. At concentrations of 2.9 mg triticonazole/L and above, signs of toxicity were evident among mysids, including loss of equilibrium and lethargy. The results are summarized in Table 8.2.4.2-1.

Table 8.2.4.2-1: Exposure 1: Acute toxicity (48 h) of triticonazole to saltwater mysids (*Americamysis bahia*)

Concentration [mg a.s./L] (nominal)	**Control**	**Solvent control**	**0.64**	**1.1**	**1.8**	**3.0**	**4.9**
Concentration [mg a.s./L] (mean measured)	**--**	**--**	**0.60**	**1.0**	**1.7**	**2.9**	**4.6**
Mortality (48 h) [%]	0	0	0	0	0	20	30
Symptoms (48 h) *	none	none	none	none	none	C, G	C, G
	Endpoints [mg triticonazole/L] (mean measured)						
LC_{50} (48 h)	> 4.6						

* Symptoms after 48 h of exposure: C = lethargy; G = loss of equilibrium

After 48 hours in the second exposure series no mortality and toxic effects were observed in the control, the solvent control and in the tested concentrations up to and including 1.1 mg triticonazole/L. Mortality rates of 55%, 20% and 40% were observed in the 2.9, 4.9 and 7.5 mg triticonazole/L test item groups after 48 hours, respectively. At the four highest test item concentrations signs of toxicity were evident among mysids, including loss of equilibrium, lethargy and erratic swimming. The results are summarized in Table 8.2.4.2-2.

Table 8.2.4.2-2: Exposure 2: Acute toxicity (48 h) of triticonazole to saltwater mysids (*Americamysis bahia*)

Concentration [mg a.s./L] (nominal)	**Control**	**Solvent control**	**1.0**	**1.7**	**2.8**	**4.7**	**7.9**
Concentration [mg a.s./L] (mean measured)	**--**	**--**	**1.1**	**1.8**	**2.9**	**4.9**	**7.5**
Mortality (48 h) [%]	0	0	0	0	55	20	40
Symptoms (48 h) *	none	none	none	C	E, C	C	G
	Endpoints [mg triticonazole/L] (mean measured)						
LC_{50} (48 h)	> 7.5						

* Symptoms after 48 h of exposure: C = lethargy; G = loss of equilibrium: E = erratic swimming

III. CONCLUSION

In a flow-through acute toxicity study with saltwater mysids (*Americamysis bahia*) the LC_{50} (48 h) for triticonazole was determined to be > 7.5 mg a.s./L based on mean measured concentrations.

The following acute toxicity study on the eastern oyster (*Crassostrea virginica*) performed with technical triticonazole is not required for registration in the EU and it has not been evaluated previously on EU level. However, the study was conducted due to U.S. data requirements and it is provided for completeness.

Report: CA 8.2.4.2/2
Dionne E., 1998a
Triticonazole technical - Acute toxicity to eastern oyster (Crassostrea virginica) under flow-through conditions
C019777
Guidelines: EPA 72-3, EPA, FIFRA
GLP: yes
(certified by United States Environmental Protection Agency)

Executive Summary

In a 96-hour acute toxicity laboratory study the effect of triticonazole on shell deposition of eastern oysters was investigated under flow-through conditions. The eastern oysters were exposed to a dilution water control, a solvent control and to nominal concentrations of 1.3, 2.1, 3.5, 5.9 and 9.9 mg triticonazole/L (corresponding to mean measured concentrations of 1.4, 2.2, 3.3, 4.9 and 8.3 mg a.s./L) in groups of 20 oysters per replicate with two replicates per treatment. Eastern oysters were observed for survival and symptoms of toxicity daily during the exposure period. Measurements of shell deposition for each oyster were made after 96 hours.

The biological results are based on mean measured concentrations of the test item. Growth among dilution water control and solvent control oysters at test termination averaged between 2.0 and 1.8 mm, respectively. After 96 hours of exposure, one mortality was observed among oysters exposed to the 1.4 mg a.s./L treatment group and the solvent control, each. No statistically significant difference in shell growth was observed between the control groups (t-test), therefore subsequent statistical analyses were performed by comparing the pooled control data to the treatment data. Statistically significant inhibition of shell growth compared to the pooled control was observed at the four highest tested concentrations (Williams' test).

In a flow-through acute toxicity study with eastern oysters (*Crassostrea virginica*), the EC_{50} (96 h) for triticonazole was 8.9mg a.s./L based on mean measured concentrations. The NOEC (96 h) was determined to be 1.4 mg a.s./L.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Triticonazole technical (BAS 595 F; Reg. no.: 4378513), batch no. OP9750057, purity: 90.52%.

B. STUDY DESIGN

Test species: Eastern oyster (*Crassostrea virginica*), juveniles, mean valve height of 42 ± 4.6 mm; source: "P. Cummins Oyster Company", Annapolis, Maryland, USA.

Test design: Flow-through system (96 hours); 5 test item concentrations plus a control and a solvent control, 2 replicates per treatment with 20 oysters per replicate (40 animals per treatment); daily assessment of mortality and symptoms of toxicity; measurements of shell deposition 96 hours after start of exposure.

Endpoints: EC_{50} and NOEC for shell growth inhibition.

Test concentrations: Control (dilution water), solvent control (0.2 mL acetone/L), 1.3, 2.1, 3.5, 5.9 and 9.9 mg triticonazole/L (nominal), corresponding to mean measured concentrations of 1.4, 2.2, 3.3, 4.9 and 8.3 mg a.s./L.

Test conditions: Glass aquaria (49.5 x 25.5 x 29 cm), test volume 18 L, dilution water: natural unfiltered seawater, flow rate: 75 ml/minute, approx. 6.0 volume additions per 24 hours; salinity: 31‰ - 32‰; temperature: 20°C - 22°C; pH 7.2 - 7.8; oxygen content: 4.4 mg/L - 7.0 mg/L; photoperiod 16 h light : 8 h dark; no aeration; supplemental feeding with algae (*Isochrysis galbana)*: 180 mL of concentrated algal suspension (10^7 cells/mL) three times daily.

Analytics: Analytical verification of test item concentrations was conducted using an HPLC-method with UV-detection.

Statistics: Descriptive statistics; t-test for comparison of shell deposition data in the control groups; EC_{50} calculation by linear regression, Williams' test for determination of the NOEC.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each concentration at test initiation and at test termination. Mean measured concentrations for triticonazole ranged from 86.4% to 109.5% of nominal concentrations at test initiation and from 79.8% to 107.7% of nominal at test termination. The following biological results are based on mean measured concentrations.

Biological results: Growth among dilution water control and solvent control oysters at test termination averaged between 2.0 and 1.8 mm, respectively. After 96 hours of exposure, one mortality was observed among oysters exposed to the 1.4 mg a.s./L treatment group and the solvent control, each. No statistically significant difference in shell growth was observed between the control groups (t-test), therefore subsequent statistical analyses were performed by comparing the pooled control data to the treatment data. Statistically significant inhibition of shell growth compared to the pooled control was observed at the four highest tested concentrations (Williams' test). The results are summarized in Table 8.2.4.2-3.

Table 8.2.4.2-3: Acute toxicity (96 h) of triticonazole to eastern oysters (*Crassostrea virginica*)

Concentration [mg a.s./L] (nominal)	**Control**	**Solvent control**	**1.3**	**2.1**	**3.5**	**5.9**	**9.9**
Concentration [mg a.s./L] (mean measured)	**--**	**--**	**1.4**	**2.2**	**3.3**	**4.9**	**8.3**
Mean shell deposition after 96 h [% of control]	--	--	6.9	22 *	26 *	35 *	50 *
Endpoints [mg triticonazole/L] (mean measured)							
EC_{50} (96 h)	8.9 (95% confidence limits: 5.6 - 17)						
NOEC (96 h)	1.4						

* Statistically significantly different compared to the pooled control (Williams' test).

III. CONCLUSION

In a flow-through acute toxicity study with eastern oysters (*Crassostrea virginica*), the EC_{50} (96 h) for triticonazole was 8.9mg a.s./L based on mean measured concentrations. The NOEC (96 h) was determined to be 1.4 mg a.s./L.

CA 8.2.5 Long-term and chronic toxicity to aquatic invertebrates

CA 8.2.5.1 Reproductive and development toxicity to *Daphnia magna*

Altogether there are four reproduction studies on *Daphnia magna* available with triticonazole. Study R013032 / 1992/7003213 gives the EU agreed endpoint so far according to EFSA Scientific Report (2005) 33. However, according to the recent guideline OECD 211 this study is not valid anymore, as the mean number of living offspring produced per parent animal surviving at the end of the test was < 60 in the control (i.e. 44) and the solvent control (i.e. 45). Thus, the lowest endpoint from the other studies on *D. magna* will be used in the risk assessment.

The following life-cycle test on the cladoceran *Daphnia magna* performed with technical triticonazole was conducted due to U.S. data requirements. It has not been evaluated previously on EU level and it is provided for completeness.

Report:	CA 8.2.5.1/1 Putt A.E., 2006a BAS 595 F (Triticonazole) - Full life-cycle toxicity test with water fleas, Daphnia magna, under static-renewal conditions 2006/7007209
Guidelines:	EPA 850.1300
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary

In a 21-day semi-static (static-renewal) toxicity test, effects of triticonazole to water fleas (*Daphnia magna*) were examined. Neonates less than 24 hours old were exposed to nominal concentrations of 0.19, 0.38, 0.75, 1.5 and 3.0 mg a.s./L, a water control and solvent control. All treatment groups and the controls consisted of 10 replicates with one parent daphnid in each. Adult survival was recorded daily over the exposure period of 21 days. Numbers of offspring were determined from day 8 (first brood release) and daily throughout the remainder of the test. Body length and weight were assessed at test termination after 21 days of exposure.

The analytical recovery of the test concentrations resulted in 91% to 100% of nominal concentrations. After 21 days of exposure, survival among the dilution water control and solvent control organisms averaged 90% and 100%, respectively, whereas 10% mortality was observed at the two highest test concentrations. The number of offspring per female varied between 113 and 137 in the test item treatments compared to 135 in the pooled control. At test end the average body length of the adult daphnids ranged from 4.55 to 4.71 mm and the average dry weight of the adult daphnids ranged from 0.96 to 1.05 mg in the test treatments. A statistically significant effect was observed at the highest tested concentrations of 3.0 mg a.s./L for reduced body length.

In a 21-day semi-static (static-renewal) toxicity study with *Daphnia magna* the NOEC of triticonazole was determined to be 1.5 mg a.s./L based on mean total body length.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Triticonazole (BAS 595 F; Reg. No. 4378513), batch no. not reported, purity: 90.3%.

B. STUDY DESIGN

Test species: *Daphnia magna*, < 24 hours old, source: Springborn Smithers culture, USA.

Test design: Static-renewal (21-days); renewal every 48 or 72 hours; 5 test concentrations plus water and solvent controls, 10 replicates per treatment with 1 daphnid in each. On day 8 the numbers of offspring were determined upon the first brood release in any vessel, and daily throughout the remainder of the test. Offspring were removed, counted and discarded at each observation interval. In addition, the number of immobilized offspring and the time to first brood release were recorded for each treatment level and the controls. At test termination (day 21), the total body length was measured. Daphnids were dried for 24 hours in a Precision Oven and dry weight of each surviving adult daphnid was measured.

Endpoints: NOEC, LOEC, MATC and EC_{50} values based on survival, behavior, reproduction and growth.

Test concentrations: 0 (control), 0.19, 0.38, 0.75, 1.5 and 3.0 mg a.s./L (nominal).
0.19, 0.37, 0.75, 1.5 and 3.0 mg a.s./L (mean measured).

Test conditions: Test vessels: 100 mL clear glass beakers, each containing 80 mL test solution.
Dilution water: fortified well water; temperature 20-21°C; pH 7.9-8.8; oxygen content 7.1-10.1 mg/L (60-105% saturation); total hardness: 160-170 mg $CaCO_3$/L; total alkalinity: 90-100 mg $CaCO_3$/L; conductivity 500 µmhos/cm.
Photoperiod: 16 hours light: 8 hours darkness (12-14 $\mu E.m^{-2}.s^{-1}$).
Feeding: unicellular green algae, *Ankistrodesmus falcatus* (4 x 10^7 cells/mL) in addition to a suspension of YCT (yeast, cereal leaves and flaked fish food).

Analytics: Exposure solution concentrations were analytically confirmed in newly prepared test solutions on days 0, 3, 17 and 19 and in aged exposure solutions on days 3, 5, 19 and test termination (day 21) using HPLC/UV method.

Statistics: Descriptive statistics; Student's t-Test for the comparison of the performance of water and solvent control organisms; Fisher´s exact test for survival; Williams' Test was used to establish treatment effects for reproduction and dry weight; Bonferroni's t-Test to evaluate length data.

II. RESULTS AND DISCUSSION

Analytical measurements: Measured concentrations were consistent between sampling intervals for both the newly prepared and aged solutions. The concentration of triticonazole in new solutions ranged from 93% to 100% of nominal concentrations while the concentration of the test substance in aged solutions (two to three days old) ranged from 91% to 100% of nominal concentrations. Based on the mean measured concentrations, the treatment levels tested were defined as 0.19, 0.37, 0.75, 1.5 and 3.0 mg a.s./L.

Biological results: After 21 days of exposure, survival among the dilution water control and solvent control organisms averaged 90 and 100%, respectively, whereas 10% mortality was observed at the two highest test concentrations of 1.5 and 3.0 mg a.s./L. All other test concentrations resulted in 0% mortality. The mean number of offspring released per female varied between 113 and 137 in the test item treatments compared to 135 offspring/female in the pooled control. At test end the average body length of the adult daphnids ranged from 4.55 to 4.71 mm and the average dry weight of the adult daphnids ranged from 0.96 to 1.05 mg in the treatments.

Statistically significant effects on reproduction were observed for body length. Williams' Test determined a significant difference in total body length among daphnids exposed to all treatment levels tested as compared to the solvent control data (i.e., 4.81 mm). A review of this data indicates that daphnid length in the four lowest treatment levels exhibited < 3% reduction in length compared to the solvent control data. The highest treatment level (3.0 mg a.s./L) established a 5.4% reduction compared to the solvent control data. The coefficient of variation (CV) for the control and solvent control daphnid length measurements was 1.0% for both groups. Review of historical daphnid length data from 10 previous studies performed at Springborn Smithers Laboratories under the same study design (static-renewal, 10 individual daphnids per treatment level or control) established that the average CV for daphnid length in the control groups was 2.1% (range of 1.3 to 6.2%, N = 14). The results of these studies also established that a reduction in daphnid length of > 3% was required to elicit a statistically significant reduction compared to the control data. Therefore, based on the observed results of this study and historical data (control CV one-half the normal average), the statistically significant reductions observed in the four lowest treatment levels (0.19 to 1.5 mg a.s./L) are not considered biologically significant. The results are summarized in Table 8.2.5.1-1.

Table 8.2.5.1-1: Effects of triticonazole (21 days) on *Daphnia magna* reproduction, growth and parent mortality

Concentration [mg a.s./L] (mean measured)	Control	Solvent control	0.19	0.37	0.75	1.5	3.0
Parent mortality [%]	10	0	0	0	0	10	10
Av. Total number of offspring released/parent	131	138	113	137	121	122	113
Av. dry weight [mg]	0.85	0.83	1.05	0.96	0.99	1.03	1.03
Av. body length [mm]	4.89	4.81	4.71*	4.70*	4.67*	4.67*	4.55**
Endpoints [mg triticonazole/L] (mean measured)							
NOEC (21 days)	1.5						

* Significantly reduced as compared to the solvent control, based on Williams' Test, but not considered biologically significant since percent reductions are < 3% of the solvent control data.
** Significantly reduced as compared to the solvent control, based on Williams' Test.

III. CONCLUSION

In a 21-day semi-static (static-renewal) toxicity study with *Daphnia magna* the NOEC of triticonazole was determined to be 1.5 mg a.s./L (mean measured) based on mean total body length.

The following life-cycle test on the cladoceran *Daphnia magna* performed with triticonazole was conducted due to U.S. data requirements and has not been evaluated previously on EU level. The study was (wrongly) not listed in the "Application" document submitted for the triticonazole AIR 3 renewal process; however, it is provided for completeness.

Report:	CA 8.2.5.1/2 Urann K., 2012a BAS 595 F - Full life cycle toxicity test with water fleas (Daphnia magna) under static-renewal conditions, following OPPTS draft guideline 850.1300 2012/7003660
Guidelines:	EPA 850.1300
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary

In a 21-day semi-static toxicity test, effects of triticonazole to water fleas (*Daphnia magna*) were examined. Neonates less than 24 hours old were exposed to nominal concentrations of 0.10, 0.20, 0.40, 0.80, 1.6 and 3.2 mg triticonazole/L. Additionally, a dilution water control and a solvent control were set up. All treatment groups and the control consisted of 10 replicates with one parent daphnid in each. Parent survival and offspring production were recorded daily over the exposure period of 21 days. Body length and weight was assessed at test termination after 21 days of exposure.

The biological results are based on mean measured concentrations. After 21 days of exposure no significant reduction of parent survival could be determined at any test item concentration compared to the dilution water control. Statistical analysis determined a significant reduction in cumulative offspring per female among daphnids exposed to the 0.88, 1.8 and 3.5 mg a.s./L treatment levels compared to the dilution water control. Total body length was statistically significantly reduced at the test item concentration of 1.8 mg a.s./L compared to the dilution water control. However, due to the lack of a clear dose response at the 3.5 mg/L treatment level, this effect was not considered to be biologically-relevant. For dry weight, no statistically significant differences compared to the dilution water control were determined at any of the concentrations tested.

In a 21-day semi-static chronic toxicity study with *Daphnia magna* the NOEC of triticonazole was determined to be 0.43 mg a.s./L based on mean measured concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Triticonazole (BAS 595 F; Reg. no.: 4378513), batch no. COD-001440, purity: 91.3%.

B. STUDY DESIGN

Test species: Water flea (*Daphnia magna* STRAUS), neonates from in-house culture; < 24 hours old at test initiation.

Test design: Semi-static system (21 days), 6 test concentrations plus water control and solvent control, 10 replicates per treatment with one parent daphnid in each; renewal of test solutions at 48- or 72-hour intervals; daily assessment of parent mortality and reproductive performance over the 21 day exposure period; assessment of body length and weight at test termination after 21 days of exposure.

Endpoints: NOEC, parent survival, reproduction, parent length and dry weight.

Test concentrations: Control, solvent control (0.1 mL DMF/L), 0.10, 0.20, 0.40, 0.80, 1.6 and 3.2 mg triticonazole/L (nominal), corresponding to mean measured concentrations of 0.11, 0.21, 0.43, 0.88, 1.8 and 3.5 mg a.s./L.

Test conditions: 100 mL glass beakers, test volume 80 mL, dilution water: fortified, filtered well water; temperature: 19°C - 21°C; pH 7.7 - 8.6; oxygen content: 7.7 mg/L - 10 mg/L (86% - 110%); total hardness: 180 - 210 mg $CaCO_3$/L, total alkalinity: 84 - 98 mg $CaCO_3$/L; conductivity: 710 - 790 µS/cm; light intensity: 71 - 88 foot-candles (760 - 950 lux); photoperiod 16 hours light : 8 hours dark; feeding: 200 µL algal suspension (*Ankistrodesmus falcatus*,4 x 10^7 cells/mL) and 50 µL a yeast, cereal leaves and digested flaked fish food suspension.

Analytics: The test item concentrations were analyzed using a HPLC-method with UV detection.

Statistics: Descriptive statistics; Fisher's Exact Test with Bonferroni-Holm's Test, Bonferroni's Adjusted t-Test and Bonferroni Adjusted Wilcoxon Test for determination of the NOEC values ($\alpha = 0.05$).

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of the test item concentrations was conducted in all treatments at day 0, 2, 5, 16, 19 and 21. Mean recoveries of triticonazole in new solutions ranged from 100% to 110% of nominal concentrations. Mean measured concentrations in aged solutions ranged from 110% to 120% of nominal concentrations. The following biological results are based on mean measured test item concentrations.

Biological results: After 21 days of exposure no significant reduction of parent survival could be determined at any test item concentration compared to the dilution water control (Fisher's Exact Test with Bonferroni-Holm's Test, α = 0.05). Statistical analysis determined a significant reduction in cumulative offspring per female among daphnids exposed to the 0.88, 1.8 and 3.5 mg a.s./L treatment levels compared to the dilution water control (Bonferroni's Adjusted t-Test, α = 0.05). Total body length was statistically significantly reduced at the test item concentration of 1.8 mg a.s./L compared to the dilution water control (Bonferroni Adjusted Wilcoxon Test, α = 0.05). However, due to the lack of a clear dose response at the 3.5 mg/L treatment level, this effect was not considered to be biologically-relevant. For dry weight, no statistically significant differences compared to the dilution water control were determined at any of the concentrations tested (Bonferroni Adjusted Wilcoxon Test, α = 0.05). The results are summarized in Table 8.2.5.1-2.

Table 8.2.5.1-2: Effects of triticonazole (21 d) on *Daphnia magna* parent survival, reproduction and growth.

Concentration [mg/L] (nominal)	**Control**	**Solvent control**	**0.10**	**0.20**	**0.40**	**0.80**	**1.6**	**3.2**
Concentration [mg/L] (mean measured)	**--**	**--**	**0.11**	**0.21**	**0.43**	**0.88**	**1.8**	**3.5**
Mean survival of parental daphnids [%]	100	90	90	100	100	100	90	100
Mean number of offspring released per daphnid ± SD	159 ± 14	137 ± 54	153 ± 11	141 ± 11 a)	143 ± 21	131 ± 17 b)	124 ± 12 b)	85 ± 21 b)
Mean total body length ± SD [mm]	4.66 ± 0.16	4.68 ± 0.20	4.62 ± 0.10	4.58 ± 0.13	4.48 ± 0.31	4.46 ± 0.40	4.38 ± 0.21 c)	4.55 ± 0.07
Mean dry weight ± SD [mg]	0.76 ± 0.10	0.86 ± 0.14	0.73 ± 0.06	0.71 ± 0.17	0.71 ± 0.22	0.82 ± 0.15	0.90 ± 0.15	0.98 ± 0.05
Endpoints [mg triticonazole/L] (mean measured)								
NOEC (21 d)	0.43							

SD - standard deviation

a) Statistically significant effect compared to the dilution water control (Bonferroni's Adjusted t-Test, α = 0.05). Due to the lack of a clear dose response at the 0.43 mg a.s./L treatment level, this effect was not considered to be biologically relevant.

b) Statistically significantly reduced compared to the dilution water control (Bonferroni's Adjusted t-Test one-sided, α = 0.05).

c) Statistically significantly reduced compared to the dilution water control (Bonferroni's Adjusted Wilcoxon Test, α = 0.05. Due to the lack of a clear dose response at the 3.5 mg/L treatment level, this effect was not considered to be biologically-relevant.

III. CONCLUSION

In a 21-day semi-static chronic toxicity study with *Daphnia magna* the NOEC of triticonazole was determined to be 0.43 mg a.s./L based on mean measured concentrations.

The following life-cycle test on the cladoceran *Daphnia magna* performed with triticonazole was conducted due to U.S. data requirements and has not been evaluated previously on EU level. The study was (wrongly) not listed in the "Application" document submitted for the triticonazole AIR 3 renewal process; however, it is provided for completeness.

Report:	CA 8.2.5.1/3 McElligott A., 1998a Triticonazole - Daphnia magna life cycle (21-day static renewal) chronic toxicity study R013169
Guidelines:	EPA 72-4, FIFRA 72-4
GLP:	yes

Executive Summary

In a 21-day semi-static toxicity test, effects of triticonazole to water fleas (*Daphnia magna*) were examined. Neonates less than 24 hours old were exposed to nominal concentrations of 0.08, 0.19, 0.48, 1.2 and 3.0 mg triticonazole/L (corresponding to mean measured concentrations of 0.08, 0.19, 0.47, 1.3 and 3.0 mg a.s./L). Additionally, a dilution water control and a solvent control were set up. All treatment groups and the controls consisted of 7 replicates containing one individually held daphnid each plus 3 replicates with groups of 5 daphnids. Biological observations (parent survival, appearance and behavior of surviving daphnids, number and survival of offspring, time of first offspring, presence of males or winter eggs, presence of eggs in brood pouch and presence of unhatched eggs) were conducted at test initiation and test termination and on days 2, 5, 7, 9, 12, 14, 16, 19 before transfer of the parent animals to fresh test solutions. Measurements of growth (body weight and length) were made at test termination.

The biological results are based on nominal concentrations. After 21 days of exposure, no statistically significant effects on parent mortality occurred at the test item concentrations of up to and including the highest concentration tested (individually held daphnids and grouped daphnids). Statistically significant differences for the number of live offspring per parent and for mean total length were observed at the highest test item concentration.

In a 21-day semi-static toxicity study with *Daphnia magna* the NOEC of triticonazole was determined to be 1.3 mg a.s./L based on nominal concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Triticonazole (BAS 595 F; Reg. No. 4378513), batch no. 013951, purity: 97.2%.

B. STUDY DESIGN

Test species: Water flea (*Daphnia magna* STRAUS), neonates from in-house culture; originally obtained from INERIS Laboratory, Vert-le-Petit, France, < 24 hours old at test initiation.

Test design: Semi-static system (21 days), 5 test concentrations plus control and solvent control, 7 replicates containing 1 individually held daphnid each plus 3 replicates with groups of 5 daphnids per treatment; biological observations (parent survival, appearance and behavior of surviving daphnids, number and survival of offspring, time of first offspring, presence of males or winter eggs, presence of eggs in brood pouch and presence of unhatched eggs) at test initiation and test termination and on days 2, 5, 7, 9, 12, 14, 16, 19 before transfer of the parent animals to fresh test solutions; determination of total body length and dry weight at test termination.

Endpoints: NOEC, parent mortality, reproduction and growth (total length and dry weight).

Test concentrations: Control (dilution water), solvent control (0.1 mL DMF/L), 0.08, 0.19, 0.48, 1.2 and 3.0 mg triticonazole/L (nominal); corresponding to mean measured concentrations of 0.08, 0.19, 0.47, 1.3 and 3.0 mg a.s./L.

Test conditions: 250 mL glass beakers; test volume: 200 mL; dilution water: reconstituted water 80% DSW (Dutch standard water) + 20% LC-oligo medium; temperature: 16.8°C - 21.1°C; pH 7.25 - 8.23; oxygen content: 7.8 mg/L - 8.8 mg/L; total hardness of dilution water: 170 ± 10 mg $CaCO_3$/L; conductivity of dilution water: 540 ± 40 µS/cm; photoperiod 16 hours light : 8 hours dark; light intensity: 400 - 800 lux; feeding: yeast suspension (7.8 ng/L), seaweed extract, unicellular green algae (*Chlorella vulgaris,* 1.25 x 10^8 cells/mL) and combination of meat extract and fish food (2 g/L and 10 g/L, respectively) three times weekly; no aeration.

Analytics: Analytical verification of test item concentrations was conducted using an HPLC-method with UV detection.

Statistics: Descriptive statistics; Fisher's exact test (two tails) for comparison of the dilution water control and solvent control data; ANOVA followed by Dunnett's test for determination of the NOEC values.

II. RESULTS AND DISCUSSION

Analytical results: Analytical verification of the active substance concentrations was conducted in all treatments in fresh and old test item solutions of the 1st, 2nd, 6th and 9th test solution renewals. Recoveries of triticonazole in fresh solutions were in the range of 90% to 108% of nominal concentrations. Measured concentrations of the test item in old solutions ranged from 92% to 108%. The following biological results are based on mean measured concentrations.

Biological results: No statistically significant differences between the dilution water control and the solvent control was observed for parental survival (individually held and grouped daphnids) and for growth data. A statistically significant difference was observed between the control and the solvent control groups reproduction data (α = 0.05). Thus, for each of the subsequent comparisons the test concentration groups were compared to the solvent control groups. After 21 days of exposure, no statistically significant effects on parent mortality occurred at the test item concentrations of up to and including the highest concentration tested (individually held and grouped daphnids). Statistically significant differences for the number of live offspring per parent and for mean total length were observed at the highest test item concentration (ANOVA followed by Dunnett's test; $p < 0.01$). The results are summarized in Table 8.2.5.1-3.

Table 8.2.5.1-3: Effects of triticonazole on *Daphnia magna* parent mortality, reproduction and growth after 21 days of exposure

Concentration [mg a.s./L] (nominal)		**Control**	**Solvent control**	**0.08**	**0.19**	**0.48**	**1.2**	**3.0**
Concentration [mg a.s./L] (mean measured)		**--**	**--**	**0.08**	**0.19**	**0.47**	**1.3**	**3.0**
Parent survival (day 21) [%]	Individually held daphnids [a)]	57	71	71	86	57	86	43
	Grouped daphnids [b)]	93	93	93	93	93	93	93
Mean number of live offspring from parent animals ± SD (day 21)		111.3 ± 12.82	129.4 ± 8.20	121.4 ± 23.14	120.0 ± 6.40 [#]	112.3 ± 16.82	125.3 ± 13.78	2.0 ± 3.46 **
Mean total length ± SD [mm]		4.29 ± 0.05	4.26 ± 0.11	4.21 ± 0.11	4.15 ± 0.19	4.18 ± 0.07	4.09 ± 0.09	3.93 ± 0.15 **
Mean dry weight ± SD [mg]		0.61 ± 0.07	0.60 ± 0.06	0.55 ± 0.07	0.53 ± 0.06	0.53 ± 0.02	0.55 ± 0.12	0.46 ± 0.11
		Endpoints [mg triticonazole/L] (mean measured)						
$NOEC_{overall}$ (21 d)		1.3						

SD = standard deviation
** Statistically significant effects compared to the solvent control (ANOVA followed by Dunnett's test; $p < 0.01$).
[#] One daphnid produced only 8 young during the test period, Dixon's test showed that this value could be considered as an outlier, therefore this value was excluded from the statistical analysis of the test data.
[a)] 7 daphnids per treatment
[b)] Groups of 5 daphnids with 3 replicates per treatment, daphnids were held for information on survival only.

III. CONCLUSION

In a 21-day semi-static toxicity study with *Daphnia magna* the NOEC of triticonazole was determined to be 1.3 mg a.s./L based on nominal concentrations.

CA 8.2.5.2 Reproductive and development toxicity to an additional aquatic invertebrate species

The following life-cycle toxicity study on the saltwater mysid *Americamysis bahia* performed with the active substance triticonazole is not required for registration in the EU and it has not been evaluated previously on EU level. However, the study was conducted due to U.S. data requirements and is provided for completeness.

Report:	CA 8.2.5.2/1 Putt A.E., 2006b BAS 595 F (Triticonazole) - Life-cycle toxicity test with Mysids (Americcamysis bahia) 2006/7007246
Guidelines:	EPA 850.1350, EPA 72-4
GLP:	yes (certified by United States Environmental Protection Agency)

The chronic toxicity of triticonazole to saltwater mysids (*Americamysis bahia*) was evaluated in a 28-day full-life cycle test under flow-through conditions. Mysids were exposed to a dilution water control, a solvent control and to nominal test item concentrations of 0.019, 0.038, 0.075, 0.15 and 0.30 mg triticonazole/L (corresponding to mean measured concentrations of 0.025, 0.041, 0.085, 0.16 and 0.32 mg a.s./L). Survival, reproductive success and symptoms of toxicity were assessed throughout the study. Total body length and dry weight of the males and females was determined at test termination.

The biological results are based on mean measured concentrations. No statistically significant differences were determined between the control and the solvent control data. Thus, the data from both control groups were pooled and the pooled data set was used for statistical evaluation of treatment related effects. Survival of saltwater mysids was statistically significantly affected compared to the pooled control at the highest test item concentrations of 0.32 mg triticonazole/L. Reproductive success showed statistically significant differences compared to the pooled control data at the test item concentrations of 0.085, 0.16 and 0.32 mg triticonazole/L. Mean total body length of male and female mysids showed no statistically significant differences from the pooled control data. Evaluation of dry weight data showed that the dry weights of the surviving adult male mysids were statistically significantly different from the pooled control data at the test item concentration of 0.32 mg triticonazole/L. Dry weight of female mysids was not statistically significantly different from the pooled control data.

In a flow-through chronic toxicity study with saltwater mysids (*Americamysis bahia*), the overall NOEC (28 d) for triticonazole was determined to be 0.041 mg a.s./L based on mean measured concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Triticonazole (BAS 595 F; Reg. no.: 4378513), purity: 90.3%.

B. STUDY DESIGN

Test species: Saltwater mysid (*Americamysis bahia*), juveniles, age: less than 24 hours old; source: in-house culture; originally obtained from "Aquatic BioSystems Inc.", Fort Collins, Colorado, USA.

Test design: Flow-through system (28 d); 5 test item concentrations plus a control and a solvent control, 2 replicates for each test item concentration, the control and the solvent control; 30 mysids per glass aquaria (15 mysids per test chamber); mysids were maintained in test chambers until sexual maturity; at time of sexual maturity (day 13) male-female pairs were transferred into pairing chambers (one pair per chamber, 10 pairing chambers per glass aquaria); remaining mysids (after isolation of male-female pairs) were pooled and placed in separate test chambers within glass aquaria to replace dead males of the paired (male/female) groups; dead parental mysids and juveniles released during the test were removed; daily assessment of survival and symptoms of toxicity, reproduction (number of offspring produced by each female) from day 13 on; determination of total body length and dry weight at test termination.

Endpoints: NOEC based on survival, reproductive success, total body length and dry weight.

Test concentrations: Control (dilution water), solvent control (82 mL triethylene glycol/L); 0.019, 0.038, 0.075, 0.15 and 0.30 mg triticonazole/L (nominal), corresponding to mean measured concentrations of 0.025, 0.041, 0.085, 0.16 and 0.32 mg triticonazole/L.

Test conditions: Glass aquaria (39 x 20 x 25 cm) with two test chambers, test chambers: glass petri dishes (10 cm diameter, 2 cm depth) with a 15 cm high nylon screen collar (210 µm mesh size) attached, pairing chambers: petri dishes (6 cm diameter) with a 13 cm high nylon screen collar (210 µm mesh size); dilution water: artificial seawater prepared by addition of a commercially prepared salt formula to laboratory well water, flow rate: 7.4 aquarium volume additions per day to provide 90% test solution replacement rate of approx. 5 hours; salinity: 19 - 21‰; temperature: 25 - 27°C; pH 8.1 - 8.4; oxygen content: 4.5 - 7.3 mg/L; photoperiod 16 h light : 8 h dark; light intensity: 260 - 620 lux; mysids were fed with live brine shrimps (*Artemia salina*) supplemented with a mixture of proteins and fatty acids twice daily.

Analytics: Analytical verification of test item concentrations was conducted using a HPLC-method with UV detection.

Statistics: Descriptive statistics, t-test (α = 0.05) for comparison of control with solvent control; Williams's test for determination of NOEC values (α = 0.05).

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each concentration at test initiation and after 6, 12, 20 and 28 days. Measured concentrations of triticonazole were between 93.3% and 115.8% of nominal at test initiation. Measured concentrations after 6, 12, and 20 days ranged from 100.0% to 121.1%, from 106.7% to 136.8% and from 105.3% to 142.1% of nominal, respectively. At test termination, measured concentrations were between 106.7% and 136.8% of nominal. The following biological results are based on mean measured concentrations.

Biological results: No statistically significant differences were determined between the control and the solvent control data (t-test; α = 0.05). Thus, the data from both control groups were pooled and the pooled data set was used for statistical evaluation of treatment related effects. Survival of saltwater mysids was statistically significantly affected compared to the pooled control at the highest test item concentrations of 0.32 mg triticonazole/L (Williams test, α = 0.05). Reproductive success showed statistically significant differences compared to the pooled control data at the test item concentrations of 0.085, 0.16 and 0.32 mg triticonazole/L (Williams test, α = 0.05). Mean total body length of male and female mysids showed no statistically significant differences from the pooled control data (Williams test, α = 0.05). Evaluation of dry weight data showed that the dry weights of the surviving adult male mysids were statistically significantly different from the pooled control data at the test item concentration of 0.32 mg triticonazole/L (Williams test, α = 0.05). Dry weight of female mysids was not statistically significantly different from the pooled control data (Williams test, α = 0.05). The results are summarized in Table 8.2.5.2-1.

Table 8.2.5.2-1: Chronic toxicity (28 d) of triticonazole to saltwater mysids (*Americamysis bahia*)

Concentration [mg a.s./L] (nominal)	**Control**	**Solvent control**	**Pooled control +**	**0.019**	**0.038**	**0.075**	**0.15**	**0.30**
Concentration [mg a.s./L] (mean measured)	**--**	**--**	**--**	**0.025**	**0.041**	**0.085**	**0.16**	**0.32**
Mean survival on day 28 [%]	94	92	93	98	89	90	89	85 *
Reproductive success [Mean number of offspring per female]	6.8	5.2	6.0	4.7	4.0	2.2 *	1.8 *	1.4 *
Mean total body length on day 28, males [mm]	7.4	7.3	7.4	7.5	7.4	7.3	7.3	7.2
Mean total body length on day 28, females [mm]	7.7	7.5	7.6	7.7	7.6	7.5	7.5	7.4
Mean dry weight on day 28, males [mg]	0.95	0.90	0.93	0.98	0.94	0.84	0.88	0.81 *
Mean dry weight on day 28, females [mg]	1.21	1.16	1.19	1.24	1.24	1.13	1.09	1.08
Endpoints [mg triticonazole/L] (mean measured)								
NOEC $_{overall}$ (28 d)	0.041							

+ Mean of control and solvent control data; control and solvent control data were not significantly different (t-test; $\alpha = 0.05$).
* Statistically significant differences compared to the pooled control (Williams test; $\alpha = 0.05$).

III. CONCLUSION

In a flow-through chronic toxicity study with saltwater mysids (*Americamysis bahia*), the overall NOEC (28 d) for triticonazole was determined to be 0.041 mg a.s./L based on mean measured concentrations.

CA 8.2.5.3 Development and emergence in Chironomus riparius

A spiked water toxicity study on *Chironomus riparius* performed with triticonazole 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 120-hour algae study on the freshwater green algae *Selenastrum capricornutum* was conducted due to U.S. data requirements. It is not required for registration in the EU and it has not been evaluated previously on EU level.
Following the recommendations and validity criteria defined in the current OECD guideline 201 for alga testing (OECD, 2011) the study is considered to be not valid because:

- The following validity criterion is not met: the mean coefficients of variation (CV) for section by section specific growth rate in both the dilution water control and the solvent control were significantly > 35% after 0 - 72 hours, 0 - 96 hours and 0 - 120 hours (*i.e.* about 70% in dilution water control and about 60% in the solvent control after 72 h, 96 h and 120 h)

- The endpoint of this study is of very limited use for the risk assessment as only one test item concentration was tested and no clear endpoint could be derived (effect < 50% at the tested concentration).

Nevertheless, a summary of the study is provided for completeness. In accordance to the EFSA Aquatic Guidance Document (EFSA, 2013) and OECD guideline 201 (2011) the 72 h endpoints obtained in the 120 h study are considered as relevant endpoint for the aquatic risk assessment, therefore both the 72 h and 120 h endpoints are reported in the study summary below.

Report: CA 8.2.6.1/1
Hoberg J.R., 1998a
Triticonazole - Toxicity to the freshwater green alga, Selenastrum capricornutum
R012017
Guidelines: EPA 122-2
GLP: yes

Executive Summary

In a 120-hour static acute toxicity laboratory study, the effect of triticonazole on the growth of the green alga *Selenastrum capricornutum* was investigated. The nominal concentration of 3.0 mg a.s./L was applied, corresponding to a mean measured concentration of 2.5 mg a.s./L. Assessment of growth was conducted 24 h, 48 h, 72 h, 96 h and 120 h after test initiation.

The biological results are based on the mean measured test item concentration. After 120 hours of exposure, no morphological effects on algae were observed in the control groups and in the test item concentration of 2.5 mg a.s./L. Cell density in the test item concentration of 2.5 mg a.s./L was significantly reduced compared to the pooled control after 120 hours of exposure.

In a 120-hour algae test with *Selenastrum capricornutum*, the 72 h and 120 h E_bC_{50} of triticonazole was determined to be > 2.5 mg a.s./L, based on mean measured concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

Test item:	Triticonazole (BAS 595 F; Reg. No. 4378513), batch no. OP9750057, purity: 90.52%.

B. STUDY DESIGN

Test species:	Unicellular fresh water green alga, *Selenastrum capricornutum* Prinz (syn. *Pseudokirchneriella subcapitata* (Reinsch) Korshikov), strain 1648, originally obtained from the University of Texas, Austin, USA.
Test design:	Static system; test duration 120 hours; 1 test item concentration, solvent control and control with 3 replicates respectively; daily assessment of growth.
Endpoint:	EC_{50} with respect to cell density after exposure over 72 h and 120 hours.
Test concentrations:	Control, solvent control (0.1 mL DMF/L), 3.0 mg a.s./L (nominal), corresponding to a mean measured concentration of 2.5 mg a.s./L.
Test conditions:	250 mL Erlenmeyer flasks; test volume 100 mL; test medium: AAP medium; pH 7.5 at test initiation and pH 8.9 at test termination; temperature: 24°C - 25°C; conductivity: 80 - 90 µS/cm; initial cell densities 0.3 x 10^4 cells/mL; continuous light at 3200 - 5400 lux; continuous shaking: 100 rpm.
Analytics:	Analytical verification of test item concentrations was conducted using an HPLC-method with UV detection.
Statistics:	Descriptive statistics, t-test to compare control, solvent control and treatment data ($p < 0.05$); empirical estimation of EC_{50} values.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of the test item concentration was conducted at the beginning and at the end of the test. The measured values of triticonazole ranged from 90% of nominal at test initiation to 77% of nominal at test termination. The following biological results are based on the mean measured test item concentration.

Biological results: After 120 hours of exposure, no morphological effects on algae were observed in the control groups and in the test item concentration of 2.5 mg a.s./L. Cell density in the test item concentration of 2.5 mg a.s./L was significantly reduced compared to the pooled control after 120 hours of exposure (t- test, $p < 0.05$). The results are summarized in Table 8.2.6.1-1.

Table 8.2.6.1-1: Effect of triticonazole on the growth of green alga *Pseudokirchneriella subcapitata*

Concentration [mg a.s./L] (nominal)	**Control**	**Solvent control**	**3.0**
Concentration [mg a.s./L] (mean measured)	**--**	**--**	**2.5**
Mean cell density (72 h) ± SD [x 10^4 cells/mL]	40 ± 4.4	40 ± 2.4	25 ± 0.80
Inhibition compared to the pooled control in 72 h (cell density) [%]	--	--	37.5 [a)]
Mean cell density (120 h) ± SD [x 10^4 cells/mL]	221 ± 6.1	226 ± 4.8	150 ± 6.2 *
Inhibition compared to the pooled control in 120 h (cell density) [%]	--	--	33 *, [a)]
	Endpoints [mg triticonazole/L] (mean measured)		
E_bC_{50} (72 h & 120 h)	> 2.5		

SD: Standard deviation.

* Statistically significantly reduced compared to the pooled control (t-test; $p < 0.05$).

a) Statistically significant differences compared to the pooled control were only determined for cell density after 120 h of exposure.

III. CONCLUSION

In a 120-hour algae test with *Selenastrum capricornutum*, the 72 h and 120 h EC_{50} of triticonazole was determined to be > 2.5 mg a.s./L, based on mean measured concentrations.

Due to the reasons given below, the algae study on *Anabaena flos-aquae* (DocID R012015 / 1998/1003127) is not considered to be valid according to current standard and is thus not considered for the aquatic risk assessment. Nevertheless, for better transparency, the summary of this study was extracted from the originally submitted EU dossier of triticonazole and is provided below.

Following the recommendations and validity criteria defined in the current OECD guideline 201 for alga testing (OECD, 2011) the study is considered to be not valid because:

- The following validity criterion is not met: the mean coefficients of variation (CV) for section by section specific growth rate in both the dilution water control and the solvent control were > 35% after 0 - 72 hours and after 0 - 120 hours (*i.e.* about 40% in dilution water control and about 50% in solvent control after 72 h and 120 h).

Executive Summary

Algae, *Anabaena flos-aquae*: The toxicity of triticonazole to the freshwater blue-green algae *Anabaena flos-aquae* was investigated at the maximum recommended annual application rate of 3.77 kg/ha (3.36 lb a.s./A.). Based on a conversion required by test guidelines, this application rate is equivalent to 2.5 mg a.s./L, when applied to a 15 cm water column. A single exposure concentration of 3.0 mg a.s./L was chosen for the definitive test. Achievement of this test concentration was aided by the preparation of a stock solution of triticonazole in dimethylformamide. Analytical verification of the test media concentration on days 0 and 5 revealed mean concentrations of 2.7 and 2.47 mg a.s./L in the three replicate test systems (overall mean 2.58 mg a.s./L).
The exposure period was 5-day duration, at 25 °C with continuous illumination at 290 to 300 foot-candles (3100 to 3200 lux), shaking rate of 100 rpm. The pH on day 0 was 7.5 and rose to 8.3 in controls and 8.2 in the test media by day 5. Conductivity during the same period rose from 80 to 90 µS/cm.
Over the 5 day test period cell density was inhibited by 20% in the test medium. The morphology of the cells in all groups appeared normal. There was no statistical difference between the control and solvent control groups and consequently these were pooled to perform the statistical analysis of the test group. The test group was found to be statistically different from the pooled control. The results are summarized in Table 8.2.6.1-2.

Table 8.2.6.1-2: Effect of triticonazole (BAS 595 F) on the growth of blue-green alga *Anabaena flos-aquae*

Mean measured concentration (mg a.s./L)	Cell density (x 10^4 cells / ml)					day 5 percent inhibition
	day 1	day 2	day 3	day4	day 5	
Control	5.3	11	29	88	153	--
Solvent control	6.0	13	28	89	153	--
2.6	2.9	8.0	21	80	123[b]	20

b = significantly reduced compared to pooled controls (t-test)

The 120-hour EC_{50} was empirically estimated to be > 2.6 mg a.s./L (the mean measured test concentration) since cell density was inhibited by only 20% at this treatment. The concentration tested exceeded the foreseeable annual maximum application rate, therefore, additional testing at a concentration range sufficient to derive an EC_{50} value was not performed. Based on a t-Test, the 5-day NOEC was determined to be <2.6 mg a.s./L.

The following 72-hour algae study on the freshwater green algae *Pseudokirchneriella subcapitata* has not been evaluated previously on EU level is provided in support of the aquatic risk assessment. The study has been performed because the older (EU agreed) study on *S. capricornutum* with triticonazole (BASF DocID 1992/1001384 / Doc Name R013056) shows some apparent deficiencies / deviations from current guidelines (*e.g.* only one test item concentration was tested; pH in the control medium increase by > 1.5 units during the test). Therefore, already during Annex I inclusion process it was stated by the zRMS that the endpoints of this study are of very limited use for the risk assessment (see zRMS Comments in DAR, Volume 3, Annex B.9., 2003). Thus, the results of the new study are considered as relevant endpoints for the risk assessment.

Report: CA 8.2.6.1/2
Seeland-Fremer A.,Wydra V., 2014a
Toxicity of Reg.No. 4378513 (Triticonazole technical (BAS 595 F)) to Pseudokirchneriella subcapitata in an algal growth inhibition test
2014/1083347

Guidelines: OECD 201 (2011), OECD Series on Testing and Assessment No. 23 (2000) - Aquatic Toxicity Testing of Difficult Substances and Mixtures, (EC) No 761/2009 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH 2009 - Part C.3: Algal Inhibition Test, 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)

GLP: yes
(certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

Executive Summary

In a 72-hour static acute toxicity laboratory study, the effect of triticonazole on the growth of the green alga *Pseudokirchneriella subcapitata* was investigated. The following nominal concentrations were applied: 0.1, 0.32, 1.0, 3.2 and 10 mg a.s./L. Additionally, a dilution water control and a solvent 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. After 72 hours of exposure, no morphological effects on algae were observed in the control groups and at test item concentrations up to and including 3.2 mg a.s./L. The shape of the algal cells was affected at 10 mg a.s./L with cells being thicker and not curved. Growth rate and yield were significantly reduced at the highest test item concentration after 72 hours of exposure.

In a 72-hour algae test with *Pseudokirchneriella subcapitata*, the E_rC_{50} and the E_yC_{50} of triticonazole were both determined to be > 10 mg a.s./L, based on nominal concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Triticonazole technical (BAS 595 F; Reg. No. 4378513), batch no. COD-001440, purity: 91.3% (± 1.0%).

B. STUDY DESIGN

Test species: Unicellular fresh water green alga, *Pseudokirchneriella subcapitata* (Reinsch) Korshikov (syn. *Selenastrum capricornutum* Prinz), specification: SAG 61.81; stock obtained from "Sammlung von Algenkulturen", Göttingen, Germany.

Test design: Static system; test duration 72 hours; 5 test item concentrations, each with 3 replicates per treatment, solvent control and control each with 6 replicates; daily assessment of growth.

Endpoints: EC_{10} and EC_{50} with respect to growth rate and yield after exposure over 72 hours.

Test concentrations: Control, solvent control (0.1 mL dimethylformamide/L), 0.1, 0.32, 1.0, 3.2 and 10 mg a.s./L (nominal).

Test conditions: 50 mL Erlenmeyer flasks; test volume 50 mL; test medium: OECD medium; pH 7.9 - 8.0 at test initiation and pH 9.3 - 9.8 at test termination; temperature: 21.3°C - 23.0°C; initial cell densities 5 x 10^3 cells/mL; continuous light at 5181 - 5960 lux; continuous stirring.

Analytics: Analytical verification of test item concentrations was conducted using an HPLC-method with UV detection.

Statistics: Descriptive statistics, probit analysis ($p < 0.05$) for determination of EC_x values, William´s t-test ($p < 0.05$) for determination of the NOEC values.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each concentration at the beginning and at the end of the test. The measured values of triticonazole ranged from 96% to 120% of nominal at test initiation and from 92% to 109% of nominal at test termination. The following biological results are based on nominal concentrations.

Biological results: After 72 hours of exposure, no morphological effects on algae were observed in the control groups and at test item concentrations up to and including 3.2 mg a.s./L. The shape of the algal cells was affected at 10 mg a.s./L with cells being thicker and not curved. Growth rate and yield were significantly reduced at the highest test item concentration after 72 hours of exposure (Williams t- test, $p < 0.05$). The effects on algal growth are summarized in Table 8.2.6.1-3.

Table 8.2.6.1-3: Effect of triticonazole (BAS 595 F) on the growth of green alga *Pseudokirchneriella subcapitata*

Concentration [mg a.s./L] (nominal)	**Solvent control**	**Control**	**0.1**	**0.32**	**1.0**	**3.2**	**10**
Inhibition in 72 h (growth rate) [%] #	--	-0.3	0.2	-0.4	-1.8	1.2	5.6 *
Inhibition in 72 h (yield) [%] #	--	-1.9	1.4	-2.7	-10.7	6.3	27.0 *
	Endpoints [mg a.s./L] (nominal)						
E_rC_{50} (72 h)	> 10						
E_yC_{50} (72 h)	> 10						
E_rC_{10} (72 h)	> 10						
E_yC_{10} (72 h)	4.39 (95% confidence limits: 2.86 - 5.46)						
NOEC (72 h) overall	3.2						

Negative values indicate stimulated growth compared to the solvent control.
* Statistically significant differences compared to the solvent control (Williams t-test, $p < 0.05$, one-sided).

III. CONCLUSION

In a 72-hour algae test with *Pseudokirchneriella subcapitata*, the E_rC_{50} and the E_yC_{50} of triticonazole were both determined to be > 10 mg a.s./L, based on nominal concentrations.

Due to the reasons given below, the algae study on *Selenastrum capricornutum* (Doc Name R013056 / DocID 1992/1001384) is not considered to be valid according to current standard and is thus not considered for the aquatic risk assessment. Nevertheless, for better transparency, the summary of this study was extracted from the originally submitted EU dossier of triticonazole and is provided below.

Executive Summary

<u>Algae, *Selenastrum capricornutum*</u>: The toxicity of triticonazole to the freshwater algae *Selenastrum capricornutum* was tested in the laboratory according to OECD guideline 201 and EPA E-72-2. Following the results of a range-finding experiment, in which no effect was found at concentrations of 0.01, 0.1 and 1.0 mg a.s./L, a limit-test design was adopted. The highest concentration tested was 1.0 mg/L. This was the highest attainable concentration achievable using tetrahydrofuran as a solvent system. 6 replicates flasks were tested along with 3 replicate controls and 3 replicate solvent controls. The temperature was 24°C and lighting was continuous at 7000 lux. All cultures were shaken on an orbital platform.
Cell density was measured at 0, 24, 48, 72 and 96 hours by measuring absorbance at 665 nm.
The measured concentrations of triticonazole in the test medium at 0 and 96 hr were found to be close to nominal. No effects were found of triticonazole on algal growth. No abnormalities of algal cells were detected. The results are summarized in Table 8.2.6.1-4.

Table 8.2.6.1-4: Effect of triticonazole (BAS 595 F) on the growth of green alga *Selenastrum capricornutum*

Mean measured concentration. (mg/L)	pH hr	Absorbance values at 665 nm					pH
		0 hr	24 hr	48 hr	72 hr	96 hr	96 hr
Control (mean 3 replicates)	7.8	0.025	0.104	0.286	0.387	0.552	9.6
Solvent control (mean 3 reps)	7.7	0.025	0.106	0.272	0.389	0.560	9.5
1.0 (mean 6 reps)	7.6	0.025	0.101	0.295	0.384	0.557	9.5

Based on biomass the E_bC_{50} (96 h) was determined to be >1.0 mg/L. The NOEC was also reported to be ≥ 1.0 mg/l. There was no difference in the rate of growth between 0 and 24 hours. Accordingly, the E_rC_{50} is ≥ 1.0 mg/L.

This study on *Selenastrum capricornutum* shows some apparent deficiencies / deviations from current guidelines (*e.g.* only one test item concentration was tested; pH in the control medium increase by > 1.5 units during the test). Therefore, already during the Annex I inclusion process it was stated by the zRMS that the endpoints of this study are of very limited use for the risk assessment (see zRMS Comments in DAR, Volume 3, Annex B.9., 2003). Thus, the results of the new study on *P. subcapitata* are considered as relevant endpoints for the risk assessment.

The following 72-hour algae study on the freshwater green algae *Pseudokirchneriella subcapitata* performed with RPA 406203 was (wrongly) not listed in the “Application” document submitted for the triticonazole AIR 3 renewal process. However, it is provided in support of the aquatic risk assessment and has not been evaluated previously on EU level.

Report:	CA 8.2.6.1/3 Hoffmann F., 2009a Effect of Reg.No. 5079359 (metabolite of BAS 595 F, Triticonazole) on the growth of the green alga Pseudokirchneriella subcapitata 2009/1050280
Guidelines:	OECD 201
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

In a 72-hour static toxicity laboratory study, the effect of RPA 406203 on the growth of the green alga *Pseudokirchneriella subcapitata* was investigated. The following nominal concentrations were applied: 0 (control), 1.56, 3.13, 6.25, 12.5, 25, 50 and 100 mg RPA 406203/L (corresponding to mean measured concentrations of 1.40, 2.54, 5.03, 10.93, 18.4, 40.5 and 79.5 mg RPA 406203/L). Assessment of growth was conducted 24, 48, and 72 h after test initiation.

The biological results are based on mean measured concentrations of the test item. No morphological effects on the algae were observed.

In a 72-hour algae test with *Pseudokirchneriella subcapitata,* the E_rC_{50} (72 h) of RPA 406203 was determined to be 73.32 mg/L based on mean measured concentrations, the E_yC_{50} (72 h) was 9.29 mg/L (mean measured).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: RPA 406203 (Reg. No. 5079359; photo-metabolite of triticonazole), batch no. BESS0578; purity: 99.9% (± 1%).

B. STUDY DESIGN

Test species: Unicellular fresh water green alga, *Pseudokirchneriella subcapitata* (Reinsch) Korshikov (syn. *Selenastrum capricornutum* Prinz); specification: SAG 61.81; stock obtained from "Sammlung von Algenkulturen", Göttingen, Germany.

Test design: Static system (72 hours); 7 test item concentrations with 5 replicates for each plus a control with 10 replicates; daily assessment of growth.

Endpoints: EC_{10} and EC_{50} with respect to growth rate and yield after exposure over 72 hours.

Test concentrations: Control, 1.56, 3.13, 6.25, 12.5, 25, 50 and 100 mg RPA 406203/L (nominal), corresponding to mean measured concentrations of 1.40, 2.54, 5.03, 10.93, 18.4, 40.5 and 79.5 mg/L.

Test conditions: 100 mL Erlenmeyer flasks, test volume: 60 mL; nutrient solution according to OECD 201; pH 8.1 at test initiation and pH 7.68 - 8.02 at test termination; temperature: 22 ± 1°C; initial cell densities: 1 x 10^4 cells/mL; continuous light at about 8000 lux, continuous shaking at about 135 rpm.

Analytics: Analytical verification of test item concentrations was conducted using an HPLC-method with MS-detection.

Statistics: Descriptive statistics, probit analysis for determination of EC_x values for growth rate and yield.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each test concentration at the beginning and at the end of the test. Mean measured values for RPA 406203 ranged from 68.2% to 102.5% of nominal concentrations at test initiation and from 68.3% to 106.6% of nominal at test termination. The following biological results are based on mean measured concentrations.

Biological results: No morphological effects on the algae were observed. The effects on algal growth are summarized in Table 8.2.6.1-5.

Table 8.2.6.1-5: Effect of RPA 406203 on the growth of the green algae *Pseudokirchneriella subcapitata*

Concentration [mg/L] (nominal)	**Control**	**1.56**	**3.13**	**6.25**	**12.5**	**25**	**50**	**100**
Concentration [mg/L] (mean measured)	**--**	**1.40**	**2.54**	**5.03**	**10.93**	**18.4**	**40.5**	**79.5**
Inhibition in 72 h (growth rate) [%] #	--	-0.4	2.7	9.4	18.2	35.7	40.3	46.9
Inhibition in 72 h (yield) [%] #	--	-1.6	11.1	33.4	54.5	79.1	82.8	87.6
Endpoints [mg RPA 406203/L] (mean measured)								
E_rC_{50} (72 h)	73.32 (95% confidence limits: 61.4 - 91.0)							
E_rC_{10} (72 h)	4.29 (95% confidence limits: 3.08 - 5.56)							
E_yC_{50} (72 h)	9.29 (95% confidence limits: 8.44 - 10.22)							
E_yC_{10} (72 h)	1.99 (95% confidence limits: 1.59 - 2.39)							

Negative values indicate stimulated growth compared to the control.

III. CONCLUSION

In a 72-hour algae test with *Pseudokirchneriella subcapitata,* the E_rC_{50} (72 h) of RPA 406203 was determined to be 73.32 mg/L based on mean measured concentrations, the E_yC_{50} (72 h) was 9.29 mg/L (mean measured).

The following 96-hour algae non-GLP study on the freshwater green algae *Pseudokirchneriella subcapitata* performed with the intermediate DMBCP (RPA 405217) has not been evaluated previously on EU level. Following the recommendations and validity criteria defined in the current OECD guideline 201 for alga testing (OECD, 2011) the study is considered to be not valid because:

- Study was not conducted under GLP conditions.
- No analytical verification of test item concentrations was conducted.

Thus, the results of this study will not be considered in the aquatic risk assessment. Nevertheless, a summary of the study is provided for completeness. In accordance to the EFSA Aquatic Guidance Document (EFSA, 2013) and OECD guideline 201 (2011) the 72 h endpoints obtained in the 96 h study are considered as relevant endpoint for the aquatic risk assessment, therefore both the 72 h and 96 h endpoints are reported in the study summary below.

Report:	CA 8.2.6.1/4 Peters A., 1992a Toxicity of RPA 405217 (DMBCP) to the Scenedesmus subspicatus CHODAT (96 hours) (according to guideline OECD No. 201 from June 1984) C039712
Guidelines:	OECD 201
GLP:	no

Executive Summary

In a 96-hour static toxicity laboratory study, the effect of dimethylbencylidenecyclopentanone (DMBCP) on the growth of the green alga *Scenedesmus subspicatus* was investigated. The following concentrations were applied: 5.00, 10.00, 20.00, 40.00, 80.00 and 160.00 mg DMBCP/L (nominal). Assessment of growth was conducted 24 h, 48 h, 72 h and 96 h after test initiation.

The biological results are based on nominal concentrations. Morphological effects on algae were not assessed. Statistically significant effects compared to the control on growth rate and biomass were not evaluated.

In a 96-hour algae test with *Scenedesmus subspicatus* the E_rC_{50} for DMBCP was determined to be 56.38 mg/L, the E_bC_{50} was 8.64 mg/L based on nominal concentrations. After 72 hours the E_rC_{50} value for DMBCP was determined to be 35.41 mg/L and the E_bC_{50} was 8.08 mg/L (nominal).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Dimethylbencylidenecyclopentanone (DMBCP; RPA 405217) (BAS 555 F; Reg. no.: 4 539 595; intermediate), batch no: DA 696; purity: 956 g/kg.

B. STUDY DESIGN

Test species: Unicellular fresh water green alga, *Scenedesmus subspicatus* CHODAT (syn. *Desmodesmus subspicatus*); specification: SAG 86.81; stock obtained from "Sammlung von Algenkulturen", Göttingen, Germany.

Test design: Static system (96 hours); 6 test concentrations plus a control and a solvent control with 4 replicates for each; daily assessment of growth.

Endpoints: EC_{10} and EC_{50} with respect to growth rate and biomass after exposure over 72 and 96 hours.

Test concentrations: Control, solvent control (0.1 g/L Tween-80), 5.00, 10.00, 20.00, 40.00, 80.00, 160.00 mg DMBCP/L (nominal).

Test conditions: 250 mL Erlenmeyer flasks; nutrient solution according to OECD 201; pH 8.8 - 8.9 at test initiation and pH 9.3 - 10.4 at test termination; water temperature: 22.5 ± 1.0°C; initial cell densities: 10000 cells/mL; continuous light, light intensity: 8000 lux, shaking of test tubes three times daily.

Analytics: No analytical verification of test item concentrations was conducted.

Statistics: Descriptive statistics, probit analysis for determination of EC_x values.

II. RESULTS AND DISCUSSION

Analytical measurements: No analytical verification of test item concentrations was conducted. The following biological results are based on nominal concentrations.

Biological results: Morphological effects on algae were not assessed. Statistically significant effects compared to the control on growth rate and biomass were not evaluated. The results are summarized in Table 8.2.6.1-6.

Table 8.2.6.1-6: Effect of DMBCP on the growth of the green alga *Scenedesmus subspicatus*

Concentration [mg/L] (nominal)	Control	Solvent control	5.00	10.00	20.00	40.00	80.00	160.00
Inhibition in 72 h (growth rate) [%]	14.92	--	11.65	21.96	38.62	63.03	76.62	67.48
Inhibition in 72 h (biomass) [%]	23.68	--	34.40	53.34	72.01	85.93	91.64	91.09
Inhibition in 96 h (growth rate) [%]	8.80	--	6.16	12.76	33.41	62.99	67.16	51.58
Inhibition in 96 h (biomass) [%]	30.88	--	30.18	49.52	74.70	90.82	94.09	92.91
Endpoints [mg DMBCP/L] (nominal) *								
E_rC_{50} (72 h)	35.41							
E_rC_{10} (72 h)	3.44							
E_bC_{50} (72 h)	8.08							
E_bC_{10} (72 h)	0.76							
E_rC_{50} (96 h)	56.38							
E_rC_{10} (96 h)	5.54							
E_bC_{50} (96 h)	8.64							
E_bC_{10} (96 h)	1.14							

* Based on arithmetical evaluation (probit analysis).

III. CONCLUSION

In a 96-hour algae test with *Scenedesmus subspicatus* the E_rC_{50} for DMBCP was determined to be 56.38 mg/L, the E_bC_{50} was 8.64 mg/L based on nominal concentrations. After 72 hours the E_rC_{50} value for DMBCP was determined to be 35.41 mg/L and the E_bC_{50} was 8.08 mg/L (nominal).

CA 8.2.6.2 Effects on growth of an additional algal species

The following 120-hour algae study on the freshwater diatom *Navicula pelliculosa* was conducted due to U.S. specific data requirements. It is not required for registration in the EU and it has not been evaluated previously on EU level.
Following the recommendations and validity criteria defined in the current OECD guideline 201 for alga testing (OECD, 2011) the study is considered to be not valid because:

- The following validity criterion is not met: the mean coefficients of variation (CV) for section by section specific growth rate in both the dilution water control and the solvent control were significantly > 35% after 0 - 72 hours, 0 - 96 hours and 0 - 120 hours (*i.e.* about 40% - 52% in dilution water control and solvent control after 72 h, 96 h and 120 h).

- No response variables (*e.g.* average specific growth rate; yield) were calculated.

Nevertheless, a summary of the study is provided for completeness. In accordance to the EFSA Aquatic Guidance Document (EFSA, 2013) and OECD guideline 201 (2011) the 72 h endpoints obtained in the 120 h study are considered as relevant endpoint for the aquatic risk assessment, therefore both the 72 h and 120 h endpoints are reported in the study summary below.

Report:	CA 8.2.6.2/1 Hoberg J.R., 1998b Triticonazole - Toxicity to the freshwater diatom, Navicula pelliculosa R012969
Guidelines:	EPA 122-2, EPA 123-2, FIFRA
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary

In a 120-hour static acute toxicity laboratory study, the effect of triticonazole on the growth of the diatom *Navicula pelliculosa* was investigated. The nominal concentrations of 0.093, 0.19, 0.39, 0.75, 1.5 and 3.0 mg a.s./L were applied (corresponding to mean measured concentrations of 0.092, 0.17, 0.27, 0.59, 1.2 and 2.5 mg a.s./L). Assessment of growth was conducted 24 h, 48 h, 72 h, 96 h and 120 h after test initiation.

The biological results are based on the mean measured test item concentration. No statistically significant differences were determined between the control and the solvent control data. Thus, the data from both control groups were pooled and the pooled data set was used for statistical evaluation of treatment related effects. After 120 hours of exposure, no morphological effects on algae were observed in the control groups and in all test item concentrations. A significant reduction in cell density in all treatment levels tested as compared to the pooled control data was detected after 120 h of exposure.

In a 120-hour algae test with *Navicula pelliculosa*, the 72 h EC_{50} value of triticonazole was determined to be 1.5 mg a.s./L based on mean measured concentrations. After 120 hours the EC_{50} value for triticonazole was determined to be 0.95 mg a.s./L (mean measured).

I. MATERIAL AND METHODS

A. MATERIALS

Test item:	Triticonazole (BAS 595 F; Reg. No. 4378513), batch no. OP9750057, purity: 90.52%.

B. STUDY DESIGN

Test species:	Fresh water diatom, *Navicula pelliculosa*, strain 153045, originally obtained from the Carolina Biological Supply, Burlington, North Carolina, USA.
Test design:	Static system; test duration 120 hours; 6 test item concentrations, plus a solvent control and a control; 3 replicates per treatment; daily assessment of growth.
Endpoint:	EC_{50} with respect to cell density after exposure over 72 h and 120 hours.
Test concentrations:	Control, solvent control (0.1 mL DMF/L), 0.093, 0.19, 0.39, 0.75, 1.5 and 3.0 mg a.s./L (nominal), corresponding to mean measured concentrations of 0.092, 0.17, 0.27, 0.59, 1.2 and 2.5 mg a.s./L.
Test conditions:	250 mL Erlenmeyer flasks; test volume 100 mL; test medium: AAP medium; pH 7.3 - 7.5 at test initiation and pH 7.4 - 8.1 at test termination; temperature: 25°C; conductivity: 80 µS/cm; initial cell densities 1.0 x 10^4 cells/mL; continuous light at 4100 - 5400 lux; continuous shaking: 100 rpm.
Analytics:	Analytical verification of test item concentrations was conducted using an HPLC-method with UV detection.
Statistics:	Descriptive statistics, t-test to compare control to solvent control data; statistically significant differences of cell densities in test item concentrations compared to pooled control were determined using Williams' test ($p < 0.05$); determination of EC_{50} values by linear regression.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of the test item concentration was conducted at the beginning and at the end of the test. The measured values of triticonazole ranged from 74.4% to 104.3% of nominal at test initiation and from 66.7% to 93.5% of nominal at test termination. The following biological results are based on the mean measured test item concentrations.

Biological results: No statistically significant differences were determined between the control and the solvent control data (t-test). Thus, the data from both control groups were pooled and the pooled data set was used for statistical evaluation of treatment related effects. After 120 hours of exposure, no morphological effects on algae were observed in the control groups and in all test item concentrations. A significant reduction in cell density in all treatment levels tested as compared to the pooled control data was detected after 120 h of exposure (Williams test, $p < 0.05$). The results are summarized in Table 8.2.6.2-1.

Table 8.2.6.2-1: Effect of triticonazole on the growth of the fresh water diatom *Navicula pelliculosa*

Concentration [mg a.s./L] (nominal)	**Control**	**Solvent control**	**Pooled control**	**0.093**	**0.19**	**0.39**	**0.75**	**1.5**	**3.0**
Concentration [mg a.s./L] (mean measured)	--	--	--	**0.092**	**0.17**	**0.27**	**0.59**	**1.2**	**2.5**
Mean cell density (72 h) ± SD [x 10^4 cells/mL] [a)]	36 ± 3.4	36 ± 1.8	36 ± 2.4	39 ± 1.4	37 ± 1.3	35 ± 0.66	33 ± 0.90	15 ± 0.75	12 ± 2.6
Inhibition compared to the pooled control in 72 h (cell density) [%] #	--	--	--	-8.3	-2.8	2.8	8.3	58	67
Mean cell density (120 h) ± SD [x 10^4 cells/mL]	155 ± 1.4	151 ± 2.5	153 ± 2.5	150 ± 1.1 *	149 ± 3.0 *	143 ± 2.3 *	122 ± 2.2 *	46 ± 2.6 *	33 ± 1.8 *
Inhibition compared to the pooled control in 120 h (cell density) [%]	--	--	--	1.9	2.3	6.7	21	70	78
Endpoints [mg triticonazole/L] (mean measured)									
EC_{50} (72 h)	1.5 (95% confidence limits: 0.72 - 3.3)								
EC_{50} (120 h)	0.95 (95% confidence limits: 0.47 - 2.0)								

SD: Standard deviation.
Negative values indicate stimulated growth compared to the pooled control.
* Statistically significantly reduced compared to the pooled control (Williams Test; $p < 0.05$).
a) Statistically significant differences compared to the pooled control were only determined for cell density after 120 h of exposure.

III. CONCLUSION

In a 120-hour algae test with *Navicula pelliculosa*, the 72 h EC_{50} value of triticonazole was determined to be 1.5 mg a.s./L based on mean measured concentrations. After 120 hours the EC_{50} value for triticonazole was determined to be 0.95 mg a.s./L (mean measured).

The following 120-hour algae study on the marine diatom *Skeletonema costatum* was conducted due to U.S. specific data requirements. It is not required for registration in the EU and it has not been evaluated previously on EU level.
Following the recommendations and validity criteria defined in the current OECD guideline 201 for alga testing (OECD, 2011) the study is considered to be not valid because:

- The following validity criterion is not met: the mean coefficients of variation (CV) for section by section specific growth rate in both the dilution water control and the solvent control were significantly > 35% after 0 - 72 hours, 0 - 96 hours and 0 - 120 hours (*i.e.* about 50% - 60% in dilution water control and 60% - 68% in solvent control after 72 h, 96 h and 120 h).

- No response variables (*e.g.* average specific growth rate; yield) were calculated.

Nevertheless, a summary of the study is provided for completeness. In accordance to the EFSA Aquatic Guidance Document (EFSA, 2013) and OECD guideline 201 (2011) the 72 h endpoints obtained in the 120 h study are considered as relevant endpoint for the aquatic risk assessment, therefore both the 72 h and 120 h endpoints are reported in the study summary below.

Report: CA 8.2.6.2/2
Hoberg J.R., 1998c
Triticonazole - Toxcity to the marine diatom, Skeletonema costatum
B004429
Guidelines: EPA 122-2, EPA 123-2
GLP: yes

Executive Summary

In a 120 h static toxicity laboratory study, the effect of triticonazole on the growth of the marine diatom *Skeletonema costatum* was investigated. The following nominal concentrations were applied: 0.031, 0.062, 0.13, 0.25, 0.50 and 0.99 mg triticonazole/L, corresponding to mean measured concentrations of 0.031, 0.066, 0.10, 0.23, 0.44 and 0.97 mg a.s./L. Additionally, a solvent control (dimethylformamide) and a dilution water control were set up. Assessment of growth was conducted after 24, 48, 72, 96 and 120 h after test initiation.

The biological results are based on mean measured concentrations of the test item. No statistically significant differences were determined between the control and the solvent control data. Thus, the data from both control groups were pooled and the pooled data set was used for statistical evaluation of treatment related effects after 120 h of exposure. Inhibition after 72 h of exposure is based on comparison to the solvent control data. After 120 hours of exposure statistically significant differences compared to the pooled control were observed for cell density in treatment levels ≥ 0.066 mg a.s./L. At test termination cells exposed to the treatment levels tested and the controls appeared normal.

In a 120 h algae test with *Skeletonema costatum*, the EC_{50} (120 h) based on cell density was determined to be 0.31 mg a.s./L (mean measured). The 72 h EC_{50} based on cell density was 0.29 mg a.s./L (mean measured).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Triticonazole (BAS 595 F; Reg. no.: 4378513), batch no. OP9750057; purity: 90.52%.

B. STUDY DESIGN

Test species: Marine diatom, *Skeletonema costatum*, strain CCMP 1332, in-house culture; stock originally obtained from Bigelow Laboratories, West Boothbay Harbor, Maine, USA.

Test design: Static system (120 hours); 6 test item concentrations plus a dilution water control and a solvent control with 3 replicates for each; daily assessment of growth.

Endpoints: EC_{50} with respect to cell density after exposure over 72 and 120 hours; NOEC after 120 hours of exposure.

Test concentrations: Control (dilution water), solvent control (0.1 mL dimethylformamide/L), 0.031, 0.062, 0.13, 0.25, 0.50 and 0.99 mg triticonazole/L (nominal), corresponding to mean measured concentrations of 0.031, 0.066, 0.10, 0.23, 0.44 and 0.97 mg a.s./L.

Test conditions: 250 mL Erlenmeyer flasks; test volume: 100 mL; artificially enriched seawater medium; pH 8.0 - 8.1 at test initiation and pH 8.3 - 8.9 at test termination; temperature: 20°C - 21°C; conductivity: 32 - 35 µS/cm; initial cell densities: 1 x 10^4 cells/mL; photoperiod: 16 hours light : 8 hours dark, light intensity: 3200 - 4900 lux, continuous shaking at 60 rpm.

Analytics: Analytical verification of test item concentrations was conducted using an HPLC method with UV detection.

Statistics: Descriptive statistics, t-test (α = 0.05) for comparison of cell densities in the control and the solvent control; EC_{50} values were determined by linear regression; NOEC (120 h) was determined using Williams Test (α = 0.05).

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each test concentration at the beginning and at the end of the test. Measured concentrations of triticonazole ranged from 84.6% to 111.3% of nominal concentrations at test initiation. At test termination measured concentrations of triticonazole were between 74.0% and 101.6% of nominal. The following biological results are based on mean measured concentrations.

Biological results: No statistically significant differences were determined between the control and the solvent control data (t-test; α = 0.05). Thus, the data from both control groups were pooled and the pooled data set was used for statistical evaluation of treatment related effects after 120 h of exposure. Inhibition after 72 h of exposure is based on comparison to the solvent control data. After 120 hours of exposure statistically significant differences compared to the pooled control were observed for cell density in treatment levels ≥ 0.066 mg a.s./L (Williams Test; α = 0.05). At test termination cells exposed to the treatment levels tested and the controls appeared normal. The effects on algal growth are summarized in Table 8.2.6.2-2.

Table 8.2.6.2-2: Effect of triticonazole on growth of the marine diatom *Skeletonema costatum*

Concentration [mg a.s./L] (nominal)	Control	Solvent control	Pooled control	0.031	0.062	0.13	0.25	0.50	0.99
Concentration [mg a.s./L] (mean measured)	**--**	**--**	**--**	**0.031**	**0.066**	**0.10**	**0.23**	**0.44**	**0.97**
Mean cell density (72 h) [x 10^4 cells/mL]	40	42	--	42	40	38	36	7.0	1.4
Inhibition of growth (72 h) [% of solvent control] [a)]	--	--	N.A	0	4.8	9.5	14	83	97
Mean cell density (120 h) [x 10^4 cells/mL]	155	156	156	156	153 *	147 *	145 *	21 *	3.8 *
Inhibition of growth (120 h) [% of pooled control] [+, a)]	--	--	--	- 0.07	2.0	5.9	7.0	87	98
Endpoints [mg triticonazole/L] (mean measured)									
EC_{50} (72 h)	0.29 (95% confidence limits: 0.14 - 0.61)								
EC_{50} (120 h)	0.31 (95% confidence limits: 0.13 - 0.76)								

N.A. = not applicable

* Statistically significantly different compared to the pooled control (Williams Test; α = 0.05).

[+] Negative values indicate stimulated growth compared to the pooled control.

[a)] Statistically significant differences compared to control data were only determined for mean cell density after 120 h of exposure.

III. CONCLUSION

In a 120 h algae test with *Skeletonema costatum*, the EC_{50} (120 h) based on cell density was determined to be 0.31 mg a.s./L (mean measured). The 72 h EC_{50} based on cell density was 0.29 mg a.s./L (mean measured).

CA 8.2.7 Effects on aquatic macrophytes

A study on duckweed *Lemna gibba* performed with triticonazole has already been evaluated during the previous Annex I inclusion process of triticonazole. No additional studies are required and no (new) study has been conducted.

CA 8.2.8 Further testing on aquatic organisms

This point is not triggered and not addressed via (new) toxicity studies.

References

EFSA (2013) EFSA Scientific Opinion. Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters. EFSA Journal 2013; 11(7): 3290.

European Commission (2013) Commission Regulation (EU) No 283/2013 of 1 March 2013 setting out the data requirements for active substances, in accordance with the Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market. OJ L 93, 3.4.2013, p. 1–84.

Hermsen, S.A.B., van den Brandhof, E.-J., van der Ven, L.T.M., Piersma, A.H. (2011) Relative embryotoxicity of two classes of chemicals in a modified zebrafish embryotoxicity test and comparison with their in vivo potencies. Toxicology in Vitro 2011; 25(3): 745-753.

OECD (2012) OECD Guideline for the Testing of Chemicals, Guideline 211, *Daphnia magna* Reproduction Test. OECD Publishing. Adopted: 02 October 2012, pp. 25.

OECD (2011) OECD Guidelines for the Testing of Chemicals, Guideline 201, Freshwater Algae and Cyanobacteria, Growth Inhibition Test. OECD Publishing. Adopted: 23 March 2006, Annex 5 corrected: 28 July 2011. pp. 25.

CA 8.3 Effects on arthropods

CA 8.3.1 Effects on bees

Since Annex I inclusion of the active substance triticonazole (BAS 595 F), new studies on honeybees have been performed with the active substance. As a result there are new endpoints, which are considered in the honeybee risk assessment. Summaries of these new studies are provided below.

Details on the EU agreed studies which have been already evaluated within the Annex I inclusion of triticonazole are provided in the EU Review documents of triticonazole (*i.e.* Draft Assessment Report (DAR), Volume 3, Annex B.9., 2003; Addendum to the DAR May, 2005; EFSA Scientific Report (2005) 33, 1 - 69).

For better transparency and traceability of the active substance history, the results of all studies are summarized in Table 8.3.1-1. Studies from the former EU review are given in grey letters.

Table 8.3.1-1: List of studies and endpoints with honeybees and the active substance triticonazole (BAS 595 F)

Substance	Test species	Endpoint	Value	Reference (BASF DocID)	EU agreed[1]
triticonazole	honeybee	48 h acute oral LD_{50}	> 155.5 µg a.s./bee	R005760	yes *EFSA Scientific Report* (2005) 33, 1-69
		48 h acute contact LD_{50}	> 100 µg a.s./bee		
		48 h acute oral LD_{50}	> 96.26 µg a.s./bee [2]	2006/1024251	no, new study (see CA 8.3.1.1)
		48 h acute contact LD_{50}	> 100 µg a.s./bee		

1) EU agreed denotes studies assessed during the previous EU evaluation process that are not necessarily listed in the list of endpoint as in the Conclusion on the peer review of triticonazole, *EFSA Scientific Report* (2005) 33, 1-69.

2) Relevant acute oral toxicity endpoint, which is used for the risk assessment.

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
Hernadi D., 2006a
Effects of BAS 595 F (acute contact and oral) on honey bees (Apis mellifera L.) in the laboratory
2006/1024251
Guidelines: OECD 213, OECD 214
GLP: yes
(certified by National Institute of Pharmacy, Budapest, Hungary)

Executive Summary

In a dose response test, worker bees (*Apis mellifera* L.) were exposed to triticonazole. The toxicity of the test product was determined in an oral test at a nominal concentration of 100 µg a.s./bee, resulting in an actual uptake of 96.26 µg a.s./bee. Additionally, honeybees were treated with dimethoate as reference standard at concentrations ranging from 0.07 to 0.30 µg dimethoate/bee (nominal) or with a solution of water and sugar as a control. The test was conducted with 5 replicates for the control and test item treatment, each of the test cages contained 10 bees. Assessment of mortality was done after 4, 24 and 48 hours.

No mortality was observed after 48 hours in the control and the test item treatment. Adverse effects on behaviour were not noticed.

In an acute oral toxicity test, the LD_{50} value (48 h) was > 96.26 µg a.s./bee.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Triticonazole (BAS 595 F, Reg. No. 4378513), batch no. COD-00601, purity: 90.3%.

B. STUDY DESIGN

Test species: Honeybee (*Apis mellifera* L.), worker bees (approx. 6 weeks); deriving from a healthy and queen-right colony, bred by Györgyi Purger Pordánné, Szentgál, Hungary, collected on the morning of use and kept under test conditions.

Test design: Limit test for oral toxicity; duration 48 h, 5 replicates for the control and the test item, 3 replicates for the reference item, each replicate consisting of 10 bees per cage; assessment of mortality after 4, 24 and 48 hours.

Endpoints: LD_{50} value, behavioural abnormalities.

Reference item: Dimethoate, 400 g/L.

Test concentrations: Control, 100.0 µg a.s./bee; resulting in an actual uptake of 96.26 µg a.s./bee.

Test conditions: Temperature: 23.8 - 25.9°C; relative humidity: 55% - 67%, photoperiod: 24 h darkness.

Statistics: Descriptive statistics.

II. RESULTS AND DISCUSSION

No mortality was observed after 48 hours in the control and the test item treatment. Adverse effects on behavior were not noticed. The results are summarized in Table 8.3.1.1.1-1.

Table 8.3.1.1.1-1: Toxicity of triticonazole to honeybees (*Apis mellifera*) in an oral toxicity test

Treatment	Uptake of test item	Mean mortality [%]	
[µg a.s./bee]	[µg a.s./bee]	24 h	48 h
Control	--	0	0
100.0	96.26	0	0
	Endpoint [µg a.s./bee]		
LD_{50}	> 96.26		

III. CONCLUSION

In an acute oral toxicity test, the LD_{50} value (48 h) was > 96.26 µg a.s./bee.

CA 8.3.1.1.2 Acute contact toxicity

Report: CA 8.3.1.1.2/1
Hernadi D., 2006a
Effects of BAS 595 F (acute contact and oral) on honey bees (Apis mellifera L.) in the laboratory
2006/1024251
Guidelines: OECD 213, OECD 214
GLP: yes
(certified by National Institute of Pharmacy, Budapest, Hungary)

Executive Summary

In a dose response test, worker bees (*Apis mellifera* L.) were exposed to triticonazole. The toxicity of the test product was determined in a contact test at a nominal concentration of 100 µg a.s./bee. Additionally, honeybees were treated with dimethoate as reference standard at concentrations ranging from 0.07 to 0.30 µg dimethoate/bee (nominal) or with a solution of water and CO_2 and a solution of CO_2 and acetone as controls. The test was conducted with 5 replicates for the controls and test item treatment, each of the test cages contained 10 bees. Assessment of mortality was done after 4, 24 and 48 hours.

No mortality was observed after 48 hours in the control and the test item treatment. Adverse effects on behavior were not noticed.

In an acute contact toxicity test, the LD_{50} (48 h) was > 100 µg a.s./bee.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Triticonazole (BAS 595 F, Reg. No. 4378 513), batch no. COD-00601, purity: 90.3%.

B. STUDY DESIGN

Test species: Honeybee (*Apis mellifera* L.), worker bees (approx. 6 weeks); deriving from a healthy and queen-right colony, bred by Györgyi Purger Pordánné, Szentgál, Hungary, collected on the morning of use and kept under test conditions.

Test design: Limit test for contact toxicity; duration 48 h, 5 replicates for the controls and the test item, 3 replicates for the reference item, each replicate consisting of 10 bees per cage; assessment of mortality after 4, 24 and 48 hours.

Endpoints: LD_{50} value, behavioral abnormalities.

Reference item: Dimethoate, 400 g/L.

Test concentrations: Control, acetone control, 100.0 µg a.s./bee

Test conditions: Temperature: 23.8 - 25.9°C; relative humidity: 55% - 67%, photoperiod: 24 h darkness.

Statistics: Descriptive statistics.

II. RESULTS AND DISCUSSION

No mortality was observed after 48 hours in the controls and the test item treatment. Adverse effects on behavior were not noticed. The results are summarized in Table 8.3.1.1.2-1.

Table 8.3.1.1.2-1: Toxicity of triticonazole to honeybees (*Apis mellifera*) in a contact toxicity test

Treatment	Mean mortality [%]	
[µg a.s./bee]	24 h	48 h
Control	0	0
Acetone control	0	0
100.0	0	0
	Endpoint [µg a.s./bee]	
LD_{50}	> 100.0	

III. CONCLUSION

In an acute contact toxicity test, the LD_{50} (48 h) was > 100 µg a.s./bee.

CA 8.3.1.2 Chronic toxicity to bees

The test was performed with the formulated product and reference is made to chapter CP 10.3.1.2 (see DocID 2014/1000023).

CA 8.3.1.3 Effects on honeybee development and other honeybee life stages

The test was performed with the formulated product and reference is made to chapter CP 10.3.1.3 (see DocID 2014/1000024).

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 with the active substance. New tier 2 studies have been performed with the formulated product and reference is made to chapter CP 10.3.2.2 (BASF DocID 2004/1025180, BASF DocID 2009/1098729, and BASF DocID 2007/1023106).

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 triticonazole (BAS 595 F), new studies on soil macro-organisms have been performed with the active substance and its relevant metabolites in soil. As a result there are new endpoints, which are considered in the respective risk assessment. Summaries of these new studies are provided below.

Details on the EU agreed studies which have been already evaluated within the Annex I inclusion of triticonazole are provided in the EU Review documents of triticonazole (*i.e.* Draft Assessment Report (DAR), Volume 3, Annex B.9., 2003; Addendum to the DAR May, 2005; EFSA Scientific Report (2005) 33, 1 - 69).

For better transparency and traceability of the active substance history, the results of all studies are summarized in Table 8.4-1. Studies from the former EU review are given in grey letters.

Table 8.4-1 Toxicity to non-target soil meso- and macrofauna of triticonazole and relevant metabolites

Substance (Reg.No, synonyms)	Species	Endpoint	Value [mg/kg dry soil]	Reference (BASF DocID)	EU agreed[1)]
triticonazole	*Eisenia fetida*	LC_{50} $LC_{50\ CORR}$	> 1000 * > 500	R013015	yes *EFSA Scientific Report* (2005) 33, 1-69
metabolite, Reg. no. 5 079 288; RPA 407922		LC_{50}	> 1000	C021833	yes *EFSA Scientific Report* (2005) 33, 1-69
metabolite, Reg. no. 5 059 144; RPA 406341		LC_{50}	> 1000	C020497	yes *EFSA Scientific Report* (2005) 33, 1-69
metabolite, Reg. no. 5 079 285; RPA 404766		LC_{50}	> 1000	C017900	yes *EFSA Scientific Report* (2005) 33, 1-69
triticonazole		NOEC NOEC CORR	≥ 500 * ≥ 250	R006093	yes *EFSA Scientific Report* (2005) 33, 1-69
metabolite, Reg. no. 5 079 288; RPA 407922		NOEC	125	2014/1000027	no, new study
metabolite, Reg. no. 5 059 144; RPA 406341		NOEC	≥ 10	2006/1030247	no, new study
metabolite, Reg. no. 5 079 285; RPA 404766		NOEC	≥ 250	2014/1000026	no, new study
triticonazole	*Folsomia candida*	NOEC	250	2014/1000021	no, new study

Substance (Reg.No, synonyms)	Species	Endpoint	Value [mg/kg dry soil]	Reference (BASF DocID)	EU agreed [1)]
metabolite, Reg. no. 5 079 288; RPA 407922		NOEC	≥ 500	2014/1000029	no, new study
metabolite, Reg. no. 5 059 144; RPA 406341		NOEC	≥ 50	2006/1031679	no, new study
metabolite, Reg. no. 5 079 285; RPA 404766		NOEC	≥ 500	2014/1000028	no, new study
triticonazole	*Hypoaspis aculeifer*	NOEC	≥ 1000	2014/1000022	no, new study
metabolite, Reg. no. 5 059 144; RPA 406341		NOEC	≥ 10	2014/1083348	no, new study

[1)] EU agreed denotes studies assessed during the previous EU evaluation process that are not necessarily listed in the list of endpoint as in the Conclusion on the peer review of triticonazole, *EFSA Scientific Report* (2005) 33, 1-69.
* Toxicity endpoint is re-adjusted using a soil factor of 2 to address the organic content of the soil (peat 10%), since the log P_{ow} of the substance is > 2.

CA 8.4.1 Earthworms – sub-lethal effects

Report: CA 8.4.1/1
Friedrich S., 2013a
Sublethal toxicity of Reg.No. 5079285 (metabolite of BAS 595 F, Triticonazole) to the earthworm Eisenia fetida in artificial soil
2014/1000026
Guidelines: OECD 222 (2004)
GLP: yes
(certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

Executive Summary

The effects of Reg. No. 5 079 285 (metabolite of BAS 595 F, triticonazole) on mortality, biomass development and reproduction of the earthworm *Eisenia fetida* (Annelida: Oligochaeta) were investigated in an extended laboratory study over 56 days. Five test concentrations (15.63, 31.25, 62.5, 125, 250 mg/kg dry soil) were incorporated into the soil (containing 10% peat) with four replicates per treatment, each containing 10 worms. An untreated control with 8 replicates was included. The reference item was tested in a separate study. Assessment of worm mortality, body weight and feeding activity was carried out after 28 days; assessment of reproduction (number of juveniles) was carried out after 56 days.

Reg. No. 5 079 285 did not show any statistically significant effects on mortality and body weight. The mortality of adult worms was about 0% – 2.5% in the treated variants and 2.5% in the control group. The weight change of adult worms was about 31.9% – 36.8% in the treated variants and 34.9% in the control group. The reproduction rates were not statistically different compared to the control up to a concentration of 250 mg/kg dry soil. No behavioral abnormalities were observed in any of the treatment groups. The feeding activity in all the treated groups was comparable to the control.

In a 56-day earthworm reproduction study with Reg. No. 5 079 285 (metabolite of BAS 595 F, triticonazole), no adverse effects on survival, biomass development and reproduction could be determined at concentrations up to and including 250 mg/kg dry soil. The NOEC for mortality, biomass and reproduction was determined to be 250 mg/kg dry soil, the highest concentration tested. The EC_{50} for reproduction was estimated to be higher than 250 mg/kg dry soil, the highest concentration tested.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Reg. No. 5 079 285 (metabolite of BAS 595 F, triticonazole; RPA 404766); batch no.: L67-148; analyzed purity: 99.3% (tolerance ± 1.0%).

B. STUDY DESIGN

Test species: Earthworm (*Eisenia fetida*), adult worms (with clitellum and weight range 302 to 450 mg), approximately 3 months old, source: W. Neudorff GmbH KG, followed by in-house culture.

Test design: 56-day test in treated artificial soil prepared according to OECD 222 (10% peat); different concentrations of the test item were incorporated into the soil; 6 treatment groups (5 test item concentrations, control); 4 replicates for the test item treatments and 8 replicates for the control with 10 worms each. Assessment of adult worm mortality, behavioral effects and biomass development was carried out after 28 days. Reproduction rate (number of offspring) was assessed after an additional 28 days (56 days after application).

Endpoints: Mortality, weight change, feeding activity and 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, 15.63, 31.25, 62.5, 125 and 250 mg Reg. No. 5 079 285/kg dry soil.

Test conditions: Artificial soil according to OECD 222 (with 10% peat); pH 6.0 – 6.06 at test initiation, pH 5.70 – 5.74 at test termination; water content 54.7% – 54.9% of maximum water holding capacity (WHC) at test initiation and 53.9% – 54.2% of the maximum WHC at test termination; temperature: 18.1 °C – 21.9 °C; photoperiod: 16 h light : 8 h dark, light intensity: 540 lux.

Statistics: Descriptive statistics, Fisher's Exact Binominal Test for mortality ($\alpha = 0.05$, one-sided greater), Williams t-test for weight change and reproduction ($\alpha = 0.05$, one-sided smaller).

II. RESULTS AND DISCUSSION

Reg. No. 5 079 285 (metabolite of BAS 595 F, triticonazole) did not show any statistically significant effects on mortality (Fisher's Exact Binominal Test for mortality, $\alpha = 0.05$, one-sided greater) and body weight (Williams t-test, $\alpha = 0.05$, one-sided smaller). The mortality of adult worms was about 0% – 2.5% in the treated variants and 2.5% in the control group. The weight change of adult worms was about 31.9% – 36.8% in the treated variants and 34.9% in the control group. The reproduction rates were not statistically different compared to the control up to a concentration of 250 mg/kg dry soil (Williams t-test, $\alpha = 0.05$, one-sided smaller). No behavioral abnormalities were observed in any of the treatment groups. The feeding activity in all the treated groups was comparable to the control. The results are summarized in Table 8.4.1-1.

Table 8.4.1-1: Effects of Reg. No. 5 079 285 on earthworms (*Eisenia fetida)* in a 56-day reproduction study

Reg. No. 5 079 285 [mg/kg dry soil]	Control	15.63	31.25	62.5	125	250
Mortality (day 28) [%]	2.5	2.5	0	0	2.5	0
Weight change (day 28) [%]	34.9	36.8	33.4	36.1	36.7	31.9
No. of juveniles (day 56)	106.0	117.3	101.0	110.3	104.5	97.5
Reproduction (day 56) [% of control]	100	110.6	95.3	104.0	98.6	92.0
Endpoints [mg/kg dry soil]						
NOEC (day 28) (mortality and weight)	≥ 250					
NOEC (day 56) (reproduction)	≥ 250					
EC_{50}	> 250					

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 72.7% and 98.8% compared to the control at the tested concentrations of 5 and 10 mg product/kg dry soil.

III. CONCLUSION

In a 56-day earthworm reproduction study with Reg. No. 5 079 285 (metabolite of BAS 595 F, triticonazole), no adverse effects on survival, biomass development and reproduction could be determined at concentrations up to and including 250 mg/kg dry soil. The NOEC for mortality, biomass and reproduction was determined to be 250 mg/kg dry soil, the highest concentration tested. The EC_{50} for reproduction was estimated to be higher than 250 mg/kg dry soil, the highest concentration tested.

Report: CA 8.4.1/2
Friedrich S., 2013b
Sublethal toxicity of Reg.No. 5079288 (metabolite of BAS 595 F, Triticonazole) to the earthworm Eisenia fetida in artificial soil
2014/1000027

Guidelines: OECD 222 (2004)

GLP: yes
(certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

Executive Summary

The effects of Reg. No. 5 079 288 (metabolite of BAS 595 F, triticonazole) on mortality, biomass development and reproduction of the earthworm *Eisenia fetida* (Annelida: Oligochaeta) were investigated in an extended laboratory study over 56 days. Five test concentrations (15.63, 31.25, 62.5, 125, 250 mg/kg dry soil) were incorporated into the soil (containing 10% peat) with four replicates per treatment, each containing 10 worms. An untreated control with 8 replicates was included. The reference item was tested in a separate study. Assessment of worm mortality, body weight and feeding activity was carried out after 28 days; assessment of reproduction (number of juveniles) was carried out after 56 days.

Reg. No. 5 079 288 did not show any statistically significant effects on mortality and body weight. The mortality of adult worms was about 0% – 5.0% in the treated variants and 0% in the control group. The weight change of adult worms was about 40.1% – 44.8% in the treated variants and 41.6% in the control group. The reproduction rates were not statistically different compared to the control up to a concentration of 250 mg/kg dry soil. No behavioral abnormalities were observed in any of the treatment groups. The feeding activity in all the treated groups was comparable to the control.

In a 56-day earthworm reproduction study with Reg. No. 5 079 288 (metabolite of BAS 595 F, triticonazole), no adverse effects on survival and biomass development could be determined at concentrations up to and including 250 mg/kg dry soil. Statistically significant effects on number of juveniles of *Eisenia fetida* were determined at the tested concentration of 250 mg/kg dry soil. The NOEC for mortality and biomass was determined to be 250 mg/kg dry soil, the highest concentration tested, whereas the NOEC for reproduction was determined to be 125 mg/kg dry soil. The EC_{50} for reproduction was estimated to be higher than 250 mg/kg dry soil, the highest concentration tested.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Reg. No. 5 079 288 (metabolite of BAS 595 F, triticonazole; RPA 407922); batch no.: 33484-39; analyzed purity: 99.5% (tolerance ± 1.0%).

B. STUDY DESIGN

Test species: Earthworm (*Eisenia fetida*), adult worms (with clitellum and weight range 284 to 468 mg), approximately 3 months old, source: W. Neudorff GmbH KG, followed by in-house culture.

Test design: 56-day test in treated artificial soil prepared according to OECD 222 (10% peat); different concentrations of the test item were incorporated into the soil; 6 treatment groups (5 test item concentrations, control); 4 replicates for the test item treatments and 8 replicates for the control with 10 worms each. Assessment of adult worm mortality, behavioral effects and biomass development was carried out after 28 days exposure of adult worms in treated artificial soil. Reproduction rate (number of offspring) was assessed after additional 28 days (assessed 56 days after application).

Endpoints: Mortality, weight change, feeding activity and 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, 15.63, 31.25, 62.5, 125 and 250 mg Reg. No. 5 079 288/kg dry soil.

Test conditions: Artificial soil according to OECD 222 (with 10% peat); pH 6.12 – 6.16 at test initiation, pH 5.71 – 5.77 at test termination; water content 54.7% – 55.0% of maximum water holding capacity (WHC) at test initiation and 54.1% – 54.5% of the maximum WHC at test termination; temperature: 18.6 °C – 21.9 °C; photoperiod: 16 h light : 8 h dark, light intensity: 520 lux.

Statistics: Descriptive statistics, Fisher's Exact Binominal Test for mortality ($\alpha = 0.05$, one-sided greater), Williams t-test for weight change and reproduction ($\alpha = 0.05$, one-sided smaller), Probit analysis.

II. RESULTS AND DISCUSSION

Reg. No. 5 079 288 (metabolite of BAS 595 F, triticonazole) did not show any statistically significant effects on mortality (Fisher's Exact Binominal Test for mortality, α = 0.05, one-sided greater) and body weight (Williams t-test, α = 0.05, one-sided smaller). The mortality of adult worms was about 0% – 5.0% in the treated variants and 0% in the control group. The weight change of adult worms was about 40.1% – 44.8% in the treated variants and 41.6% in the control group. The reproduction rates were not statistically different compared to the control up to a concentration of 250 mg/kg dry soil (Williams t-test, α = 0.05, one-sided smaller). No behavioral abnormalities were observed in any of the treatment groups. The feeding activity in all the treated groups was comparable to the control. The results are summarized in Table 8.4.1-2.

Table 8.4.1-2: Effects of Reg. No. 5 079 288 on earthworms (*Eisenia fetida*) in a 56-day reproduction study

Reg. No. 5 079 288 [mg/kg dry soil]	Control	15.63	31.25	62.5	125	250
Mortality (day 28) [%]	0	0	0	5.0	0	5.0
Weight change (day 28) [%]	41.6	40.8	42.5	41.1	44.8	40.1
No. of juveniles (day 56)	122.9	117.3	121.8	116.8	106.8	90.3 *
Reproduction (day 56) [% of control]	100	95.4	99.1	95.0	86.9	73.4
Endpoints [mg/kg dry soil]						
NOEC (day 28) (mortality and weight)	≥ 250					
NOEC (day 56) (reproduction)	125					
EC_{50} (day 56)	> 250					

* Statistically significantly different from the control (Williams t-test, α = 0.05).

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 72.7% and 98.8% compared to the control at the tested concentrations of 5 and 10 mg product/kg dry soil.

III. CONCLUSION

In a 56-day earthworm reproduction study with Reg. No. 5 079 288 (metabolite of BAS 595 F, triticonazole), no adverse effects on survival and biomass development could be determined at concentrations up to and including 250 mg/kg dry soil. Statistically significant effects on number of juveniles of *Eisenia fetida* were determined at the tested concentration of 250 mg/kg dry soil. The NOEC for mortality and biomass was determined to be 250 mg/kg dry soil, the highest concentration tested, whereas the NOEC for reproduction was determined to be 125 mg/kg dry soil. The EC_{50} for reproduction was estimated to be higher than 250 mg/kg dry soil, the highest concentration tested.

Report: CA 8.4.1/3
Wolf A., 2006a
Effects of Reg.No. 5059144 (RPA 406341, metabolite of BAS 595 F) on growth and reproduction of earthworms (Eisenia fetida) in artificial soil with 5% peat
2006/1030247
Guidelines: OECD 222
GLP: yes
(certified by Landesamt fuer Umweltschutz und Gewerbeaufsicht, Mainz, Germany Fed.Rep.)

Executive Summary

The effects of RPA 406341 on mortality, biomass development and reproduction of the earthworm *Eisenia fetida* were investigated in a 56-days extended laboratory study. Two application rates (1 and 10 mg RPA 406341/kg dry soil) were incorporated into the soil (5% peat) with four replicates per treatment (each containing 10 worms). An untreated control with 8 replicates was included. Assessment of adult worm mortality, behavioral effects and biomass development was carried out after 28 days, assessment of reproduction rate (number of juveniles) was carried out after 56 days.

No mortality was observed in the control and all test item treatment groups. No statistically significant effects on mortality, body weight and reproduction were observed up to the highest rate tested.

In a 56-day reproduction study with RPA 406341, no statistically significant effects on mortality, growth and reproduction of earthworms (*Eisenia fetida*) were observed up to a rate of 10 mg RPA 406341/kg dry soil, the highest rate tested. Therefore the NOEC was determined to be 10 mg RPA 406341/kg dry soil.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Reg. No. 5 059 144 (metabolite of BAS 595 F, triticonazole; RPA 406341); batch BESS0541; purity 99%.

B. STUDY DESIGN

Test species: Earthworm (*Eisenia fetida*), adult worms (with clitellum and weight of 300 - 500 mg), less than 1 year old; source: in-house culture

Test design: Different concentrations of the test item are mixed homogeneously into the soil. 4 treatment groups (2 test item rates, control, reference item); 4 replicates/group with 10 worms each; 8 replicates for the control. Assessment of worm mortality, behavioral effects and weight change after 28 days of exposure, after additional 28 days (56 days after application) determination of number of offspring.

Endpoints: Mortality, weight change, behavioral effects, reproduction rate.

Reference item: Benlate (Benomyl 50% nominal). The reference item was tested at 5 mg/kg dry soil.

Test rates: Control, 1 and 10 mg RPA 406341/kg dry soil.

Test conditions: Artificial soil according to OECD 222 (with a reduced content of peat of 5%); pH 5.7 – 5.9 at test initiation, 6.2 – 6.3 at test termination; water content 60% at test termination; temperature: 19 °C – 21 °C; photoperiod: 16 hours light : 8 hours dark, light intensity: 400 lux – 700 lux. Feeding with cow manure.

Statistics: Descriptive statistics; Bonferroni-t-test, for weight change and reproduction data, $\alpha = 0.05$.

II. RESULTS AND DISCUSSION

No mortality was observed in the control and all test item treatment groups. No statistically significant effects on mortality, body weight and reproduction were observed up to the highest rate tested (Bonferroni-t-test, $\alpha = 0.05$). The results are summarized in Table 8.4.1-3.

Table 8.4.1-3: Effect of RPA 406341 on earthworm (*Eisenia fetida*) in a 56-day reproduction study

RPA 406341 [mg/ kg dry soil]	Control	1	10
Mortality (28 d) [%]	0	0	0
Weight change (28 d) [%]	+59.96	+61.33	+57.87
Number of juveniles (56 d)	105.00	91.75	112.00
Reproduction in percent of control (56 d) [%]	--	87.38	106.67
	Endpoints [mg RPA 406341/kg dry soil]		
NOEC (56 d)	≥ 10		
EC_{50}	> 10		

III. CONCLUSION

In a 56-day reproduction study with RPA 406341, no statistically significant effects on mortality, growth and reproduction of earthworms (*Eisenia fetida*) were observed up to a rate of 10 mg RPA 406341/kg dry soil, the highest rate tested. Therefore the NOEC was determined to be 10 mg RPA 406341/kg dry soil.

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
Friedrich S., 2013c
Effects of BAS 595 F (Triticonazole) on the reproduction of the collembolan Folsomia candida
2014/1000021
Guidelines: OECD 232 (2009), ISO 11267 (1999)
GLP: yes
(certified by Saechsisches Staatsministerium fuer Umwelt und Landesentwicklung, Dresden, Germany)

Executive Summary

The effects of BAS 595 F on mortality and reproduction of the collembola *Folsomia candida* were investigated in a chronic laboratory study over 28 days. Five application rates (62.5, 125, 250, 500 and 1000 mg a.s./kg dry soil) were incorporated into the soil with 4 replicates per test item treatment. An untreated control and a solvent control with acetone, each with 8 replicates, were included. All replicates contained 10 collembolans. Assessment of mortality, reproduction rate (number of juveniles) and behavior was carried out after 28 days.

Statistically significant effects on mortality compared to the solvent control was observed at concentrations of 500 and 1000 mg a.s./kg dry soil. Mortality rates of 2.5% - 37.5% were recorded in the test item treatment groups. In the untreated control and solvent control the mortality rates were 1.3% and 2.5%, respectively. Statistically significant effects on the number of juveniles compared to the solvent control was recorded at a concentration of 1000 mg a.s./kg dry soil. The mean reproduction in the untreated control and solvent control reached 1303 and 1276 juveniles, respectively. Reproduction rates at 62.5, 125, 250, 500 and 1000 mg a.s./kg dry soil were 1271, 1312, 1227, 1282 and 1003 juveniles, respectively.

In a 28-day Collembola reproduction study with BAS 595 F (triticonazole), the LC_{50} and the EC_{50} could not be calculated, but it can be concluded that the LC_{50} and the EC_{50} are higher than 1000 mg a.s./kg dry soil. The NOEC for mortality was determined to be 250 mg a.s./kg dry soil, respectively. The NOEC for reproduction was determined to be 500 mg a.s./kg dry soil, respectively.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 595 F (triticonazole, Reg. No. 4 378 513), batch no. COD-001440, analyzed purity: 91.3% (tolerance ± 1.0%).

B. STUDY DESIGN

Test species: Collembolans (*Folsomia candida)*, juveniles (9 - 12 days old); source: in-house culture.

Test design: 28-day chronic laboratory test in treated artificial soil according to OECD 232 and ISO 11267. Different concentrations of the test item were mixed homogenously into artificial soil and filled into glass vessels after which collembolans were introduced on top of the soil; 7 treatment groups (5 test item concentrations, control, solvent control); 4 replicates for each test item treatment and 8 replicates for the control groups, each containing 10 collembolans. Assessment of adult mortality, reproduction (number of juveniles) and behavioral effects after 28 days.

Endpoints: Mortality, reproduction rate after 28 days.

Reference item: Boric acid (100% analyzed). The effects of the reference item were investigated in a separate study.

Test concentrations: Control (untreated soil), solvent control (with acetone), 62.5, 125, 250, 500 and 1000 mg BAS 595 F/kg dry soil.

Test conditions: Artificial soil according to OECD 232 (peat: 5%); pH 6.06 – pH 6.14 at test initiation, pH 5.75 – pH 5.80 at test termination; water content at study initiation 56.8% – 57.1% of maximum water holding capacity and 55.9% – 56.4% of maximum WHC at test termination; temperature: 18.3 °C – 21.9 °C; photoperiod: 16 h light : 8 h dark, light intensity: 520 lux.

Statistics: Descriptive statistics, Fisher's Exact Binominal Test with Bonferroni Correction for mortality (one-sided greater, $\alpha = 0.05$), Williams-t-test for reproduction data (one-sided smaller, $\alpha = 0.05$).

II. RESULTS AND DISCUSSION

Statistically significant effects on mortality compared to the solvent control was observed at concentrations of 500 and 1000 mg a.s./kg dry soil (Fisher's Exact Binomial Test with Bonferroni Correction, $\alpha = 0.05$, one-sided greater). Mortality rates of 2.5% - 37.5% were recorded in the test item treatment groups. In the untreated control and solvent control the mortality rates were 1.3% and 2.5%, respectively.
Statistically significant effects (Williams-t-test, $\alpha = 0.05$, one-sided smaller) on the number of juveniles compared to the solvent control was recorded at a concentration of 1000 mg a.s./kg dry soil. The mean reproduction in the untreated control and solvent control reached 1303 and 1276 juveniles, respectively. Reproduction rates at 62.5, 125, 250, 500 and 1000 mg a.s./kg dry soil were 1271, 1312, 1227, 1282 and 1003 juveniles, respectively. The results are summarized in Table 8.4.2.1-1.

Table 8.4.2.1-1: Effects of BAS 595 F on Collembola (*Folsomia candida)* in a 28-day reproduction study

BAS 595 F [mg a.s./kg dry soil]	Control	Solvent control	62.5	125	250	500	1000
Mortality (day 28) [%]	1.3	2.5	2.5	2.5	12.5	17.5 *	37.5 *
No. of juveniles (day 28)	1303	1276	1271	1312	1227	1282	1003 *
Reproduction (day 28) [% of control]	--	100	100	103	96	100	79
Endpoints [mg a.s./kg dry soil]							
NOEC $_{mortality}$	250						
NOEC $_{reproduction}$	500						
LC_{50}	> 1000						
EC_{50}	> 1000						

* Statistically significant differences compared to the control (Fisher's Exact Binominal Test with Bonferroni Correction for mortality, one-sided greater, $\alpha = 0.05$, Williams-t-test for reproduction data, one-sided smaller, $\alpha = 0.05$).

In a separate study, the $EC_{50\ reproduction}$ of the reference item boric acid was calculated to be 108 mg a.s./kg dry soil.

III. CONCLUSION

In a 28-day Collembola reproduction study with BAS 595 F (triticonazole), the LC_{50} and the EC_{50} could not be calculated, but it can be concluded that the LC_{50} and the EC_{50} are higher than 1000 mg a.s./kg dry soil. The NOEC for mortality was determined to be 250 mg a.s./kg dry soil, respectively. The NOEC for reproduction was determined to be 500 mg a.s./kg dry soil, respectively.

Report: CA 8.4.2.1/2
Schulz L., 2014a
Effects of BAS 595 F (Triticonazole) on the reproduction of the predatory mite Hypoaspis aculeifer
2014/1000022
Guidelines: OECD 226 (2008)
GLP: yes
(certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

Executive Summary

The effects of BAS 595 F on mortality and reproduction of the predatory mite *Hypoaspis aculeifer* were investigated in a chronic laboratory study over 14 days. Five application rates (62.5, 125, 250, 500 and 1000 mg a.s./kg dry soil) were incorporated into the soil with 4 replicates per test item treatment. An untreated control and a solvent control with acetone, each with 8 replicates, were included. All replicates contained 10 predatory mites. Assessment of adult mortality and reproduction effects was carried out after 14 days.

Mortality rates of 0.0% - 5.0% were recorded in the test item treatment groups. In the untreated control and in the solvent control the mortality rate was 2.5% and 3.8%, respectively. The observed mortality rates for adult mortality in the test item treatment groups compared to the solvent control were not statistically significant. Differences between the behavior and the morphology of the mites in the solvent control and the test item treatment groups could not be observed. Reproduction rates in the 62.5, 125, 250, 500 and 1000 mg a.s./kg dry soil were 270.3, 276.0, 256.0, 249.8 and 203.8 juveniles, respectively. The mean reproduction in the untreated control and in the solvent control reached 249.3 and 246.9 juveniles, respectively. The test item showed no statistically significant adverse effects on reproduction at all tested concentrations compared to the solvent control.

In a 14-day *Hypoaspis aculeifer* reproduction study, the LC_{50} and EC_{50} are estimated to be higher than 1000 mg a.s./kg dry soil. The NOEC for mortality and for reproduction was determined to be ≥ 1000 mg a.s./kg dry soil, the highest concentration tested.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 595 F (triticonazole, Reg. No. 4 378 513), batch no. COD-001440, analyzed purity: 91.3% (tolerance ± 1.0%).

B. STUDY DESIGN

Test species: Predatory mites (*Hypoaspis aculeifer)*, adult mites with an age difference of 2 days; source: in-house culture.

Test design: 14-day chronic laboratory test in treated artificial soil according to OECD 226. Different concentrations of the test item were mixed homogenously into artificial soil and used to fill vessels after which mites were introduced on top of the soil; 7 treatment groups (5 test item concentrations, control, solvent control); 4 replicates for each test item treatment and 8 replicates for the control groups, each containing 10 mites. Assessment of adult mortality and reproduction effects was carried out after 14 days.

Endpoints: Mortality, reproduction rate after 14 days.

Reference item: Dimethoate EC 400 (411.7 g/L analyzed). The effects of the reference item were investigated in a separate study.

Test concentrations: Control (untreated soil), solvent control (with acetone), 62.5, 125, 250, 500 and 1000 mg BAS 595 F/kg dry soil.

Test conditions: Artificial soil according to OECD 226; pH 5.7 – pH 5.8 at test initiation, pH 5.8 – pH 5.9 at test termination; water content at study initiation 56.67% – 59.43% of maximum water holding capacity and 53.56% – 57.61% of maximum WHC at test termination; temperature: 19.5 °C – 21.2 °C; photoperiod: 16 h light : 8 h dark, light intensity: 511 lux.

Statistics: Descriptive statistics, Fisher's Exact Binominal Test with Bonferroni Correction for mortality (one-sided greater, $\alpha = 0.05$), Williams-t-test for reproduction data (one-sided smaller, $\alpha = 0.05$).

II. RESULTS AND DISCUSSION

Mortality rates of 0.0% - 5.0% were recorded in the test item treatment groups. In the untreated control and in the solvent control the mortality rate was 2.5% and 3.8%, respectively. 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). Differences between the behavior and the morphology of the mites in the solvent control and the test item treatment groups could not be observed.
Reproduction rates in the 62.5, 125, 250, 500 and 1000 mg a.s./kg dry soil were 270.3, 276.0, 256.0, 249.8 and 203.8 juveniles, respectively. The mean reproduction in the untreated control and in the solvent control reached 249.3 and 246.9 juveniles, respectively. The test item showed no statistically significant adverse effects on reproduction at all tested concentrations compared to the solvent control (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 595 F on predatory mite (*Hypoaspis aculeifer*) in a 14-day reproduction study

BAS 595 F [mg a.s./kg dry soil]	Control	Solvent control	62.5	125	250	500	1000
Mortality (day 14) [%]	2.5	3.8	2.5	5.0	0.0	2.5	2.5
No. of juveniles (day 14)	249.3	246.9	270.3	276.0	256.0	249.8	203.8
Reproduction (day 14) [% of control]	--	100	109	112	104	101	83
Endpoints [mg a.s./kg dry soil]							
$NOEC_{mortality}$	≥ 1000						
$NOEC_{reproduction}$	≥ 1000						
LC_{50}	> 1000						
EC_{50}	> 1000						

In a separate study, the $EC_{50\ reproduction}$ of the reference item Dimethoate EC 400 was calculated to be 6.64 mg a.s./kg dry soil.

III. CONCLUSION

In a 14-day *Hypoaspis aculeifer* reproduction study, the LC_{50} and EC_{50} are estimated to be higher than 1000 mg a.s./kg dry soil. The NOEC for mortality and for reproduction was determined to be ≥ 1000 mg a.s./kg dry soil, the highest concentration tested.

Report: CA 8.4.2.1/3
Friedrich S., 2013d
Effects of Reg.No. 5079285 (metabolite of BAS 595 F, Triticonazole) on the reproduction of the collembolan Folsomia candida
2014/1000028

Guidelines: OECD 232 (2009), ISO 11267 (1999)

GLP: yes
(certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

Executive Summary

The effects of Reg. No. 5 079 285 on mortality and reproduction of the Collembola *Folsomia candida* were investigated in a chronic laboratory study over 28 days. Five application rates (31.25, 62.5, 125, 250 and 500 mg/kg dry soil) were incorporated into the soil with 4 replicates per test item treatment. An untreated control and a solvent control with acetone, each with 8 replicates, were included. All replicates contained 10 collembolans. Assessment of mortality, reproduction rate (number of juveniles) and behavior was carried out after 28 days.

No statistically significant effect on parental mortality compared to the solvent control group was found for any concentration tested. Mortality rates of 0% - 5.0% were recorded in the test item treatment groups. In the untreated control and solvent control the mortality rate was 3.8%. No statistically significant effects on the number of juveniles compared to the solvent control group were recorded at any concentration tested. The mean reproduction in the untreated control and solvent control reached 973 and 972 juveniles, respectively. Reproduction rates at 31.25, 62.5, 125, 250 and 500 mg/kg dry soil were 1009, 949, 1005, 995 and 981 juveniles, respectively.

In a 28-day Collembola reproduction study with Reg. No. 5 079 285 (metabolite of BAS 595 F, triticonazole), the LC_{50} and the EC_{50} could not be calculated, but it can be concluded that LC_{50} and EC_{50} are higher than 500 mg/kg dry soil. The NOEC for mortality and reproduction was determined to be 500 mg/kg dry soil, the highest concentration tested.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Reg. No. 5 079 285 (metabolite of BAS 595 F, triticonazole; RPA 404766); batch no.: L67-148; analyzed purity: 99.3% (tolerance ± 1.0%).

B. STUDY DESIGN

Test species: Collembolans (*Folsomia candida)*, juveniles (9 - 12 days old); source: in-house culture.

Test design: 28-day chronic laboratory test in treated artificial soil according to OECD 232 and ISO 11267. Different concentrations of the test item were mixed homogenously into artificial soil and used to fill glass vessels after which collembolans were introduced on top of the soil; 7 treatment groups (5 test item concentrations, control, solvent control); 4 replicates for each test item treatment and 8 replicates for the control groups, each containing 10 collembolans. Assessment of adult mortality, reproduction (number of juveniles) and behavioral effects after 28 days.

Endpoints: Mortality, reproduction rate after 28 days.

Reference item: Boric acid (100% analyzed). The effects of the reference item were investigated in a separate study.

Test concentrations: Control (untreated soil), solvent control (with acetone), 31.25, 62.5, 125, 250 and 500 mg Reg. No. 5 079 285/kg dry soil.

Test conditions: Artificial soil according to OECD 232 (with 5% peat); pH 6.03 – pH 6.07 at test initiation, pH 5.82 – pH 5.87 at test termination; water content at study initiation 56.8% – 57.3% of maximum water holding capacity and 55.7% – 56.8% of maximum WHC at test termination; temperature: 18.1 °C – 21.9 °C; photoperiod: 16 h light : 8 h dark, light intensity: 540 lux.

Statistics: Descriptive statistics, Fisher's Exact Binominal Test with Bonferroni Correction for mortality (one-sided greater, $\alpha = 0.05$), Williams-t-test for reproduction data (one-sided smaller, $\alpha = 0.05$).

II. RESULTS AND DISCUSSION

No statistically significant effect (Fisher's Exact Binomial Test with Bonferroni Correction, $\alpha = 0.05$, one-sided greater) on parental mortality compared to the solvent control group was found for any concentration tested. Mortality rates of 0% - 5.0% were recorded in the test item treatment groups. In the untreated control and solvent control the mortality rate was 3.8%.
No statistically significant effects (Williams-t-test, $\alpha = 0.05$, one-sided smaller) on the number of juveniles compared to the solvent control group were recorded at any concentration tested.
The mean reproduction in the untreated control and solvent control reached 973 and 972 juveniles, respectively. Reproduction rates at 31.25, 62.5, 125, 250 and 500 mg/kg dry soil were 1009, 949, 1005, 995 and 981 juveniles, respectively. The results are summarized in Table 8.4.2.1-3.

Table 8.4.2.1-3: Effects of Reg. No. 5 079 285 on Collembola (*Folsomia candida)* in a 28-day reproduction study

Reg. No. 5 079 285 [mg/kg dry soil]	Control	Solvent control	31.25	62.5	125	250	500
Mortality (day 28) [%]	3.8	3.8	5.0	0.0	2.5	5.0	5.0
No. of juveniles (day 28)	973	972	1009	949	1005	995	981
Reproduction (day 28) [% of control]	--	100	104	98	103	102	101
Endpoints [mg/kg dry soil]							
$NOEC_{mortality}$	≥ 500						
$NOEC_{reproduction}$	≥ 500						
LC_{50}	> 500						
EC_{50}	> 500						

In a separate study, the $EC_{50\ reproduction}$ of the reference item boric acid was calculated to be 108 mg a.s./kg dry soil.

III. CONCLUSION

In a 28-day Collembola reproduction study with Reg. No. 5 079 285 (metabolite of BAS 595 F, triticonazole), the LC_{50} and the EC_{50} could not be calculated, but it can be concluded that LC_{50} and EC_{50} are higher than 500 mg/kg dry soil. The NOEC for mortality and reproduction was determined to be 500 mg/kg dry soil, the highest concentration tested.

Report: CA 8.4.2.1/4
Royer S., 2006a
Effect of Reg.No. 5059144 (RPA 406341, metabolite of BAS 595 F) on the reproduction of the collembola Folsomia candida in artificial soil with 5% peat
2006/1031679
Guidelines: ISO 11267
GLP: yes
(certified by Landesamt fuer Umweltschutz und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

The effects of RPA 406341 on mortality and reproduction of Collembola *(Folsomia candida)* were investigated in a laboratory study over 28 days. Three application rates (1, 10 and 50 mg RPA 406341 per kg dry soil) were incorporated into the soil with 5 replicates per treatment (each containing 10 juvenile collembolans). An untreated control with 5 replicates was included. Assessment of adult springtail mortality, behavioral effects and reproduction rate (number of juveniles) was carried out after 28 days.

After 28 days of exposure, no significant mortality was observed in the test item groups. Mortality rates ranged from 0% to 6% in the test item groups. A mortality of 2% was observed in the control, no mortality was observed in the solvent control. The reproduction was not statistically significant different compare to the solvent control in the test item groups (Dunnett-test, α = 0.05). In the control a mean of 434 juveniles was counted and 356 in the solvent control. In the treatment groups a mean number of juveniles of 333 to 369 were counted.

In a 28-day *Folsomia candida* reproduction study with RPA 406341 the NOEC based on mortality and reproduction was 50 mg RPA 406341/kg dry soil. The LC_{50} and EC_{50} were determined to be > 50 mg RPA 406341/kg dry soil, respectively.

I. MATERIAL AND METHODS

A. MATERIALS

Test item:	Reg. No. 5 059 144 (metabolite of BAS 595 F, triticonazole; RPA 406341); batch BESS0541; purity 99%.

B. STUDY DESIGN

Test species:	Collembola (*Folsomia candida)*, juveniles (10-12 days old); source: in-house culture.
Test design:	28-day test; artificial soil filled in glass vessels was treated with different concentrations of the test item before collembolans were introduced; 5 treatment groups (3 test item concentrations, control, solvent control); 5 replicates for each and each containing 10 juvenile collembolans. Assessment of adult collembolans mortality, behavioral effects and reproduction rate (number of juveniles) after 28 days.
Endpoints:	Mortality, behavioral effects, reproduction rate.
Test concentrations:	Control, solvent control, 1, 10 and 50 mg RPA 406341 per kg dry soil (nominal).
Reference item:	BAS 337 01 H (Phenmedipham, 157 g/L nominal). The effects of the reference item were investigated in a separate study.
Test conditions:	Artificial soil according to ISO 11267 with a reduced content of peat (5%); pH 5.55 – 5.65 at test initiation, 5.34 – 5.66 at test termination; water content at study initiation 60% of maximum water holding capacity at test termination; temperature: 20 °C ± 2 °C; photoperiod: 16 h light : 8 h dark, light intensity: 450 to 780 lux; food: 2 mg granulated dry yeast at the start of the test and after 14 days.
Statistics:	Descriptive statistics, Dunnett-test for reproduction data ($\alpha = 0.05$).

II. RESULTS AND DISCUSSION

After 28 days of exposure, no significant mortality was observed in the test item groups. Mortality rates ranged from 0% to 6% in the test item groups. A mortality of 2% was observed in the control, no mortality was observed in the solvent control. The reproduction was not statistically significant different compare to the solvent control in the test item groups (Dunnett-test, $\alpha = 0.05$). In the control a mean of 434 juveniles was counted and 356 in the solvent control. In the treatment groups a mean number of juveniles of 333 to 369 was counted. The results are summarized in Table 8.4.2.1-4.

Table 8.4.2.1-4: Effect of RPA 406341 on Collembola (*Folsomia candida)* in a 28-day reproduction study

RPA 406341 [mg/kg dry soil]	Control	Solvent control	1	10	50
Mortality (day 28) [%]	2.0	0.0	6.0	0.0	4.0
No. of juveniles (day 28)	434.3	356.1	332.8	369.1	350.9
Reproduction in [%] of control (day 28)	--	--	93.5	103.7	98.5
Endpoints [mg RPA 406341/kg dry soil]					
NOEC (day 28)	≥ 50				
EC_{50}	> 50				
LC_{50}	> 50				

III. CONCLUSION

In a 28-day *Folsomia candida* reproduction study with RPA 406341 the NOEC based on mortality and reproduction was 50 mg RPA 406341/kg dry soil. The LC_{50} and EC_{50} were determined to be > 50 mg RPA 406341/kg dry soil, respectively.

Report: CA 8.4.2.1/5
Ganssmann M., 2014a
Effect of Reg. No. 5059144 (RPA 406341, metabolite of BAS 595 F) on the reproduction of Hypoaspis aculeifer in artificial soil with 5% peat
2014/1083348

Guidelines:

GLP: no
(certified by)

Executive Summary

The effects of Reg. No. 5 059 144 on mortality and reproduction of the predatory mite *Hypoaspis aculeifer* were investigated in a laboratory study over 14 days. Five application rates 0.625, 1.25, 2.5, 5.0 and 10 mg Reg. No. 5 059 144/kg dry soil were incorporated into the soil with four replicates per treatment, each containing 10 mites. An untreated control with 8 replicates was included.

A slight mortality of up to 13% was observed in the test item treated groups, which was not statistically significantly different compared to the control, where 13% of the adult mites died. Reproduction of the predatory mites exposed to Reg. No. 5 059 144 was not statistically significantly different compared to the control up to and including the highest test concentration of 10 mg/kg dry soil. No behavioral abnormalities were observed in any of the treatment groups.

Reg. No. 5 059 144 caused no statistically significant effects on mortality or reproduction of *Hypoaspis aculeifer* up to and including the highest tested concentration of 10 mg/kg dry soil. Therefore, the overall No Observed Effect Concentration (NOEC) was determined to be 10 mg/kg dry soil.

I. MATERIAL AND METHODS

A. MATERIALS

Test item:	Reg. No. 5 059 144 (metabolite of BAS 595 F, triticonazole; RPA 406341); batch no.: L74-160; analyzed purity: 91.8%.

B. STUDY DESIGN

Test species:	Predatory mites (*Hypoaspis aculeifer)*, adult females, approximately 14 days after reaching the adult stage; source: in-house culture.
Test design:	14-day chronic laboratory test in treated artificial soil. Different concentrations of the test item were mixed homogenously into artificial soil and used to fillglass vessels after which mites were introduced on top of the soil; 6 treatment groups (5 test item concentrations, control); 4 replicates for each test item treatment and 8 replicates for the control groups, each containing 10 mites. Assessment of adult mortality and reproduction was performed after 14 days.
Endpoints:	Mortality, reproduction rate (number of juveniles) after 14 days.
Reference item:	Perfekthion (a.s.: dimethoate, 411.7 g/L analyzed). The effects of the reference item were investigated in a separate study.
Test concentrations:	Control (untreated soil), 0.625, 1.25, 2.5, 5.0 and 10.0 mg Reg. No. 5 059 144/kg dry soil.
Test conditions:	Artificial soil according to OECD 226; pH 6.1 – pH 6.2 at test initiation, pH 6.3 at test termination; water content at study initiation 56.4% - 57.5% of maximum water holding capacity and 55.0% – 57.0% of maximum WHC at test termination; temperature: 18 °C – 22 °C; photoperiod: 16 h light : 8 h dark, light intensity: 400 – 800 lux.
Statistics:	Descriptive statistics, Fisher's Exact Test for mortality (one-sided greater, $\alpha = 0.05$), Williams-t-test for reproduction data (one-sided smaller, $\alpha = 0.05$), calculation of the LC_{50} by Probit analysis.

II. RESULTS AND DISCUSSION

A slight mortality of up to 13% was observed in the test item treated groups, which was not statistically significantly different compared to the control, where 13% of the adult mites died (Fisher's Exact Test, $\alpha = 0.05$, one-sided greater). Reproduction of the predatory mites exposed to Reg. No. 5 059 144 was not statistically significantly different compared to the control up to and including the highest test concentration of 10 mg/kg dry soil (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 8.4.2.1-5.

Table 8.4.2.1-5: Effects of Reg. No. 5 059 144 on predatory mite (*Hypoaspis aculeifer*) in a 14-day reproduction study

Reg. No. 5 059 144 [mg/kg dry soil]	Control	0.625	1.25	2.5	5	10
Mortality (day 14) [%]	13	8	5	13	5	8
No. of juveniles (day 14)	161	189	189	198	186	175
Reproduction (day 14) [% of control]	--	117	117	123	116	109
Endpoints [mg/kg dry soil]						
$NOEC_{mortality}$	≥ 10					
$NOEC_{reproduction}$	≥ 10					
LC_{50}	> 10					
EC_{50}	> 10					

In a separate study, the reference item dimethoate showed statistically significant effects on reproduction at a concentration of 3.0 mg dimethoate/kg dry soil and above. The $EC_{50\ reproduction}$ was 4.2 mg dimethoate/kg dry soil.

III. CONCLUSION

Reg. No. 5 059 144 caused no statistically significant effects on mortality or reproduction of *Hypoaspis aculeifer* up to and including the highest tested concentration of 10 mg/kg dry soil. Therefore, the overall No Observed Effect Concentration (NOEC) was determined to be 10 mg/kg dry soil.

Report:	CA 8.4.2.1/6 Friedrich S., 2013e Effects of Reg.No. 5079288 (metabolite of BAS 595 F, Triticonazole) on the reproduction of the collembolan Folsomia candida 2014/1000029
Guidelines:	OECD 232 (2009), ISO 11267 (1999)
GLP:	yes (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

Executive Summary

The effects of Reg. No. 5 079 288 (metabolite of BAS 595 F, triticonazole) on mortality and reproduction of the Collembola *Folsomia candida* were investigated in a chronic laboratory study over 28 days. Five application rates (31.25, 62.5, 125, 250 and 500 mg Reg. No. 5 079 288/kg dry soil) were incorporated into the soil with 4 replicates per test item treatment. An untreated control and a solvent control, each with 8 replicates, were included. All replicates contained 10 collembolans. Assessment of mortality, reproduction rate (number of juveniles) and behavior was carried out after 28 days.

No statistically significant effect on parental mortality compared to the solvent control group was found for any concentration tested. Mortality rates of 2.5% - 10.0% were recorded in the test item treatment groups. In the untreated control and solvent control the mortality rate was 2.5%.
No statistically significant effects on the number of juveniles compared to the solvent control group were recorded at any concentration tested. The mean reproduction in the untreated control and solvent control reached 704 and 720 juveniles, respectively. Reproduction rates at 31.25, 62.5, 125, 250 and 500 mg Reg. No. 5 079 288/kg dry soil were 724, 721, 742, 718 and 611 juveniles, respectively.

In a 28-day Collembola reproduction study with Reg. No. 5 079 288 (metabolite of BAS 595 F, triticonazole), the LC_{50} and the EC_{50} could not be calculated, but it can be concluded that LC_{50} and EC_{50} are higher than 500 mg Reg. No. 5 079 288/kg dry soil. The NOEC for mortality and reproduction was determined to be 500 mg Reg. No. 5 079 288/kg dry soil, the highest concentration tested.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Reg. No. 5 079 288 (metabolite of BAS 595 F, triticonazole; RPA 407922); batch no.: 33484-39; analyzed purity: 99.5% (tolerance ± 1.0%).

B. STUDY DESIGN

Test species: Collembolans (*Folsomia candida)*, juveniles (9 - 12 days old); source: in-house culture.

Test design: 28-day chronic laboratory test in treated artificial soil according to OECD 232 and ISO 11267. Different concentrations of the test item were homogeneously mixed into the soil which was then used to fill glass vessels after which the Collembola were introduced on top of the soil; 7 treatment groups (5 test item concentrations, control, solvent control); 4 replicates for each test item treatment and 8 replicates for the control groups, each containing 10 collembolans. Assessment of adult mortality, reproduction (number of juveniles) and behavioral effects after 28 days.

Endpoints: Mortality, reproduction rate after 28 days.

Reference item: Boric acid (100% analyzed). The effects of the reference item were investigated in a separate study.

Test concentrations: Control (untreated soil), solvent control (with acetone), 31.25, 62.5, 125, 250 and 500 mg Reg. No. 5 079 288/kg dry soil.

Test conditions: Artificial soil according to OECD 232 (with 5% peat); pH 6.12 – pH 6.20 at test initiation, pH 5.88 – pH 5.96 at test termination; water content at study initiation 57.1% of maximum water holding capacity and 55.9% – 56.6% of maximum WHC at test termination; temperature: 18.1 °C – 21.9 °C; photoperiod: 16 h light : 8 h dark, light intensity: 540 lux.

Statistics: Descriptive statistics, Fisher's Exact Binominal Test with Bonferroni Correction for mortality (one-sided greater, $\alpha = 0.05$), Williams-t-test for reproduction data (one-sided smaller, $\alpha = 0.05$).

II. RESULTS AND DISCUSSION

No statistically significant effect (Fisher`s Exact Binomial Test with Bonferroni Correction, $\alpha = 0.05$, one-sided greater) on parental mortality compared to the solvent control group was found for any concentration tested. Mortality rates of 2.5% - 10.0% were recorded in the test item treatment groups. In the untreated control and solvent control the mortality rate was 2.5%.
No statistically significant effects (Williams-t-test, $\alpha = 0.05$, one-sided smaller) on the number of juveniles compared to the solvent control group were recorded at any concentration tested. The mean reproduction in the untreated control and solvent control reached 704 and 720 juveniles, respectively. Reproduction rates at 31.25, 62.5, 125, 250 and 500 mg Reg. No. 5 079 288/kg dry soil were 724, 721, 742, 718 and 611 juveniles, respectively. The results are summarized in Table 8.4.2.1-6.

Table 8.4.2.1-6: Effects of Reg. No. 5 079 288 on Collembola (*Folsomia candida)* in a 28-day reproduction study

Reg. No. 5 079 288 [mg/kg dry soil]	Control	Solvent control	31.25	62.5	125	250	500
Mortality (day 28) [%]	2.5	2.5	2.5	2.5	2.5	2.5	10.0
No. of juveniles (day 28)	704	720	724	721	742	718	611
Reproduction (day 28) [% of control]	--	100	101	100	103	100	85
Endpoints [mg/kg dry soil]							
NOEC $_{mortality}$	≥ 500						
NOEC $_{reproduction}$	≥ 500						
LC_{50}	> 500						
EC_{50}	> 500						

In a separate study, the $EC_{50\ reproduction}$ of the reference item boric acid was calculated to be 108 mg a.s./kg dry soil.

III. CONCLUSION

In a 28-day Collembola reproduction study with Reg. No. 5 079 288 (metabolite of BAS 595 F, triticonazole), the LC_{50} and the EC_{50} could not be calculated, but it can be concluded that LC_{50} and EC_{50} are higher than 500 mg Reg. No. 5 079 288/kg dry soil. The NOEC for mortality and reproduction was determined to be 500 mg Reg. No. 5 079 288/kg dry soil, the highest concentration tested.

CA 8.5 Effects on nitrogen transformation

Since Annex I inclusion of the active substance triticonazole (BAS 595 F) new studies on nitrogen transformation have been performed with the metabolites RPA 407922, RPA 406341 and RPA 404766. As a result, these new endpoints are considered in the risk assessment. Summaries of the new studies are provided below in this chapter.

Details on the EU agreed studies which have been already evaluated within the Annex I inclusion of triticonazole are provided in the EU Review documents of triticonazole (*i.e.* Draft Assessment Report (DAR), Volume 3, Annex B.9., 2003; Addendum to the DAR May, 2005; EFSA Scientific Report (2005) 33, 1 - 69).

For better transparency and traceability of the active substance history, the results of all studies are summarized in Table 8.5-1. Studies from the former EU review are given in grey letters.

Table 8.5-1: Toxicity to nitrogen transformation of triticonazole and metabolites

Test substance	Endpoint	Endpoint (< 25% effect) [mg/kg dry soil]	Reference (BASF DocID)	EU agreed[1)]
triticonazole	Effects on nitrogen transformation	≥ 2.13	R013039 (+ amendment C017774)	yes *EFSA Scientific Report* (2005) 33, 1-69
metabolite, Reg. no. 5 059 144; RPA 406341	Effects on nitrogen transformation	≥ 10	2006/1028713	no, new study
metabolite, Reg. no. 5 079 285; RPA 404766	Effects on nitrogen transformation	≥ 1.0	2013/1103636	no, new study
metabolite, Reg. no. 5 079 288; RPA 407922	Effects on nitrogen transformation	≥ 1.0	2014/1000025	no, new study

[1)] EU agreed denotes studies assessed during the previous EU evaluation process that are not necessarily listed in the list of endpoint as in the Conclusion on the peer review of triticonazole, *EFSA Scientific Report* (2005) 33, 1-69.

Report: CA 8.5/1
Royer S., 2006b
Effect of Reg.No. 5059144 (RPA 406341, metabolite of BAS 595 F) on soil microorganisms: Nitrogen transformation test
2006/1028713
Guidelines: OECD 216 (2000)
GLP: yes
(certified by Landesamt fuer Umweltschutz und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

The effect of RPA 406341 on nitrogen transformation was tested in a lucerne-enriched silty sand soil. RPA 406341 was applied to samples of the soil, in a laboratory, at nominal application rates of 1 mg/kg and 10 mg/kg dry soil. The treated soils and untreated controls were incubated at approx. 20 °C in the dark for 28 days. Triplicate samples of each treatment were removed for analysis of NH_4-nitrogen and NO_3-nitrogen, 7, 14 and 28 days after application.

No adverse effects of RPA 406341 on nitrogen transformation in soil were observed in both test item concentrations after 28 days.

Based on the results of this study, in accordance with OECD guideline 216, RPA 406341 caused no short-term and no long-term effects on the soil nitrogen transformation (measured as NO_3-N production) in a field soil tested up to a concentration of 10 mg/kg dry soil.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: RPA 406341 (metabolite of triticonazole (BAS 595 F); Reg. No. 5 059 144), batch BESS0541; purity 99%.

B. STUDY DESIGN

Test species: Biologically active agricultural soil: silty sand, pH 6.7, 1.12% C_{org}, WHC 34.2 g/100 g dry soil.

Test design: Determination of the N-transformation (NO_3-nitrogen production) in soil enriched with lucerne meal (concentration in soil 0.5%). Comparison of test item treated soil with a non-treated soil. 3 replicates per treatment and concentration. NH_4-nitrogen formed from organically bound nitrogen and NO_3-nitrogen from the nitrification process was determined by using ion-selective electrodes and an Orion expandable ion-analyzer EA 940. Sampling scheme: 0, 7, 14 and 28 days after treatment, subsamples were withdrawn from the bulk batches and subjected to the measurement.

Endpoints: Effects on the NO_3-nitrogen production after 28 day exposure.

Test rates: Control, 1 mg RPA 406341 per kg dry soil and 10 mg RPA 406341 per kg soil. Test concentrations related to a soil depth of 5 cm and a soil density of 1.5 g/cm^3.

Reference item: Dinoterb (purity: 98.0 ± 0.5%). The reference item was applied at a rate of 6.7 and 13.3 mg/kg dry soil.

Test conditions: Soil moisture: 55% of its maximum water holding capacity; pH 6.65 - 7.04. Soil samples were incubated at 20°C ± 2°C while stored in glass bottles in the dark.

Statistics: Descriptive statistics.

II. RESULTS AND DISCUSSION

No adverse effects of RPA 406341 on nitrogen transformation in soil were observed in both test item concentrations after 28 days. The results are summarized in Table 8.5-2.

Table 8.5-2: Effects of RPA 406341 on soil micro-organisms (nitrogen transformation) on days 7, 14 and 28 of incubation

Time interval (days)	Control	1 mg RPA 406341 per kg dry soil		10 mg RPA 406341 per kg dry soil	
	NO_3-N [mg/kg dry soil]	NO_3-N [mg/kg dry soil]	% Deviation from the control [1)]	NO_3-N [mg/kg dry soil]	% Deviation from the control [1)]
0-7	2.0	1.8	-10.0	1.8	-10.0
0-14	4.2	4.2	0.0	3.6	-14.3
0-28	14.6	13.2	-9.6	12.8	-12.3

[1)] Based on NO_3-nitrogen production; - = inhibition, + = stimulation.

The reference item Dinoterb produced a clear effect (+28.77% and +39.73% at 6.7 and 13.3 mg/kg dry soil).

III. CONCLUSION

Based on the results of this study, in accordance with OECD guideline 216, RPA 406341 caused no short-term and no long-term effects on the soil nitrogen transformation (measured as NO_3-N production) in a field soil tested up to a concentration of 10 mg/kg dry soil.

Report: CA 8.5/2
Stojanowitsch M., 2015a
Reg.No. 5079285 (metabolite of BAS 595 F, Triticonazole): Effects on the activity of the soil microflora under laboratory conditions (nitrogen transformation)
2013/1103636

Guidelines: OECD 216 (2000)

GLP: yes
(certified by Landesanstalt fuer Umwelt, Messungen und Naturschutz Baden-Wuerttemberg, Karlsruhe, Germany)

Executive Summary

In a soil microbial activity study, the effects of Reg. No. 5 079 285 (metabolite of BAS 595 F, triticonazole) on the nitrogen transformation were investigated in a medium loamy sand soil. Reg. No. 5 079 285 was applied to samples of the soil at nominal test concentrations of 0.1 mg/kg and 1.0 mg/kg dry soil. Reg. No. 5 079 285 treated soils and untreated controls were incubated at 20°C ± 2 °C in the dark for 42 days. Triplicate samples of each treatment were removed for determination of nitrogen transformation (measured as NO_3-nitrogen production) 0, 7, 14, 28 and 42 days after application.

No adverse effects of Reg. No. 5 079 285 on nitrogen transformation (> 25% deviation from control) in soil could be observed in both test concentrations, 0.1 mg/kg dry soil and 1.0 mg/kg dry soil, after 42 days (time interval 0-42). A deviation of 26.8% and 26.9% (at 0.1 and 1.0 mg Reg. No. 5 079 285/kg dry soil, respectively) could be seen at day 28. However, by study termination, the percent deviation was < 25%, thereby indicating no long term effects from exposure to Reg. No. 5 079 285.

Exposure of Reg. No. 5 079 285 (metabolite of BAS 595 F, triticonazole) in a field soil up to a test concentration of 1.0 mg Reg. No. 5 079 285/kg dry soil caused no adverse effects (deviation from control < 25%, OECD 216) on the soil nitrogen transformation (measured as NO_3-N production) at the end of the 42-day incubation period.

I. MATERIALS AND METHODS

A. MATERIALS

Test item: RPA 404766 (metabolite of triticonazole (BAS 595 F); Reg. No. 5 079 285); batch no. L67-148; analyzed purity: 99.3% (±1.0%).

B. STUDY DESIGN

Test species: Biologically active agricultural soil: medium loamy sand soil: pH 7.0 ($CaCl_2$), 1.05% TOC, 39.86% water holding capacity max (WHC_{max}).

Test design: Determination of the N-transformation (NO_3-nitrogen production) in soil enriched with lucerne meal (concentration in the soil 0.5%). Comparison of test item treated soil with a non-treated soil. NO_3-nitrogen formed from the nitrification process was determined using an Ion analyzer. Sampling dates: 0, 7, 14, 28 and 42 days after treatment. Sub-samples (3 replicates per treatment) were withdrawn from the soil bulk batches and subjected to the measurement.

Endpoints: Effects on NO_3-nitrogen production 0, 7, 14, 28 and 42 days after application.

Test concentrations: Control (deionized water), 0.1 mg Reg. No. 5079285/kg dry soil, 1.0 mg Reg. No. 5079285/kg dry soil.

Reference item: Sodium Chloride. The reference item was tested in a separate study at a concentration of 20 g a.s./kg dry soil.

Test conditions: Soil moisture: approximately 42% of its maximum water holding capacity, measured water content: 13.2 – 14.4g/100 g soil; pH 7.0 – 7.4. Soil samples were incubated at 19.4°C – 21.1°C in glass flasks in the dark.

Statistics: Descriptive statistics.

II. RESULTS AND DISCUSSION

No adverse effects of Reg. No. 5 079 285 on nitrogen transformation (> 25% deviation from control) in soil could be observed in both test concentrations, 0.1 mg/kg dry soil and 1.0 mg/kg dry soil, after 42 days (time interval 0-42). A deviation of 26.8% and 26.9% (at 0.1 and 1.0 mg Reg. No. 5 079 285/kg dry soil, respectively) could be seen at day 28. However, by study termination, the percent deviation was < 25%, thereby indicating no long term effects from exposure to Reg. No. 5 079 285. The results are summarized in Table 8.5-3.

Table 8.5-3: Effects of Reg. No. 5 079 285 on soil micro-organisms (nitrogen transformation) on days 0-7, 0-14, 0-28 and 0-42 of incubation

Time interval (days)	Control	0.1 mg Reg. No. 5 079 285/kg dry soil		1.0 mg Reg. No. 5 079 285/kg dry soil	
	NO_3-N [mg/kg dry soil]	NO_3-N [mg/kg dry soil]	% Deviation from the control [1)]	NO_3-N [mg/kg dry soil]	% Deviation from the control [1)]
0-7	-2.38	-2.62	-10.1	-2.12	+10.7
0-14	-1.24	-1.44	-16.0	-1.33	-7.41
0-28	-0.337	-0.428	-26.8	-0.428	-26.9
0-42	0.560	0.534	-4.73	0.421	-24.8

[1)] Based on mean NO_3^--N formation rate; a negative value indicates inhibition; a positive value indicates stimulation.

In a separate study, the reference item sodium chloride produced significant effects on the soil nitrogen turnover (170% nitrogen increase, decrease of nitrate transformation rate of 1860% during the 0-28 days interval) and short-term respiration (88.5% inhibition) at 20 g a.s./kg dry soil after 28 days of incubation

III. CONCLUSION

Exposure of Reg. No. 5 079 285 (metabolite of BAS 595 F, triticonazole) in a field soil up to a test concentration of 1.0 mg Reg. No. 5 079 285/kg dry soil caused no adverse effects (deviation from control < 25%, OECD 216) on the soil nitrogen transformation (measured as NO_3-N production) at the end of the 42-day incubation period.

Report: CA 8.5/3
Schulz L., 2013b
Effects of Reg.No. 5079288 (metabolite of BAS 595 F, Triticonazole) on the activity of soil microflora (Nitrogen transformation test)
2014/1000025
Guidelines: OECD 216 (2000)
GLP: yes
(certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

Executive Summary

In a soil microbial activity study, the effects of Reg. No. 5 079 288 (metabolite of BAS 595 F, triticonazole) on the nitrogen transformation were investigated in a loamy sand soil. Reg. No. 5 079 288 was applied to samples of the soil at nominal test concentrations of 0.1 mg/kg and 1.0 mg/kg dry soil.

No adverse effects of Reg. No. 5 079 288 on nitrogen transformation in soil could be observed in either test concentration (0.1 mg/kg dry soil and 1.0 mg/kg dry soil) after 28 days.

Exposure of Reg. No. 5 079 288 (metabolite of BAS 595 F, triticonazole) in a field soil up to a test concentration of 1.0 mg /kg dry soil caused no adverse effects (deviation from control < 25%, OECD 216) on the soil nitrogen transformation (measured as NO_3-N production) at the end of the 28-day incubation period.

I. MATERIAL AND METHODS

A. MATERIALS

Test item:	RAP 407922 (metabolite of triticonazole (BAS 595 F); Reg. No. 5 079 288); batch no.: 33484-39; analyzed purity: 99.5% (tolerance ± 1.0%).

B. STUDY DESIGN

Test species:	Biologically active agricultural soil: loamy sand soil: pH 6.5, 1.47% C_{org}, 36.71 g/100 g water holding capacity (WHC).
Test design:	Determination of the N-transformation (NO_3-nitrogen production) in soil enriched with lucerne meal (concentration in the soil 0.5%). Comparison of test item treated soil with a non-treated soil. NH_4-nitrogen formed from organically bound nitrogen in the soil and NO_3-nitrogen from the nitrification process was determined using an Autoanalyzer (Bran and Luebbe). Sampling dates: 0, 7, 14 and 28 days after treatment. Sub-samples (3 replicates per treatment) were withdrawn from the soil bulk batches and subjected to the measurement.
Endpoints:	Effects on NO_3-nitrogen production 0, 7, 14 and 28 days after application.
Test concentrations:	Control, 0.1 mg Reg. No. 5 079 288/kg dry soil, 1.0 mg Reg. No. 5 079 288/kg dry soil.
Reference item:	Dinoterb (purity: 98.0% analyzed). The reference item was applied at rates of 6.80, 16.00 and 27.00 mg/kg in a separate study.
Test conditions:	Soil moisture: approximately 45% of its maximum water holding capacity, measured water content: 16.75% – 17.87% dry soil; pH 6.2 – 6.4. Soil samples were incubated at 19.0°C – 20.8°C in glass flasks in the dark.
Statistics:	Descriptive statistics.

II. RESULTS AND DISCUSSION

No adverse effects of Reg. No. 5 079 288 on nitrogen transformation in soil could be observed in both test concentrations (0.1 mg/kg dry soil and 1.0 mg/kg dry soil) after 28 days. The results are summarized in Table 8.5-4.

Table 8.5-4: Effects of Reg. No. 5 079 288 on soil micro-organisms (nitrogen transformation) on days 7, 14 and 28 of incubation

Time interval (days)	Control	0.1 mg Reg. No. 5 079 288/kg dry soil		1.0 mg Reg. No. 5 079 288/kg dry soil	
	NO_3-N [mg/kg dry soil]	NO_3-N [mg/kg dry soil]	% Deviation from the control [1)]	NO_3-N [mg/kg dry soil]	% Deviation from the control [1)]
0-7	26.57	28.10	+5.8	26.60	+0.1
0-14	32.13	34.47	+7.3	34.07	+6.0
0-28	47.07	50.60	+7.5	48.73	+3.5

[1)] Based on respiration rate; - = inhibition; + = stimulation.

In a separate study the reference item Dinoterb produced, after 28 days of incubation, a stimulation of nitrogen transformation of +33.7% and +42.6% at 16.00 and 27.00 mg/kg dry soil, respectively.

III. CONCLUSION

Exposure of Reg. No. 5 079 288 (metabolite of BAS 595 F, triticonazole) in a field soil up to a test concentration of 1.0 mg /kg dry soil caused no adverse effects (deviation from control < 25%, OECD 216) on the soil nitrogen transformation (measured as NO_3-N production) at the end of the 28-day incubation period.

CA 8.6 Effects on terrestrial non-target higher plants

No new studies are available.

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. A study was performed with the formulated product and reference is made to chapter CP 10.6.2 (BASF DocID 2013/1003205).

CA 8.7 Effects on other terrestrial organisms (flora and fauna)

The following study is no data requirement for the risk assessment and is presented as additional information to the dossier.

Report:	CA 8.7/1 Royer S., 2006d Effect of Reg.No. 5059144 (RPA 406341, metabolite of BAS 595 F) on soil microorganisms: Carbon transformation test 2006/1028714
Guidelines:	OECD 217 (2000)
GLP:	yes (certified by Landesamt fuer Umweltschutz und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

The effect of RPA 406341 on carbon transformation was investigated in a loamy sand soil. RPA 406341 was applied to samples of the soil, in a laboratory, at nominal application rates of 1 mg/kg and 10 mg/kg dry soil. RPA 406341 treated soils and untreated controls were incubated at approximately 20°C in the dark for 28 days. Three replicate samples of each treatment were removed for analysis of carbon transformation (oxygen consumption), using a “BSB-digi” respirometer system, 7, 14 and 28 days after application.

No adverse effects of RPA 406341 on carbon transformation in soil were observed in both test item concentrations after 28 days.

Based on the results of this study, in accordance with OECD guideline 217, RPA 406341 caused no short-term and long-term effects on carbon transformation in a field soil tested up to a concentration of 10 mg/kg dry soil.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: RPA 406341, metabolite of triticonazole (BAS 595 F), Reg. No. 5 059 144, batch BESS0541; purity 99%.

B. STUDY DESIGN

Test species: Biologically active agricultural soil: silty sand, pH 6.7, 1.12% C_{org}, WHC 34.2 g/100 g dry soil.

Test design: Determination of carbon-transformation in soil after addition of glucose (concentration in soil: 0.4%). Comparison of test item treated soil with a non-treated. 4 replicates per concentration. A “BSB-digi” respirometer system was used to measure the oxygen consumption over a period of maximum 20 hours at different sampling intervals. Sampling scheme: 0, 7, 14 and 28 days after treatment, subsamples were withdrawn from the bulk batches and subjected to the measurement.

Test rates: Control, 1 mg RPA 406341 per kg dry soil and 10 mg RPA 406341 per kg soil. Test concentrations related to a soil depth of 5 cm and a soil density of 1.5 g/cm^3.

Endpoints: Effects on O_2 consumption after 28 day of exposure.

Reference item: Dinoterb (purity: 98.0 ± 0.5%). The reference item was applied at a rate of 6.7 and 13.3 mg/kg dry soil.

Test conditions: Soil moisture: 50% of its maximum water holding capacity; pH 6.57 - 6.74. Soil samples were incubated at 20°C ± 2°C while stored in glass bottles in the dark.

Statistics: Descriptive statistics.

II. RESULTS AND DISCUSSION

No adverse effects of RPA 406341 on carbon transformation in soil were observed in both test item concentrations after 28 days. The results are summarized in Table 8.7-1.

Table 8.7-1: Effects of RPA 406341 on soil micro-organisms (carbon transformation) on days 7, 14 and 28 of incubation

Soil (days)	Control	1 mg RPA 406341 per kg dry soil		10 mg RPA 406341 per kg dry soil	
	O_2 consumption [mg/h/kg dry soil]	O_2 consumption [mg/h/kg dry soil]	% Deviation from the control [1)]	O_2 consumption [mg/h/kg dry soil]	% Deviation from the control [1)]
Silty sand soil (7 d)	6.4	6.0	-6.25	6.1	-4.69
Silty sand soil (14 d)	6.1	5.9	-3.28	5.9	-3.28
Silty sand soil (28 d)	5.3	5.0	-5.66	4.9	-7.55

[1)] Based on O_2 consumption; - = inhibition, + = stimulation

In a separate study the reference item Dinoterb produced the expected level of effect (-41.51%, -33.96% inhibition).

III. CONCLUSION

Based on the results of this study, in accordance with OECD guideline 217, RPA 406341 caused no short-term and long-term effects on carbon transformation in a field soil tested up to a concentration of 10 mg/kg dry soil.

CA 8.8 Effects on biological methods for sewage treatment

The results of the already peer-reviewed and accepted study are still valid and they 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 595 F (triticonazole)	Respiration inhibition test (inhibition of oxygen consumption activated sludge from wastewater plant)	No inhibitory effect on microbial respiration up to 1000 mg a.s./L	C011667	yes

CA 8.9 Monitoring data

According to the knowledge of the applicant, there are currently no published ecotoxicological monitoring data available for triticonazole or its metabolites, which would provide additional knowledge on the ecotoxicological assessment not covered by this dossier.

Triticonazole

Document M-CA, Section 9

LITERATURE DATA

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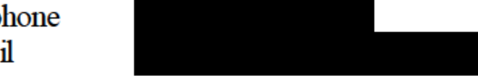

Version history[1]

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 9 LITERATURE DATA

A literature search on Triticonazole and the common product trade names was performed by the BASF Group Information Center. The Literature Search Report on Triticonazole 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 2015/1216973).

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; a list is available upon request.

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:

Analytics:	Triticonazole - Literature Analytics
Ecotoxicology:	Triticonazole - Literature Ecotoxicology aquatic
	Triticonazole - Literature Ecotoxicology general
	Triticonazole - Literature Ecotoxicology terrestric
	Triticonazole - Literature Ecotoxicology wildlife
E-fate:	Triticonazole - Literature E-fate
Impurities:	Triticonazole - Literature Impurities
Metabolism and Residues:	Triticonazole - Literature Metabolism and Residues in Animals
	Triticonazole - Literature Metabolism and Residues in Plants
Product Chemistry:	Triticonazole - Literature Product Chemistry
Toxicology:	Triticonazole - Literature Toxicology

Triticonazole

Document M-CA, Section 10

CLASSIFICATION AND LABELLING OF 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

[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

The following harmonized classification and labelling was adopted for triticonazole:

Legislation	Classification	Labelling	Concentration limits
Regulation (EC) No 1272/2008	No classification for health hazards. Aquatic Chronic 2 Hazard statement code: H411	Hazard statement code: H411	