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Boscalid

Document M-CA, Section 1

IDENTITY 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

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CA 1 IDENTITY OF THE ACTIVE SUBSTANCE

CA 1.1 Applicant

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CA 1.2 Producer

Producer of Boscalid

BASF SE
67056 Ludwigshafen
Germany

Contact person: Please refer to CA 1.1 Applicant.

Location of the manufacturing site for Boscalid

CONFIDENTIAL information - data provided separately (Document J)

CA 1.3 Common Name Proposed or ISO-accepted and synonyms

ISO common name: Boscalid

CA 1.4 Chemical Name (IUPAC and CA nomenclature)

IUPAC name: 2-chloro-N-(4′-chloro[1,1′-biphenyl]-2-yl)pyridine-3-carboxamide
also acceptable:
2-chloro-N-(4'-chlorobiphenyl-2-yl)nicotinamide

CA nomenclature: 2-chloro-N-(4′-chloro[1,1′-biphenyl]-2-yl)-3-pyridinecarboxamide

CA 1.5 Producer's Development Code Numbers

BASF Number: BAS 510 F
BASF Registry Number: Reg.No. 300355

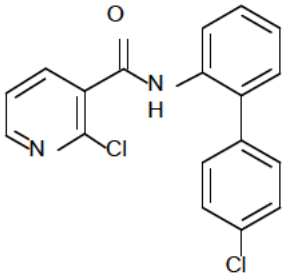
CA 1.6 CAS, EC and CIPAC Numbers

CAS No.: 188425-85-6
CIPAC No.: 673
EC No.: Not assigned

CA 1.7 Molecular and Structural Formula, Molar Mass

Molecular formula: $C_{18}H_{12}Cl_2N_2O$

Structural formula:



Molar mass: 343.21 g/mol

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: 960 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

CONFIDENTIAL information - data provided separately (Document J)

CA 1.11 Analytical Profile of Batches

CONFIDENTIAL information - data provided separately (Document J)

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Document M-CA, Section 2

PHYSICAL AND CHEMICAL PROPERTIES OF THE ACTIVE SUBSTANCE

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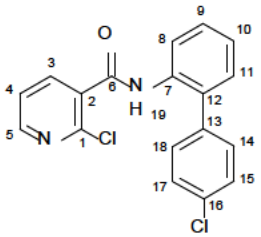
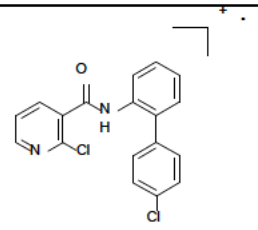
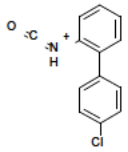
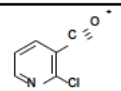
CA 2 PHYSICAL AND CHEMICAL PROPERTIES OF THE ACTIVE SUBSTANCE

Test or Study & Data Point	Guideline and method	Test material purity and specification	Findings	GLP Y/N	Reference
CA 2.1 Melting point and boiling point	EEC A.1	COD-001415: 98.9 %	Differential Scanning Calorimetry (EEC A.1 4.4.2) An endothermic effect was observed at onset temperature of 140 °C (peak: 149 °C). An exothermic effect was observed starting at onset temperature of 245 °C. Conclusion: the test substance is melting at 140 - 149 °C and decomposing at 245 °C.	Y	[see KCA 2.1/1 2013/1164740]
	EEC A.1	99.7 %	Information previously reported and peer-reviewed: capillary method, 143-144 °C Differential Scanning Calorimetry 145 °C	Y	Review report boscalid, SANCO/3919/2007 - rev. 5, 21 January 2008, Appendix I
CA 2.2 Vapour pressure, volatility	Weight loss per area and time (internal method)	99.4 %	Information previously reported and peer-reviewed: Vapour pressure 7.2×10^{-7} Pa at 20 °C 1.5×10^{-6} Pa at 25 °C	Y	Review report boscalid, SANCO/3919/2007 - rev. 5, 21 January 2008, Appendix I Draft Assessment Report, Vol. 3, Annex B.2, 2002/1014099
	Calculation		Information previously reported and peer-reviewed: Henry's law constant 5.178×10^{-8} (kPa m^3/mol)		Review report boscalid, SANCO/3919/2007 - rev. 5, 21 January 2008, Appendix I

<table>
<tr><th>Test or Study & Data Point</th><th>Guideline and method</th><th>Test material purity and specification</th><th>Findings</th><th>GLP Y/N</th><th>Reference</th></tr>
<tr><td rowspan="2">CA 2.3 Appearance (Physical state, colour)</td><td>Visual examination</td><td>99.4 %</td><td><u>Information previously reported and peer-reviewed:</u>
White, odourless crystalline solid.</td><td>Y</td><td>Review report boscalid, SANCO/3919/2007 - rev. 5, 21 January 2008, Appendix I</td></tr>
<tr><td>Visual examination</td><td>98.18 %</td><td><u>Information previously reported and peer-reviewed:</u>
White, faint smoky powdery solid.</td><td>Y</td><td>Draft Assessment Report, Vol. 3, Annex B.2, 2002/1014099</td></tr>
<tr><td>CA 2.4 Spectra (UV/VIS, IR, NMR, MS), molar extinction at relevant wavelengths, optical purity</td><td>UV/VIS</td><td>99.4 %</td><td><u>Information previously reported and peer-reviewed:</u>
Solvent: Methanol
<table><tr><th>λ_{max} [nm]</th><th>ε [L mol^{-1} cm^{-1}]</th></tr><tr><td>207</td><td>31534</td></tr><tr><td>228</td><td>19834</td></tr><tr><td>290</td><td>1529</td></tr><tr><td>300</td><td>531</td></tr></table></td><td>Y</td><td>Review report boscalid, SANCO/3919/2007 - rev. 5, 21 January 2008, Appendix I</td></tr>
</table>

Test or Study & Data Point	Guideline and method	Test material purity and specification	Findings	GLP Y/N	Reference
	IR, NMR, MS	99.4 %	Information previously reported:  ^{1}H-NMR ($CDCl_3$, TMS): 8.39 ppm (dd, 1H, H5) 8.35 ppm (d, 1H, H8) 8.08 ppm (s, 1H, H19) 8.07 ppm (dd, 1H, H3) 7.40 ppm (t, 1H, H9) 7.37 ppm (m, 2H, H14+18) 7.28 ppm (m, 2H ,H15+17) 7.20 ppm (m, 2H ,H10+11, overlapping) IR (KBr pellet): 3252 cm^{-1} (ν N-H, amide) 3022 cm^{-1} (ν C-H, aromatic) 1652 cm^{-1} (ν C=O, amide) 1580 cm^{-1} (ν C=C, aromatic ring) 1400 cm^{-1} (δ N-H, amide)	Y	1999/10832 (Daum A., 1999) reported and reviewed in Draft Assessment Report, Vol. 3, Annex B.2, 2002/1014099

<table>
<tr><th>Test or Study & Data Point</th><th>Guideline and method</th><th>Test material purity and specification</th><th>Findings</th><th>GLP Y/N</th><th>Reference</th></tr>
<tr><td></td><td></td><td></td><td>MS (Electron Ionization):<table>
<tr><td>m/z 342</td><td>  </td></tr>
<tr><td>m/z 307</td><td>$(342 - Cl)^+$</td></tr>
<tr><td>m/z 230</td><td>  </td></tr>
<tr><td>m/z 140</td><td>  </td></tr>
<tr><td>m/z 112</td><td>  </td></tr>
<tr><td>m/z 76</td><td>$(112 - HCl)^+$</td></tr>
</table>Spectra are consistent with given structure of Boscalid.</td><td></td><td></td></tr>
<tr><td>CA 2.5 Solubility in water</td><td>EEC A.6</td><td>99.4 %</td><td><u>Information previously reported and peer-reviewed:</u>
4.6 mg/L (20 °C)
No dissociation in water, therefore no pH dependency</td><td>Y</td><td>Review report boscalid, SANCO/3919/2007 - rev. 5, 21 January 2008, Appendix I</td></tr>
</table>

<table>
<tr><th>Test or Study & Data Point</th><th>Guideline and method</th><th>Test material purity and specification</th><th>Findings</th><th>GLP Y/N</th><th>Reference</th></tr>
<tr><td>CA 2.6 Solubility in organic solvents</td><td>CIPAC MT 181</td><td>99.4 %</td><td><u>Information previously reported and peer-reviewed:.</u>
<table><tr><th>Solvent</th><th>Solubility (20 °C) (g/L solvent)</th></tr><tr><td>n-Heptane</td><td>< 10</td></tr><tr><td>Toluene</td><td>20-25</td></tr><tr><td>Dichloromethane</td><td>200-250</td></tr><tr><td>Methanol</td><td>40-50</td></tr><tr><td>Acetone</td><td>160-200</td></tr><tr><td>Ethyl acetate</td><td>67-80</td></tr><tr><td>N,N-Dimethylformamide</td><td>> 250</td></tr><tr><td>Acetonitrile</td><td>40-50</td></tr><tr><td>1-Octanol</td><td>< 10</td></tr><tr><td>2-Propanol</td><td>< 10</td></tr><tr><td>olive oil</td><td>< 10</td></tr></table></td><td>N</td><td>Review report boscalid, SANCO/3919/2007 - rev. 5, 21 January 2008, Appendix I</td></tr>
<tr><td>CA 2.7 Partition co-efficient n-octanol/water</td><td>OECD 117</td><td>99.4 %</td><td><u>Information previously reported and peer-reviewed:</u>

log P_{OW} = 2.96 at 21 °C (pH 7.1).

No dissociation in water, therefore no pH dependency.</td><td>Y</td><td>Review report boscalid, SANCO/3919/2007 - rev. 5, 21 January 2008, Appendix I</td></tr>
</table>

Test or Study & Data Point	Guideline and method	Test material purity and specification	Findings	GLP Y/N	Reference
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	99.4 %	Information previously reported and peer-reviewed: No dissociation in water.	Y	Review report boscalid, SANCO/3919/2007 - rev. 5, 21 January 2008, Appendix I
CA 2.9 Flammability and self-heating	EEC A.10	COD-001415: 98.9 %	Flammability Preliminary test: negative (No burning). Main test omitted due to result of preliminary test. Conclusion: The test substance is not considered highly flammable.	Y	[see KCA 2.9/1 2013/1164740]
	EEC A.16	COD-001415: 98.9 %	Relative self-ignition temperature Result: no self-heating detected.	Y	[see KCA 2.9/1 2013/1164740]
CA 2.10 Flash point	EEC A.9		Not applicable, since melting point is higher than 40 °C.	Y	Draft Assessment Report, Vol. 3, Annex B.2, 2002/1014099
CA 2.11 Explosive properties	EEC A.14	COD-001415: 98.9 %	Pre-test via DSC: 1st reaction: onset 245°C, peak 282°C, energy release >920 J/g (exothermal) Testing on explosive properties (thermal and mechanical sensitivity and friction): negative Conclusion: The test substance is not considered to exhibit a danger of explosion in the sense of the directive.	Y	[see KCA 2.11/1 2013/1164740]

Test or Study & Data Point	Guideline and method	Test material purity and specification	Findings	GLP Y/N	Reference
CA 2.12 Surface Tension	EEC A.5 1.6.1	L71-168: 99.0%	73.6 mN/m at 90 % of saturation solubility in water at 20 °C Conclusion: The pure active ingredient Boscalid is considered a non surface active material.	Y	[see KCA 2.12/1 2015/1099102]
CA 2.13 Oxidizing properties	EEC A.17	COD-001415: 98.9 %	Highest burning rate of reference mixture $BaNO_3$/cellulose: 3.7 mm/s with a mixture containing 60 % weight of oxidizer. Highest burning rate of test mixtures: 0.67 mm/s with a mixture containing 10 % weight of test substance. Conclusion: The test substance is not considered an oxidizing substance because the maximum burning rate of the mixtures tested is lower than the maximum burning rate of the reference mixture.	Y	[see KCA 2.13/1 2013/1164740]

Test or Study & Data Point	Guideline and method	Test material purity and specification	Findings	GLP Y/N	Reference
CA 2.14 Other studies					
vapor pressure of the metabolites Reg.No. 309572 (= M510F64), Reg.No. 391572 (= M510F49), Reg.No. 107371 (= M510F47)	EEC A.4 OECD 104	Reg.No. 107371 (= M510F47) Batch No. 01174-232 (99.8 %)	$1.1 \cdot 10^{-5}$ Pa at 20 °C $2.6 \cdot 10^{-5}$ Pa at 25 °C		[see KCA 2.14/1 2014/1145903]
		Reg. No. 391572 (= M510F49) Batch No. L71-12 (99.7 %)	$1.3 \cdot 10^{-13}$ Pa at 20 °C $4.4 \cdot 10^{-13}$ Pa at 25 °C		[see KCA 2.14/2 2014/1145904]
		Reg. No. 309572 (= M510F64) Batch No. AC11643-77 (98.4 %)	$4.0 \cdot 10^{-4}$ Pa at 20 °C $8.2 \cdot 10^{-4}$ Pa at 25 °C		[see KCA 2.14/3 2014/1145905]

Test or Study & Data Point	Guideline and method	Test material purity and specification	Findings	GLP Y/N	Reference
water solubility of the metabolites Reg.No. 309572 (= M510F64), Reg.No. 391572 (= M510F49), Reg.No. 107371 (= M510F47)	OECD 105	Reg. No. 309572 (= M510F64) Batch No. AC11643-77 (98.4 %)	Milli-RO water = 89.5 ± 9.2 mg/L pH 4.0 Buffer = 118.4 ± 7.2 mg/L pH 7.0 Buffer = 6.340 ± 0.355 g/L pH 9.0 Buffer = 11.008 ± 0.214 g/L		[see KCA 2.14/4 2015/1204790]
		Reg. No. 391572 (= M510F49) Batch No. L71-12 (99.7 %)	Milli-RO water = 0.435 ± 0.067 mg/L pH 4 Buffer = 0.393 ± 0.046 mg/L pH 7 Buffer = 0.335 ± 0.029 mg/L pH 9 Buffer = 0.411 ± 0.065 mg/L		[see KCA 2.14/5 2015/1204789]
		Reg.No. 107371 (= M510F47) Batch No. L80-188 (100.0 %)	Milli-RO water = 1.642 ± 0.040 g/L pH 4 Buffer = 6.377 ± 0.041 g/L pH 7 Buffer = 5.991 ± 0.062 g/L pH 9 Buffer = 4.302 ± 0.097 g/L		[see KCA 2.14/6 2015/1204788]

Test or Study & Data Point	Guideline and method	Test material purity and specification	Findings	GLP Y/N	Reference
log P_{OW} of the metabolites Reg.No. 309572 (= M510F64), Reg.No. 391572 (= M510F49), Reg.No. 107371 (= M510F47)	OECD 117 Shake flask method	Reg. No. 309572 (= M510F64) Batch No. AC11643-77 (98.4 %)	Log P_{OW} Milli-RO water = 1.99 ± 0.03 Log P_{OW} pH 4.0 Buffer = 2.37 ± 0.01 Log P_{OW} pH 7.0 Buffer = -0.02 ± 0.10 Log P_{OW} pH 10.0 Buffer = -0.75 ± 0.07		[see KCA 2.14/4 2015/1204790]
	OECD 117 HPLC method	Reg. No. 391572 (= M510F49) Batch No. L71-12 (99.7 %)	Log Pow pH 4 Buffer = 3.20 ± 0.00 Log Pow pH 7 Buffer = 3.04 ± 0.01 Log Pow pH 10 Buffer = 2.48 ± 0.01		[see KCA 2.14/5 2015/1204789]
	OECD 117 Shake flask method	Reg.No. 107371 (= M510F47) Batch No. L80-188 (100.0 %)	Log Pow Milli-RO water = -0.29 ± 0.05 Log Pow pH 4 Buffer = -0.40 ± 0.10 Log Pow pH 7 Buffer = -0.69 ± 0.22 Log Pow pH 10 Buffer = -0.85 ± 0.16		[see KCA 2.14/6 2015/1204788]

Test or Study & Data Point	Guideline and method	Test material purity and specification	Findings	GLP Y/N	Reference
pKa values of the metabolites Reg.No. 309572 (= M510F64), Reg.No. 391572 (= M510F49), Reg.No. 107371 (= M510F47)	OECD 112	Reg. No. 309572 (= M510F64) Batch No. AC11643-77 (98.4 %)	Dissociation Constant pKa = 4.01 ± 0.06		[see KCA 2.14/4 2015/1204790]
		Reg. No. 391572 (= M510F49) Batch No. L71-12 (99.7 %)	No dissociation constant could be established employing the titration method due to the low water solubility of the test item. The expected pKa value was calculated using ACD Lab Version 12.01 to be pKa = 7.0. However, the water solubility was low and no remarkable pH-dependence of the water solubility was found.		[see KCA 2.14/5 2015/1204789]
		Reg.No. 107371 (= M510F47) Batch No. L80-188 (100.0 %)	From the base titration attempts, it could be determined that Reg.No. 107371 exhibits a pKa of 2.3. This result confirms the calculation of the pKa values using ACD Lab Version 12.01 (pKa1 = 2.07). On the other hand, from the acid titration attempts, no reliable pKa can be derived for the protonation of the nitrogen atom. The expected pKa value was calculated using ACD Lab Version 12.01 to be pKa2 = -1.32, which cannot be determined by normal acid titration. Since both pKa values are not relavant in an environmental context, no further investigations were conducted.		[see KCA 2.14/6 2015/1204788]

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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

Boscalid, a member of the fungicide group succinate dehydrogenase inhibitors (SDHI), is used worldwide in several crops for the control of a broad range of important pathogens. Boscalid is active against different fungal stages both on the plant surface and in the plant tissue. After application to the plant, the active ingredient is taken up via the leaf and then translocated via the transpiration flow. Due its mobility, it shows local systemic and translaminar activity. By that, it can control fungal stages, which have already become established in deeper tissue layers. As a result, Boscalid has preventative and curative activity.
Since the vapour pressure of boscalid is very low, activity via the gas phase is not relevant.

CA 3.2 Function

Boscalid is used as a fungicide to control harmful diseases in a broad range of crops.

CA 3.3 Effects on Harmful Organisms

Boscalid is active against different fungal stages on and in the plant. When applied protectively, boscalid inhibits spore germination and further development of germinated fungal spores. Due to its ability to penetrate into the leaf and its further translocation, it can also control fungal stages that have already become established in deeper tissue layers. Boscalid is effective under preventative and curative conditions.

CA 3.4 Field of Use Envisaged

Agriculture

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

Boscalid is used to control a broad range of important fungal diseases such as

- *Alternaria* spp.
- *Ascochyta* spp.
- *Blumeriella* spp.
- *Botrytis* spp.
- *Cercospora* spp.
- *Colletotrichum* spp.
- *Diaporthe* ssp.
- *Drechslera* spp.
- *Erysiphe* ssp.
- *Fusarium* spp.
- *Gnomonia* spp.
- *Guignardia* spp.
- *Leptosphaeria* / *Plenodomus* spp.
- *Microdochium* spp.
- *Monilinia* spp.
- *Mycosphaerella* spp.
- *Phyllosticta* spp.
- *Podosphaera* spp.
- *Puccinia* spp.
- *Pyrenopeziza* ssp.
- *Pyrenophora* spp.
- *Ramularia* spp.
- *Sclerotinia* spp.
- *Septoria* spp.
- *Sphaerotheca* spp.
- *Stemphylium* spp.
- *Uncinula* spp.
- *Uromyces* spp.
- *Venturia* spp.
- *Wilsonomyces* spp.

Boscalid is used in a wide range of crops such as (representative uses in bold):

Cereals

Legume crops
- Dry pulses (dry harvest)
  - Beans: field beans
  - Peas: chickpeas, field peas, chickling vetch
- **Legume vegetables** (fresh harvest)
  - Beans (with and without pods)
  - Peas (with and without pods)

Oilseed Rape (Winter and Spring oilseed rape)

Sunflower

Vegetables
- Brassica vegetables
  - Head cabbage
  - Leafy cabbage
  - Flowering cabbage
  - Brussel sprouts
- Bulb vegetables
  - Onions (incl. spring onions)
  - Shallot
  - Garlic
- Root and tuber vegetables
  - Carrots
  - Potato
  - Radish
  - Parsnip
- Stem vegetables
  - Leek
  - Celery
  - Asparagus
  - Fennel
- Fruiting vegetables
  - Cucurbits with edible peel (cucumber, gherkins, courgette)
  - Cucurbits with inedible peel (melon, pumpkin, zucchini)
  - Tomato
  - Aubergine
  - Pepper
- Leafy vegetables and herbs
  - Lettuce and similar
  - Fresh herbs
  - Chicory, witloof

Perennial crops

- Berries and small fruits
  - Strawberries
  - Currants
  - Raspberries
- **Grapes**
- Hop
- Nuts
- Pomefruit
- Stonefruit

Others

- Ornamentals
- Turf

CA 3.6 Mode of Action

Boscalid is a member of the fungicide group succinate dehydrogenase inhibitors (SDHI) and the mode of action at the molecular level is the inhibition of the enzyme succinate dehydrogenase (SDH), also known as complex II in the mitochondrial electron transport chain (Kulka and von Schmeling 1995). Such as other complexes of the respiratory chain, this enzyme is a component of the inner mitochondrial membrane. It consists of four nucleus-encoded subunits (SDH A, B, C, D). Two of these polypeptides (SDH C, D) anchor the complex in the membrane whilst the others project into the mitochondrial matrix where they catalyze the oxidation of succinate to fumarate as part of the tricarboxylic acid (TCA) cycle. The electrons so released are channeled into the electron transport chain via the co-substrate ubiquinol. Complex II occupies a key function in fungal metabolism. It delivers high-energy electrons for energy production and, additionally, forms an essential junction where components of the TCA cycle can be diverted to become the building blocks for amino acids and lipids. Through its inhibition of complex II, boscalid disrupts fungal growth by preventing energy production and also by eliminating the availability of the chemical building blocks for the synthesis of other essential cellular components.

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

Studies on earlier SDHIs showed that single site mutations in the SDH genes were responsible for the loss of fungicide efficacy (Keon et al. 1991, Skinner et al. 1998, Matsson et al. 1998, 2001, Ito et al. 2004). With investigations of SDHI resistance moving forward after the introduction of broad spectrum SDHIs such as boscalid, a complex picture is forming. Several mutations in the target protein at different positions in three SDH subunits B, C and D were detected in field isolates of some plant pathogens such as *Botrytis cinerea* (Stammler et al. 2007, Veloukas et al. 2011), *Corynespora cassiicola* (Miyamoto et al. 2009, 2010a), *Alternaria alternata* (Avenot and Michaelidis 2007), *Alternaria solani* (Miles et al. 2014), *Didymella bryoniae* (Avenot et al. 2012, Fernandez-Ortuno et al. 2012), *Podosphaera xanthii* (Miyamoto et al. 2010b), *Pyrenophora teres* (Stammler et al. 2014) and *Sclerotinia sclerotiorum* (Glaettli et al. 2009), and in laboratory mutants of *Zymoseptoria tritici* (=*Mycosphaerella graminicola*) (Skinner et al. 1998, Stammler et al. 2010, Fraaije et al. 2012, Scalliet et al. 2012). Different mutations were found at one gene location (e.g. B-P225L/F/T or B-H272Y/R/L/V in *B. cinerea*), and at different gene locations in different subunits (e.g. B-H277Y, C-N75S, C-G79R, C-H134R, C-S135R, D-D124N/E, D-H143R, D-D145G in *Pyrenophora teres*, Stammler et al. 2014).

Table 3.7-1 provides an overview of mutations so far detected in field and laboratory isolates. The numbering of mutations needs some explanation. Several of these amino acids, which have been found to be exchanged in different species, are homologous, but have different numbers, since the number of amino acids ahead in the protein may vary in different species. Homologous mutations found in several species but with different numbering are described in Table 3.7-2. This diversity of mutations complicates the interpretation of sensitivity findings in an unprecedented manner. There are mutations with amino acid exchanges leading to more or less complete resistance to all SDHIs, but some exchanges have also been detected which affect sensitivity to different SDHIs differently. Mutations at homologous sites may even have different effects in different fungal species (e.g. mutations at P220 in Z. tritici and at the homologous site P225 in *B. cinerea* as described by Scalliet et al. 2012). A general cross-resistance can be postulated for SDHIs, even if the effects may be somewhat different in some cases. However, there are exceptions, where no cross-resistance seems to be present (Ishii et al. 2011, Veloukas et al. 2013, Semar et al. 2014). In addition, uses of specific SDHIs also influence which mutation will occur in various fungal species (Table 3.7-3), and variability in the frequency of mutations is therefore not only a result of the fungal species but also of the use of various SDHIs.
However, monitoring studies were made for many target pathogens of boscalid without any finding of any isolate with a sensitivity outside the baseline. These include *Puccinia* spp., *Erysiphe* spp., *Puccinia* spp., *Microdochium* spp., *Podosphaera* spp., *Monilinia* spp., *Leptosphaeria maculans* and *Leptosphaeria biglobosa*.

Further information on SDHI resistance in target pathogens with more details on frequency of resistance also on a regional level are provided on the FRAC webpage (www.frac.info).

Table 3.7-1: List of cases of SDHI-resistant fungal plant pathogen species, their origin, and mutations found conferring SDHI resistance. Letter codes given for species are used in Table 3.7-2. Table reflects the list published on the FRAC webpage (Status July 2014) with some updates based on BASF, unpublished data.

Species name		Reported from host	Origin	Resistance mechanism (Subunit-mutation)
Ustilago maydis	a	(Laboratory)	Lab	B-H257L
Aspergillus oryzae	b	(Laboratory)	Lab	B-H249Y/L/N, C-T90I, D-D124E
Zymoseptoria tritici	c	(Laboratory)	Lab	B-N225I, B-H267Y/R/L, B-I269V, C-A84V, C-H152R, C-T79I, C-N86K, C-G90R, D-H129E and several others
Zymoseptoria tritici	d	Wheat	Field	B-N225T, C-T79N, C-W80S, C-N86S
Pyrenophora teres	e	Barley	Field	B-H277Y, C-N75S, C-G79R, C-H134R, C-S135R, D-D124N/E, D-H134R, D-D145G
Botrytis cinerea	f	various	Field	B-P225L/T/F, B-H272Y/R/L/V, B-N230I, D-H132R, C-A85V
Botrytis elliptica	g	Lillies	Field	B-H272Y/R
Alternaria alternata	h	Pistachio	Field	B-H277Y/R, C-H134R, D-D123E, D-H133R
Alternaria solani	i	Potatoes	Field	B-H277Y/R, D-H133R
Corynespora cassiicola	j	Cucurbits	Field	B-H278Y/R, C-S73P, D-S89P, D-G109V
Didymella bryoniae	k	Cucurbits	Field	B-H277R/Y
Podosphaera xanthii	l	Cucurbits	Field	B-H->Y (homologous to H272 in *B. cinerea*)
Sclerotinia sclerotiorum	m	Oilseed rape	Field	B-H273Y, C-H146R, D-H132R
Stemphylium vesicarium	n	Asparagus	Field	B-P225L, H272Y/R
Venturia inaequalis	o	Apple	Field	C-H151R

Table 3.7-2: Amino acids in the different SDH subunits, which were found to be exchanged and to influence SDHI sensitivity (first column). Homologous position (number) in different species (Letter codes see Table 3.7-1) is given in the second column; e.g. P220 in *Z. tritici* is homologous to P225 in *B. cinerea* and P225 in *S. vesicarium*.

Amino acid	Homologous positions
B-Proline	220c, 225 f,n
B-Histidine	257a, 249b, 267c, 277e,h,I,k, 272f,g,n, 278j, 273m
B-Asparagine	225c,d, 230f
C-Alanine	84c, 85f , also homologous position to S73 in *C. cassiicola*
C-Asparagine	86c,d, 75e
C-Glycine	90c, 79e
C-Histidine	145c, 134e,h, 146m
C-Histidine	152c, 151o
D-Asparagine acid	124e, 123h
D-Histidine	118c, 132f,m, 134e, 133h

Table 3.7-3: Frequency of *B. cinerea* strains with B-H272Y and B-P225L, without Chemical control and after 4 applications of two different SDHIs, in a strawberry trial with sampling after last application. Analysis of mutations in strains was done by pyrosequencing.

Mutation	Untreated	SDHI 1	SDHI 2
B-P225L	<2%	3%	56%
B-H272Y	<2%	92%	29%

Management strategies

The objective of anti-resistance management strategies is the reduction of selection pressure to avoid or delay the occurrence of resistance or to keep the frequency of resistant isolates in a population low. 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).

Limiting the number of sprays is also an important factor in delaying the build-up of resistant pathogen populations. The number of applications of boscalid containing fungicides will be restricted depending on the target pathogen.

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. Therefore, boscalid containing products should be applied in a preventive manner following the recommendations on the label. An optimal timing is also an effective resistance management (van den Berg *et al.* 2013).

BASF is a member of the FRAC SDHI Working Group and will promote effective anti-resistance management strategies. The current FRAC recommendations for resistance management of SDHI-fungicides are:

- Strategies for the management of SDHI fungicide resistance, in all crops are based on the statements listed below. These statements serve as a fundamental guide for the development of local resistance management programs.
- Resistance management strategies have been designed in order to be proactive and to prevent or delay the development of resistance to SDHI fungicides.
- A fundamental principle that must be adhered to when applying resistance management strategies for SDHI fungicides is that:
    - The SDHI fungicides (benodanil, bixafen, boscalid, carboxin, fenfuram, fluopyram, flutolanil, furametpyr, isopyrazam, mepronil, oxycarboxin, penthiopyrad, sedaxane, thifluzamide) are in the same cross-resistance group.
- Fungicide programs must deliver effective disease management. Apply SDHI fungicide based products at effective rates and intervals according to manufacturers' recommendations.
- Effective disease management is a critical component to delay the buildup of resistant pathogen populations.
- The number of applications of SDHI fungicide based products within a total disease management program must be limited.
- When mixtures are used for SDHI fungicide resistance management, applied as tank mix or as a co-formulated mixture, the mixture partner:
    - should provide satisfactory disease control when used alone on the target disease
    - must have a different mode of action
- SDHI fungicides should be used preventively or at the early stages of disease development.

For specific recommendation for the different target pathogens please refer to the FRAC web page (www.frac.info).

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

Exposure Controls / Personal Protection

Control parameters

Components with occupational exposure limits

No occupational exposure limits known.

Exposure controls

Personal protective equipment

Respiratory protection:
Respiratory protection not required.

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

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

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

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

Handling and Storage

Precautions for safe handling

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

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

Conditions for safe storage, including any incompatibilities

Segregate from foods and animal feeds.
Further information on storage conditions: Keep away from heat. Protect against moisture. Protect from direct sunlight.

Storage stability:
Storage duration: 24 Months

Protect from temperatures above: 40 °C
Changes in the properties of the product may occur if substance/product is stored above indicated temperature for extended periods of time.

First-Aid Measures

Description of first aid measures

Remove contaminated clothing.

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

On skin contact:
Wash thoroughly with soap and water.

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

On ingestion:
Rinse mouth and then drink plenty of water.

Most important symptoms and effects, both acute and delayed

Symptoms: No significant reaction of the human body to the product known.

Indication of any immediate medical attention and special treatment needed

Treatment: Symptomatic treatment (decontamination, vital functions).

Transport Information

Land transport

ADR

UN number:	UN3077
UN proper shipping name:	ENVIRONMENTALLY HAZARDOUS SUBSTANCE, SOLID, N.O.S. (contains BOSCALID 96%)
Transport hazard class(es):	9, EHSM
Packing group:	III
Environmental hazards:	yes
Special precautions for user:	Tunnel code: E

RID

UN number:	UN3077
UN proper shipping name:	ENVIRONMENTALLY HAZARDOUS SUBSTANCE, SOLID, N.O.S. (contains BOSCALID 96%)
Transport hazard class(es):	9, EHSM
Packing group:	III
Environmental hazards:	yes
Special precautions for user:	None known

Inland waterway transport

ADN

UN number :	UN3077
UN proper shipping name:	ENVIRONMENTALLY HAZARDOUS SUBSTANCE, SOLID, N.O.S. (contains BOSCALID 96%)
Transport hazard class(es):	9, EHSM
Packing group:	III
Environmental hazards:	yes
Special precautions for user:	None known
Transport in inland waterway vessel:	Not evaluated

Sea transport

IMDG

UN number:	UN 3077
UN proper shipping name:	ENVIRONMENTALLY HAZARDOUS SUBSTANCE, SOLID, N.O.S. (contains BOSCALID 96%)
Transport hazard class(es):	9, EHSM
Packing group:	III
Environmental hazards:	yes
Marine pollutant:	YES
Special precautions for user:	None known

Air transport

IATA/ICAO

UN number:	UN 3077
UN proper shipping name:	ENVIRONMENTALLY HAZARDOUS SUBSTANCE, SOLID, N.O.S., (contains BOSCALID 96%)
Transport hazard class(es):	9, EHSM
Packing group:	III
Environmental hazards:	yes
Special precautions for user:	None known

Transport in bulk according to Annex II of MARPOL73/78 and the IBC Code

Regulation:	Not evaluated
Shipment approved:	Not evaluated
Pollution name:	Not evaluated
Pollution category:	Not evaluated
Ship Type:	Not evaluated

Fire-Fighting Measures

Extinguishing media
Suitable extinguishing media: water spray, foam, dry powder
Unsuitable extinguishing media for safety reasons: carbon dioxide

Special hazards arising from the substance or mixture
The substances/groups of substances mentioned can be released in case of fire:
carbon monoxide, hydrogen chloride, carbon dioxide, nitrogen oxides, organochloric compounds

Advice for fire-fighters
Special protective equipment:
Wear self-contained breathing apparatus and chemical-protective clothing.

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

CA 3.9 Procedures for Destruction or Decontamination

Waste treatment methods

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

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

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

CA 3.10 Emergency Measures in Case of an Accident

Personal precautions, protective equipment and emergency procedures

Use personal protective clothing. Avoid contact with the skin, eyes and clothing. Avoid dust formation.

Environmental precautions

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

Methods and material for containment and cleaning up

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

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Boscalid

Document M-CA, Section4

ANALYTICAL METHODS

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Version history[1]

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

[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

Table of Contents

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

Report: CA 4.1.1/1
Anonymous, 2015a
Boscalid 673, CIPAC method
2015/1042146
Guidelines: none
GLP: no

Principle of the methods

CIPAC method 673 describes the determination of the content of the active substance boscalid (Reg. No. 300355) in technical grade active ingredient BAS 510 F (TGAI) using high performance liquid chromatography (HPLC) with external standardization and UV detection.

Method parameters (according to DocID 2015/1042146)

<table>
<tr><td>Column</td><td>stainless steel, 250 x 4.6 mm (i.d), packed with J'sphere ODS-H80, 4 µm, or equivalent material with the same selectivity</td></tr>
<tr><td>Eluent A</td><td>water / acetonitrile / ammonium acetate, 350 ml /650 ml / 770 mg</td></tr>
<tr><td>Eluent B</td><td>water / acetonitrile / ammonium acetate, 100 ml / 900 ml / 770 mg</td></tr>
<tr><td>Gradient</td><td>
<table>
<tr><th>time [min]</th><th>eluent A [mL]</th><th>eluent B [mL]</th><th>flow rate [mL/min]</th></tr>
<tr><td>0</td><td>100</td><td>0</td><td>1</td></tr>
<tr><td>14.0</td><td>100</td><td>0</td><td>1</td></tr>
<tr><td>14.5</td><td>0</td><td>100</td><td>1</td></tr>
<tr><td>15.0</td><td>0</td><td>100</td><td>2</td></tr>
<tr><td>22.0</td><td>0</td><td>100</td><td>2</td></tr>
<tr><td>22.5</td><td>0</td><td>100</td><td>1</td></tr>
<tr><td>23.0</td><td>100</td><td>0</td><td>1</td></tr>
<tr><td>26.0</td><td>100</td><td>0</td><td>1</td></tr>
</table>
</td></tr>
<tr><td>Column temperature</td><td>ambient (approx.. 23 °C)</td></tr>
<tr><td>Flow rate</td><td>1-2 mL/min (see gradient)</td></tr>
<tr><td>Detection</td><td>UV, 260 nm</td></tr>
<tr><td>Injection volume</td><td>5 µL</td></tr>
<tr><td>Analysis time</td><td>approx. 26 min</td></tr>
<tr><td>Retention time</td><td>Reg.No. 300355 – approx. 6.8 min</td></tr>
</table>

Identity
The identity of the active ingredient boscalid in technical grade technical grade active ingredient BAS 510 F is confirmed by reverse phase high performance liquid chromatography (HPLC) and infrared spectroscopy (IR). The identity was confirmed by coincidence of retention times in the test item and the authentic reference standard and by comparison of the IR spectra, respectively.

Validation
The method was peer-reviewed in a CIPAC ring test with the following results:

Repeatability r = 15 to 21 g/kg at 981 g/kg active ingredient content
Reproducibility R = 17 to 22 g/kg at 981 g/kg active ingredient content

Conclusion
The CIPAC method 673 is suitable for the determination of boscalid (Reg. No. 300355) in technical grade active ingredient BAS 510 F (TGAI). According to SANCO/3030/99 Rev. 4 CIPAC methods are deemed to be validated.

(b) Determination of significant and relevant impurities and additives (such as stabilisers) in the active substance as manufactured
Technical grade boscalid does not contain impurities of toxicological, eco-toxicological or environmental concern.

Information on significant impurities and additives is provided in document JCA (CONFIDENTIAL information).

CA 4.1.2 Methods for risk assessment

In case the order of the study summaries is differing compared to the information given in the application submitted for renewal of approval, then this is pointed out by an additional comments addressing this topic at the respective section of the dossier. If amendments to studies are issued which provide additional information to the original study report, then they are not listed in individual summaries, but the information from the study and its amendment are summarised and presented in one study summary. Whenever this is the case, an appropriate comment is made.

Only new, not already peer-reviewed, studies are summarised and presented in chapters M-CA 4.1.2 and 4.2. However, an overview of already peer-reviewed studies is provided for each relevant matrix for the reviewer's convenience.

An overview of the changes in order made to chapter 4.1.2 compared to the Application is given in the table below for the reviewer's convenience:

Table 4.1.2-1: Overview of changes of documents submitted compared to originally listed documents in the Application

Data point in Application	Data point in current dossier	DocID	Changes to Application	Reason for change
4.1.2/1	4.1.2/1	2008/1084832	no change	n.a.
n.a.	4.1.2/2	2015/1174527	not listed in Application	Amendment to M-CA 4.1.2/1 (2008/1084832): 2nd mass transition reported; summarised together with the information form 4.2.12/1 (2008/1084832)
4.1.2/2	4.1.2/3	2010/1046613	Renumbering of M-CA point	Renumbering of order of studies as additional study included
4.1.2/3	4.1.2/4	2013/1415720	Renumbering of M-CA point	Renumbering of order of studies as additional study included
4.1.2/4	4.1.2/5	2015/1109589	Renumbering of M-CA point	Renumbering of order of studies as additional study included
n.a.	4.1.2/6	2001/1000114	not listed in Application	Summary of the methods used for analysis of samples in toxicological studies which are used under DocID of toxicological study or risk assessment (end points)
n.a.	4.1.2/7	2001/1000115	not listed in Application	Summary of the methods used for analysis of samples in toxicological studies which are used under DocID of toxicological study or risk assessment (end points)
n.a.	4.1.2/8	2000/1016881	not listed in Application	Summary of the methods used for analysis of samples in toxicological studies which are used under DocID of toxicological study or risk assessment (end points)

Data point in Application	Data point in current dossier	DocID	Changes to Application	Reason for change
n.a.	4.1.2/9	2015/1204964	not listed in Application	Summary of the methods used for analysis of samples in toxicological studies which are used under DocID of toxicological study or risk assessment (end points)
4.1.2/5	4.1.2/10	2006/1039427	Renumbering of M-CA point	Renumbering of order of studies as additional study included
4.1.2/6	4.1.2/11	2015/1091103	Renumbering of M-CA point	Renumbering of order of studies as additional study included
n.a.	4.1.2/12	2001/5001020	not listed in Application	Data generation method in support of field trials
4.1.2/7	4.1.2/13	2000/1017223	Renumbering of M-CA point	Renumbering of order of studies as additional study included
n.a.	4.1.2/14	2003/1021922	not listed in Application	Amendment to already peer-reviewed study 2000/1017223: Statement on stability of reference solutions added
n.a.	4.1.2/15	2015/1174463	not listed in Application	Amendment to 4.1.2/10, reporting the 2nd mass transition and addressing matrix effects
n.a.	4.1.2/16	1999/10170	not listed in Application	Data generation method used in support of ecotoxicological studies
n.a.	4.1.2/17	1998/10700	not listed in Application	Data generation method used in support of ecotoxicological studies
n.a.	4.1.2/18	2001/500881	not listed in Application	Data generation method used in support of ecotoxicological studies
n.a.	4.1.2/19	2009/1132364	not listed in Application	Additional information form literature search: Possibility of multimethod approach for analysis of bee relevant matrices.

An overview of the metabolites of relevance for the analytical methods is given together with other structures of relevance in Document N. The respective table also contains detailed information on different metabolite codes used due to historic reasons.

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

The following analytes are included in the residue analytical methods in support of pre-registration data-requirements. The compounds are also summarized in Document N 1, chapter 8.5, and discussed in M-CA 7.4.

Soil: BAS 510 F (Boscalid; Reg. No. 300355); 2-chloro-N-(4'-chlorobiphenyl-2-yl) nicotinamide; M510F47 (Reg. No. 107371; 2-chloronicotinic acid); M510F49 (Reg. No. 391572; N-(4'-chlorobiphenyl-2-yl)-2-hydroxynicotinamide)

Sediment: As no aquatic field studies were conducted, no separate stand-alone method validation for the determination of unlabeled boscalid or any of its metabolites was required. Due to similarity to soil matrix, determination of such, if required, any analysis required can be accomplished by applying the fully validated soil method.

Water: BAS 510 F (Boscalid; Reg. No. 300355); 2-chloro-N-(4'-chlorobiphenyl-2-yl) nicotinamide; M510F47 (Reg. No. 107371; 2-chloronicotinic acid); M510F49 (Reg. No. 391572; N-(4'-chlorobiphenyl-2-yl)-2-hydroxynicotinamide)

Air: BAS 510 F (Boscalid; Reg. No. 300355); 2-chloro-N-(4'-chlorobiphenyl-2-yl) nicotinamide

A brief summary of the methods can be found in the respective section of Doc N 1, chapter 5.1.3.

Soil

Analytical methods for the determination of Boscalid residues in soil were evaluated in the context of the inclusion in Annex I of Directive 91/414/EEC. Methods evaluated are summarized in Table 4.1.2-2 for the reviewer's convenience. The respective studies are listed as fully peer-reviewed if they were part of the Monograph (2002) or its Addendum (2006).

Studies submitted during the evaluation process and were not cited in the Monograph are also listed in the table below, but not considered as EU-peer reviewed.

In the following two tables an overview of already peer-reviewed as well as the new methods submitted for review are summarised for the reviewer's convenience. These overviews do not differentiate between data-generation methods for pre-registration purposes and monitoring methods for enforcement purposes (post-registration); hence a similar comparison will not be listed again in chapter M-CA 4.2.

Table 4.1.2-2: Overview of *already peer-reviewed* residue analytical methods for determination of boscalid residues in soil

Method. No.	DocID	Matrix	Method principle	Target analytes	LOQ [mg/kg]	year	EU reviewed
408/1	1998/11314	Soil, sediment	GC-MS	Boscalid	0.01	1998	yes (2002)
408/1	2003/1000977	Soil, sediment	GC-MS	Boscalid	0.01	2003	yes (Addendum 2006)

Table 4.1.2-3: Summary *of newly submitted* residue analytical methods for determination of boscalid residues in soil

Method. No.	DocID	Matrix	Method principle	Target analytes	LOQ [mg/kg]	year	EU reviewed
L0096/01	2008/1084832	soil	LC-MS/MS	BAS 510 F	0.01	2008	new soil method
L0096/01	2015/1174527	soil	LC-MS/MS	BAS 510 F	0.01	2015	Amendment adding information on 2nd mass transition and matrix effects
./.	2010/1046663	buffer solution	LC-MS/MS	BAS 510 F	n.a.	2010	stability of analyte in buffer solutions
./.	2013/1415720	soil	LC-MS/MS	M510F47, M510F49	0.01	2013	soil method for metabolites

A new data generation method has been developed for the determination of Boscalid in soil using highly selective and specific HPLC-MS/MS detection allowing the direct determination of Boscalid in the soil extracts without any further sample clean-up. The method described in M-CA 4.1.2/1 and 4.1.2/2 is the proposed current data generation method. It is also fully suitable as monitoring method for enforcement purposes. Stability in standard solutions was also addressed and is summarised separately.

Furthermore, an additional method for the determination of two metabolites of Boscalid, M510F47 and M510F49, was developed. This method was not used for data generation purposes, but is considered as additional information. If required, this method is fully suitable to be used as monitoring method for enforcement purposes. For completeness, this method is summarised and presented in chapter M-CA 4.1.2.

Extraction efficiency was assessed by comparison of amounts of extractable residue applying the proposed data generation method with the amount of residues extracted from soil matrix applying the various extraction schemes of the aerobic and anaerobic soil metabolism studies.

Report:	CA 4.1.2/1 Obermann M., 2009a Validation of analytical method L0096/01: Determination of Boscalid Reg.No. 300355 in soil using HPLC/MS-MS 2008/1084832
Guidelines:	EEC 91/414 Annex IIA, EEC 91/414 Annex IIIA, 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)

AND

Report:	CA 4.1.2/2 Obermann M., Studenroth S., 2015a Report Amendment No. 1 to Final Report "Validation of analytical method L0096/01: Determination of Boscalid Reg.No. 300355 in soil using HPLC/MS-MS" 2015/1174527
Guidelines:	EEC 91/414 Annex IIA, EEC 91/414 Annex IIIA, 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)

Remark: The study information of 2008/1084832 and 2015/1174527 is summarised and presented together in one study summary. The amendment issued (2015/1174527) provides additional information on the second mass transition of the LC-MS/MS method applied, as well as information on the assessment of matrix effects, which were not included in the originally issued final report.

Principle of the method

Analytical method L0096/01 is developed for the determination of boscalid (BAS 510 F) in soil by HPLC-MS/MS with a limit of quantification of 0.01 mg kg^{-1}.

Boscalid is extracted from 5 g soil samples with 50 mL of methanol/aqueous acetate buffer (80/20, v/v) by shaking at 225 rpm for about 60 min. Approximately 5 mL of the suspension is centrifuged for 5 min at 4000 rpm and 20°C.

Final determination is performed by HPLC-MS/MS using a Betasil C_{18} analytical column and a gradient mixture of water/formic acid (1000/1) and methanol/formic acid (1000/1) at a flow rate of 600 µL min^{-1}. Detection is accomplished using the protonated molecular ion at mass transitions 343→271 and 343→307 for quantification and confirmation.

Recovery findings

The results show that the method is suitable to determine residues of boscalid in soil. Samples spiked with the analyte at the limit of quantification of 0.01 mg kg^{-1} and ten times higher (0.10 mg kg^{-1}) have average recovery values (mean of five/seven replicates per fortification level and analyte) between 70% and 110%. The detailed results are given in the table below (Table 4.1.2-4).

Table 4.1.2-4: Results of the method validation for the determination of boscalid in soil (LUFA 2.2 and LUFA 2.3)

Analyte	Soil	m/z	Fortification level [mg kg^{-1}]	Number of Replicates	Mean recovery [%]	RSD [%]	Overall Recovery [%]	RSD [%]
boscalid	LUFA 2.2	343->271	0.01	7	94.6	2.2	94.2	2.0
			0.1	5	93.7	1.9		
		343->307	0.01	7	94.1	1.6	93.2	2.0
			0.1	5	92.1	1.8		
	LUFA 2.3	343->271	0.01	7	97.1	4.1	95.4	3.9
			0.1	5	93.1	1.6		
		343->307	0.01	7	97.0	2.5	95.2	3.2
			0.1	5	92.8	2.3		

RSD = Relative standard deviation

Linearity

Good linearity ($r > 0.999$) was observed in the concentration range of 0.25 ng mL^{-1} to 5 ng mL^{-1} for boscalid. At least five calibration standards prepared in methanol/acetate buffer solution (80/20, v/v) were used.

Specificity

Under the described conditions the method is specific for the determination of boscalid in soil. Significant interferences (> 30% of LOQ) were not observed at the retention time of boscalid in the untreated soil control samples.

Due to the high selectivity and specificity of HPLC-MS/MS an additional confirmatory technique was not necessary. Two mass transitions of boscalid were quantified.

Matrix Effects

Matrix effects were tested by comparing solvent-based standard solutions (prepared in methanol/acetate buffer solution, 80/20, v/v) with matrix-matched standards (quality control samples) at a concentration of 0.5 ng mL^{-1}. The findings showed that the matrix load in the tested quality control samples had negligible influence on the analysis of boscalid, therefore, the use of matrix-matched standards is not needed.

Limit of Quantification

The method has a limit of quantification (LOQ) of 0.01 mg kg^{-1}, corresponding to the lowest fortification level.

Limit of Detection The limit of detection (LOD) is 0.25 ng mL^{-1}, which is the lowest calibration standard.

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

Standard Stability The standard stability of boscalid in methanol/acetate buffer solution (80/20, v/v) at concentration levels of 0.1 µg mL^{-1} and 1.0 ng mL^{-1} was determined within BASF DocID 2010/1046613 [*see CA 4.1.2/3*]. The standard solutions were stable over a time period of at least 30 days when stored under refrigerated conditions at 4°C in the dark.

Extract Stability The extract stability was not determined within the validation study, but it was assessed in CA 4.1.2/5 (DocID 2015/1109589). Soil extracts are stable samples over a time period of 6 weeks, when stored refrigerated at 4°C in methanol/acetate buffer pH 4.65 (80/20, v/v).

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

Conclusion **The method L0096/01 for analysis of boscalid in soil used HPLC-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, limit of quantification and recoveries and is therefore applicable to correctly determine boscalid in soils.

Report:	CA 4.1.2/3 Penning H., 2010a Standard stability of BAS 510 F in methanol / acetate buffer solution 2010/1046613
Guidelines:	OECD Principles of Good Laboratory Practice; GLP Principles of the German „Chemikaliengesetz“ (Chemicals Acts)
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

To comply with the requirements of the current test guidelines, stability in standard solutions was additionally assessed and reported in a separate study report. The findings are summarised below.

Executive Summary

The stability of boscalid (BAS 510 F) standard solutions in methanol / acetate buffer solution (80/20, v/v; pH 4.65) was tested.
Standard solutions were stored at 4°C in the dark and analyzed at day 0 and after 7, 14, and 30 days against a calibration curve of freshly prepared standards.
Stability of BAS 510 F at 0.1 µg mL^{-1} and 1.0 ng mL^{-1} in methanol / acetate buffer solution (80/20, v/v; pH 4.65) is given at 4°C in the dark for at least 30 days.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

Internal code:	BAS 510 F
Common name:	boscalid
Reg. No.:	300355
Chemical name (IUPAC):	2-chloro-N-(4’-chloro-biphenyl-2-yl)nicotinamide
Molar mass:	343.2 g mol^{-1}
Batch No.:	01 893-55
Chemical Purity:	100.0%

B. STUDY DESIGN

1. Experimental Conditions

Standard solutions were prepared according to BASF method L0096/01 [*BASF DocID 2008/1084832, CA 4.1.2/1 and BASF DocID 2015/1174527, CA 4.1.2/2*]. Briefly, a stock solution of 1 mg L^{-1} boscalid in methanol was prepared. The stock solution was then further diluted with methanol / acetate buffer solution (80/20, v/v; pH 4.65). The stability of boscalid was tested in standard solutions of 0.1 µg mL^{-1} and 1.0 ng mL^{-1}.

Standard solutions were stored at 4°C in the dark and measured at day 0 and after 7, 14 and 30 days against a calibration curve of freshly prepared standards.

2. Description of analytical procedures

The standard solutions in methanol / acetate buffer solution (80/20, v/v; pH 4.65) were analyzed for boscalid with BASF method L0096/01, which was previously validated [*BASF DocID 2008/1084832, CA 4.1.2/1 and BASF DocID 2015/1174527, CA 4.1.2/2*].

Principle of the method:

Boscalid is extracted from soil samples by methanol / aqueous acetate buffer solution. Final determination of boscalid is performed by HPLC-MS/MS monitoring two parent-daughter ion transitions for quantification and confirmation.

Within this standard stability study, no extraction was necessary, standard solutions were only diluted and analyzed by HPLC-MS/MS.

II. RESULTS AND DISCUSSION

Stability of BAS 510 F standard solutions in methanol / acetate buffer solution (pH 4.65) (80/20, v/v) was tested at 0.1 µg mL^{-1} and 1.0 ng mL^{-1}at 4°C in the dark for up to 30 days. Concentrations of boscalid at the tested time intervals are given in Table 4.1.2-5 and Table 4.1.2-6 for the solutions of 0.1 µg mL^{-1} and 1.0 ng mL^{-1}, respectively. Quantification was done for both mass transitions of boscalid validated in BASF method L0096/01.

Recoveries at both concentration levels at all time points ranged between 93% and 102% of applied. For evaluation of the stability test of boscalid, an analysis was performed assuming first order degradation kinetics (details given in study report). Degradation was found to be 2.5% and -5% of applied after 30 days for 0.1 µg mL^{-1} and 1.0 ng mL^{-1}, respectively. This is within the analytical uncertainty of the analytical method and cannot be assigned to degradation.

Table 4.1.2-5: Standard Stability of boscalid in methanol/ acetate buffer (80/20, v/v; pH 4.65) at 0.1 µg mL^{-1} after storage at 4°C in the dark

Days of storage	Transition *m/z* 343 → 307		Transition *m/z* 343 → 271	
	[µg mL^{-1}]	Recovery of 0.1 µg mL [%]	[µg mL^{-1}]	Recovery of 0.1 µg mL^{-1}
0	0.104		0.099	
	0.099		0.088	
	0.102	**101.7**	**0.093**	**93.4**
7	0.095		0.103	
	0.097		0.097	
	0.096	**96.2**	**0.100**	**100.2**
14	0.095		0.094	
	0.095		0.094	
	0.095	**95.0**	**0.094**	**93.8**
30	0.099		0.098	
	0.097		0.095	
	0.098	**97.9**	**0.097**	**96.7**

Table 4.1.2-6: Standard Stability of boscalid in methanol/ acetate buffer (80/20, v/v; pH 4.65) at 1.0 ng mL^{-1} after storage at 4°C in the dark

Days of storage	Transition *m/z* 343 → 307		Transition *m/z* 343 → 271	
	[ng mL^{-1}]	Recovery of 1.0 ng mL^{-1} [%]	[ng mL^{-1}]	Recovery of 1.0 ng mL^{-1}
0	0.884		0.984	
	0.978		1.040	
	0.931	**93.1**	**1.012**	**101.2**
7	1.010		1.000	
	0.873		0.995	
	0.942	**94.2**	**0.998**	**99.8**
14	0.941		0.935	
	0.928		0.914	
	0.935	**93.5**	**0.925**	**92.5**
30	0.992		1.000	
	0.966		0.942	
	0.979	**97.9**	**0.971**	**97.1**

III. CONCLUSION

Stability of boscalid at 0.1 µg mL^{-1} and 1.0 ng mL^{-1} in methanol / acetate buffer solution (80/20, v/v; pH 4.65) is given at 4°C in the dark for at least 30 days.

Report:	CA 4.1.2/4 Kreidler D., 2013a Validation of analytical method for determination of residues of M510F47 and M510F49 in soil 2013/1415720
Guidelines:	SANCO/825/00 rev. 8.1 (16 November 2010)
GLP:	yes (certified by Landesanstalt fuer Umwelt, Messungen und Naturschutz Baden-Wuerttemberg, Karlsruhe, Germany)

Remark: This method was not used for data generation purposes, but is considered as additional information of the analytical methodology. If required, this method is fully suitable to be used as monitoring method for enforcement purposes. For completeness, this method is summarised and presented in chapter M-CA 4.1.2. At time of development and validation, the residue method has not been allocated a BASF method number. As unique identifier, the method was allocated the BASF method number *L0096/02*.

Principle of the method

An analytical method was developed for the determination of boscalid (BAS 510 F) metabolites M510F47 (Reg. No. 107371) and M510F49 (Reg. No. 391572) in soil by LC-MS/MS.
10 g soil samples are extracted with methanol by shaking for 30 min on a horizontal shaker, followed by centrifugation for 5 min at 3000 rpm. After decantation of the supernatant into a volumetric flask, the extraction is repeated once with methanol/water (1/1, v/v) and the extracts are combined. 5 mL of the combined extract are diluted with 3 mL water including 0.1% formic acid and 4 mM ammonium formate, sonicated, and shaken for homogenization. An aliquot thereof is filtered with a single-use syringe and analyzed by LC-MS/MS using a Phenomenex Prodigy ODS3 column (with guard column) and a gradient mixture of water (0.1% formic acid), 10 mM ammonium formate and methanol (0.1% formic acid). Detection is accomplished in ESI negative mode at mass transitions 156→112 (M510F47) and 323→94 (M510F49) for quantification and 155→112 (M510F47) and 323→202 (M510F49) for confirmation.

Recovery findings

The results show that the method is suitable to determine residues of boscalid metabolites M510F47 and M510F49 in soil. Samples spiked with the analytes at the limit of quantification of 0.01 mg kg^{-1} and ten times higher (0.10 mg kg^{-1}) have average recovery values (mean of five replicates per fortification level and analyte) between 70% and 110%. The detailed results are given in the table below (Table 4.1.2-7).

Table 4.1.2-7: Results of the method validation for the determination of boscalid metabolites M510F47 and M510F49 in soil LUFA 2.2

Soil	Analyte	m/z	Fortification level [mg kg^{-1}]	Number of Replicates	Mean Recovery [%]	RSD [%]	Overall Recovery [%]	RSD [%]
LUFA 2.2	M510F47	156 → 112	0.01	5	85	2	85	3
			0.10	5	84	4		
		155 → 112	0.01	5	83	6	85	5
			0.10	5	86	3		
	M510F49	323 → 94	0.01	5	88	7	87	6
			0.10	5	85	5		
		323 → 202	0.01	5	86	6	87	5
			0.10	5	88	4		

RSD = Relative standard deviation

Linearity

The detector response was linear within the range from 0.5 to 100 ng mL^{-1} with $r \geq 0.999$ for M510F47 and second order within the range from 0.5 to 100 ng mL^{-1} with $r \geq 0.999$ for M510F49. At least eight calibration levels were used for each metabolite.

Specificity

LC-MS/MS is a highly specific self-confirmatory technique. Under the described conditions the method is specific for the determination of the two metabolites in soil. Significant interferences (> 30% of LOQ) were not observed at the retention times and mass transitions of the analytes.

Due to the high selectivity and specificity of LC-MS/MS an additional confirmatory technique was not necessary.

Matrix Effects

Matrix effects were tested preparing matrix-matched standards for each matrix and analyte. No significant matrix effects occurred. Therefore, the calibration standards were prepared in water including 0.1% formic acid and 4 mM ammonium formate/methanol (40/60; v/v).

Limit of Quantification

The method has a limit of quantification (LOQ) of 0.01 mg kg^{-1} per analyte, corresponding to the lowest fortification level.

Limit of Detection

The limit of detection (LOD) was defined as 30% of the LOQ with 0.003 mg kg^{-1}.

Repeatability

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

Standard Stability

The stability of standard solutions was not determined within the validation study as standards solutions and extracts contained the same solvent, except that no matrix was present. Hence, stability was confirmed to be at least as long as extract stability (7 days).

Extract Stability	The test items are stable in fortified combined methanol/water extracts of matrix soils for at least 7 days when stored refrigerated (nominally 1 – 10°C) in the dark.
Reproducibility	Reproducibility of the method was not determined within this validation study as not required.
Conclusion	**The method for analysis of boscalid metabolites M510F47 (Reg. No. 107371) and M510F49 (Reg. No. 391572) 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, limit of quantification and recoveries and is therefore applicable to correctly determine residues of boscalid metabolites M510F47 and M510F49 in soils.**

Report: CA 4.1.2/5
Ertunc T., Studenroth S., 2015
Comparative Analysis of Extraction Procedures on Boscalid (BAS 510 F) originating from a Field Accumulation and Dissipation study in Northern Italy
2015/1109589

Guidelines: OECD Principles of Good Laboratory Practice; GLP Principles of the German „Chemikaliengesetz“ (Chemicals Acts); SANCO/3029/99 rev. 4 (11/07/00)

GLP: yes
(certified by Landesamt für Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Rheinland-Pfalz)

Principle of the methods

The extraction efficiency of residue analytical method L0096/01 using methanol/acetate buffer pH 4.65 (80/20, v/v) as extraction solvent was compared with the extraction procedures of two aerobic and one anaerobic soil metabolism studies applying sequential extraction schemes using methanol and a mixture of methanol/pure water (50/50, v/v) at different ratios of soil to extraction solvent. Selected field soil samples generated during a field accumulation and dissipation study [*BASF DocID 2015/1178191 CA 7.1.2.2.2*] performed with boscalid (BAS 510 F) were extracted with each of the four extraction procedures. The analysis of boscalid in the different soil extracts obtained by applying the four different extraction procedures was accomplished by LC-MS/MS, based on the principles described in residue analytical method L0096/01. The results of residue analytical method L0096/01 (set to 100% reference) were compared to the extraction procedures used in the aerobic and anaerobic soil metabolism studies.

Extraction Procedure 1: Extraction Procedure of Residue Analytical Method L0096/01 [*BASF DocID 2008/1084832, CA 4.1.2/1 and BASF DocID 2015/1174527, CA 4.1.2/2*]:

5 g of a wet soil sample were extracted with 50 mL of a mixture of methanol and acetate buffer pH 4.65 (80/20, v/v). The sample was shaken for 60 min at 225 rpm. Phase separation was accomplished by centrifugation for 5 min at 4000 rpm and 20°C. A final volume of 50 mL of the decanted supernatant, which corresponds to soil control matrix in methanol/acetate buffer pH 4.65 (80/20, v/v) with boscalid, was adequate for residue levels between 0.01 mg kg^{-1} (limit of quantification; LOQ) to 0.05 mg kg^{-1} (5xLOQ). Soil extracts were diluted with appropriate amounts of methanol/acetate buffer pH 4.65 (80/20, v/v) at higher residue levels.

Extraction Procedure 2: based on Aerobic Soil Metabolism Study [*BASF DocID 1999/11807*]:

5 g of a wet soil sample were sequentially extracted 3 times with 9 mL pure methanol, followed by 3 times 9 mL methanol/pure water (50/50, v/v). The sample was shaken for 30 min at 225 rpm. After each extraction, the samples were centrifuged; the methanol extracts were centrifuged for 5 min at 3000 rpm and 20°C and methanol/pure water extracts were centrifuged for 10 min at 3000 rpm and 20°C. All individual extracts were combined in a 100 mL flask by filtration over cotton wool and filled up to the mark with methanol/pure water (75/25, v/v). Prior to quantitative determination by LC-MS/MS, an aliquot of 5 mL was taken and centrifuged for 5 min at 4000 rpm and 20°C. Further dilutions were made as appropriate.

Extraction Procedure 3: based on Anaerobic Soil Metabolism Study [*BASF DocID 2000/1014990*]:

10 g of a wet soil sample was suspended with 5 mL pure H_2O, then 6 mL pure methanol were added. The samples were shaken for 40 min at 225 rpm. Phase separation was accomplished by centrifugation for 10 min at 3000 rpm and 20°C. Then, the soil remnant was further sequentially extracted twice with 10 mL pure methanol and then 3 times with 10 mL of a mixture of methanol/pure water (50/50, v/v). Phase separation was accomplished as described above. All individual extracts were combined in a 100 mL flask by filtration over cotton wool and filled up to the mark with methanol/pure water (75/25, v/v). Prior to quantitative determination by LC-MS/MS, an aliquot of 5 mL was taken and centrifuged for 5 min at 4000 rpm and 20°C. Further dilutions were made as appropriate.

Extraction Procedure 4: based on Aerobic Soil Metabolism Study [*BASF DocID 2002/5002772, CA 7.1.1.1*]:

5 g of a wet soil sample was sequentially extracted 3 times with 12.5 mL pure methanol, followed by 3 times 12.5 mL methanol/pure water (50/50 v/v). Extraction was accomplished by shaking for 45 min at 225 rpm. After each extraction, the samples were centrifuged; the methanol extracts were centrifuged for 5 min at 3000 rpm and 20°C and the methanol/pure water (50/50, v/v) extracts were centrifuged for 10 min at 3000 rpm and 20°C. All individual extracts were combined in a 100 mL flask by filtration over cotton wool and filled up to the mark with methanol/pure water (75/25, v/v). Prior to quantitative determination by LC-MS/MS, an aliquot of 5 mL was taken and centrifuged for 5 min at 4000 rpm and 20°C. Further dilutions were made as appropriate.

Limit of Quantification The validated LOQ of the residue analytical method L0096/01 is 0.01 mg kg^{-1}.

Extractability The results of the residue analytical method L0096/01 (extraction procedure 1 – set as 100% reference) showed good comparability with the extraction efficiencies of the aerobic and anaerobic soil metabolism studies (extraction procedures 2, 3, and 4). The results ranged from 95.2% to 100.8% for field sample L1503380002 and from 101.5% to 103.2% for field sample L1503380003. A summary of the residue results of the extraction procedures is given in Table 4.1.2-8.

Table 4.1.2-8: Summary of residue results of boscalid

Sample No.	Extraction procedure	boscalid [mg kg^{-1}]	% of residue analytical method L0096/01
L1503380002	1	2.00	
	2	2.01	100.8
	3	1.90	95.2
	4	1.97	98.8
L1503380003	1	1.58	
	2	1.63	103.2
	3	1.62	102.3
	4	1.61	101.5

Extract Stability The stability of boscalid in field soil extracts, prepared according to residue analytical method L0096/01, was investigated. Boscalid was stable in the final extracts of fortified and treated field soil samples over a time period of 6 weeks, when stored refrigerated at 4°C in methanol/acetate buffer pH 4.65 (80/20, v/v). Extract stability was conducted to obtain additional information for CA 4.1.2/1 and 4.1.2/2 (method validation for the determination of boscalid in soil).

Matrix Effects Matrix effects were assessed by comparison of solvent standards prepared in methanol/acetate buffer pH 4.65 (80/20, v/v) against standards prepared in soil control matrix according to residue analytical method L0096/01. The matrix load in the tested soil samples had negligible influence on the analysis of boscalid; therefore, no matrix matched standards are needed.

Conclusion **The results of the reference residue analytical method L0096/01 (set as 100% reference) showed good comparability with the extraction efficiencies of the aerobic and anaerobic soil metabolism studies. Overall, the results for boscalid ranged from 95.2% to 103.2%, hence the proposed residue analytical method removes incurred residue to a satisfactory high extent.**

Water

Analytical methods for the determination of Boscalid residues in water were evaluated in the context of the inclusion in Annex I of Directive 91/414/EEC. Methods evaluated are summarized in Table 4.1.2-9 for the reviewer's convenience. The respective studies are listed as fully peer-reviewed if they were part of the Monograph (DAR, 2002) or its Addendum (2006).

Studies submitted during the evaluation process and were not cited in the draft risk assessment report are also listed in the table below, but not considered as EU-peer reviewed.

In the following two tables below, an overview of already peer-reviewed methods versa newly submitted ones are summarised for the reviewer's convenience.

In the following two tables an overview of already peer-reviewed as well as the new methods submitted for review are summarised for the reviewer's convenience. These overviews do not differentiate between data-generation methods for pre-registration purposes and monitoring methods for enforcement purposes; hence a similar comparison will not be listed again in chapter M-CA 4.2.

Table 4.1.2-9: Summary of *already peer-reviewed* residue analytical methods for determination of boscalid residues in water

Method. No.	DocID	Matrix	Method principle	Target analytes	LOQ [µg/L]	year	EU reviewed
411	1998/10922	Water	GC-MS	Boscalid	0.05	2001	yes (2002)
411	2003/1000976	Water	GC-MS	Boscalid	0.05	2003	yes (Addendum2006)
411/0	2001/1008955	Water	GC-MS	Boscalid	0.05	1998	yes (2002)
411/0	2003/1000975	Water	GC-MS	Boscalid	0.05	2003	yes (Addendum2006)

Table 4.1.2-10: Summary of *newly submitted* residue analytical methods for determination of boscalid residues in water

Method. No.	DocID	Matrix	Method principle	Target analytes	LOQ [µg/L]	year	EU reviewed
L0127/01	2008/1086809	water	LC-MS/MS	BAS 510F	0.03	2008	no; new method
L0127/01	2015/1174526	water	LC-MS/MS	BAS 510 F	0.03	2015	Amendment adding information on 2nd mass transition and matrix effects
L0127/02	2015/1109588	water	LC-MS/MS	M510F47, M510F49	0.03	2015	Method for metabolites
L0127/01 and L0127/02	2015/1114688	water	LC-MS/MS	BAS 510 F, M510F47, M510F49	0.03	2015	ILV for methods L0127/01 and L0127/02

No data generation for water were required as no aquatic field dissipation study or in support of any other environmental fate study was conducted. However, the monitoring method summarised and submitted in chapter M-CA 4.2 can also be used for the purpose of pre-registration data generation and is the proposed data generation method. Details of the methods can be found in chapter M-CA 4.2.

Air

Analytical methods for the determination of Boscalid residues in air were evaluated in the context of the inclusion in Annex I of Directive 91/414/EEC. Methods evaluated are summarized in below for the reviewer's convenience. The respective studies are listed as fully peer-reviewed if they were part of the Draft Assessment Report (DAR, 2002) or its Addendum (2006).

Studies submitted during the evaluation process and were not cited in the draft risk assessment report are also listed in the table below, but not considered as EU-peer reviewed.

In the following two tables an overview of already peer-reviewed as well as the new methods submitted for review are summarised for the reviewer's convenience. These overviews do not differentiate between data-generation methods for pre-registration purposes and monitoring methods for enforcement purposes; hence a similar comparison will not be listed again in chapter M-CA 4.2.

Table 4.1.2-11: Summary of *already peer-reviewed* residue analytical methods for determination of boscalid residues in air

Method. No.	DocID	Matrix	Method principle	Target analytes	LOQ [µg/m^3]	year	EU reviewed
460	2000/1014992	Air	GC-MS	BAS 510 F	1.5	2000	yes (2002)

No new methods for air were required for risk assessment in support of environmental fate studies. A short summary of the already peer-reviewed validated analytical method for post-approval and monitoring purposes is described in chapter M-CA 4.2.

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

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

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

Analytical methods used in support of dietary and gavage studies (non-radiolabeled) have been established and validated in separate study reports. The relevant methods are presented in this section. The methods are presented in detail, although no separate validation report has been issued. The summary is presented under the unique identifier number of the toxicological study.

General remark: Both methods described below in detail (M-CA 4.1.2/3 and 4) use HPLC coupled with UV-detection for determination and quantification of the analyte of interest in the dietary feeding and/ or gavage capsules. As it was shown that the methods used are specific for the analyte (Boscalid) and as the source of the analyte is known, an additional confirmatory technique is not required. This is different to the other data-generation methods (used for analysis of residues) presented in this chapter M-CA 4.1.2, where the source of the analyte could potentially be unknown, such as in soil matrix analysed in field studies.

Report:	CA 4.1.2/6 [redacted] 2001a BAS 510 F – Chronic Toxicity Study in Wistar rates; Administration in the diet for 24 months 2001/1000114
Guidelines:	EEC Commission Directive 87/302; OECD Guideline No. 452, U.S. EPA $ 83-1, Japan/JMAFF
GLP:	yes (certified by Landesanstalt fuer Pflanzenschutz Rheinland-Pfalz, Mainz, Germany)

AND

Report:	CA 4.1.2/7 [redacted] 2001b BAS 510 F – Carcinogenicity Study in Wistar rates; Administration in the diet for 24 months 2001/1000115
Guidelines:	EEC Commission Directive 87/302; OECD Guideline No. 452, U.S. EPA $ 83-1, Japan/JMAFF
GLP:	yes (certified by Landesanstalt fuer Pflanzenschutz Rheinland-Pfalz, Mainz, Germany)

Remark: For both studies [*BASF DocID 2001/1000114 and 2001/1000115*] the same analytical procedure was used. An overview of the analytical procedure given below is done under the unique document identifier of the original toxicological study.

Principle of the method

Analysis of Stability: Feed samples were extracted with a mixture of acetonitrile/bidistilled water/0.5M sulfuric acid (800/200/5, v/v/v). Then, the extracts were analyzed by HPLC with UV-detection at 210 nm. The chromatography was performed on a Polygosil 60 5 C18 column with a flow rate of 1.2 mL min^{-1} of 60% acetonitrile + 0.5M sulfuric acid and 40% bidistilled water + 0.5M sulfuric acid.

Homogeneity and Concentration Control Analysis: Feed Samples (carrier: feed "Kliba 343 Mehl") were pre-incubated with bidistilled water and extracted with acetonitrile. Then, the extracts were analyzed by HPLC with UV-detection at 274 nm. The chromatography was performed on a Polygosil 60 5 C18 column with a flow rate of 1.2 mL min^{-1} of 60% acetonitrile + 0.5M sulfuric acid and 40% bidistilled water + 0.5M sulfuric acid.

Recovery findings

The described method is suitable to determine residues of boscalid in feed. Samples were spiked with boscalid with 100 mg kg^{-1}, 500 mg kg^{-1}, 2500 mg kg^{-1} and 15000 mg kg^{-1} and analyzed in the respective dose week. All average recovery values were between 70% and 110%. The detailed results are given in Table 4.1.2-12.

Table 4.1.2-12: Recovery results of boscalid in feed

Matrix	Fortification level [mg kg^{-1}]	n [a]	Mean recovery [b] [%]	Average recovery [c] [%]	RSD [c] [%]
Feed	100	22	101.6, 102.9, 95.8, 103.4, 100.8, 104.2, 89.9, 96.5, 102.6, 102.9, 98.6	99.9	4.4
	500	14	98.4, 94.6, 91.6, 95.6, 93.8, 95.8, 98.4	95.5	2.6
	2500	14	94.4, 104.5, 90.5, 92.9, 94.0, 100.2, 98.1	96.4	5.0
	15000	20	94.3, 96.9, 97.5, 94.8, 92.9, 91.8, 93.2, 92.4, 97.5, 98.9	95.0	2.6

RSD = Relative standard deviation
[a] n = number of single replicates
[b] Mean of two replicates, data sets were analyzed at different dose weeks
[c] Average recoveries and RSD data are based on calculations using data originating from the study report.

Linearity The analytical concentrations of the samples were calibrated with 3 standard concentrations. However, quantification of the unknown sample was done by relating the peak area to the standard concentration of the expected concentration. Therefore, the determination of a correlation coefficients r was not relevant. Nevertheless, when applying a linear fit to the peak areas obtained, good linearity was observed (r>0.99), was obtained (*information derived from the study raw data*).

Specificity The identification and quantification of boscalid were based on the selected wavelength at 210 nm and 274 nm and the retention time. Under the described conditions the method is specific for the determination of boscalid in feed. As the method is only used for dose verification of the known substance and known nominal concentrations, no additional confirmatory technique is necessary.

Matrix Effects As no interference at the elution time of the analyte of interest was observed in the UV-traces, no adverse effects of any matrix occurred.

Interference No significant interferences were observed in the control samples.

Limit of Quantification The limit of quantification was defined by the lowest fortification level of 100 mg kg^{-1} for boscalid.

Limit of Detection Good detectability is achieved at a signal to noise ratio of 3:1, which is defined as the limit of detection (LOD).

Repeatability The relative standard deviation (RSD, %) with respect to recoveries following fortifications at the different dose levels were < 20% for boscalid.

Stability The analysis of the stability demonstrate that boscalid was stable in feed over a period of 32 days, when stored at ambient temperature (Table 4.1.2-13).

Table 4.1.2-13: Stability results of boscalid in feed

Nominal content [mg kg^{-1}]	Time after starting [days]	Analytical value [mg kg^{-1}]	Mean value [mg kg^{-1}]	% of initial content
100	0	102.3 98.9 101.1 97.5	99.9	100.0
	13	99.6 95.4 97.1	97.4	97.5
	32	99.2 99.6 94.5	97.8	97.9

Reproducibility The data in Table 4.1.2-12 are mean values of replicates measured at different days. The data are comparable, so a reproducibility is given.

Conclusion **The described method is considered suitable for the quantitative analysis of boscalid in feed.**

Report: CA 4.1.2/8
[redacted] 2000a
BAS 510 F - Chronic oral toxicity study in Beagle dogs - Administration in the diet for 12 months
2000/1016881

Guidelines: EEC Commission Directive 87/302; OECD Guideline No. 452, OPPTS 870.4100, Japan/JMAFF

GLP: yes
(certified by Landesanstalt fuer Pflanzenschutz Rheinland-Pfalz, Mainz, Germany)

Principle of the method

Analysis of Stability: Feed samples (dog feed wetted with drinking water, 1/1, v/v) were extracted with acetonitrile. Then, the extracts were analyzed by HPLC with UV-detection at 210 nm. The chromatography was performed on a Nucleosil 7 C18 column with a flow rate of 1.2 mL min^{-1} of 70% acetonitrile + 0.5M sulfuric acid and 30% bidistilled water + 0.5M sulfuric acid.

Homogeneity and Concentration Control Analysis: Feed Samples (dog feed "Kliba Haltungsdiät Hund"; *engl. translation: "Kliba, dog maintenance diet"*) were pre-incubated with water and extracted with acetonitrile. Then, the extracts were analyzed by HPLC with UV-detection at 210 nm. The chromatography was performed on a Polygosil 60 5 C18 column with a flow rate of 1.0 mL min^{-1} of 70% acetonitrile + 0.5M sulfuric acid and 30% bidistilled water + 0.5M sulfuric acid.

Recovery findings

The described method is suitable to determine residues of boscalid in wetted dog feed. Samples were spiked with boscalid with 200 mg kg^{-1}, 800 mg kg^{-1}, 2000 mg kg^{-1} and 20000 mg kg^{-1}. All average recovery values were between 70% and 110%. The detailed results are given in Table 4.1.2-14.

Table 4.1.2-14: Recovery results of boscalid in wetted dog feed

Matrix	Fortification level [mg kg^{-1}]	n [a]	Mean recovery [b] [%]	Average recovery [c] [%]	RSD [c] [%]
Wetted Dog Feed	200	14	96.0, 101.0, 102.5, 105.0, 115.5, 104.5, 102.0	103.8	5.7
	800	10	98.1, 96.5, 101.9, 101.6, 90.8	97.8	4.6
	2000	10	97.0, 92.2, 100.7, 101.6, 97.4	97.8	3.8
	20000	14	98.5, 101.5, 102.7, 94.8, 100.6, 100.8, 93.2	98.9	3.6

RSD = Relative standard deviation
[a] n = number of single replicates
[b] Mean of two replicates, data sets were analyzed at different days
[c] Average recovery and RSD values are based on calculations of the data originating from the study report.

Linearity	The analytical concentrations of the samples were calibrated with 3 standard concentrations. However, quantification of the unknown sample was done by relating the peak area to the standard concentration of the expected concentration. Therefore, the determination of a correlation coefficients r was not relevant. Nevertheless, when applying a linear fit to the peak areas obtained, good linearity was observed (r>0.99), was obtained (*information derived from the study raw data*).
Specificity	The identification and quantification of boscalid were based on the selected wavelength at 210 nm and the retention time. Under the described conditions the method is specific for the determination of boscalid in dog feed. As the method is only used for dose verification of the known substance and known nominal concentrations, no additional confirmatory technique is necessary.
Matrix Effects	As no interference at the elution time of the analyte of interest was observed in the UV-trace, no adverse effects of any matrix occurred.
Interference	No significant interferences were observed in the control samples.
Limit of Quantification	The limit of quantification was defined by the lowest fortification level of 200 mg kg^{-1} for boscalid.
Limit of Detection	Good detectability is achieved at a signal to noise ratio of 3:1, which is defined as the limit of detection (LOD).
Repeatability	The relative standard deviation (RSD, %) with respect to recoveries following fortifications at the different dose levels were < 20% for boscalid.
Stability	The analysis of the stability demonstrate that boscalid was stable in wetted dog feed over a period of 24 hours, when stored at ambient temperature (Table 4.1.2-15).

Table 4.1.2-15: Stability results of boscalid in wetted dog feed

Nominal content [mg kg^{-1}]	Time after starting [hours]	Analytical value [mg kg^{-1}]	Mean value [mg kg^{-1}]	% of initial content
104	0	106.1 101.9 103.7 104.9	104.2	100.0
	1	109.5 111.5 113.0	111.3	106.8
	2	114.5 110.0 114.0	112.8	108.3
	24	112.5 114.5 116.0	114.3	109.7

Reproducibility The data in Table 4.1.2-14 are mean values of replicates measured at different days. The data are comparable, so a reproducibility is given. No independent laboratory validation needs to be conducted for a data generation method / method for concentration control.

Conclusion **The described method is considered suitable for the quantitative analysis of boscalid in wetted dog feed.**

Report: CA 4.1.2/9
[redacted] et al, 2015a
Reg. No. 391572 – Repeated dose 90-day toxicity study in Wistar rats; Administration via the diet
2015/1204964

Guidelines: OECD 408, OPPTS 870.3100, Commission Regulation (EC) No 440/2008, JMAFF No. 12-Nousan-8147

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

Remark: In CA-4.1.2 the analytical report is summarised under the DocID of the original study. The toxicological study can be found in chapter CA-5.8.1/7.

Principle of the method

Stability analysis in rat diet and homogeneity and concentration control: The samples (Ground Kliba maintenance diet mouse/rat "GLP" meal) were weighed into a centrifuge tube and extracted 3 times with 30 mL acetonitrile for 30 min on a lab shaker. After centrifugation at 4500 rpm for 5 min, the supernatants were collected and the combined extracts were diluted with acetonitrile to 100 mL. The samples were filtered (cellulose filter, 0.2 µm) prior to HPLC analysis. Residues of M510F49 (Reg.No. 391572) were analyzed by HPLC with UV-detection at 335 nm. The chromatography was performed on an Ascentis Express C_{18} column with a flow rate of 1 mL min^{-1} of acetonitrile and water, each with formic acid as modifier.

Recovery findings

The described method is suitable to determine residues of M510F49 in rat diet. Samples were spiked with the analyte in concentrations of 150 ppm, 1500 ppm and 15000 ppm. All mean recovery values were between 90% and 110% of the nominal concentrations. Considering the low relative standard deviation in the homogeneity analysis, it was concluded that M510F49 was distributed homogeneously in the rat diet samples. The detailed results are given in Table 4.1.2-16.

Table 4.1.2-16: Recovery results of M510F49 in rat diet

Matrix	Nominal Concentration [ppm]	Nominal Concentration [%]	Mean Nominal Concentration [%]	RSD [%]
Rat diet	150	102.2, 99.3, 103.4	101.6	2.1
	1500	98.8	--	--
	15000	94.5, 90.1, 95.5	93.3	3.1

RSD = Relative standard deviation

Linearity The linearity was tested at concentrations between about 4 mg L^{-1} and 50 mg L^{-1}, prepared in matrix solution (preparation procedure according to the description in principle of the methods). Linear correlation with coefficients of $r > 0.995$ were obtained. Six calibration standards distributed over the range given above were used.

Specificity The identification and quantification of M510F49 were based on the selected wavelength at 335 nm and the retention time. Under the described conditions, the method is specific for the determination of M510F49 in rat diet. As the method is only used for dose verification of known substances and known nominal concentrations, no additional confirmatory technique is necessary.

Matrix Effects Matrix-matched calibration solutions were used for quantification of M510F49, hence any potentially occurring matrix effect has already been accounted for by adjusting the matrix in the standard solutions used for calibration.

Interference No test substance was detected in the vehicle control samples with a concentration of ≥ 30% of the lowest calibration solution.

Limit of Quantification The limit of quantification was defined by the lowest fortification level of 150 ppm for M510F49.

Limit of Detection The limit of detection was defined by the lowest calibration standard of 4 mg L^{-1} for M510F49.

Stability Analysis It was shown that M510F49 was stable in rat diet over a time period of 4 days stored at room temperature (Table 4.1.2-17). The stability of M510F49 was given over 9 days under freezer conditions, followed by 4 days stored at room temperature (Table 4.1.2-18).

Table 4.1.2-17: Results of stability analysis of M510F49 in rat diet stored at room temperature over 10 days

Matrix	Time after starting [days]	Nominal Concentration [ppm]	Nominal Concentration [%]	Mean Nominal Concentration [%]	RSD [%]
Rat diet	0	50	101.5, 100.6, 103.7, 104.0, 101.5	102.3	1.5
	4	50	94.8, 97.4, 95.0, 95.4, 93.6	95.2	1.4
	10	50	75.9, 85.4, 87.0, 85.5, 85.7	83.9	5.4

RSD = Relative standard deviation

Table 4.1.2-18: Results of stability analysis of M510F49 in rat diet stored 9 days under freezer conditions followed by 4 days at room temperature

Matrix	Time after starting [days]	Nominal Concentration [ppm]	Nominal Concentration [%]	Mean Nominal Concentration [%]	RSD [%]
Rat diet	0	47	102.9, 96.6, 100.8, 104.0, 95.7	100.0	3.7
	0	47	98.9, 98.0, 102.0, 101.9, 100.2	100.2	1.8
	1	47	93.9, 93.8, 92.1	93.3	1.1
	2	47	89.0, 92.0, 90.8, 92.0, 90.4	90.8	1.4
	3	47	96.5, 96.4, 94.0, 98.7, 97.3	96.6	1.8
	4	47	98.9, 95.4, 99.4, 97.1, 98.1	97.8	1.6

RSD = Relative standard deviation

Repeatability The relative standard deviation (RSD, %) with respect to recoveries following fortifications at the different fortification levels were < 10% for M510F49.

Reproducibility Reproducibility was not tested within the concentration control study.

Conclusion **The described analytical method is considered suitable for the quantitative analysis of M510F49 (Reg.No. 391572) in rat diet.**

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

Where necessary, analytical method parameters and assessment of their suitability are addressed within the respective study.

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

The following methods cover the compounds of the data generation methods used for pre-registration purposes for analysis of samples of plant and animal origin as discussed in CA 6.3, 6.4 and 6.6:

Plant: BAS 510 F (Boscalid; Reg. No. 300355); 2-chloro-N-(4'-chlorobiphenyl-2-yl) nicotinamide

Animal: BAS 510 F (Boscalid; Reg. No. 300355); 2-chloro-N-(4'-chlorobiphenyl-2-yl) nicotinamide; M510F01 (Reg. No. 398794; 2-chloro-N-(4'chloro-5-hydroxy-biphenyl-2-yl)-nicotineamide

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

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

Plant

Analytical methods for the determination of Boscalid residues in plant matrices were evaluated in the context of the inclusion in Annex I of Directive 91/414/EEC. Methods evaluated are summarised in Table 4.1.2-19 below for the reviewer's convenience. The respective studies are listed as fully peer-reviewed if they were part of the Draft Assessment Report (DAR, 2002) or its Addendum (2006).

Studies submitted during the evaluation process and were not cited in the draft risk assessment report are also listed in the table below, but not considered as EU-peer reviewed.

In the following two tables an overview of already peer-reviewed as well as the new methods submitted for review are summarised for the reviewer's convenience. These overviews do not differentiate between data-generation methods for pre-registration purposes and monitoring methods for enforcement purposes; hence a similar comparison will not be listed again in chapter M-CA 4.2.

Table 4.1.2-19: Summary of *already peer-reviewed* residue analytical methods for determination of boscalid residues in plant matrices

Method. No.	DocID	Matrix	Method principle	Target analytes	LOQ [mg/kg]	year	EU reviewed
DFG S19	1999/11461	tomatoe, lemon, grain, oilrape seed	GC-MS	BAS 510 F	0.01 and 0.02	1999	yes (2002)
ILV of DFG S19	2000/1014886	apple, sour cherry, grapes, strawberry, carrot, onion, tomato, broccoli, cabbage, leek, dwarf beans, oilrape seed	LC-MS/MS	BAS 510 F	0.05	2001	yes (2002)
DFG S19	2000/1017227	white cabbage, lettuce oilrape seed hops	GC-MS	BAS 510 F, M510F01	0.01	2001	yes (2002)
445/0	2000/1012404	apple, sour cherry, grapes, strawberry, carrot, onion, tomato, broccoli, cabbage, leek, dwarf beans, oilrape seed	LC-MS/MS	BAS 510 F	0.05	2000	yes (2002)
./.	2000/1014856	solvents - stability	HPLC-UV	BAS 510 F	n.a.	2000	yes (2002)
./.	2000/1017225	solvents - stability	HPLC-UV	BAS 510 F, M510F01, M510F49, M510F51, M510F53	n.a.	2000	yes (2002)
./.	2001/1001739	plant - extractability	^{14}C-HPLC	BAS 510 F	n.a.	2001	yes (2002)

Table 4.1.2-20: Summary of *newly submitted* residue analytical methods for determination of boscalid residues in plant matrices

Method. No.	DocID	Matrix	Method principle	Target analytes	LOQ [mg/kg]	year	EU reviewed
535/1	2006/1039427	wheat, lettuce, lemon, oil seed rape, tomato, onion	LC-MS/MS	BAS 510 F	0.01	2006	new method
L0076/01	2015/1091103	hops, spices, herbal infusions	LC-MS/MS	BAS 510 F	0.01	2015	new method for difficult matrices
D9908	2001/5001020	almond, plum, onion	LC-MS/MS	BAS 510 F	0.02	2001	data generation method used for analysis of field trials
L0328/01	2015/1114667	wheat grain, lemon, dry peas, oilseed rape seed, tomato	LCMS/MS	BAS 510 F	0.01	2015	multimethod based on QuEChERS

The currently proposed data generation method for the determination of Boscalid in plant matrices is BASF residue analytical method 535/1.

Additionally, as the method from Reichert, 2001 (2000/1017227), was not considered valid due to low recoveries for the determination of boscalid in hops, a new method was developed and validated for the determination of boscalid in generally as difficult considered plant matrices. The method proposed is validated in tea, herbal infusions, as well as hops. The method L0076/01 is suitable as data generation method for pre-registration purposes, as well, if required for monitoring purposes, if required.
The following method (BASF method 535/1) is the current data generation method used in field trials (chapter M-CA-6.3) and in processing studies (chapter M-CA-6.5).

Remark on Extraction Efficiency – Plant matrices

Extraction efficiency has been assessed during the Annex I inclusion process in study 2001/1001739. It could be shown that the extraction solvent used in residue analytical method 445/0 (mixture of methanol, water and hydrochloric acid) as well as acetone and water used in the multimethod approach DFG S19 removed comparable amounts of residues than the metabolism extraction scheme(refer to already peer-reviewed studies 2000/1014861, 2000/1014860, 2000/1014862, and 1999/11240). The newly submitted residue analytical methods 535/1, L0076/01, and D/9908 use an identical extraction liquid than method 445/0, hence suitability of this extraction liquid is also fully confirmed.

Report:	CA 4.1.2/10 Mackenroth C.,Lehmann A., 2007a Validation of BASF method No. 535/1 in plant matrices 2006/1039427
Guidelines:	EPA 860.1340, SANCO/825/00 rev. 6 (20 June 2000), SANCO/3029/99 rev. 4 (11 July 2000), EEC 6/46, EEC 91/414 Annex III (Part A Section 5)
GLP:	yes (certified by Landesamt fuer Umweltschutz und Gewerbeaufsicht, Mainz, Germany Fed.Rep.)

Principle of the method: In method 535/1 residues of boscalid (BAS 510 F) are extracted from wheat (plant, straw, grain), lemon, lettuce, oilseed rape (seed), tomato and onion using a mixture of methanol, water and hydrochloric acid. An aliquot of the extract is centrifuged and partitioned at alkaline conditions against cyclohexane. After evaporation of cyclohexane, the residues are dissolved in methanol/water (50/50, v/v). Detection was accomplished by electrospray ionization in positive mode at mass transition 343→271 for quantification and 343→307 for confirmation. Final determination of the analytes is performed by HPLC-MS/MS using a HP 1100 Series HPLC system (Betasil Cl8 100*2.1 mm column), equipped with PE Sciex API 3000 and applying a water-methanol gradient with 0.1% formic acid as modifier. The results are calculated by direct comparison of the sample peak responses of the external standards.

Recovery findings: In both matrices tested, the mean recovery values were between 70% and 120%. The detailed results are given in the table below.

Table 4.1.2-21: Validation results of method 535/1: boscalid (BAS 510 F) (HPLC-MS)

Test substance	Crop	Fortification level (mg/kg)	No of analyses	Average recovery (%)		Relative standard deviation (%)	
Transition				343→307	343→271	343→307	343→271
Boscalid (BAS 510 F)	Wheat plant*	0.01	5	93	92	5.2	16.6
		0.1	5	83	84	5.6	7.7
	Wheat grain	0.01	5	84	100	7.3	5.2
		0.1	5	84	82	4.3	13.9
	Wheat straw	0.01	5	86	86	7.3	11.1
		0.1	5	87	82	11.6	9.4
	Lemon	0.01	5	88	89	2.1	17.4
		0.1	5	82	77	8.7	14.8
	Lettuce	0.01	5	82	92	6.0	5.3
		0.1	5	82	81	8.8	3.0
	Oilseed rape seed	0.01	5	80	80	7.1	12.9
		0.1	5	84	86	6.5	18.2
	Tomato	0.01	5	86	81	5.0	18.4
		0.1	5	86	82	3.2	9.2
	Onion	0.01	5	88	83	7.0	10.3
		0.1	5	88	81	8.8	9.2

* Without roots

Linearity: The linearity was tested using 4 standards at concentrations between 0.05-0.5 ng/ml. Standards dissolved in methanol/water were injected in triplicate and the response was plotted against the concentration. Linear correlations with coefficients of $r \geq 0.99$ were obtained for boscalid (BAS 510 F).

Specificity: HPLC-MS/MS is a highly specific analytical technique. Under the described conditions the method is highly specific for the determination of boscalid in plant matrices. Analysis is possible at two different mass transitions for each analyte.
Due to the high selectivity and specificity of HPLC-MS/MS an additional confirmatory technique was not necessary as two different mass transitions were evaluated and fully validated.

Matrix Effects: No matrix matched standards have been used and no matrix effects >20% were reported.

Interference: No significant interference was observed at elution times of the analytes of interest (interference <30% LOQ).

Limit of Quantitation: The limit of quantitation was defined by the lowest fortification level successfully tested and was 0.01 mg/kg in all matrices.

Repeatability: The relative standard deviations (RSD, %) for both commodities and fortification levels were <20% and therefore in accordance with SANCO/825/00 rev. 8.1 and SANCO/12571/2013. The detailed values are shown in Table 4.1.2-21.

Reproducibility: As the method is used as data generation method, no independent laboratory validation is required.

Stability of Solutions: The stability of the analytes in working solutions, or extracts was no subject of this study. BAS 510 F is stable in methanol and in the sample solvent over a time interval of 60 days (see Method 445, 2000/1012404) and solvent stability study (2000/1014856).

Conclusion: **Analytical method 535/1 is considered fully suitable for the analysis of boscalid in plant matrices to determine the analytes of interest in wheat (grain, straw), lemon, lettuce, oilseed rape (seed), tomato and onion with an LOQ of 0.01 mg/kg.**

Report:	CA 4.1.2/11 Austin R., 2015a Validation of BASF Analytical Method Number L0076/01 for the Determination of BAS 510 F (Boscalid) in Hops, Spices and Herbal Infusions 2015/1091103
Guidelines:	EU Commission Regulation No. 283/2013 setting out the data requirements, in accordance with Regulation (EC) No 1107/2009; EU Guidance Document SANCO/825/00 rev. 8.1, 16 Nov 2010; EC Guidance Document SANCO/3029/99 rev 4, 11 Jul 2000; OECD Guidance Document on Pesticide Residue Analytical Methods ENV/JM/MONO(2007)17, 13 Aug 2007; US EPA Guideline OPPTS 860.1340, Aug 1996
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Residue analytical method *L0076/01* is the current proposed data generation method for determination of boscalid metabolites in some plant matrices, such as hops, spices and herbal infusions.

Principle of the method:	Residues of boscalid (BAS 510 F) are extracted from hops, spices and herbal infusions (green tea) with a mixture of methanol, water and hydrochloric acid (70:25:5, v/v/v). An aliquot of the extract was centrifuged and partitioned in alkaline conditions against cyclohexane, evaporated to dryness and dissolved in methanol/water (1:1, v/v) for analyses. The final determination of BAS 510 F (boscalid) was performed by LC-MS/MS monitoring selective ion mass transitions using positive electrospray ionization. In Table 4.1.2-22 the mass transitions for all matrices regarding quantitation and confirmation are given. Analysis of hops and herbal infusions was accomplished on a Betasil C18 column applying a methanol-water gradient using 0.1% formic acid as modifier, whereas, chromatography of spice matrices is performed on a Synergi Polar-RP 100 Å column.
Recovery findings:	In all matrices tested, the mean recovery values were between 70% and 110%. Detailed results are given in Table 4.1.2-22.

Table 4.1.2-22: Validation results of method L0076/01: BAS 510 F (boscalid) in matrices of hops, spices and herbal infusions

Test substance	Crop	Fortification level (mg/kg)	No of replicates	Average recovery (%)		Relative standard deviation (%)	
Transition				**343→272**	**343→140**	**343→272**	**343→140**
BAS 510 F	Hops	0.01	5	83.2	79.5	5.8	4.2
		0.1	5	90.2	86.8	2.1	3.0
		Mean	10	86.7	83.2	5.8	5.7
Transition				**343→271**	**343→140**	**343→271**	**343→140**
	Spices	0.01	5	80.7	82.5	4.6	4.4
		0.1	5	77.1	77.1	2.5	1.6
		Mean	10	78.9	79.8	4.3	4.7
Transition				**343→307**	**343→271**	**343→307**	**343→271**
	Herbal infusions	0.01	5	83.0	84.8	2.3	2.9
		0.1	5	89.9	89.7	8.7	9.2
		Mean	10	86.5	87.2	7.5	7.2

Linearity: Good linearity was observed in the range tested (0.040-10.0 ng/mL). Linear correlations with coefficients ≥0.99 were obtained. At least 5 calibration points distributed over the range given above were used. Calibration standards were prepared in an aqueous mixture of methanol and water (1:1, v/v).

Specificity: LC-MS/MS is a highly specific detection technique and therefore a confirmatory technique is not required. Analysis was fully validated for two different mass transitions for each analyte.

Matrix Effects: The matrix effect was tested for each matrix. Significant matrix effects (≥ ±20%) on LC-MS/MS response were observed in hops and spices and therefore, calibration solutions in matrix were used for quantification of BAS 510 F. In contrast, solvent standards were used for quantification of BAS 510 F in herbal infusions as matrix effects were insignificant (<±20%)

Interference: Significant interferences (> 30% of the limit of quantification) of BAS 510 F measured in the control samples were not observed at the retention time of BAS 510 F.

Limit of Quantitation: The limit of quantitation was 0.01 mg/kg for BAS 510 F in all matrices tested. The limit of detection (LOD) was set at 0.002 mg/kg for BAS 510 F in all matrices. This was the equivalent of the lowest calibration standard and is equivalent to 20% of the LOQ.

Repeatability: The relative standard deviations (RSD, %) at each fortification level and the overall RSD for each matrix was <20%. The detailed values are shown in Table 4.1.2-22.

Stability of Solutions: BAS 510 F is demonstrated to be stable in working solutions for at least 62 days when stored refrigerated in the dark. The final sample extracts in were re-injected after at least 8 days of storage under refrigerated conditions. Re-injection of final extracts resulted in recoveries within the acceptable range of 70-110%.

Conclusion: **Analytical method L0076/01 successfully validated and fulfils the requirements with regard to specificity, linearity, limit of quantification, recoveries and repeatability for the analysis of BAS 510 F (boscalid) in difficult plant matrices. The method is considered suitable for the determination of residues of BAS 510 F in hops, spices and herbal infusions over the concentration range tested (0.01 - 0.1 mg/kg). As quantitation is accomplished by MS/MS detection, no confirmatory technique is required as two different, selective mass transitions have been validated.**

Report: CA 4.1.2/12
Jones J.E. 2001a
Method for determination of BAS 500 F, BF 500-3 and BAS 510 F residues in plant matrices using LC/MS/MS (Validation of Method D9908)
2001/5001020
Guidelines: US EPA OPPTS 860.1340
GLP: yes
(certified by United States Environmental 'Protection Agency)

Principle of the method: In method D9908 residues of boscalid (BAS 510 F) were extracted from almond with acetonitrile, cleaned by a liquid/liquid partition with hexane, and further purified. Residues in plum are extracted using a mixture of methanol, water and hydrochloric acid and further cleaned via SPE. Residues in onions are extracted using a mixture of methanol, water and hydrochloric acid and an aliquot was cleaned by liquid/liquid partitioning using cyclohexane, followed by purification via SPE. Detection was accomplished by electrospray ionization in positive mode at mass transition 343→307 for quantification. Final determination of the analytes is performed by HPLC-MS/MS using a HPLC system (Phenomenex Columbus C18 2mm*100mm column), equipped with PE Sciex API 3000 mass spectrometer, and applying a water-methanol gradient, containing ammonium formate with 0.1% formic acid as modifier. The results are calculated by direct comparison of the sample peak responses of the external standards.

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

Table 4.1.2-23: Validation results of method D9908: boscalid (BAS 510 F) (LC-MS/MS)

Test substance	Crop	Fortification level (mg/kg)	No of analyses	Average recovery (%)	Relative standard deviation (%)
Transition				**343→307**	
Boscalid (BAS 510 F)	Almond	0.05	2	79	n.a.
		3.0	2	84	n.a.
		0.05, 3.0	4	81	6.7
	Plum	0.05	2	97	n.a.
		3.0	2	94	n.a.
		0.05, 3.0	4	95	2.2
	Onion	0.05	4	86	26.7
		3.0	2	85	n.a.
		0.05, 3.0	6	86	21.0

n.a. Not applicable

Linearity: The linearity was tested using 5 standards at concentrations between 0.5-10 ng/ml. Standards dissolved in acetonitrile were injected and the response was plotted against the concentration. Linear correlations with coefficients of $r \geq 0.98$ were obtained for boscalid (BAS 510 F).

Specificity: HPLC-MS/MS monitoring two mass transitions is a highly specific analytical technique. Analysis was performed using only one mass transitions for each analyte.

Matrix Effects: No matrix matched standards have been used as no matrix effects were observed.

Interference: No significant interference was observed at elution times of the analytes of interest (interference <30% LOQ).

Limit of Quantitation: The limit of quantitation was defined by the lowest fortification level successfully tested and was 0.05 mg/kg in all matrices.

Repeatability: The relative standard deviations (RSD, %) for almond and plum, calculated for both fortification levels were <10% and therefore in accordance with SANCO/825/00 rev. 8.1 and SANCO/12571/2013. The relative standard deviations (RSD, %) for onion, calculated for both fortification levels is >10%. The detailed values are shown in Table 4.1.2-23.

Reproducibility: As the method is used as data generation method, no independent laboratory validation is required.

Stability of Solutions: The stability of the analytes in working solutions, or extracts was no subject of this study.

Conclusion: **Analytical method D9908 is considered fully suitable for the analysis of boscalid in plant matrices to determine the analytes of interest in almond, plum and onion with an LOQ of 0.05 mg/kg.**

Animal

Analytical methods for the determination of Boscalid residues in animal matrices were evaluated in the context of the inclusion in Annex I of Directive 91/414/EEC. Methods evaluated are summarized in Table 4.1.2-24 below for the reviewer's convenience. The respective studies are listed as fully peer-reviewed if they were part of the Draft Assessment Report (DAR, 2002) or its Addendum (2006).

Studies submitted during the evaluation process and were not cited in the Monograph are also listed in the table below, but not considered as EU-peer reviewed.

In the following two tables an overview of already peer-reviewed as well as the new methods submitted for review are summarized for the reviewer's convenience. These overviews do not differentiate between data-generation methods for pre-registration purposes and monitoring methods for enforcement purposes; hence a similar comparison will not be listed again in chapter M-CA 4.2.

Table 4.1.2-24: Summary of *already peer-reviewed* residue analytical methods for determination of boscalid residues in animal matrices

Method. No.	DocID	Matrix	Method principle	Target analytes	LOQ [mg/kg]	year	EU reviewed
DFG S19	2000/1017227	milk, muscle, fat, kidney, liver (cow) egg (hen)	GC-ECD and GC-MS	Boscalid, M510F01	0.01 (milk) and 0.025(others)	2001	yes (2002)
DFG S19	2000/1017226	milk, liver (cow)	GC-ECD	Boscalid, M510F01	0.01 (milk) and 0.025 (others)	2001	yes (2002)
471/0	2000/1017223	milk, cream, egg, muscle, liver, kidney, fat	LC-MS/MS	Boscalid, M510F01	0.01 (milk, cream, eggs) and 0.025 (others)	2000	yes (2002)
476/0	2000/1017224	milk, liver (cow)	GC-MS	M510F53	0.01 (milk) and 0.05 (liver)	2000	yes (2002)

Table 4.1.2-25: Summary of *newly submitted* residue analytical methods for determination of boscalid residues in animal matrices

Method. No.	DocID	Matrix	Method principle	Target analytes	LOQ [mg/kg]	year	EU reviewed
471/0	2003/1021922	milk, cream, egg, muscle, liver, kidney, fat	LC-MS/MS	Boscalid, M510F01	0.01 (milk, cream, eggs) and 0.025 (others)	2000	no; 1st Amendment to 2000/1017223; information on stability
471/0	2015/1174463	milk, cream, egg, muscle, liver, kidney, fat	LC-MS/MS	Boscalid, M510F01	0.01 (milk, cream, eggs) and 0.025 (others)	2015	no; 2nd Amendment to 2000/1017223; information on 2nd mass transition and matrix effect

As no new feeding studies were conducted, no new stand-alone validated analytical methods were developed for animal matrices. However, an amendment was issued for the already peer-reviewed data generation methods 471/0 (Grosshans F., 2001; 2000/1017223 and its first amendment 2003/1021922) to additionally report the second mass transition and to address matrix effects. The results of this amendment are summarized together with the amended final report, although method 471/0 has already been fully peer-reviewed. For completeness, method 471/0 is summarized again for the reviewer's convenience to present the results from both, the original validation report and both amendments. Method 471/1 has been fully peer-reviewed as is considered as acceptable for the determination of boscalid in animal matrices.

Method 471/0 is also the proposed monitoring method for enforcement purposes. The respective independent laboratory validation is summarized and submitted in chapter 4.2.

Report:	CA 4.1.2/13 Grosshans F., 2001 a The validation of BASF method 471/0: The determination of BAS 510 F and the metabolite M510F01 in animal matrices 2000/1017223
Guidelines:	EPA 860.1340, Guidance Document on Residue Analytical Methods (SANCO/3029/99 rev.4)
GLP:	yes (certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

AND

Report:	CA 4.1.2/14 Grosshans F., 2004 a Report amendment No. 1: The validation of BASF method 471/0: The determination of BAS 510 F and the metabolite M510F01 in animal matrices 2003/1021922
Guidelines:	EPA 860.1340, Guidance Document on Residue Analytical Methods (SANCO/3029/99 rev.4)
GLP:	yes (certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

AND

Report:	CA 4.1.2/15 Courtois J. et al., 2015 a Report Amendment No.2: The validation of BASF method 471/0: The determination of BAS 510 F and the metabolite M510F01 in animal matrices 2015/1174463
Guidelines:	EPA 860.1340, Guidance Document on Residue Analytical Methods (SANCO/3029/99 rev.4)
GLP:	yes (certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Principle of the method

Method Number 471/0:
BAS 510 F (Boscalid) and its metabolite M510F01 were extracted from animal matrices using methanol. An aliquot of the extract was evaporated to dryness, redissolved in buffer solution and incubated with ß-glucuronidase/arylsulfatase to cleave the glucuronide M510F02 to M510F01. Then a liquid/liquid partition with ethyl acetate is carried out and the organic phase is purified on SPE C18 and if necessary on SPE silica gel columns. The final determination of the analytes BAS 510 F and M510F01 was performed by HPLC/MS/MS.
The LC-MS/MS method allows to quantify and to confirm boscalid (BAS 510 F) and its metabolite using two mass transitions (ESI+):
Boscalid (BAS 510 F) for milk, liver, muscle, egg, cream and fat: (343 m/z→307 m/z and 343 m/z→140 m/z).
Boscalid (BAS 510 F) for kidney: 343 m/z→307 m/z and 343 m/z→271 m/z.
Boscalid metabolite M510F01: 359 m/z→323 m/z and 359 m/z→140 m/z for quantitative confirmation.

Recovery findings

The average recoveries for the two parent-daughter ion transitions monitored were within the acceptable range of 70% to 110%. The detailed results are given in the table below.

Table 4.1.2-26: Validation results of method 471/0: boscalid (BAS 510 F) in animal matrices

Test substance	Sample Matrix	Fortification level (mg/kg)	No of analyses	Average recovery (%)		Relative standard deviation (%)	
Boscalid (BAS 510 F)	**Transition**			**343→307**	**343→140**	**343→307**	**343→140**
	Cow, milk	0.01	5	86.0	86.2	3.6	6.7
		0.1	5	88.7	87.6	7.7	11.9
	Cow, cream	0.01	5	72.2	73.4	1.5	9.6
		0.1	5	89.9	83.5	4.7	8.7
	Cow, muscle	0.025	5	86.4	107	4.0	3.4
		0.25	5	94.5	93.1	1.5	1.8
	Cow, fat	0.025	5	80.0	79.9	5.4	5.8
		0.25	5	81.0	80.9	8.5	6.6
	Cow, liver	0.025	5	86.7	74.2	6.3	8.7
		0.25	5	96.0	90.9	8.7	8.8
	Hen, egg	0.01	5	82.5	88.2	3.8	4.9
		0.1	5	93.1	93.3	3.1	5.3
	Transition			**343→307**	**343→271**	**343→307**	**343→271**
	Cow, kidney	0.025	5	83.3	84.5	1.9	27.7
		0.25	5	90.6	92.3	3.9	7.6
M510F01	**Transition**			**359→323**	**359→140**	**359→323**	**359→140**
	Cow, milk	0.01	5	88.4	83.3	5.8	5.6
		0.1	5	84.9	82.8	8.6	11.5
	Cow, cream	0.01	5	89.5	83.9	1.7	3.2
		0.1	5	94.2	93.9	2.3	6.0
	Cow, muscle	0.025	5	89.3	106	2.1	2.9
		0.25	5	86.3	88.6	1.4	4.0
	Cow, fat	0.025	5	81.0	79.5	4.0	2.6
		0.25	5	82.6	82.3	7.4	5.5
	Cow, kidney	0.025	5	81.6	73.2	2.5	7.0
		0.25	5	82.2	78.7	4.6	4.2
	Cow, liver	0.025	5	90.9	91.9	10.3	11.6
		0.25	5	91.5	91.0	6.2	5.1
	Hen, egg	0.01	5	82.7	82.5	6.1	4.2
		0.1	5	89.1	88.5	8.2	6.8

Linearity

Good linearity was observed in the range of 0.1 to 1.0 ng/mL (for milk, liver, muscle) or 0.25 ng/mL to 2.5 ng/mL (for egg, muscle, cream, fat and kidney) using 4 calibration points, respectively. Linear correlations with coefficients >0.95 were obtained. Calibration standards were prepared in acetonitrile.

Specificity

LC-MS/MS is a highly specific detection technique and therefore no confirmatory technique is required. Analysis is possible at two different mass transitions, hence no additional confirmatory analytical technique is required.

Matrix Effects:	Matrix effects were assessed by preparation of quality control standards, containing at the level equivalent to the LOQ in each analytical measurement queue. No matrix effects (>20%) were observed for BAS 510 F, except for milk at the mass transition 343/307, where the matrix effect accounted for 24%. The use of matrix-matched standards is therefore not considered imperative. No matrix effects (>20%) were observed for the metabolite M510F01.
Interference:	No significant interference was observed. At elution time of the signals of interest, interference was below the required limit of 30% LOQ in all cases, as no signal was observed at elution times of the analytes of interest.
Limit of Quantitation:	The limit of quantitation of the analytical method is 0.01 mg/kg in eggs, milk and cream and 0.025 mg/kg[1] for muscle, fat, kidney and liver.
Repeatability:	The relative standard deviations (RSD, %) for all matrices, except the confirmatory mass transition of the kidney at fortification level 0.025 mg/kg, with an RSD of 15% at the fortification level of >0.1 to ≤1.0 mg/kg. The detailed values are shown in Table 4.1.2-26.
Reproducibility	Reproducibility of the method was not determined within this validation study.
Stability of Solutions:	Stability of standards as extract stability, storage and volume stability were not investigated during the study.
Conclusion:	**It could be demonstrated that the method 471/0 fulfills the requirements with regard to linearity, specificity, repeatability, limit of quantification, and recoveries and is therefore applicable and suitable to correctly determine residues of boscalid (BAS 510 F) and its metabolite M510F01 in foodstuffs of animal origin (exemplified with milk, cream, egg, muscle, fat, liver and kidney).**

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

In general, methods for the determination of concentration, whenever necessary, are reported along with the respective ecotoxicological study.

Furthermore, three individually validated analytical methods were used in support of ecotoxicological studies

1. *BASF-method CP-No. 327*, was used for analysis of samples originating from ecotoxicological studies (refer to chapter M-CA 8.2). The method was validated as a stand-alone method, but was adapted to the technical set-up of the respective testing facility where the studies were conducted. Additional data from these concurrent validation are reported in detail in the respective study reports. The method described below in detail uses HPLC coupled with UV-detection for determination and quantification of the analyte of interest in the water phase. As it was shown that the method used are specific for the analyte (boscalid) and as the source of the analyte is known, an additional confirmatory technique is not required. This is different to the other data-generation methods presented in this chapter where the source of the analyte could potentially be unknown, such as in soil matrix analysed in open field studies.

2. *BASF-method 408/01* which allows the determination of boscalid in soil with a LoQ of 0.01 mg/kg using GC-MS.

3. *BASF-method D0004 (equivalent to L0122/01)* which allows the determination of boscalid and its metabolites M510M47 and M510M49 in soil with a LoQ of 0.01 mg/kg per analyte using LC-MS/MS.

Summaries of those three methods are given below in CA 4.1.2-16 to 18.

Report: CA 4.1.2/16
Petersen-Thiery M., 1999 a
Validation of analytical method CP 327: Bestimmung von BAS 510 F in Wasser by HPLC
1999/10170
Guidelines: Appendix 1 to § 19 a Section 1 Chemikaliengesetz of 25th July 1994
GLP: yes
(certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Remark: BASF analytical method CP 327 was developed to determine residues of boscalid (BAS 510 F) in tap water using HPLC-UV. The results of the validation studies of the analytical method are summarized below.

Principle of the method The water samples were diluted with 20 vol % acetonitrile and then without further work-up directly concentrated on a reversed-phase column and analyzed by HPLC with UV-detection at 233 nm. The chromatography was performed on a YMC J'sphere ODS H80 column with a flow rate of 1 mL min^{-1} of acetonitrile / water / 0.5 M sulfuric acid (600/400/5, v/v/v).

Recovery findings The described method is suitable to determine residues of boscalid in tap water. Samples were spiked with boscalid with a set of low concentrations of 5 µg L^{-1}, 60 µg L^{-1} and 100 µg L^{-1} and a set of high concentrations of 100 µg L^{-1}, 500 µg L^{-1} and 1000 µg L^{-1}. All mean recovery values were between 70% and 110%. The detailed results are given in Table 4.1.2-27.

Table 4.1.2-27: Recovery results of boscalid in tap water

Matrix	Fortification level [µg L^{-1}]	Number of Replicates	Mean Recovery [a] [%]	RSD [a] [%]	Overall Recovery [a] [%]	RSD [a] [%]
tap water (low concentration)	5	5	106.9	1.11	104.1	3.93
	60	5	106.4	1.43		
	100	5	98.9	2.14		
tap water (high concentration)	100	5	106.6	1.10	105.2	2.40
	500	5	105.0	3.51		
	1000	5	103.9	1.62		

RSD = Relative standard deviation
[a] Mean/average recoveries and RSD data are based on calculations using data originating from the study report.

Linearity	For the low concentration range, the linearity was tested using four standards (double injected) at concentrations between 2 µg L^{-1} to 114 µg L^{-1} yielding a coefficient of correlation of r > 0.99997. For the high concentration range, three standards in the concentration range of 0.08 mg L^{-1} to 1.42 mg L^{-1} were tested and showed good linearity with a coefficient of correlation of r > 0.99997.
Specificity	The UV-wavelength chosen is specific for boscalid. The identification and quantification were based on the selected wavelength and the retention time. Under the described conditions the method is specific for the determination of boscalid in tap water.
Matrix Effects	As no interference at the elution time of the analyte of interest was observed in the UV-trace, no adverse effects of any matrix occurred.
Interference	No significant interferences were observed in the blank carriers.
Limit of Quantification	The limit of quantification was defined by the lowest fortification level of 5 µg L^{-1} for boscalid.
Limit of Detection	The limit of detection was defined by the lowest calibration standard of 2 µg L^{-1} for boscalid.
Repeatability	The relative standard deviation (RSD, %) with respect to recoveries following fortifications at the different fortification levels were < 10% for boscalid.
Reproducibility	The results in Table 4.1.2-27 showed mean values of five independent samples of each concentration with different amounts of boscalid. The data are comparable, so a reproducibility is given.
Conclusion	**The described analytical method CP 327 is considered suitable for the quantitative analysis of boscalid in tap water.**

Report: CA 4.1.2/17
Keller W., 1998a
Validation of Analytical Method No. 408; Determination of BAS 510 Active Ingredient Residues in Soil
1998/10700
Guidelines: EPA 171-4(c), EPA 171-4(d), BBA Merkblatt Nr. 58 (1983)
GLP: yes
(certified by Ministerium für Arbeit, Soziales und Gesundheit, Mainz, Rheinland-Pfalz, Germany)

Remark: BASF analytical method No. 408 was developed to determine residues of boscalid (BAS 510 F) in soil using GC-MS. The results of the validation study of the analytical method are summarized below.

Principle of the method A 50 g soil sample is extracted with 100 mL toluene and 50 mL water. After separation of the toluene extract by centrifugation and clean-up step by silica gel chromatography, boscalid is quantified by GC-MS. The chromatography is performed on a DB-5 capillary column using helium as carrier gas. Detection is accomplished by electron ionization (EI) in the mass range 115 to 350.

Recovery findings The described method is suitable to determine residues of boscalid in soil. Samples were spiked with boscalid at concentration levels of 0.01 mg kg^{-1}, 0.1 mg kg^{-1} and 1.0 mg kg^{-1}. All mean recovery values were between 70% and 110%. The detailed results are given in Table 4.1.2-28.

Table 4.1.2-28: Recovery results of boscalid in German standard soils 2.1 and 2.2

Matrix	Fortification level [mg kg^{-1}]	Number of Replicates	Mean Recovery [%]	RSD [%]	Overall Recovery [%]	RSD [%]
German standard soil 2.1	0.01	5	96.7	5.6	93.2	7.3
	0.1	5	85.9	3.4		
	1.0	5	97.1	5.0		
German standard soil 2.2	0.01	5	91.7	4.4	91.2	7.2
	0.1	5	85.3	7.7		
	1.0	5	96.5	3.6		

RSD = Relative standard deviation

Linearity	Good linearity ($r > 0.99$) was observed in a concentration range between 0.05 µg mL^{-1} to 1.0 µg mL^{-1}. Five calibration levels, prepared in acetone, distributed over the concentration range given above were used with Reg.No. 304813 (2-chloro-N-(4'-methyl-biphenyl-2-yl)-nicotinamide) as internal standard.
Specificity	The method allows for the determination of boscalid using GC-MS, which is a highly selective and self-confirmatory detection technique. Therefore, no confirmatory technique is required.
Matrix Effects	No interferences at the retention time of interest were reported.
Stability of Working Solutions	Standard solutions of boscalid (prepared in acetone) were stable for at least 30 days, when stored in a refrigerator at 4°C [*BASF Doc ID 1998/11206*].
Extract Stability	Extract stability was not determined within the study.
Interference	No significant interferences were observed at the retention time of boscalid in the control samples.
Limit of Quantification	The limit of quantification was defined by the lowest fortification level of 0.01 mg kg^{-1} for boscalid.
Limit of Detection	Good detectability is achieved at a signal to noise ratio of 3:1, which is defined as the limit of detection (LOD).
Repeatability	The relative standard deviation (RSD, %) with respect to recoveries following fortifications at the different fortification levels were < 20% for boscalid.
Reproducibility	Reproducibility was not determined within the study.
Conclusion	**The described analytical method No. 408 is considered suitable for the quantitative analysis of boscalid in soil.**

Report:	CA 4.1.2/18 Saha M.G., 2001a Validation of BASF method no. D0004 and D0004 /1: The determination of residues of BAS 510 F and its metabolites in soil using LC-MS/MS 2001/5000881
Guidelines:	EPA 164-1
GLP:	yes (certified by United States Protection Agency)

Remark: BASF method D0004 was developed to determine residues of boscalid (BAS 510 F) and its metabolites in soil using LC-MS/MS. The method was further modified (BASF method D0004/1) to determine residues of boscalid and additional metabolites in soil using LC-MS/MS. As the methods D0004 and D0004/1 were only used for the determination of boscalid, the results of the validation of boscalid are summarized below.

Principle of the method

A 10 g soil sample aliquot is extracted with methanol, followed by extraction with methanol/water (50/50, v/v). An aliquot of the extract is diluted with water containing 0.1% formic acid and 4 mM ammonium formate for determination of HPLC-MS/MS.
Method D0004: Analysis was accomplished using an Inertsil ODS-3 column with a flow rate of 0.4 mL min^{-1} and a gradient mixture of water (4 mM ammonium formate and 0.1% formic acid) and methanol (4 mM ammonium formate and 0.1% formic acid). Quantification was accomplished in positive ion mode using atmospheric-pressure chemical ionization (APCI) with heated nebulizer (400°C) with mass transitions 343→140 for boscalid.
Method D0004/1: Analysis was accomplished using a Phenomenex Polar RP 80A column with a flow rate of 0.4 mL min^{-1} and a gradient mixture of water (4 mM ammonium formate and 0.1% formic acid) and methanol (4 mM ammonium formate and 0.1% formic acid). Quantification was accomplished in positive ion mode using APCI with heated nebulizer (400°C) with mass transitions 343→140 for boscalid.

Recovery findings

The described method is suitable to determine residues of boscalid in soil. Samples were spiked with the analyte with concentrations of 0.01 mg kg^{-1} (limit of quantification; LOQ), 0.1 mg kg^{-1} and 1.0 mg kg^{-1}. All mean recovery values were between 70% and 120%. The detailed results are given in Table 4.1.2-29.

Table 4.1.2-29: Recovery results of boscalid in soil

Method	Analyte	Fortification level [mg kg^{-1}]	Number of Replicates	Mean Recovery [a] [%]	RSD [%]	Overall Recovery [a] [%]	RSD [%]
D0004	boscalid (m/z 343→140)	0.01	14	96	3.3	96	3.7
		0.1	14	97	3.8		
		1.0	14	94	4.1		
D0004/1	boscalid (m/z 343→140)	0.01	12	96	6.8	95	5.2
		0.1	12	95	2.9		
		1.0	12	94	5.4		

RSD = Relative standard deviation

[a] Method D0004: Recovery data determined in five soil samples: silt loam, clay, loamy sand, sandy loam, organic soil; method D0004/1: Mean recovery data determined in four soil types: sandy loam, clay loam, loam with high organic matter and loamy sand.

Linearity During validation of method D0004, good linearity ($r > 0.996$) was observed in the concentration range of 1.25 ng mL^{-1} to 10 ng mL^{-1}. Standard solutions were prepared in methanol/water with 0.1% formic acid and 4 mM ammonium formate (50/50, v/v). During validation of method D0004/1, good linearity ($r \geq 0.998$) was observed in the concentration range of 1.25 ng mL^{-1} to 25 ng mL^{-1}. Standard solutions were prepared in methanol/water with 0.3% formic acid and 4 mM ammonium formate (80/20, v/v). Four concentration levels distributed over the calibration ranges given above were used.

Specificity The method allows the determination of boscalid using LC-MS/MS, which is a highly selective and self-confirmatory detection technique. Therefore, no confirmatory technique is required.

Matrix Effects As no interference at the elution time of the analyte of interest was observed in the chromatograms, no adverse effects of any matrix occurred.

Stability of Working Solutions Stock and fortification solutions of boscalid (prepared in methanol) were stable for 85 days and 30 days, respectively, when stored in a refrigerator at 4°C. Calibration standards of boscalid (prepared in methanol/water with 0.3% formic acid and 4 mM ammonium formate (80/20, v/v)) were stable for 30 days, when stored in a refrigerator at 4°C.

Extract Stability Analyte boscalid in soil extracts prepared in methanol and water/methanol (50/50, v/v) and in soil extracts prepared in methanol/water with 0.1% formic acid and 4 mM ammonium formate (50/50, v/v)) were stable for 21 days, when stored in a refrigerator at 4°C.

Interference	No significant interferences were observed in the control samples.
Limit of Quantification	The limit of quantification of both methods was defined by the lowest fortification level of 0.01 mg kg^{-1} for each analyte.
Limit of Detection	Good detectability is achieved at a signal to noise ratio greater than 3:1, which is defined as the limit of detection (LOD).
Repeatability	The relative standard deviation (RSD, %) with respect to recoveries following fortifications at the different fortification levels were ≤ 20% for boscalid.
Reproducibility	Reproducibility of method D0004/1 was successfully tested within an independent laboratory validation study [*BASF DocID 2001/5000882*]. However, an independent laboratory validation is not required for soil matrices for data generation purposes; therefore this information is only listed for completeness of information.
Conclusion	**The described methods D0004 and D0004/1 are considered suitable for the quantitative analysis of boscalid in soil.**

No stand-alone validation of an analytical method for bee-relevant matrices was conducted. Validation of bee-relevant matrices were validated within studies 2014/1000868 (effect study), 2014/1000867 (effect study; 2015/1107611 – Amendment), and 2015/1000383. Details considering the residue analytical method can be found in the analytical phase reports of the respective studies.

A short overview of the main parameters (recoveries of fortified samples) is given below:

- 2014/1000181: Boscalid can be determined in pollen, nectar (surrogate), and inflorescences with a limit of quantification of 0.01 mg/kg and a limit of detection of 0.001 mg/kg using highly selective LC-MS/MS following an extraction using a mixture of methanol/water/2 N HCl (70/25/5 v/v/v) . Average recoveries for all matrices range from 73% to 78% with a maximum relative standard deviation of 5% when preparing fortified samples at levels from 0.01 mg/kg and 0.1 mg/kg. Detector linearity (r>0.99) was given over a concentration range of 0.021 ng/mL to 4.21 ng/mL. Full details are provided in the analytical phase report in the respective study 2014/10001811.

- 2014/1000868: Validation of the analytical method was performed under study 2014/1000867. The limit of quantification in all matrices was 0.01 mg/kg with an LOD of 0.003 mg/kg.

- 2014/1000867: see overview 2014/1000181.

- 2015/1000383: Average recoveries were 87.5% with a relative standard deviation of 11%. The validation of the methodology was done under study 2014/1000181.

Additional information from scientific literature:

In the public literature, there is additional information available on the suitability of applying a multimethod approach for the analysis of bee-relevant matrices for boscalid. A summary of the proposed methodology is given below in CA-4.1.2./19.

Report: CA 4.1.2/19
Walorczyk S.,Gnusowski B., 2009 a
Development and validation of a multi-residue method for the determination of pesticides in honeybees using acetonitrile-based extraction and gas chromatography-tandem quadrupole mass spectrometry
2009/1132364
Guidelines: none
GLP: no

Principle of the method: Honeybees were homogenized and extracted with acetonitrile/water (2:1, v/v) and several salts. Clean-up was achieved by freezing-out and dispersive solid-phase extraction. After acidification, the extract was evaporated and the residue re-dissolved in toluene. Final analysis was performed by GC-MS/MS. Separations were carried out on a J & W DB-5 MS column; helium was used as carrier gas and argon as collision gas.

Recovery findings: In honeybees the mean recovery values were between 70% and 110%. The detailed results are given below.

Table 4.1.2-30: Validation results: boscalid in honeybees

Test substance	Matrix	Fortification level (mg/kg)	No of analyses	Average recovery (%)		Relative standard deviation (%)	
Transition				140 → 112	140 → 76	140 → 112	140 → 76
Boscalid	Honeybees	0.01	n r.	87	n.r.	20	n.r.
		0.05	n r.	85	n.r.	7	n.r.
		0.5	n r.	88	n.r.	6	n.r.

n.r. not reported

Linearity: Linearity of calibration curves was studied in the concentration range between 10 and 500 ng/mL using five calibration standards. Good linearity was observed for boscalid with correlation coefficient $r \geq 0.999$.

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

Matrix effects:	Matrix matched standards were used to consider matrix effects.
Limit of Quantitation:	The lowest tested fortification level by this method was 0.01 mg/kg.
Repeatability:	The relative standard deviations (RSD, %) were ≤20% for fortification level of 0.01 mg/kg and <10% for fortification levels 0.05 and 0.5 mg/kg. The detailed values are shown in Table 4.1.2-30
Reproducibility:	Analysis of real samples proved the method's feasibility for the intended purpose.
Conclusion:	**The GC-MS/MS multi residue analytical method can be used for the analysis of boscalid in honeybees if deemed necessary.**

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

Where necessary, this information is reported along with the respective method validation studies.

CA 4.2 Methods for post-approval control and monitoring purposes

General remark

The order of the study summaries is differing compared to the information given in the application submitted for renewal of approval.
An overview of the changes made to chapter 4.2 compared to the Application is given in Table 4.2-1 for the reviewer's convenience:

Table 4.2-1: Overview of changes of documents submitted compared to originally listed documents in the Application

Data point in Application	Data point in current dossier	DocID	Changes to Application	Reason for change
4.2./1	4.2./1	2015/1114667	no change	./.
n.a.	4.2./2	2009/1132362	Not in Application	Additional information from Literature
n.a.	4.2./3	2009/132363	Not in Application	Additional information from Literature
n.a.	4.2./4	2008/1103137	Not in Application	Additional information from Literature
n.a.	4.2./5	2015/1240157	Not in Application	Additional information from Literature
n.a.	4.2./6	2012/1369202	Not in Application	Additional information from Literature
n.a.	4.2./7	2012/1369183	Not in Application	Additional information from Literature
4.2/2	4.2./8	2015/1114666	Shift due to renumbering	Renumbering due to literature included
n.a	4.2/9	2015/1251211	Not in Application	Amendment contains additional information
n.a.	4.2./10	2012/1369182	Not in Application	Additional information from Literature
4.2./3	4.2./11	2008/1086809	Shift due to renumbering	Renumbering due to literature included
n.a.	4.2./12	2015/1174526	Not in Application	Amendment contains additional information
4.2/4	4.2./13	2015/1109588	Shift due to renumbering	Additional information from Literature
4.2./5	4.2./14	2015/1114668	Shift due to renumbering	Additional information from Literature
n.a.	4.2./15	2007/1071245	Not in Application	Additional information from Literature
n.a.	4.2./16	2000/1014992	Not in Application	Executive summary of air method included in dossier, although peer-reviewed, for completeness of monitoring methods in environmental matrices
n.a.	4.2./17	2015/1240162	Not in Application	Additional information from Literature
n.a.	4.2./18	2010/1233452	Not in Application	Additional information from Literature
n.a.	4.2./19	2008/1103136	Not in Application	Additional information from Literature

Data point in Application	Data point in current dossier	DocID	Changes to Application	Reason for change
n.a.	4.2./20	2014/1327592	Not in Application	Additional information from Literature
n.a.	4.2./21	20141327593	Not in Application	Additional information from Literature
n.a.	4.2./22	2001/1298011	Not in Application	Additional information from Literature

n.a. not applicable; study was not included in the original Application.

An overview of the metabolites of relevance for the analytical methods is given together with other structures of relevance in Document N3. The respective table also contains detailed information on different metabolite codes used due to historic reasons.

Based on the current residue definition for MRL setting and enforcement, residue analytical methods are required for the parent molecule boscalid in food of plant origin.

(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

Food of plant origin

An overview of already peer-reviewed as well as the newly submitted monitoring methods for plant matrices is listed in detail in M-CA 4.1.2. In chapter M-CA 4.2 a detailed description of the new residue methods proposed for monitoring and enforcement purposes for plant matrices is given.

During review of the Maximum Residue Levels (MRLs) currently established at European level, it was pointed out that validated methods for enforcement of boscalid in hops, spices and herbal infusions are required. To close this data gap, a new method for the determination of boscalid in such matrices was developed and validated. As the residue analytical method is equally suitable for pre-registration data generation as well as for monitoring purposes, the Applicant provides a detailed description of this method in chapter M-CA 4.1.2/11 together with the other data generation methods for pre-registration purposes as the method is suitable for both, data generation and monitoring purposes.

Although the already peer-reviewed enforcement methods for plant matrices (based on the DFG S19 approach: Weeren and Pelz; DocID 1999/11461, 1999, Reichert, 2001; DocID 2001/1014886) were considered fully valid and can be used for enforcement purposes (EFSA Reasoned Opinion, EFSA Journal 2014; 12(7): 3799), a new monitoring method based on QuECHERs approach was successfully developed and validated to account for the widely used QuECHERs approach for monitoring purposes. The relevant residue for enforcement and risk assessment in the proposed residue definition is boscalid only, with an LOQ of 0.01 mg/kg in crop commodities of high water content, high oil content, acidic and dry commodities (EFSA Journal 2014;12(7):3799). As a vast number of validated enforcement methods using QuECHERs approach is available from the EURL (European Reference Laboratories for Residues of Pesticides) Data Pool, no additional ILV (independent laboratory validation) is required. An overview of the methods available from the data pool as well as additional information found in the scientific literature, is given further below.

Report: CA 4.2/1
Schernikau N., 2015a
Validation of BASF method L0328/01 for the determination of BAS 510 F in plant matrices by LC-MS/MS
2015/1114667

Guidelines: SANCO/825/00 rev. 8.1 (16 November 2010), SANCO/3029/99 rev. 4 (11 July 2000), OECD-ENV/JM/MONO/(2007)17, EPA 860.1340 (1996)

GLP: yes
(certified by Freie und Hansestadt Hamburg, Behörde für Gesundheit und Verbraucherschutz, Hamburg, Germany)

Principle of the method: Samples of homogenized plant (wheat grain, lemon, dry peas, oilseed rape seed and tomato) were extracted with acetonitrile after addition of water to the plant matrix. After addition of a buffer salt mixture, containing magnesium sulphate, sodium chloride and sodium citrate, the extract was shaken. After centrifugation, an aliquot of the acetonitrile phase was cleaned by dispersive solid phase extraction, using primary secondary amine (PSA). The samples were analyzed using an 1200 Binary Rapid Resolution LC System equipped with an API 5000 detector system.
The LC-MS/MS method allows to quantify and to confirm boscalid using two mass transitions (343→307 m/z and 343→271 m/z) (ESI+).

Recovery findings: The mean recovery values of the validation experiments were between 70% and 110%, which fulfils the legal requirements for mean recovery values per fortification level. Detailed results are given in table below.

Table 4.2-2: Validation results of method L0328/01 (QuEChERS): boscalid (BAS 510 F) in plant matrices

Test substance	Matrix	Fortification level (mg/kg)	No of analyses	Average recovery (%)		Relative standard deviation (%)	
Transition				343→307	343→271	343→307	343→271
Boscalid (BAS 510 F)	Tomato (Fruit)	0.01	5	109	109	3.7	3.8
		0.1	5	102	103	5.8	4.3
	Lemon (Fruit)	0.01	5	103	105	2.6	3.5
		0.1	5	101	101	1.5	2.2
	Wheat (Grain)	0.01	5	94.4	97.3	2.8	1.6
		0.1	5	93.4	93.4	4.3	3.9
	Dry Pea	0.01	5	107	106	3.6	3.2
		0.1	5	106	104	2.4	3.4
	Oilseed rape (OSR) seed	0.01	5	86.6	87.9	3.2	3.5
		0.1	5	83.8	83.7	6.1	5.6

Linearity: Good linearity was observed in the range tested (0.15 ng/mL to 10.0 ng/mL). Linear correlations with coefficients ≥0.99 were obtained. Seven calibration points distributed over the range given above were used. Calibration standards were prepared in a mixture of acetonitrile and formic acid (1/1, v/v).

Specificity: LC-MS/MS monitoring two mass transitions is a highly specific detection technique and therefore an additional confirmatory technique is not required. Recovery data was reported for each mass transition and matrix considered.

Matrix Effects: Matrix effects on the detection of BAS 510 F (Boscalid) in extracts of plant (wheat grain, lemon, dry peas, oilseed rape seed and tomato) were found to be insignificant (<20%). Therefore, solvent standards were used for quantification.

Interference: No significant interference above 30 % of LOQ was detected at the retention times and mass transitions of any of the control specimen extracts of each matrix (wheat grain, lemon, dry peas, oilseed rape seed and tomato).

Limit of Quantitation: The limit of quantitation was 0.01 mg/kg in all matrices tested, corresponding to a concentration of 0.5 ng/mL in the extract.

Repeatability: The relative standard deviations (RSD, %) for all commodities and fortification levels were <7%. The detailed values are shown in the table above.

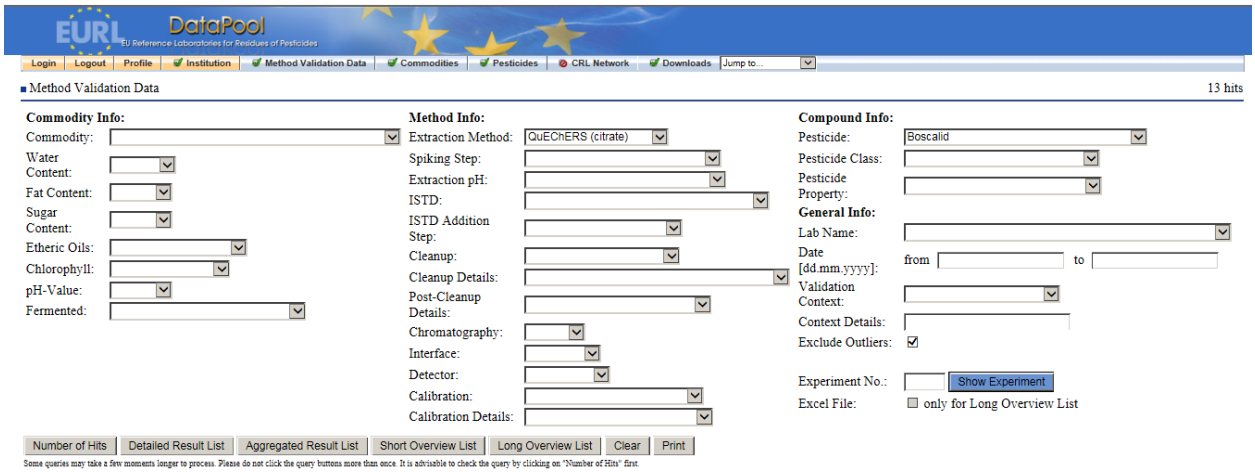
Reproducibility: Reproducibility of the method was not determined within this validation study. The high number of validation data available in the EURL data pool for QuEChERS methodology for each of the relevant crop commodities, provides the required information equivalent to an independent laboratory validation.

Stability of Solutions: Analytical standards of the analyte were found to be stable for at least 10 days when stored refrigerated (5°C ± 4°C) in the dark when prepared in acetonitrile/0.1% formic acid (1/1, v/v). After extraction, the analyte was found to be stable in the final extract of all matrices for at least 9 days when stored refrigerated (5°C ± 4°C) in the dark.

Conclusion: **The analytical method L0328/01 (QuEChERS methodology) could be demonstrated to fulfill the requirements with regard to specificity, repeatability, limit of quantification and recoveries and is therefore considered fully suitable for the analysis of boscalid in different plant matrices (wheat grain, lemon, dry peas, oilseed rape seed and tomato) with an LOQ of 0.01 mg/kg for enforcement purposes**.

The EURL data pool provides information on a vast number of analytical methods based on QuEChERS in various matrix types, e.g. spices, herbs and tea (difficult plant matrices), matrices of high water, high oil, high acid content, as well as high starch/protein and sugar containing plant matrices. The EURL data pool is accessible via internet (http://www.eurl-pesticides-datapool.eu/). An overview of the validation data available for the different crop commodities in the CRL data pool is given below. Based on the large number of methods published in the EURL data pool, no additional ILV for the proposed enforcement method is required.
In addition to QuECHERS approach, ChemElut is another widely used approach for the monitoring of boscalid. An overview is also given below.

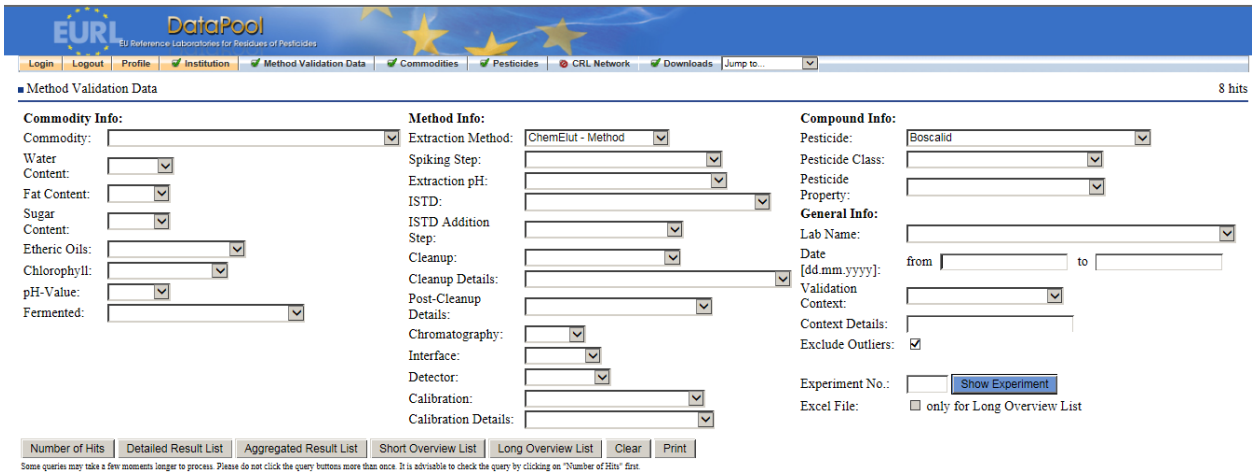
Overview of validation data available for boscalid analysis using *QuEChERS* (citrate) in the EURL data pool



Long Overview List

Pesticide	Chr	Matrix Type	Level min	Level max	Rec Median	Rec Mean	CV [%]	# of rec	% Rec (70-120%)	# of Labs
Boscalid			0,001	1,6	**99**	98	10,8	1260	**98**	11
	GC	Dry (spices, herbs, tea)	0,01	0,1	**76**	78	11	60	**88**	1
	GC	Water containing	0,101	0,101		79	9,4	2	**100**	1
	LC	Acidic	0,002	0,1	**99**	99	9,5	317	**99**	8
	LC	Dry (cereals, dry pulses)	0,01	0,2	**99**	98	10	113	**97**	7
	LC	Dry (spices, herbs, tea)	0,01	0,1	**100**	97	9,6	10	**100**	2
	LC	Fatty (oils)	0,01	0,01		97	1,5	2	**100**	1
	LC	Fatty, dry (oil seeds, nuts)	0,01	0,1	**92**	92	4,8	14	**100**	3
	LC	Fatty, wet (oily fruits)	0,01	0,1	**98**	98	8,4	18	**100**	4
	LC	Other	0,02	0,025	**95**	94	7,1	6	**100**	3
	LC	Sugar containing	0,01	0,1	**101**	99	9,5	116	**98**	7
	LC	Water containing	0,001	1,6	**99**	100	9,8	594	**97**	9
	LC	Water containing, extract rich	0,02	0,05	**102**	101	8,8	8	**100**	2

Overview of validation data available for boscalid analysis using *ChemElut* in the EURL data pool



Long Overview List

Pesticide	Chr	Matrix Type	Level min	Level max	Rec Median	Rec Mean	CV [%]	# of rec	% Rec (70-120%)	# of Labs
Boscalid			0,01	0,1	**83**	89	25,2	71	**86**	5
	LC	Acidic	0,01	0,1	**79**	81	16,5	24	**79**	3
	LC	Dry (cereals, dry pulses)	0,01	0,1	**78**	81	11,6	9	**100**	1
	LC	Fatty, wet (oily fruits)	0,01	0,1	**153**	141	30,6	5	**40**	1
	LC	Other	0,025	0,025		74	6,6	2	**100**	1
	LC	Sugar containing	0,01	0,1	**99**	96	10,9	9	**100**	1
	LC	Water containing	0,01	0,1	**92**	89	15,5	21	**95**	4
	LC	Water containing, extract rich	0,025	0,025		51		1	**0**	1

In the scientific literature, also a vast number of publications can be found applying multimethod approaches, either using acetonitrile (QuECHERs) or methanol for the monitoring of boscalid in various plant matrices. Hence, a sufficiently large number of under GLP validated QuEChERS methods as well as accessible scientific literature provides the required information to consider the enforcement methods, based on QuEChERS, as fully independently validated.

Overview of scientific literature:

In addition to the information available on validated methods for the enforcement of boscalid as available from the EURL data pool, information published in the scientific literature was also assessed and are summarised below in CA 4.2/2 – 4.2/7.

Report: CA 4.2/2
Gonzalez-Rodriguez R.M. et al., 2009 a
Multiresidue determination of 11 new fungicides in grapes and wines by liquid-liquid extraction/clean-up and programmable temperature vaporization injection with analyte protectants/gas chromatography/ion trap mass spectrometry
2009/1132362
Guidelines: none
GLP: no

Principle of the method: Residues of boscalid were extracted from homogenized grape samples and wine samples (previously evaporated to dryness) with ethyl acetate/hexane (1:1, v/v). After phase partitioning with sodium chloride and anhydrous sodium sulfate, an aliquot of the organic layer was evaporated and redissolved in acetonitrile. After clean up by SPE using acetonitrile/toluene (3:1, v/v), the eluate was evaporated and reconstituted in acetone (containing analyte protectants) and mixed with triphenylphosphate as internal standard. The final determination was performed by GC-MS equipped with a Supelco SPB-5 fused silica capillary column. Helium was used as carrier gas, and selected ion monitoring (SIM) was performed for determination of residues. For quantitation and confirmation of boscalid, ions at m/z 140, 307 and 342 were selected.

Recovery findings: A set of 5 samples fortified at 0.05 mg/kg comparing the extraction with and without analyte protectants in acetone was analyzed. The mean recovery values were between 70% and 120% for grapes and with the use of analyte protectants in red wine. In wine without analyte protectants, average recoveries were 131-137%, while in white wine with analyte protectants, the required range was only slightly exceeded with 124%. Detailed results are given in table below.

Table 4.2-3: Validation results of GC-MS multi-residue method: boscalid in grapes and wine

Test substance	Matrix	Fortification level (mg/kg)	No of analyses	Average recovery (%)		Relative standard deviation (%)	
		Use of stabilizers in acetone:		Without	With	Without	With
Boscalid (BAS 510 F)	White grapes	0.05	5	97	118	4	9
	Red grapes	0.05	5	106	112	6	4
	White wine	0.05	5	137	124	4	12
	Red wine	0.05	5	131	119	9	5

Linearity:	The linearity of the GC-MS detector was tested using eight standards at concentrations between 0.001-0.2 mg/kg for grapes and 0.002-0.1 mg/L for wine. Linear correlations with coefficients ≥0.99 were obtained for boscalid.
Specificity:	GC-MS is a highly specific detection technique and therefore a confirmatory technique is not needed. Analysis is possible for three ions.
Matrix Effects:	To avoid matrix effects, analyte protectants were used.
Interference:	Triphenylphosphate was used as internal standard to correct for the variability in GC injection and MS detection response. No interference was observed for Boscalid.
Limit of Quantitation:	The LOQ was calculated by the signal-to-noise ratios obtained by analyzing unspiked grape and wine samples. For boscalid, an LOQ of 0.001 mg/kg was indicated for white grapes, of 0.002 mg/kg for red grapes and white wine and of 0.003 mg/kg for red wine. The lowest tested fortification level by this method was 0.05 mg/kg for boscalid.
Limit of Detection:	The LOD was calculated by the signal-to-noise ratios obtained by analyzing unspiked grape and wine samples. For boscalid, an LOD of <0.001 mg/kg was indicated for white grapes and of 0.001 mg/kg for red grapes and wine.
Repeatability:	The relative standard deviations (RSD, %) for all commodities and fortification levels were <20%. The detailed values are shown in the table above.
Reproducibility:	The method was successfully applied to real samples treated with pesticides.
Stability of Solutions:	All standard solutions were stored in dark vials at 4°C. Grapes were stored at -18°C until use.
Conclusion:	**The GC-MS multi residue analytical method can be used for the analysis of boscalid in plant matrices with high acid content.**

Report: CA 4.2/3
Lee S.J. et al., 2008 a
Multiresidue analysis of pesticides with hydrolyzable functionality in cooked vegetables by liquid chromatography tandem mass spectrometry
2009/1132363
Guidelines: none
GLP: no

Principle of the method: Residues of boscalid were extracted from boiled or finely chopped cucumber, onion, potato, sweet potato and radish samples with acetonitrile. The extract was filtered before phase partitioning with sodium chloride. An aliquot of the acetonitrile layer was evaporated and redissolved in methanol. Thereafter the aliquot was filtered. The final determination was performed by LC-MS/MS equipped with an Agilent Zorbox XDB-C_{18} column with a water/methanolic ammonium formate gradient. For quantitation and confirmation of boscalid, mass transitions m/z 343→307 and 343→140 are used.

Recovery findings: Two sets of samples fortified at 0.1 mg/kg and 1 mg/kg were analyzed. The mean recovery values were between 70% and 120% for 0.1 mg/kg. At the fortification level of 1.0 mg/kg only radish was in the tolerated range of 70%-110%. For cucumber, onion and sweet potato only slight exceedances were observed with 112-117%, while the average recovery for potato was 125%. Detailed results are given in table below.

Table 4.2-4: Validation results of LC-MS/MS multi-residue method: boscalid in vegetables

Test substance	Matrix	Fortification level (mg/kg)	No of analyses	Average recovery (%)		Relative standard deviation (%)	
Transition				**343→307**	**343→140**	**343→307**	**343→140**
Boscalid (BAS 510 F)	Cucumber	0.1	n r	93	n.r	12	n.r
		1.0	n r	112	n.r	6	n.r
	Onion	0.1	n r	103	n.r	12	n.r
		1.0	n r	113	n.r	12	n.r
	Potato	0.1	n r	113	n.r	3	n.r
		1.0	n r	125	n.r	2	n.r
	Sweet potato	0.1	n r	101	n.r	6	n.r
		1.0	n r	117	n.r	22	n.r
	Radish	0.1	n r	90	n.r	15	n.r
		1.0	n r	94	n.r	8	n.r

n.r. not reported

Linearity:	The linearity was tested using five calibration samples at concentrations between 0.01-2.0 µg/mL. Linear correlations with coefficients ≥0.995 were obtained for all testes substances.
Specificity:	LC-MS/MS is a highly specific detection technique and therefore a confirmatory technique is not required. Analysis is possible at two ion transitions.
Matrix Effects:	Signal enhancement (19%) was found for boscalid in the study; cooked radish was used as representative matrix. To minimize matrix effects, matrix-matched standards were used.
Limit of Quantitation:	The LOQ was calculated by the signal-to-noise ratios obtained by analyzing unspiked samples. The lowest tested fortification level by this method was 0.1 mg/kg for boscalid.
Limit of Detection:	The LOD was calculated by the signal-to-noise ratios obtained by analyzing unspiked samples. For boscalid, an LOD of 0.0015, 0.0023, 0.003, 0.0042 and 0.0016 mg/kg was indicated for cucumber, onion, potato, sweet potato and radish, respectively.
Repeatability:	The relative standard deviations (RSD, %) for all commodities were ≤15% except for sweet potato with 22% at the 1.0 mg/kg fortification level. The detailed values are shown in the table above.
Reproducibility:	The method was successfully applied to real vegetable samples
Stability of Solutions:	The final extracts were stored at 4°C before injection.
Conclusion:	**The LC-MS/MS multi residue analytical method can be used for the analysis of boscalid in several plant matrices.**

Report: CA 4.2/4
Walorczyk S., 2008 a
Development of a multi-residue method for the determination of pesticides in cereals and dry animal feed using gas chromatography-tandem quadrupole mass spectrometry: II. improvement and extension to new analytes
2008/1103137
Guidelines: none
GLP: no

Principle of the method: Pesticides were extracted from finely ground sub-samples after addition of triphenylphosphate (internal standard) and acetonitrile using a modified QuEChERS method. After adding several salts, the mixture was shaken and centrifuged. At this stage, an optional low-temperature clean step was performed prior to dispersive-SPE for the most complex matrices such as maize and compound feeds. Therefore, an aliquot of the supernatant was transferred into a glass test tube and stored for at least 2 hours in a freezer (−26°C). The extract was then separated from the precipitates by simple decantation. An aliquot of the extract was transferred into a polypropylene centrifuge tube and dispersive SPE using magnesium sulphate, C18, PSA and acetonitrile was conducted. The supernatant was acidified by adding acetonitrile with 5% (v/v) formic acid. After evaporation of the extract, the residue was re-dissolved in toluene. A second internal standard (PCB 153) was added.
The final determination was performed by GC-MS/MS (Varian CP-3800) equipped with electronic flow control, a 1079 universal capillary injector, and a CP-8400 autosampler. The GC system was interfaced with a model 1200 triple quadrupole mass spectrometer (Varian) operating in electron ionization mode. For quantification and confirmation of boscalid, ions at m/z 140, 112 and 76 were used.

Recovery findings: Recoveries of pesticides from wheat grain were determined for six replicates at four spiking levels of 0.01, 0.05, 0.1 and 0.5 mg/kg. Recoveries from a feed mixture were determined for five replicates at two spiking levels of 0.01 and 0.1 mg/kg. In wheat grain and feed mixture the mean recovery values were between 70% and 120%. The detailed results are given below.

Table 4.2-5: Validation results of boscalid in wheat grain and feed mixture.

Test substance	Matrix	Fortification level (mg/kg)	No of analyses	Average recovery (%)		Relative standard deviation (%)	
		Transition		140→112	140→76	140→112	140→76
Boscalid (BAS 510 F)	Wheat grain	0.01	6	98	n r	13	n r
		0.05	6	108	n r	3	n r
		0.1	6	94	n r	5	n r
		0.5	6	99	n r	2	n r
	Feed mixture	0.01	5	119	n r	1	n r
		0.1	5	100	n r	8	n r

Linearity: The linearity of the calibration curves was evaluated at a concentration range between 0.0075 and 0.75 mg/kg by duplicate analyses of seven calibration standards prepared in blank matrix extract in toluene. Good linearity was observed for boscalid with correlation coefficients ≥0.99.

Specificity: GC-MS/MS using three ions is a highly specific detection technique and therefore a confirmatory technique is not required *(SANCO/825/00rev. 8, 16/11/2010)*. Although, only data for one mass transition were presented.

Matrix effects: To minimize matrix effects, matrix-matched calibration standards were used.

Limit of Quantitation: Limit of quantification was defined as the lowest spiking level, at which the validation was achieved. The lowest tested fortification level by this method was 0.01 mg/kg.

Repeatability: The relative standard deviations (RSD, %) were <20% for fortification level of 0.01 mg/kg and below 10% for fortification levels 0.05, 0.1 and 0.5 mg/kg. The detailed values are shown in the table above.

Conclusion: **The multi residue method fulfills the requirements with regard to the specificity, repeatability, replications and recoveries.**

Report: CA 4.2/5
Hanot V. et al., 2015 a
A simple multi-residue method for the determination of pesticides in fruits and vegetables using a methanolic extraction and ultra-high-performance liquid chromatography-tandem mass spectrometry: Optimization and extension of scope
2015/1240157
Guidelines: none
GLP: no

Principle of the method: Residues of boscalid were extracted from homogenized lettuce and orange samples using ammonium acetate in methanol/water (95:5, v/v). After several filtration steps and a dilution step right before injection, analysis was performed by UPLC-MS/MS in ESI+ mode. Analysis was accomplished using an Acquity BEH C18 column and a water/methanol gradient with ammonium acetate as modifier.

Recovery findings: The mean recovery values were between 70% and 110%, except for orange at 0.01 mg/kg fortification with 111.8%.

Table 4.2-6: Validation results of UPLC-MS/MS method: boscalid (BAS 510 F) in plant matrices

Test substance	Matrix	Fortification level (mg/kg)	No of analyses	Average recovery (%)		Relative standard deviation (%)	
Transition				343 → 307	343 → 271	343 → 307	343 → 271
Boscalid (BAS 510 F)	Lettuce	0.01	6	80.3	n.r.	8.2	n r.
		0.10	6	86.9	n.r.	2.7	n r.
	Orange	0.01	6	111.8	n.r.	7.4	n r.
		0.10	6	86.5	n.r.	5.4	n r.

n.r. not reported

Linearity: Linearity was tested over a concentration range of 0 to 0.1 µg/mL (equal to 0 to 1 mg/kg matrix equivalent) (7 concentration levels). A correlation coefficient of at least 0.99 was targeted.

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

Matrix effects: Matrix effect was evaluated by comparing calibration curve from standard solutions in solvent with calibration curve in matrix extract at 7 identical concentration levels. Despite the dilution step before injection, a small matrix effect still occurs for most of the pesticides. Matrix matched standards are therefore needed for correct quantitation.

Interference: No interference peak was observed at a level higher than the LOQ.

Limit of Quantitation: The limit of quantitation was 0.01 mg/kg.

Repeatability: The relative standard deviations (RSD, %) were <10%.

Reproducibility: The described method is used routinely for the European coordinated control program. Several quality assurance criteria have been set, i.e. internal standard is used to check that samples have been properly injected, and calibration curves have to demonstrate a good correlation fit (≥0.995).

Conclusion: **The UPLC-MS/MS multi-residue analytical method can be used for the analysis of boscalid in plant matrices with high water and high acid content.**

Report: CA 4.2/6
Munoz E. et al., 2011 a
Multiresidue method for pesticide residue analysis in food of animal and plant origin based on GC or LC and MS or MS/MS
2012/1369202
Guidelines: none
GLP: no

Principle of the method: Residues of boscalid were extracted from diverse homogenized food matrices of plant origin with low fat content using accelerated solvent extraction and ethyl acetate as extraction solvent. Samples such as fat and oil were dissolved with dichloromethane. Subsequently, aliquots were evaporated to dryness – or, if required (i.e. chlorophyll or high fat content) were purified by gel permeation chromatography using ethyl acetate and cyclohexane, and/or – dependent on chlorophyll content – by SPE. Final determination was performed by UPLC-MS/MS in ESI+ mode. Analysis was accomplished using a Waters Acquity UPLC BEH C_{18} column and a water/methanol gradient with formic acid as modifier. For quantitation of boscalid mass transition 343→307 was used (ESI+). The results were confirmed by mass transition 343→140.

Recovery findings: Recovery was tested at three levels (0.01, 0.02 and 0.4 mg/kg) in a minimum of five replicates per level for representative matrices of both, plant and animal origin (apple or pear, cucumber, orange or lemon, spinach, raisins, bean or flour, biscuits, tea, pepper, fish, cheese, hazelnuts, oil, honey). Baby food was tested at 3 levels (0.003 or 0.006, 0.015 and 0.03 mg/kg) with five replicates per level (baby milk, baby cereals, baby food and fruit, baby food, vegetables and fish). The mean recovery values were between 70% and 130%. No detailed results were reported.

Linearity: Linearity was tested over a concentration range of 0.003-0.1 mg/kg for baby food and 0.01-0.4 mg/kg for all other matrices using five concentration levels, respectively. A correlation coefficient of >0.98 was achieved.

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

Matrix effects: Matrix matched standards were used for quantitation.

Limit of Quantitation: The limit of quantitation was 0.01 mg/kg.

Repeatability: The relative standard deviations (RSD, %) were <20%.

Reproducibility: Recovery determination was performed on at least three days for all matrices applying the same procedure as described under recovery finding above. Results were within 70-130% recovery and <20% relative standard deviation.

Conclusion: **The UPLC-MS/MS multi-residue analytical method can be used for the analysis of boscalid in diverse food matrices of animal and plant origin.**

Report: CA 4.2/7
Walorczyk S.,Drozdzynski D., 2012 a
Improvement and extension to new analytes of a multi-residue method for the determination of pesticides in cereals and dry animal feed using gas chromatography-tandem quadrupole mass spectrometry revisited
2012/1369183
Guidelines: none
GLP: no

Principle of the method: Samples were extracted with water/acetonitrile mixture (2:3, v/v) and several salts. Clean-up was achieved by dispersive solid-phase extraction. An aliquot of the extract was evaporated and reconstituted in toluene for GC-MS/MS analysis. Analytes were separated on an Agilent DB-5 MS column. Helium was used as carrier gas and argon as collision gas.

Recovery findings: The mean recovery values were between 70% and 110%. The detailed results are given below.

Table 4.2-7: Validation results of GC-MS/MS multi-residue method: boscalid in buckwheat and rye

Test substance	Matrix	Fortification level (mg/kg)	No of analyses	Average recovery (%)		Relative standard deviation (%)	
Transition				140 → 112	140 → 76	140 → 112	140 → 76
Boscalid	Buckwheat	0.01	6	99	n r.	5	n r.
		0.05	6	96	n r.	4	n r.
		0.25	6	100	n r.	2	n r.
	Rye	0.01	6	98	n r.	4	n r.
		0.05	6	100	n r.	5	n r.
		0.25	6	97	n r.	2	n r.

n.r. not reported

Linearity: Linearity of calibration curves was studied in the concentration range between 0.005 and 0.5 µg/mL using seven calibration standards. Good linearity was observed for a vast majority of the compounds with correlation coefficient >0.99.

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

Matrix effects: Matrix matched calibration was used for quantification purposes.

Limit of Quantitation: The limit of quantification (LOQ) was 0.01 mg/kg for boscalid.

Repeatability: The relative standard deviations (RSD, %) were ≤10%. The detailed values are shown in Table 4.2-7.

Reproducibility: The applicability of the method to routine analysis of roughly 900 real samples with satisfactory results of quality control samples clearly demonstrated that on-going performance and ruggedness of the developed approach was highly satisfactory 22% recovery with 22% RSD for 20 samples in case of boscalid.

Conclusion: **The GC-MS/MS multi residue analytical method can be used for the analysis of boscalid in cereals and animal feeds.**

Food of animal origin

For food of animal origin, the relevant residue for both enforcement and risk assessment is defined as boscalid in muscle, fat, milk and eggs and as the sum of boscalid and its hydroxy metabolite M510F01 (free and conjugated), expressed as boscalid, in liver and kidney using residue analytical method 471/0. Successful cleavage of the conjugate was confirmed as part of the validation of method 471/0, using isolated ^{14}C-labelled metabolite M510F02. Potential impact of the enzyme load on the LC-MS/MS analysis was additionally assessed and validated as part of residue analytical method 471/0.

Method 476/0 allows the determination of bound residues as metabolite M510F53 in liver.
Although methods are considered fully valid, a new ILV (independent laboratory validation) of BASF method 471/0 was conducted to comply with the latest requirement of the SANCO guideline 825/00 rev. 8.1. Within this ILV, stability aspects of standards and extracts, as well as a detailed assessment of matrix effects, are addressed.

Both analytical methods, 471/0 and 476/0, are peer-reviewed and considered fully valid for determination of residues of boscalid and its relevant metabolites in food of animal origin (EFSA Journal 2014;12(7): 3799). As the methods are already peer-reviewed, they are not summarised again in detail.

Enforcement levels are in food of animal origin with an LOQ of 0.01 mg/kg in milk and eggs, and an LOQ of 0.025 mg/kg in muscle and fat. Boscalid and its metabolite M510F01 (free and conjugated) can be enforced in liver and kidney with an LOQ of 0.025 mg/kg for each compound (EFSA Journal 2014;12(7):3799 15). Additionally, the ILV conducted also validated a lower fortification level of 0.01 mg/kg also in liver and kidney. This is considered as additional information.

Report:	CA 4.2/8 Weber H.,Zetzsch A., 2015 a Independent laboratory validation of the BASF method L0041/01 (471/0) for the determination of BAS 510 F (Boscalid) and metabolite M510F01 in animal matrices 2015/1114666
Guidelines:	SANCO/825/00 rev. 8.1 (16 November 2010), SANCO/3029/99 rev. 4 (11 July 2000), OECD Guidance Document on Pesticide Residue Analytical Methods (ENV/JM/MONO(2007)17 - 13-Aug-07, EPA 860.1340 (1996)
GLP:	yes (certified by Freie und Hansestadt Hamburg, Behoerde fuer Gesundheit und Verbraucherschutz, Hamburg, Germany)

AND

Report:	CA 4.2/9 Weber H., 2015 a Amendment No. 1 - Independent laboratory validation of the BASF method L0041/01 (471/0) for the determination of BAS 510 F (Boscalid) and metabolite M510F01 in animal matrices 2015/1251211
Guidelines:	SANCO/825/00 rev. 8.1 (16 November 2010), SANCO/3029/99 rev. 4 (11 July 2000), OECD Guidance Document on Pesticide Residue Analytical Methods (ENV/JM/MONO(2007)17 - 13-Aug-07, EPA 860.1340 (1996)
GLP:	yes (certified by Freie und Hansestadt Hamburg, Behoerde fuer Gesundheit und Verbraucherschutz, Hamburg, Germany)

Remark: BASF residue analytical method L0041/01 (471/0) is used as data generation method for pre-registration purposes. Although an independent laboratory validation exists, a new ILV was conducted for method 471/0 to comply with the latest requirements of the updated SANCO guideline 825/00 rev. 8.1. This ILV addresses stability assessment in standard solutions and extracts as well as a detailed assessment of matrix effects in the various animal matrices. A detailed summary of the ILV is given below.

Principle of the method: Residues of boscalid and its metabolite M510F01 were extracted from homogenized samples of animal matrices (muscle, kidney, liver, fat, milk, cream and egg) with methanol. An aliquot of the methanol extract was evaporated to dryness, re-dissolved in buffer solution and incubated with ß-glucuronidase/arylsulfatase. Then a liquid/liquid partition with ethyl acetate was carried out and the organic phase was purified on SPE C18. The final determination was performed by LC-MS/MS (1200 Binary Rapid Resolution LC System) equipped with a Nucleosil 100-5 C18 column and an API 4000 ESI detector (AB Sciex).

For quantitation of boscalid and M510F01 mass transitions 343→307 and 359→323 were used (ESI+), respectively. The results were confirmed by mass transitions 343→271 and 359→140 (ESI+), respectively.

Recovery findings: For validation, untreated livestock samples (muscle, kidney, liver, fat, cream milk and egg) were fortified with boscalid and M510F01 and analyzed according to the established method validation guidelines. The method was proved to be suitable to determine residues of BAS 510 F in all matrices tested (muscle, kidney, liver, fat, milk, cream and egg), as the mean recovery values per fortification level were between 70% and 100%. Detailed results are given in table below.

Table 4.2-8: Validation results of Boscalid in different animal matrices

Test substance	Matrix	Fortification level (mg/kg)	No of analyses	Average recovery (%)		Relative standard deviation (%)	
Transition				343→307	343→271	343→307	343→271
Boscalid (BAS 510 F)	Muscle	0.01	5	83.3	80.7	4.8	5.1
		0.025	5	78.8	80.7	3.5	6.1
		0.25	5	80.0	80.2	4.7	4.7
	Kidney	0.01	5	77.7	77.5	4.6	8.3
		0.025	5	78.1	78.9	4.0	4.8
		0.25	5	74.0	72.5	2.9	3.2
	Liver	0.01	5	71.6	78.3	3.1	4.9
		0.025	5	70.8	73.4	8.2	11
		0.25	5	71.9	72.8	5.4	4.4
	Fat	0.01	5	87.6	87.5	8.2	8.0
		0.025	5	84.4	83.9	6.2	9.8
		0.25	5	80.9	80.4	7.3	9.5
	Cream	0.01	5	73.5	71.2	3.3	4.2
		0.10	5	76.2	78.2	5.8	6.3
	Milk	0.01	5	75.6	72.5	5.3	5.0
		0.10	5	85.9	86.8	2.6	5.6
	Egg	0.01	5	75.7	74.7	2.1	5.5
		0.10	5	89.9	89.1	3.0	2.4
M510F01	Muscle	0.01	5	78.3	76.4	6.3	7.0
		0.025	5	81.7	82.6	4.7	3.8
		0.25	5	82.6	83.0	4.8	4.1
	Kidney	0.01	5	83.1	81.9	2.1	6.7
		0.025	5	79.6	82.0	4.3	5.5
		0.25	5	73.8	75.3	2.2	3.0
	Liver	0.01	5	75.1	79.6	3.5	5.7
		0.025	5	74.7	75.4	5.6	6.3
		0.25	5	80.9	82.2	6.6	6.1
	Fat	0.01	5	84.5	82.1	6.6	6.4
		0.025	5	86.8	84.0	1.8	3.8
		0.25	5	80.6	80.9	3.3	4.2
	Cream	0.01	5	79.3	83.4	3.3	5.0
		0.10	5	86.7	87.0	6.6	6.8
	Milk	0.01	5	85.4	80.8	3.2	5.3
		0.10	5	86.8	85.8	11	10
	Egg	0.01	5	81.7	79.3	6.0	2.2
		0.10	5	94.5	96.7	2.8	1.9

Linearity: The linearity of the LC-MS/MS detector was tested using nine calibration standards at concentrations between 1.25 ng/ml and 500 ng/ml for both analytes. Each run at least 5 standards were injected and the response plotted against concentration. Linear correlations with coefficients ≥0.99 were obtained for boscalid and M510F01.

Specificity: LC-MS/MS using two mass transitions is a highly specific detection technique and therefore a confirmatory technique is not required.

Matrix Effects: Matrix effects on the detection of BAS 510 F (Boscalid) in extracts of animal matrices (muscle, kidney, liver, fat, milk, cream and egg) were found to be insignificant (<20%). Therefore, solvent standards were used for quantification.

Interference: No significant interference above 30% of LOQ was detected in at the retention times and mass transitions of any of the control specimen extracts of each matrix (muscle, kidney, liver, fat, cream milk and egg).

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

Repeatability: The relative standard deviations (RSD, %) for all commodities and fortification levels were <15%. The detailed values are shown in the table above.

Reproducibility: This study represents the independent laboratory validation and proves the reproducibility of method L0041/01 (471/0).

Stability of Solutions: Analytical standards of boscalid and M510F01 were found to be stable for at least 21 days when stored refrigerated (5°C ± 4°C) in the dark when prepared in acetonitrile.

After extraction, both analytes were found to be stable in the final extract of all matrices for at least 9 days when stored refrigerated (5°C ± 4°C) in the dark.

Conclusion: **The analytical method L0041/01 (471/0) could be demonstrated to fulfill the requirements with regard to specificity, repeatability, limit of quantification and recoveries and is therefore considered fully suitable for the analysis of boscalid and M510F01 in animal matrices (muscle, kidney, liver, fat, milk, cream and egg) with an LOQ of 0.01 mg/kg for enforcement purposes**.

Additional information from scientific literature:

In the literature search conducted for boscalid, additional information on the analysis of boscalid in various animal matrices were found. They were considered as useful additional information to the methodology provided. The relevant scientific literature presenting a multimethod approach using ethyl acetate extraction for chicken tissue and liver tissue is summarised below.

Report: CA 4.2/10
Taylor M.J. et al., 2012 a
A liquid chromatography-electrospray tandem mass spectrometry method for the determination of multiple pesticide residues involved in suspected poisoning of non-target vertebrate wildlife, livestock and pets
2012/1369182
Guidelines: none
GLP: no

Principle of the method: Residues of boscalid were extracted from homogenized samples with ethyl acetate. After filtration, the extract was evaporated and diluted in cyclohexane and ethyl acetate. An aliquot was diluted in methanol, evaporated again and diluted with methanol containing ammonium acetate. After filtration, analysis was performed by LC-MS/MS in the positive ionization mode. Analysis was performed on a Thermo Fisher Scientific Hypersil Gold C_{18} BDS column using a methanol/ aqueous methanol gradient with ammonium acetate as modifier. For quantitation and confirmation of boscalid, mass transitions m/z 343→307 and 343→140 are used, respectively.

Recovery findings: Recovery was determined following analysis of samples fortified with 0.1 and 1.0 mg/kg. The mean recovery values were between 70% and 110% for boscalid. Detailed results are given in table below.

Table 4.2-9: Validation results of LC-MS/MS method: boscalid (BAS 510 F) in chicken matrices

Test substance	Matrix	Fortification level (mg/kg)	No of analyses	Average recovery (%)		Relative standard deviation (%)	
			Transition:	343→307	343→140	343→307	343→140
Boscalid (BAS 510 F)	Liver	0.1	5	70	n.r.	n.r.	n r.
		1.0	6	87	n.r.	n.r.	n r.
	Muscle	0.1	6	72	n.r.	n.r.	n r.
		1.0	6	80	n.r.	n.r.	n r.

n.r. not reported

Linearity:	The linearity was tested using four standards at concentrations between 0.025 mg/L-0.50 mg/L. Linear correlations with coefficients ≥0.96 were obtained.
Specificity:	LC-MS/MS is a highly specific detection technique and therefore a confirmatory technique is not required. Analysis is possible at two different mass transitions.
Matrix Effects:	To minimize matrix effects, matrix matched standards were used. Signal to noise ratio was larger than 5 to 1.
Limit of Quantification:	The lowest level tested by fortification experiments is 0.1mg/kg.
Limit of Detection:	The lowest calibration standard, equivalent the LOD is 0.025 mg/L.
Repeatability:	The relative standard deviations (RSD, %) for both matrices and fortification levels were <10%. The detailed values are shown in the table above.
Reproducibility:	The method was found also suitable for the analysis of digestive tract content and blood, vomit and fecal specimens. In addition, the method is routinely and successfully applied to the analysis of carcasses, meat or eggs.
Stability of Solutions:	Extract stability was assessed for some analytes not including boscalid for a period of 7 days at 5°C.
Conclusion:	**The LC-MS/MS multi-residue analytical method can be used for the analysis of boscalid in animal matrices for enforcement purposes.**

(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

Soil

No specific monitoring method, e.g. based on a multimethod approach was developed. The residue analytical method described in chapter M-CA 4.1.2/2 (update the most recent guidelines) is fully suitable as monitoring method and hence proposed as monitoring method for soil.

Water

Report:	CA 4.2/11 Penning H., 2009a Validation of analytical method L0127/1 for the determination of BAS 510 F (Boscalid) residues in surface water and groundwater 2008/1086809
Guidelines:	EEC 91/414 Annex IIA, EEC 91/414 Annex IIIA, SANCO/3029/99 rev. 4 (11 July 2000)
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

AND

Report:	CA 4.2/12 Ertunc T.,Studenroth S., 2015 c Report Amendment No. 1 to final report: Validation of analytical method L0127/1 for the determination of BAS 510 F (Boscalid) residues in surface water and groundwater 2015/1174526
Guidelines:	EEC 91/414 Annex IIA, EEC 91/414 Annex IIIA, SANCO/3029/99 rev. 4 (11 July 2000)
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Remark: The Amendment issued in 2015 contains additional information on matrix effects and reports the results of the second mass transition. Information from the originally issues report and the amendment are summarised together.

Principle of the method

Analytical method L0127/01 is developed for the determination of boscalid (BAS 510 F) in soil by HPLC-MS/MS with a limit of quantification of 0.03 µg kg^{-1}.
10 mL of a water sample is extracted on a pre-conditioned C18 SPE column. After drying the column with a stream of N_2 at 30°C for 45 min, the column is prewashed with cyclohexane, which is discarded. Then, boscalid is eluted from the column with 2 x 2.5 mL cyclohexane/ethyl acetate (1/1, v/v). The collected eluates are evaporated to dryness in the evaporator at 40°C water bath temperature. The residues are dissolved in methanol/water (80/20, v/v).
Final determination is performed by HPLC-MS/MS using a Betasil C_{18} analytical column and a gradient mixture of water/formic acid (1000/1) and methanol/formic acid (1000/1) at a flow rate of 0.6 mL min^{-1}. Detection is accomplished in ESI positive mode at mass transitions 343→271 and 343→307 for quantification and confirmation.

Recovery findings

The results show that the method is suitable to determine residues of boscalid in water. Samples spiked with the analyte at the limit of quantification of 0.03 µg kg^{-1} and ten times higher (0.30 µg kg^{-1}) have average recovery values (mean of five replicates per fortification level and analyte) between 70% and 110%. The detailed results are given in the table below (Table 4.2-10).

Table 4.2-10: Results of the method validation for the determination of boscalid in ground- and surface water

Analyte	Soil	m/z	Fortification level [µg kg^{-1}]	Number of Replicates	Mean recovery [%]	RSD [%]	Overall Recovery [%]	RSD [%]
Boscalid	Ground-water	343 → 271	0.03	5	89.9	3.8	88.7	6.2
			0.3	5	87.6	8.4		
		343 → 307	0.03	5	90.8	5.6	90.2	6.0
			0.3	5	89.6	6.9		
	Surface water	343 → 271	0.03	5	90.1	4.5	92.7	5.2
			0.3	5	95.2	4.5		
		343 → 307	0.03	5	86.9	3.2	91.0	7.0
			0.3	5	95.1	6.9		

RSD = Relative standard deviation

Linearity

Good linearity (r > 0.999) was observed in the range of 0.025 ng mL^{-1} to 0.5 ng mL^{-1} for two mass transitions of boscalid in two different water types (surface water and groundwater). At least seven calibration standards prepared in methanol/water (80/20, v/v) were used.

Specificity	Under the described conditions the method is specific for the determination of boscalid in water. Significant interferences (> 30% of LOQ) were not observed at the retention time of boscalid in the untreated ground- and surface water control samples. Due to the high selectivity and specificity of HPLC-MS/MS an additional confirmatory technique was not necessary. Two mass transitions of boscalid were quantified.
Matrix Effects	Matrix effects were tested by comparing solvent-based standard solutions (prepared in methanol/water, 80/20, v/v) with matrix-matched standards (quality control samples) at a concentration of 0.15 ng mL^{-1}. The findings showed that the matrix load in the tested quality control samples had only negligible influence on the analysis of boscalid, therefore, the use of matrix-matched standards is not needed.
Limit of Quantification	The method has a limit of quantification (LOQ) of 0.03 µg kg^{-1} boscalid in water, corresponding to the lowest fortification level.
Limit of Detection	The limit of detection (LOD) is 0.025 ng mL^{-1}, which is the lowest calibration standard.
Repeatability	The relative standard deviations (RSD, %) for all fortification levels were below 20%.
Standard Stability	The standard stability of boscalid in methanol/water (80/20, v/v) was determined within BASF DocID 2000/1014856, assessing stability of various analytes in solvents, including boscalid, in standard solutions. The standard solutions were stable over a time period of at least 4 weeks when stored under refrigerated conditions at 4°C in the dark. Stability of calibration standards was additionally assessed in the independent laboratory validation BASF DocID 2015/111468 (see M-CA 4.2/13)
Extract Stability	The extract stability was not determined within the validation study.
Reproducibility	Reproducibility of the method was not determined within this validation study but in the independent laboratory validation successfully carried out (see M-CA 4.2/13).
Conclusion	**The method L0127/01 for analysis of boscalid in surface water and groundwater used HPLC-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, limit of quantification and recoveries and is therefore applicable to correctly determine boscalid in ground- and surface water.**

Report:	CA 4.2/13 Ertunc T.,Studenroth S., 2015 b Validation of analytical method L0127/02 for the determination of M510F47 (Reg.No. 107371) and M510F49 (Reg.No. 391572) in surface and groundwater 2015/1109588
Guidelines:	SANCO/825/00 rev. 8.1 (16 November 2010), SANCO/3029/99 rev. 4 (11 July 2000), EPA 850.6100, OECD-ENV/JM/MONO/(2007)17 (OECD No. 39), OECD-ENV/JM/MONO/(2007)17 (OECD No. 72)
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Principle of the method

The analytical method L0127/02 is developed for the determination of boscalid (BAS 510 F) metabolites M510F47 (Reg. No. 107371) and M510F49 (Reg. No. 391572) in surface and groundwater by LC-MS/MS.

The enrichment and clean-up of both analytes is accomplished using solid-phase extraction (SPE).

For analysis of M510F47, a 50 mL water sample is concentrated using OASIS HLB SPE-cartridges. The analyte is eluted from the column using methanol, with the eluate being evaporated to dryness and re-constituted in 1 mL pure water. Prior to sample concentration over a SPE-column, the natural water sample needs to be acidified to a pH $\leq$ 2 by addition of 6 M HCl.

For analysis of M510F49, a 10 mL water sample is concentrated using Octadecyl (C18) Bakerbond™ SPE columns. The analyte is eluted from the column using methanol. The eluate is evaporated to dryness and re-constituted in 2 mL of a mixture of methanol/pure water (80/20, v/v).

Analysis is accomplished by LC-MS/MS using a XSelect HSST3 column (M510F47) or Betasil C18 column (M510F49) using a gradient mixture of water (0.1% formic acid) and methanol (0.1% formic acid). Detection is accomplished in ESI positive mode for M510F47 and in ESI negative mode for M510F49 at mass transitions 158→122 (M510F47) and 323→202 (M510F49) for quantification and 158→94 (M510F47) and 323→94 (M510F49) for confirmation.

Recovery findings

The results show that the method is suitable to determine residues of boscalid metabolites M510F47 and M510F49 in water. Samples spiked with the analytes at the limit of quantification of 0.03 µg L^{-1} and ten times higher (0.30 µg L^{-1}) have average recovery values (mean of five replicates per fortification level and analyte) between 70% and 110%. The detailed results are given in the table below (Table 4.2-11).

Table 4.2-11: Results of the method validation for the determination of boscalid metabolites M510F47 and M510F49 in surface and groundwater

Analyte	Water	m/z	Fortification level [µg L^{-1}]	Number of Replicates	Mean Recovery [%]	RSD [%]	Overall Recovery [%]	RSD [%]
M510F47	Surface Water	158 → 122	0.03 0.3	5 5	94.0 89.1	2.1 4.4	91.5	4.3
		158 → 94	0.03 0.3	5 5	90.8 86.9	6.0 3.0	88.9	5.0
	Ground-water	158 → 122	0.03 0.3	5 5	88.3 89.1	2.8 2.7	88.7	2.6
		158 → 94	0.03 0.3	5 5	89.6 88.1	1.7 1.7	88.9	1.8
M510F49	Surface Water	323 → 202	0.03 0.3	5 5	79.3 77.7	3.8 4.4	78.5	4.0
		323 → 94	0.03 0.3	5 5	79.9 78.3	3.7 5.1	79.1	4.3
	Ground-water	323 → 202	0.03 0.3	5 5	78.1 73.6	4.8 9.1	75.8	7.5
		323 → 94	0.03 0.3	5 5	77.6 73.0	6.9 9.6	75.3	8.4

RSD = Relative standard deviation

Linearity

Good linearity of r > 0.9989 was observed in the range of 0.35 ng mL^{-1} to 10 ng mL^{-1} for the two mass transitions of M510F47 and r > 0.998 was observed in the range of 0.01 ng mL^{-1} to 1.0 ng mL^{-1} for the two mass transitions of M510F49. At least seven calibration points distributed over each tested concentration range were used.

Specificity

LC-MS/MS is a highly specific self-confirmatory technique. Under the described conditions the method is specific for the determination of the two metabolites in water. Significant interferences (> 30% of LOQ) were not observed at the retention times and mass transitions of the analytes.

Due to the high selectivity and specificity of LC-MS/MS an additional confirmatory technique was not necessary.

Matrix Effects

Matrix effects were tested preparing matrix-matched standards for each matrix and analyte. It was shown that the matrix-load in the tested matrix-matched standards of M510F47 had an influence on the detection. Therefore, matrix-matched standards of M510F47 are needed for further experiments.

Limit of Quantification

The method has a limit of quantification (LOQ) of 0.03 µg L^{-1} per analyte, corresponding to the lowest fortification level.

Limit of Detection	The limit of detection (LOD) for M510F47 is 0.007 µg L^{-1} and the LOD for M510F49 is 0.002 µg L^{-1}.
Repeatability	The relative standard deviations (RSD, %) for all fortification levels were < 20%.
Standard Stability	The storage stability of standard solutions of M510F47 (prepared in pure water) and M510F49 (prepared in methanol/water (80/20, v/v)), was investigated. The results demonstrate stability of both analytes for a maximum duration of 29 days when stored refrigerated at 4 ± 2°C in the dark.
Raw Extract Stability	Analytes M510F47 and M510F49 were considered stable in methanol extracts of surface and groundwater samples over a time period of 7 days, when stored refrigerated at 4 ± 2°C in the dark.
Final Extract Stability	Analytes M510F47 and M510F49 were stable in the final extracts of surface and groundwater samples over a time period of 7 days, when stored refrigerated at 4 ± 2°C in the dark. M510F47 extracts were prepared in pure water and M510F49 extracts were prepared in methanol/pure water (80/20, v/v).
Reproducibility	Reproducibility of the method was determined within an independent laboratory validation study summarized in section CA 4.2/13 [*BASF DocID 2015/1114668*].
Conclusion	**The method for analysis of boscalid metabolites M510F47 (Reg. No. 107371) and M510F49 (Reg. No. 391572) in water 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, limit of quantification and recoveries and is therefore applicable to correctly determine residues of boscalid metabolites M510F47 and M510F49 in surface and groundwater.**

Report:	CA 4.2/14 Goecer M., 2015 a Independent laboratory validation (ILV) of the BASF method L0127 for the determination of Boscalid (BAS 510 F) and two of its metabolites M510F47 (Reg.No. 107371) and M510F49 (Reg.No. 391572) in surface water and groundwater 2015/1114668
Guidelines:	SANCO/3029/99 rev. 4 (11 July 2000), SANCO/825/00 rev. 8.1 (16 November 2010), EPA 850.6100 (2012), EC 1107/2009 of the European Parliament and of the Council of 21 Oct 2009
GLP:	yes (certified by Landesanstalt fuer Umwelt, Messungen und Naturschutz Baden-Wuerttemberg, Karlsruhe, Germany)

Remark: The objective of the study was to independently validate the determination of boscalid (BAS 510 F) and two of its metabolites M510F47 (Reg. No. 107371) and M510F49 (Reg. No. 391572) with LC-MS/MS in surface water and ground water, hence method version L0127/01 and L0127/02 were together independently validated in study 2015/1114668.

Principle of the method

Boscalid: The enrichment of the analyte was achieved by adsorption on a C_{18} SPE column. After elution with cyclohexane/ethyl acetate (1/1, v/v), the eluate was evaporated to dryness and the residues were re-dissolved in methanol/water (80/20, v/v) and determined using LC-MS/MS. The chromatography was performed on a Thermo Betasil C_{18} column with a flow rate of 0.6 mL min^{-1} and a water/methanol gradient with formic acid as modifier.

M510F47: The water samples were acidified to about pH 2 and extracted using OASIS SPE cartridges. The analyte was eluted from the column with methanol. After evaporation to dryness, the residues were re-dissolved in pure water and analyzed using LC-MS/MS. The chromatography was performed on a Waters XSelect HSS T3 column with a flow rate of 0.3 mL min^{-1} and a water/methanol gradient with formic acid as modifier.

M510F49: The water samples were extracted using C_{18} SPE columns. The analyte was eluted from the column with methanol. After evaporation to dryness, the residues were re-dissolved in methanol/water (80/20, v/v) and analyzed using LC-MS/MS. The chromatography was performed on a Thermo Betasil C_{18} column with a flow rate of 0.6 mL min^{-1} and a water/methanol gradient with formic acid as modifier.

Detection of boscalid, M510F47 and M510F49 was accomplished by electrospray ionization at mass transitions 343→271 (positive mode), 158→122 (positive mode) and 323→202 (negative mode) for quantification and 343→307 (positive mode), 158→94 (positive mode) and 323→94 (negative mode) for confirmation, respectively.

Recovery findings

The method proved to be suitable to determine boscalid and its metabolites M510F47 and M510F49 in surface water and ground water. Samples were spiked at the limit of quantification of 0.03 µg L^{-1} and ten times higher (0.3 µg L^{-1}). All average recovery values (mean of at least five replicates per fortification level and analyte) were between 70% and 110%. The detailed results are given in Table 4.2-12.

Table 4.2-12: Results of the method validation for the determination of boscalid and its metabolites M510F47 and M510F49 in surface and ground water

Matrix	Analyte	m/z	Fortification level [µg L^{-1}]	Number of Replicates	Mean Recovery [%]	RSD [%]	Overall Recovery [%]	RSD [%]
Surface water	Boscalid	343→271	0.03	5	109	7	99	12
			0.3	5	90	5		
		343→307	0.03	5	107	8	99	11
			0.3	5	90	4		
	M510F47	158→122	0.03	5	103	1	103	3
			0.3	5	103	5		
		158→94	0.03	5	102	1	101	2
			0.3	5	100	3		
	M510F49	323→202	0.03	5	89	3	91	6
			0.3	5	94	6		
		323→94	0.03	5	89	3	91	4
			0.3	5	93	4		
Ground water	Boscalid	343→271	0.03	5	108	4	99	11
			0.3	5	89	3		
		343→307	0.03	5	108	4	98	11
			0.3	5	89	4		
	M510F47	158→122	0.03	5	101	1	98	4
			0.3	5	96	4		
		158→94	0.03	5	100	1	98	4
			0.3	5	96	5		
	M510F49	323→202	0.03	5	89	6	90	5
			0.3	5	91	4		
		323→94	0.03	5	92	8	92	6
			0.3	5	93	3		

RSD = Relative standard deviation

Linearity

Good linearity ($r > 0.995$) was observed in the range of 0.025 ng mL^{-1} to 0.50 ng mL^{-1} for the two mass transitions of boscalid, in the range of 0.35 ng mL^{-1} to 10 ng mL^{-1} for the two mass transitions of M510F47, and 0.010 ng mL^{-1} to 1.0 ng mL^{-1} for the two mass transitions of M510F49. At least six calibration points distributed over the concentration ranges tested were used.

Specificity	LC-MS/MS is a highly specific self-confirmatory technique. Under the described conditions the method is specific for the determination of all three analytes in surface and ground water. Due to the high selectivity and specificity of LC-MS/MS an additional confirmatory technique is not necessary. No significant interferences (> 30% LOQ) at the retention times and mass transitions were observed.
Matrix Effects	No significant matrix effects (< 20%) on the detection of boscalid and M510F49 were found. Significant matrix effects were observed for M510F47 (mean > 20% for surface water and about 20% for ground water), so matrix-matched standards were used for quantification of M510F47 in both matrices.
Stability of Working Solutions	Standard solutions of boscalid, M510F47 and M510F49 were stable for at least 35 days, when stored at 1-10°C in the dark.
Extract Stability	Analytes boscalid and M510F49 in extracts prepared in methanol and methanol/water (80/20, v/v) and analyte M510F47 in extracts prepared in pure water were stable for at least 7 days, when stored at 1-10°C.
Interference	No significant interferences were observed in the control samples.
Limit of Quantification	The method has a limit of quantification (LOQ) of 0.03 µg L^{-1}, resulting from the lowest concentration level successfully tested within recovery experiments.
Limit of Detection	The method has a limit of detection (LOD) of 0.005 µg L^{-1} for boscalid and 0.009 µg L^{-1} for M510F47 and M510F49.
Repeatability	The relative standard deviations (RSD, %) for all fortification levels were below 20%.
Reproducibility	These results of the independent laboratory validation confirm the results of the validation studies and is reported in sections CA 4.2/10 [*BASF DocID 2008/1086809*], CA 4.2/11 [*BASF DocID 2015/1174526*] and CA 4.2/12 [*BASF DocID 2015/1109588*].
Conclusion	**The described method L0127/01 is considered suitable for the quantitative analysis of boscalid (BAS 510 F) and its metabolites M510F47 (Reg. No. 107371) and M510F49 (Reg. No. 391572) in surface and ground water.**

Additional information from the scientific literature:

In the scientific literature, a publication addressing the analysis of p-chlorobenzoic acid in water was found and is summarised as additional information. Although this analyte (M510F64) is not relevant for the risk assessment, hence not relevant for monitoring (refer to chapter M-CA 7.4), the publication provides useful information on the analysis of this metabolite (M510F64). The method is considered as useful additional information.

Report: CA 4.2/15
Vanderford B.J. et al., 2007 a
Analysis of p-chlorobenzoic acid in water by liquid chromatography-tandem mass spectrometry
2007/1071245
Guidelines: none
GLP: no

Principle of the Method Deionized water and samples and wastewater samples were fortified with *para*-chlorobenzoic acid (*p*-CBA) at a concentration level of 100 ng L^{-1} and analyzed by LC-MS/MS. The chromatography was performed on a Luna C_{18} (2) column with a flow rate of 0.8 mL min^{-1} using a gradient consisting of 5 mM ammonium acetate in water and methanol. Detection was accomplished by electrospray ionization (ESI) using mass transition 155→111 for quantification and mass transitions 155→35 and 157→37 for confirmation.

Recovery Findings The described method is suitable to determine residues of *para*-chlorobenzoic acid (*p*-CBA) in water at a concentration level of 100 ng L^{-1}. Average recovery values were between 70% and 110%. The detailed results are given in Table 4.2-13.

Table 4.2-13: Recovery results of *p*-CBA in water

Matrix	Spike Concentration [ng L^{-1}]	Recovery [%]	Average Recovery [%]	RSD [a] [%]
Deionized water	100	103, 106, 104, 103, 99, 106, 109, 118, 118, 107	107	5.9
Wastewater effluent	100	90, 87, 94, 92, 96, 93, 90, 102, 100, 97	94	4.9

RSD = Relative standard deviation
[a] RSD values are based on calculations of the data originating from the study report.

Linearity	Linear regression with $1/x^2$ weighting was used and correlation coefficients were exceeded 0.995. Seven calibration standards distributed over a concentration range between 0.1 µg L^{-1} to 100 µg L^{-1} were used.
Specificity	LC-MS/MS is a highly specific self-confirmatory technique. Under the described conditions, the method is specific for the determination of *p*-CBA in water samples. Due to the high selectivity and specificity of LC-MS/MS an additional confirmatory technique was not necessary.
Matrix Effects	The results indicate the method was not susceptible to significant matrix interferences in wastewater, as the average recovery was 94%.
Limit of Detection	The limit of detection (LOD) of the method is 100 ng L^{-1}.
Repeatability	The relative standard deviation (RSD, %) for the concentration level were < 20% for *p*-CBA.
Reproducibility	Reproducibility was not tested within the study.
Conclusion	**The described analytical method is considered suitable for the quantitative analysis of *para*-chlorobenzoic acid (*p*-CBA) in water using LC-MS/MS.**

(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

The residue analytical method 460 for the determination of boscalid in air was already peer-reviewed and evaluated as fully valid for monitoring purpose in air. However, for completeness an executive summary of the method is presented again for the reviewer's convenience.

Report: CA 4.2/16
Zangmeister W., 2000 a
Validation of analytical method 460: Determination of BAS 510 F (Reg.No. 300 355) in air by GC-MS
2000/1014992
Guidelines: EEC 91/414
GLP: yes
(certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

For determination of boscalid in air the BASF-method 460 (Zangmeister, 2000) was developed. After sampling of approximately 540 L air by sucking air (1.5 L/min) for approximately 6 hours through a Tenax absorber tube, the absorber tube was closed with 2 plastic caps and transported to the laboratory for analysis or stored at + 6°C (storage stability for 3 days proven). For analysis, the Tenax adsorbent was extracted with acetone. The solvent was evaporated to dryness and the residue is redissolved in acetone. The final determination was done by GC-MS on a DB-XLB column using the ions m/z = 140, 142, and 342 against calibration standards prepared in acetone with linearity of r >0.999. No significant interferences were observed.
The analyte is stable on the adsorber material for at least 3 days when stored at 6°C in the dark. Stability of the analyte in acetone was assessed and confirmed in study 1998/11206.
As three fragments are considered highly selective and furthermore no confirmatory methods are required for the determination of residues in air if sufficient confirmatory methods are available for the determination in soil or water. The latter is the case as fully valid residue analytical methods are provided for both matrices, soil and water.

Table 4.2-14: Validation results for the determination of boscalid in air using analytical method

Matrix	Fortification level [µg m^{-3}]	Overall Recovery [%]	RSD [%]	Number of Replicates
Air	1.5	100	2.7	5
	133	92	4.7	6

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

No analytical method is required for boscalid in body fluids and tissues as the compound is not regarded as toxic or very toxic, according to the most recent SANCO/825/00 rev. 8.1. Nevertheless, screening methods for boscalid and multiple other compounds in body fluids / tissues are described in the public literature. For completeness of information, those publications are discussed below.

Based on the available amount of information provided by the public scientific literature, enough information is provided to screen for Boscalid in various body fluids, if required.

Report: CA 4.2/17
Termopoli V. et al., 2015 a
Occurence of specific environmental risk factors in brain tissues of sudden infant death and sudden unexpected death victims assessed with gas chromatography-tandem mass spectrometry
2015/1240162
Guidelines: none
GLP: no

Principle of the method: Human and lamb brain homogenates were extracted with n-hexane and purified through a Florisil cartridge. After concentration, the extract was injected into the GC–MS/MS system (EI-MRM). Separation was performed on an Agilent fused-silica HP-5MS capillary column. Helium was used as carrier gas and nitrogen as collision gas. For quantitation and confirmation of boscalid, ion transitions m/z 140 → 76 and 140 → 112 were selected.

Recovery findings: Samples were spiked at three concentrations (0.001, 0.01 and 0.05 mg/kg), each three times which gives a total number of 9 repetitions. Most compounds showed satisfactory recoveries with 70-120%. Details for boscalid were not reported.

Linearity: Linearity of calibration curves was studied in the concentration range between 0.2 and 200 µg/kg using seven calibration standards. Good linearity was observed with correlation coefficient >0.99.

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

Interference: An internal standard (chlorfenvinfos in case of boscalid) was used.

Limit of Quantitation: The limit of quantification (LOQ) was 0.17 µg/kg for boscalid.

Limit of Detection: The limit of detection (LOD) was 0.03 µg/kg for boscalid.

Repeatability: The relative standard deviations (RSD, %) were <10%.

Reproducibility: A 50 pg/µL standard solution was prepared daily for the interday repeatability studies, and five injections were made each day (5 days, n=25). The interday repeatability ranged from 8 to 18% for all compounds.
Furthermore, the method was successfully applied to the analysis of real samples (14 human cerebral cortexes).

Stability of Solutions: All standard solutions were stored in brown glass vials at 4°C.

Conclusion: **The GC-MS/MS multi residue analytical method can be used for the analysis of boscalid in human brain tissue if deemed necessary.**

Report:	CA 4.2/18 Dulaurent S. et al., 2009 a Screening of pesticides in blood with liquid chromatography-linear ion trap mass spectrometry 2010/1233452
Guidelines:	none
GLP:	no

Briefly, after solid-phase extraction (SPE) from whole blood, the compounds were separated by liquid chromatography (LC) using a reversed-phase column and identified by mass spectrometry (MS). The mass spectrometer was operated in the full-scan MS mode, in the positive and negative polarities, followed by MS scanning of ions selected in data-dependent acquisition. The detection limit for boscalid in the dual-polarity MS mode was 0.1 mg/L. Boscalid was also detectable at 0.01 mg/L in the single polarity MS mode. The results obtained with the screening method were satisfactory in terms of sensitivity, selectivity and specificity for the identification of unknown pesticides, including boscalid, in a complex matrix such as blood.

Report:	CA 4.2/19 Wang Y. et al., 2008 a Novel, fully automatic hydrophilic interaction/reversed-phase column-switching high-performance liquid chromatographic system for the complementary analysis of polar and apolar compounds in complex samples 2008/1103136
Guidelines:	mone
GLP:	no

Briefly, after solid-phase extraction (SPE) from rat urine, the compounds were separated by high-performance liquid chromatographic (HPLC) using a hydrophilic interaction chromatographic (HILIC) column and in addition a reversed-phase (RP) column, thus preventing the loss of analytes eluting at dead time in common single-column methods. Residues were identified either by a UV or by a mass spectrometric detector. No data about boscalid were reported.

Report: CA 4.2/20
Cappiello A. et al., 2014 a
Determination of selected endocrine disrupting compounds in human fetal and newborn tissues by GC-MS
2014/1327592
Guidelines: none
GLP: no

Principle of the method: Residues of boscalid were extracted from liver and brain using a liquid-solid extraction with hexane. After SPE clean-up, the extracts in hexane/dichlormethane (1:1, v/v) were evaporated and final determination was performed by GC-MS equipped with an Agilent HP-5MS column. Helium was used as carrier gas. For quantitation and confirmation of boscalid, three ions (m/z 140 as quantifier, 112 and 342 as qualifier) were selected.

Recovery findings: Recovery was determined following analysis of samples from animal tissue (swine liver and lamb brain), fortified at 0.008, 0.12 and 8 mg/kg. The mean recovery values were in the range of 70% to 110%. Detailed results are given in table below.

Table 4.2-15: Validation results of liquid-solid extraction method: boscalid (BAS 510 F) in liver and brain

Test substance	Matrix	Fortification level (mg/kg)	No of analyses	Average recovery (%)	Relative standard deviation (%)
Boscalid (BAS 510 F)	Liver	0.008	n r	87	6
		0.12	n r	90	4
		8	n r	99	4
	Brain	0.008	n r	94	4
		0.12	n r	94	6
		8	n r	97	8

n.r. Not reported

Linearity: The linearity of the GC-MS detector was tested using five concentrations between 0.0004-8.0 mg/kg in liver matrix and 0.004-4.0 mg/kg in brain matrix. Linear correlations with coefficients ≥0.99 were obtained for boscalid.

Specificity: GC-MS is a highly specific detection technique and therefore a confirmatory technique is not provided. Analysis is possible for three ions.

Interference: An internal standard (Captan D6 in case of boscalid) was used.

Limit of Quantitation: The LOQ for boscalid was 0.004 mg/kg.

Limit of Detection: The LOD for boscalid was 0.0012 mg/kg.

Repeatability: The relative standard deviations (RSD, %) for all matrices were ≤10% for all fortification levels. The detailed values are shown in the table above.

Reproducibility: The method was successfully applied to the analysis of fetal and newborn liver and brain tissues collected during autopsies of four sudden infant death syndrome (SIDS) cases and six sudden intrauterine unexplained death syndrome (SIUDS) cases.

Conclusion: **The GC-MS multi residue analytical method can be used for the analysis of boscalid in human brain tissue if deemed necessary.**

Report: CA 4.2/21
Kim H.-S. et al., 2014 a
General unknown screening for pesticides in whole blood and Korean gastric contents by liquid chromatography-tandem mass spectrometry
2014/1327593
Guidelines: none
GLP: no

Principle of the method: Residues of boscalid were extracted from blood and gastric contents using methanol/acetonitrile (30:70, v/v) with formic acid (0.4%). Extraction buffer salt was added and the mixture was centrifuged. For cleanup, an SPE sorbent was used. The clean extract was mixed with water and filtered before UPLC-MS/MS in ESI+ mode. Analysis was accomplished using an Acquity UPLC BEH C18 column and a water/methanol gradient with formic acid as modifier. Quantitation and confirmation of boscalid residues was performed using two mass transitions (343→307 m/z and 343→271 m/z).

Recovery findings: The mean recovery values were between 70% and 110%.

Table 4.2-16: Validation results of mini-QuEChERS method: boscalid (BAS 510 F) in blood and gastric contents

Test substance	Matrix	Fortification level (ng/mL)	No of analyses	Average recovery (%)		Relative standard deviation (%)	
Transition				343 → 307	343 → 272	343 → 307	343 → 272
Boscalid (BAS 510 F)	Blood	50	3	73.49	n.r.	n.r.	n.r.
		100	3	73.18	n.r.	n.r.	n.r.
	Gastric contents	50	3	73.53	n.r.	n.r.	n.r.
		100	3	73.26	n.r.	n.r.	n.r.

n.r. Not reported

Linearity: Good linearity was observed over a concentration range of 5 to 200 µg/L (6 concentration levels) for boscalid with correlation coefficients ≥0.988 for the whole blood and ≥0.986 for gastric contents.

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

Matrix effects: A modified QuEChERS that uses dispersive solid phase extraction for a small amount of sample, mini-QuEChERS, was applied to blood and gastric contents to reduce matrix effect.

Limit of Quantitation: The limit of quantitation was 5 µg/L for blood and 10 µg/L for gastric contents.

Repeatability: The relative standard deviations (RSD, %) were not reported.

Reproducibility: The linear range and correlation coefficient (r2) by Q/TOF mass spectrometry were not much inferior to triple quadrupole mass spectrometry.

Conclusion: **The HPLC-MS/MS multi-residue analytical method can be used for the analysis of boscalid in blood if deemed necessary.**

Report: CA 4.2/22
Cazorla-Reyes R. et al., 2011 a
Single solid phase extraction method for the simultaneous analysis of polar and non-polar pesticides in urine samples by gas chromatography and ultra high pressure liquid chromatography coupled to tandem mass spectrometry
2011/1298011
Guidelines: none
GLP: no

Principle of the method: For simultaneous extraction of polar and non-polar pesticides urine samples were passed across a pre-conditioned C18 SPE column. After drying the cartridges, the retained analytes were eluted with dichloromethane. The extracts were evaporated to dryness and the residue was dissolved in ethyl acetate solution of the internal standard (IS, caffeine ^{13}C, 0.5mg/L). An aliquot of the extract was taken directly for chromatographic analysis by GC-MS/MS.
For GC-amenable pesticides (e.g. boscalid), chromatographic analyses were performed by GC-MS/MS system Varian 3800, equipped with electronic flow control and fitted with a Saturn 2000 ion-trap mass spectrometer. A fused silica untreated capillary column from Supelco was used as guard column connected to a Factor Four Capillary Column VF-5ms. The ion-trap mass spectrometer was operated in the electron impact (EI) mode. For quantification and confirmation of boscalid, ions at m/z 342, 307 and 230 were monitored.

Recovery findings: Recovery studies were carried out at three concentration levels (0.005, 0.01 and 0.05 mg/kg), performing 5 replicates at each level. In urine the mean recovery values were between 70% and 120%. The detailed results are given below.

Table 4.2-17: Validation results of GC-MS/MS method: boscalid (BAS 510 F) in urine

Test substance	Matrix	Fortification level (mg/L)	No of analyses	Average recovery (%)	Relative standard deviation (%)
Boscalid	Urine	0.005	5	78	15
		0.01	5	113	16
		0.05	5	93	11

Linearity: The linearity was evaluated using spiked extracted blank urine samples with seven different concentrations of pesticides ranging from 0.1 to 100 µg/L. Linear calibration graphs were plotted by least-squares regression of relative peak area (analyte/IS) versus concentration of the calibration standards. Linear correlations with coefficients ≥0.99 were obtained for boscalid.

Specificity:	GC-MS/MS monitoring three ions is a highly specific detection technique and therefore a confirmatory technique is not required. Although, only data for combined mass transition were presented.
Matrix effects:	The calibration curves obtained from spiked (5-100µg/L) blank extracted urine were significantly different for that obtained by the use of standard solutions for GC-amenable pesticides (strong matrix effect). In order to compensate this effect, matrix matched calibration was used for quantification purposes.
Interference:	Caffeine ^{13}C was used as internal standard for the determination of the pesticides, because it has similar physico-chemical properties than some pesticides, and its chromatographic properties are well known.
Limit of Quantitation:	The publication describes LOQ and LOD calculated analyzing blank samples spiked aM510t 0.001, 0.01, 0.1, 0.5, 1, 2 and 5 µg/L, and determined as the lowest concentration of analyte for which signal-to-noise ratios were 3 and 10, respectively. For boscalid a LOQ of 0.025 µg/L was calculated.
Stability:	Standard solutions of individual compounds in acetone for GC-amenable pesticides were prepared and stored under refrigeration (T<5◦C).
Repeatability:	Repeatability was evaluated at the three concentration levels of the recovery studies, performing five replicates at each level. The relative standard deviations (RSD, %) were lower than 20% for all fortification levels.
Reproducibility:	No independent laboratory validation is published. Interday precision was studied at 5 µg/L, analyzing daily spiked samples for a period of 5 days. Interday precision was lower than 20%.
Conclusion:	**The multi residue method fulfills the requirements with regard to the specificity, repeatability, replications and recoveries.**

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Document M-CA, Section 5

TOXICOLOGICAL AND METABOLISM STUDIES ON THE ACTIVE SUBSTANCE

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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 5 TOXICOLOGICAL AND METABOLISM STUDIES ON THE ACTIVE SUBSTANCE

For a list of metabolites occurring in rats, please refer to Document N3 and see table 5.1.1-1 below.

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

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

Studies already peer-reviewed and presented in the original dossier for Annex I inclusion:

For the determination of the toxicokinetic properties of boscalid, two studies are available (see table below) with the test substance radiolabeled in the diphenyl or pyridine ring system. One study (BASF DocID 2000/1014183) investigated absorption, distribution and excretion in rats after single oral low dose (50 mg/kg bw) and single oral high dose (500 mg/kg bw) while the other study (BASF DocID 2000/1017220) covers the metabolism after single oral low (50 mg/kg bw) or high doses (500 mg/kg bw). Both studies have been part of the previous evaluation and are therefore not submitted again in this dossier. A detailed evaluation has been provided in the Monograph (November 2002). For reasons of convenience, a short summary of the main results is given below.

Table 5.1.1-1: Studies already peer-reviewed and presented in the original Annex I dossier

Category of test	Dose ranges	Results	Reference (BASF DocID)
Biokinetics of ^{14}C-boscalid in rats	50/500 mg/kg bw (single ^{14}C-exposure)	After oral administration to rats, boscalid was rapidly but incompletely absorbed from the gastrointestinal tract, widely distributed and rapidly eliminated from the body. Tissue distribution determined 8 h after administration revealed highest amounts of radioactivity in the GI-tract, liver and adipose tissue in low-dose rats. In the high-dose group, a similar distribution was observed in males, while in females, highest concentrations were found in the GI-tract, liver, thyroid and kidney. There was no evidence of a cumulative potential of boscalid. Approx. 99% of the administered low dose was recovered in excreta within 7 days (17% via urine and 82% via feces). At the high dose level of 500 mg/kg bw, total excretion was similar (96.1%), while only 3.5% AD was eliminated via the urine. There were no significant differences in the excretory pattern with regard to sex, radiolabel used or frequency of application.	2000/1014183 Amendment: 2002/1006141
Metabolism of ^{14}C-boscalid in rats	50/500 mg/kg bw (single ^{14}C-exposure)	After oral administration to male and female rats, the systematically available portion of boscalid was rapidly and intensively metabolized to a large number of biotransformation products. The hydroxylation of the diphenyl moiety was the quantitatively most important pathway. Second important was the substitution of the Cl of the 2-chloropyridine part against SH by conjugation with glutathione. Partial cleavage of the glutathione moiety afforded the cysteine conjugate and finally the SH-compound which was subsequently methylated or oxidized. In addition, the introduction of glutathione and a second hydroxy group into the diphenyl part of the molecule was observed. Combinations of these reactions and the conjugation of the OH-groups with glucuronic acid or sulfate and the conjugation of the SH-group with glucuronic acid led to the large number of metabolites. The cleavage of the amide bond is negligible because the 2-chloronicotinic acid was detected only in trace amounts. No major differences were observed with regard to label, sex, and dose level.	2000/1017220 Amendment: 2002/1005447

Study submitted in this supplementary dossier (not yet peer-reviewed):

In addition, excretion and metabolism of BAS 510 F (boscalid) was further investigated (BASF DocID 2003/1012629) in male and female rats after multiple oral dosing with unlabeled BAS 510 F for 14 or 28 consecutive days, both followed by a single radiolabeled dose with [diphenyl-U-^{14}C]-BAS 510 F, each 500 mg/kg. Yet, the study has not been peer reviewed and is therefore reported below.

Report:	CA 5.1.1/1 [redacted] et al., 2003 a 14C-BAS-510 F: Investigation of the metabolic profiles in urine and feces of rats after multiple dosing 2003/1012629
Guidelines:	none
GLP:	yes (certified by Landesamt fuer Umweltschutz und Gewerbeaufsicht, Mainz, Germany)

The study consisted of two phases. In phase A, beside the control group, in two treatment groups rats were dosed with 500 mg/kg unlabeled BAS 510 F for 14 or 28 consecutive days. In phase B, the same group, including the untreated control, were dosed with 500 mg/kg diphenyl-labeled U-^{14}C-BAS 510 F. Only phase B is reported in the following.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description: Boscalid (BAS 510 F)
Lot/Batch #: N46 (BAS 510 F), 641-2017 (diphenyl-U-^{14}C)
Purity: 95.7% (BAS 510 F), >99% (diphenyl-U-^{14}C), >99% (radiochemical)
CAS#: 188425-85-6
Development code: Not reported
Stability of test compound: Stable during dosing period

2. Vehicle and/or positive control: 0.5% aqueous CMC (carboxymethyl cellulose) solution, containing 1% Cremophor EL)

3. Test animals

Species: Rat
Strain: Wistar rats (CrlGlxBrlHan:Wi), Charles River, Sulzfeld, Germany
Age: 7 weeks
Sex: Male and female
Number of animals: 24 (4 males + 4 females for each dose)
Weight at dosing: 187.8±8.1 g (males); 142.9±5.5 (females)
Acclimation period: 1 day
Diet: Ground Kliba maintenance diet rat/mouse/hamster, meal, *ad libitum*
Water: Drinking water (bottles), *ad libitum*
Husbandry:
Housing: After application of ^{14}C labeled BAS 510 F, rats were placed separately in metabolism cages.
Environmental conditions:
Temperature: 22±2°C
Humidity: 30-70%
Air changes: Not reported
Photoperiod: Alternating 12-hour light and dark cycles

4. Preparation of dosing solutions

The dose formulations for the diphenyl-label were prepared by mixing non-labeled BAS 510 F and [diphenyl-U-^{14}C]-labeled BAS 510 F using a dilution factor of 12.54 (for administration at 500 mg/kg), leading to target specific activities of ca.13.89 µCi/mg for the dose group 0M/0F and 1M/1F and 13.46 µCi/mg for the dose group 2M/2F. Thereby, the calculated dose was suspended in an aqueous solution of 0.5% CMC containing 1% Cremophor EL. The formulations were stirred during application. The single oral doses of these formulations (about 1.0 mL/l00 g bw) were weighed into plastic disposable syringes and administered orally by gavage. The achieved actual mean doses and specific radioactivity per dose group are listed in Table 5.1.1-2

B. STUDY DESIGN AND METHODS

1. Dates of work: April 8, 2003-May 28, 2003

The excretion and metabolism of BAS 510 F (boscalid) was investigated in male and female rats after multiple oral dosing with unlabeled BAS 510 F for 14 consecutive days (test group 1) or 28 consecutive days (test group 2), both followed by one single radiolabeled dose with [diphenyl-U-^{14}C]-BAS 510 F, each 500 mg/kg.

Urine, feces and cage wash were collected from male and female rats of all dose groups in the time interval of 0 to 48 hours post application. Samples of several animals were combined for each sex and test group and stored at -20°C until analysis.

Table 5.1.1-2: Summary of dose groups and dosing parameters

Test group	**0M**	**0F**	**1M**	**1F**	**2M**	**2F**
Phase A: Test substance (unlabeled)	Control*	Control*	BAS 510 F	BAS 510 F	BAS 510 F	BAS 510 F
Number of animals	4	4	4	4	4	4
No. of doses	14	14	14	14	28	28
Nominal dose (mg/kg bw)	0	0	500	500	500	500
Phase B: Test substance (radioactive label)	Diphenyl	Diphenyl	Diphenyl	Diphenyl	Diphenyl	Diphenyl
No. of doses	1	1	1	1	1	1
Route of administration	Oral	Oral	Oral	Oral	Oral	Oral
Nominal dose (mg/kg bw)	500	500	500	500	500	500
Mean dose achieved (mg/kg bw)	**527**	**506**	**505**	**509**	**505**	**501**
Specific radioactivity (dpm/µg)	30864	30864	30864	30864	29901	29901
Dilution factor	12.54	12.54	12.54	12.54	12.54	12.54
Sampling	Urine Feces Cage wash	Urine Feces Cage wash	Urine Feces Cage wash	Urine Feces Cage wash	Urine Feces Cage wash	Urine Feces Cage wash

* VehicIe control: 0.5% aqueous CMC solution [carboxymethyl cellulose], containing 1% Cremophor EL)

The metabolite patterns in urine, feces and cage wash samples were analyzed using two different HPLC (LC 01 and LC 02) systems. Metabolites were identified by LC-MS/MS and by comparison of the retention times and metabolic profiles with the rat metabolism study conducted at [redacted], see BASF DocID 2000/1017220.
Aliquots of urine pool samples were analyzed by HPLC directly after thawing and dilution with phosphate buffer. Metabolites in feces were isolated after drying, homogenization and methanolic extraction. For quantitation only HPLC (LC 01) was used. Aliquots were analyzed for metabolic patterns by HPLC.

II. RESULTS AND DISCUSSION

Storage stability
The stability of the test substance in aqueous CMC-solution was proven over a period of 4 days at room temperature in previous studies conducted at [redacted].

Excretion
The excretion of radioactive residues was rapid and comparable for both sexes. Over an observation period of about 48 hours, the majority of the radioactive residues (approx. 68-76% of dose) were excreted via feces. Smaller portions were excreted via urine (approx. 9-11% of the dose for males and approx. 14-18% for females). Excretion via urine and feces was nearly complete within 48 hours after dosing for all dose groups.

Table 5.1.1-3: Excretion of radioactivity after single oral administration of ^{14}C-BAS 510 F to rats at a rate of nominally 500 mg/kg

Matrix	[% of the administered radioactivity]					
	Male (0M)	Female (0F)	Male (1M)	Female (1F)	Male (2M)	Female (2F)
Urine (0-48 h)	**10.21**	**17.68**	**8.61**	**13.97**	**10.53**	**14.76**
Feces (0-48 h)	**75.54**	**71.55**	**75.65**	**69.19**	**67.67**	**69.84**
Other sources						
Cage wash (0-48 h)	**0.53**	**0.97**	**0.55**	**0.42**	**0.51**	**0.91**
Total (0-48 h)	**86.28**	**90.20**	**84.81**	**83.58**	**78.71**	**85.51**

Extractability
With methanol, 88.8-90.4% of the radioactive residues of BAS 510 F in feces of both sexes could be extracted.

Table 5.1.1-4: Extractability of feces samples with solvent (methanol) after single dosing of rats with [diphenyl-U-^{14}C]-BAS 510 F

Matrix (time interval)	Feces activity	Extracted radioactivity		Unextracted radioactivity	
	% dose	% dose	% feces activity	% dose	% feces activity
Single dose of 500 mg/kg bw; male rats (0M)					
time interval: 0-48 h	75.5	68.7	90.9	7.8	10.3
Single dose of 500 mg/kg bw; female rats (0F)					
time interval: 0-48 h	71.6	67.5	94.3	8.3	11.7
Repeated doses of 500 mg/kg bw (14 days) and single dose of 500 mg/kg bw; male rats (1M)					
time interval: 0-48 h	75.7	67.2	88.8	8.7	11.5
Repeated doses of 500 mg/kg bw (14 days) and single dose of 500 mg/kg bw female rats (1F)					
time interval: 0-48 h	69.2	62.3	90.1	5.7	8.2
Repeated doses of 500 mg/kg bw (28 days) and single dose of 500 mg/kg bw; male rats (2M)					
time interval: 0-48 h	67.7	60.6	89.6	9.3	13.8
Repeated doses of 500 mg/kg bw (28 days) and single dose of 500 mg/kg bw; female rats (2F)					
time interval: 0-48 h	69.8	67.0	96.0	7.4	10.5

Metabolism
The major transformation steps in the metabolic pathway of BAS 510 F are

- Hydroxylation of the parent compound
- Formation of glucuronide conjugates of metabolites via hydroxylation of the parent compound

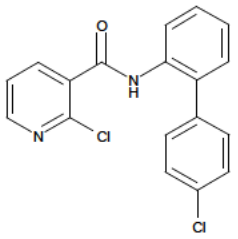
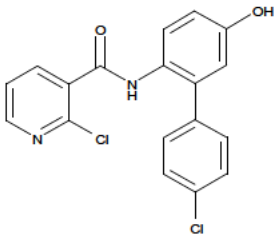
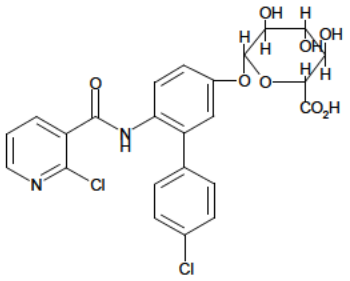
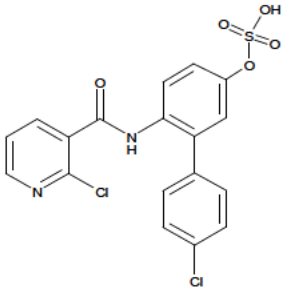
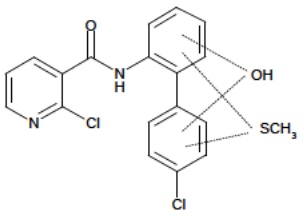
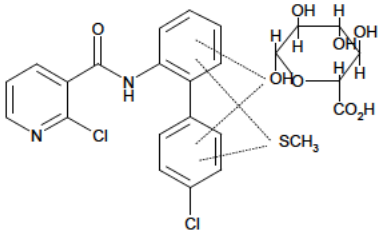
In urine, of male and female rats of all test groups metabolites and components were identified and ranged from 0.17% to 38%. The metabolite patterns of all test groups and both sexes showed four main metabolites, M510F02, M510F42, M510F03 and M510F01. These metabolites are either glucuronide or sulfate conjugates, except for M510F001 which was generated by hydroxylation of the parent compound.
The metabolite patterns in urine were largely comparable for both sexes, but the amount of some of the major metabolites varied in between both genders. For the hydroxylated M510F01, the percentage of dose excreted in the period 0-48 hours is increased in repeated dose groups compared to single dose group from 1.4% to 2.9% in females and decreased from 3% to 0.8% in males. For M510F02, the most abundant metabolite in urine, there was an increase of the percentage of dose value from 2.8% (single dose group) to 4.3% and 5.6% (repeated dose groups) in male rats, whereas the metabolite decreased from 14.3% (single dose group) to 9.55% and 10.75% (repeated dose groups) of the dose in female rats. Other metabolites, M510F42, a glucuronic acid conjugate, M510F03, a sulfate conjugate and M510F20 were only present at minor amounts. The parent compound BAS 510 F was not present in urine.

In feces, two metabolites and parent compound were identified for male and female rats of all dose groups and ranged from 2.8% to 37.9% of the dose. Comparable metabolites and components were detected in the major peaks for both genders, but the amount of the major metabolites and parent compound varied in between both genders. The parent BAS 510 F was the predominant compound in feces and ranged from 29.4% to 38.0% of the dose. The hydroxylated metabolite M510F01 was identified as the most abundant metabolite in feces of both sexes and of all dose groups (up to 13% and 21% of the dose for males and females of the single dose group and up to 13% and 23-25% of the dose for males and females of the repeated dose groups, respectively). No significant differences could be detected between metabolite patterns of the different test groups. The glucuronic acid conjugate M510F02 was not detected in any feces extracts.

Table 5.1.1-5: Identified metabolites and components in urine and feces

Metabolite / Component	Urine 0M	Urine 0F	Urine 1M	Urine 1F	Urine 2M	Urine 2F	Feces 0M	Feces 0F	Feces 1M	Feces 1F	Feces 2M	Feces 2F
	Composition of radioactive residues in % of the dose											
BAS 510 F	-	-	-	-			36.32	34.02	37.98	30.22	29.35	29.71
M510F02	2.78	14.33	4.29	9.55	5.56	10.75	-	-				
M510F42	0.32	0.45	0.79	0.35	1.45	0.55	-	-		-		
M510F03	1.63	0.17	1.07	0.16	1.18	0.14	-			-		
M5I0F01	2.96	1.44	1.35	2.89	0.77	2.47	13.01	21.02	12.93	23.05	13.32	24.51
M510F20	0.45	-	0.31	0.17	0.27	0.19	4.20	2.80	7.19	3.27	8.32	4.88

Table 5.1.1-6: Structures of metabolites identified in rat matrices

Metabolite designation				Structure/Name
Substance Code	Synonym	Reg. No	CAS-No	
BAS 510 F	Boscalid M510F00	300355	188425-85-6	
M510F01	N/A	398794	661463-87-2	
M510F02	N/A	-	661463-88-3	
M510F03	N/A	-	-	
M510F20	N/A	-	-	
M510F42	N/A	-	-	

N/A Not applicable

III. CONCLUSION

Radioactive residues of BAS 510 F were excreted rapidly and efficiently within 48 hours. Thereby, the metabolite patterns in urine and feces of rats that received one single dose of 500 mg/kg BAS 510 F are comparable to the metabolite patterns of urine and feces samples of animals that received multiple doses of the test compound. Comparable metabolite patterns of male and female rats in urine and feces samples were detected, but the amount of some of the major metabolites varied in between both genders. Main metabolites in urine were M510F02 and M510F01.
The main residues identified in feces were the parent BAS 510 F and the hydroxylated metabolite M510F01. There were no significant deviations of the recorded metabolic patterns of the different test groups in feces.

An overview of all metabolites identified in rat metabolism studies is presented in the table below.

Table 5.1.1-7: Overview of all metabolites identified in rat metabolism studies

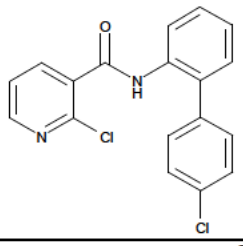
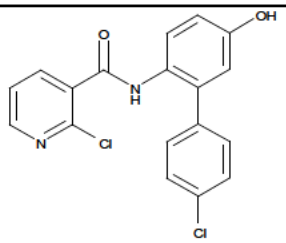
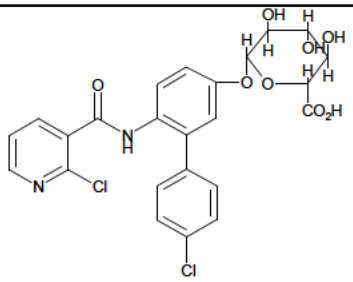
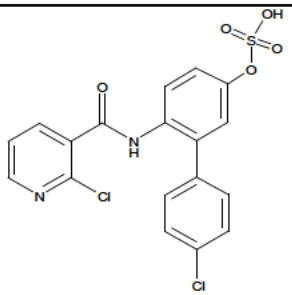
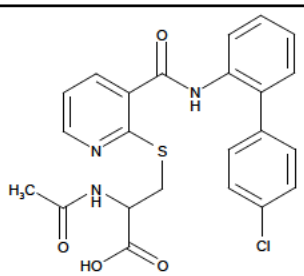
Substance Code	Study	Occurrence (Compartment)	Chemical structure
BAS 510 F	Rat	Urine, feces, plasma, kidney, liver	
M510F01	Rat	Urine, feces, plasma, bile, kidney, liver	
M510F02	Rat	Urine, plasma, bile, kidney, liver	
M510F03	Rat	Urine, bile, kidney	
M510F04	Rat	Urine	

Table 5.1.1-7: Overview of all metabolites identified in rat metabolism studies

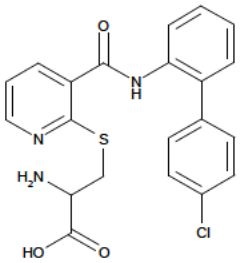
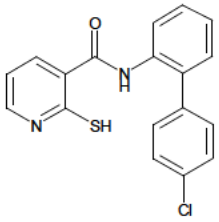
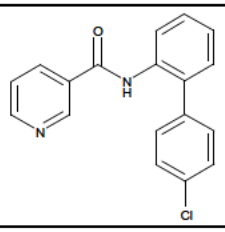
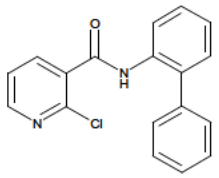
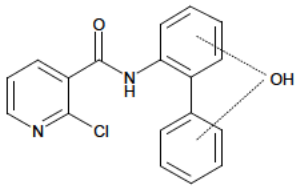
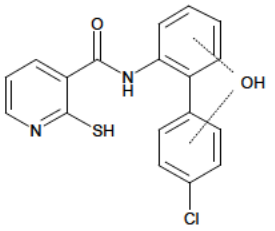
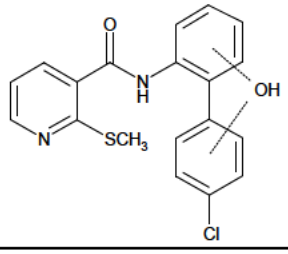
Substance Code	Study	Occurrence (Compartment)	Chemical structure
M510F05	Rat	Urine, feces, bile, kidney, liver	
M510F06	Rat	Urine, feces, plasma, kidney, liver	
M510F08	Rat	Urine	
M519F09	Rat	Feces	
M510F10	Rat	Urine	
M510F11	Rat	Feces	
M510F12	Rat	Urine	

Table 5.1.1-7: Overview of all metabolites identified in rat metabolism studies

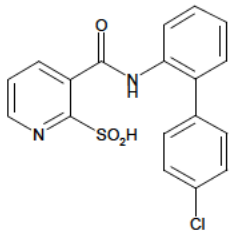
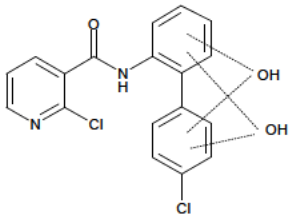
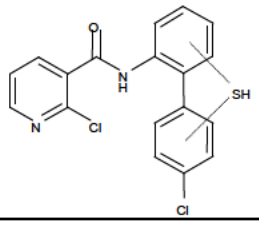
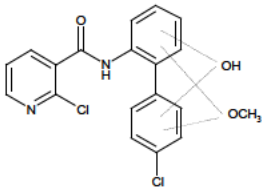
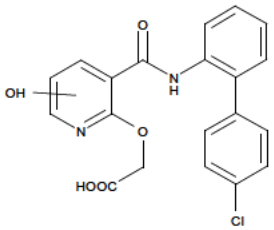
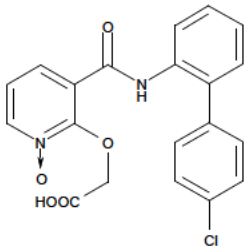
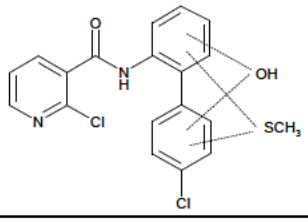
Substance Code	Study	Occurrence (Compartment)	Chemical structure
M510F13	Rat	Urine	
M510F14	Rat	Urine	
M510F15	Rat	Urine	
M510F16	Rat	Urine	
M510F18	Rat	Urine	
M510F19	Rat	Urine	
M510F20	Rat	Urine, feces	

Table 5.1.1-7: Overview of all metabolites identified in rat metabolism studies

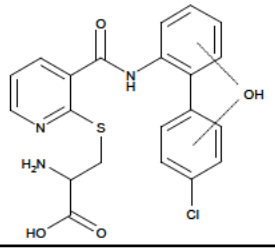
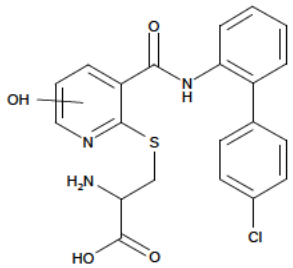
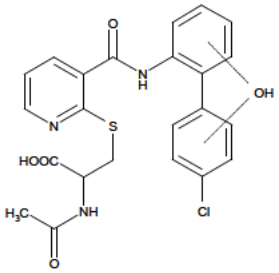
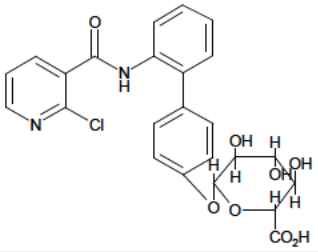
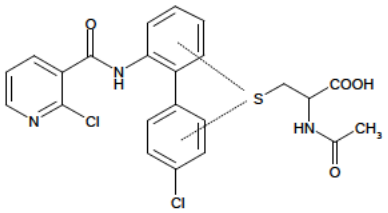
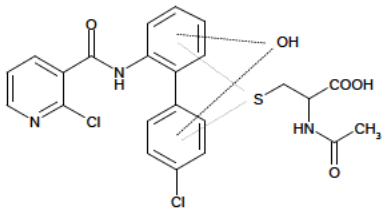
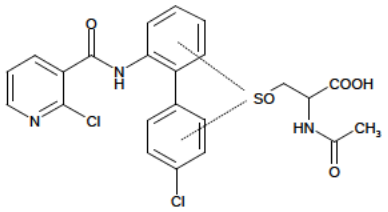
Substance Code	Study	Occurrence (Compartment)	Chemical structure
M510F22	Rat	Urine, bile	
M510F23	Rat	Bile	
M510F28	Rat	Urine	
M510F29	Rat	Urine	
M510F32	Rat	Urine	
M510F33	Rat	Urine	
M510F34	Rat	Urine	

Table 5.1.1-7: Overview of all metabolites identified in rat metabolism studies

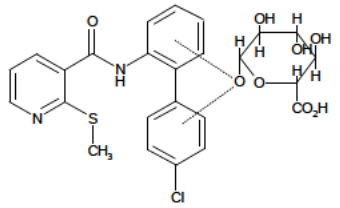
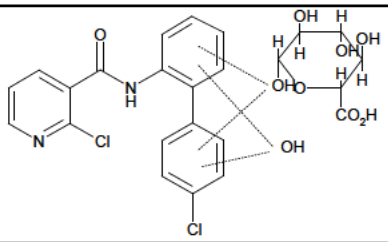
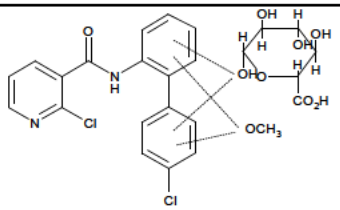
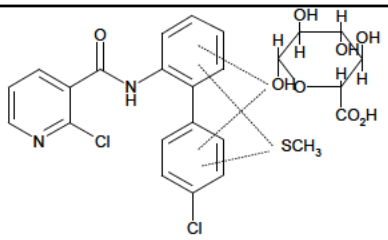
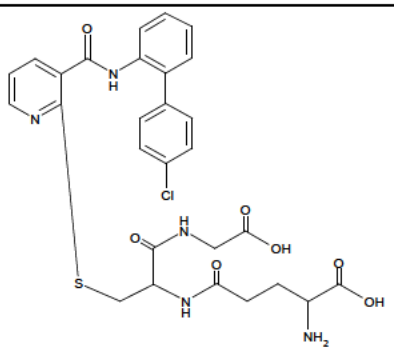
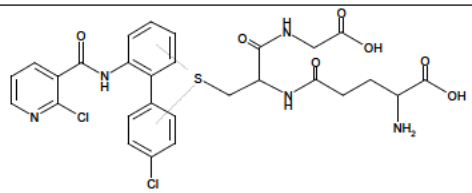
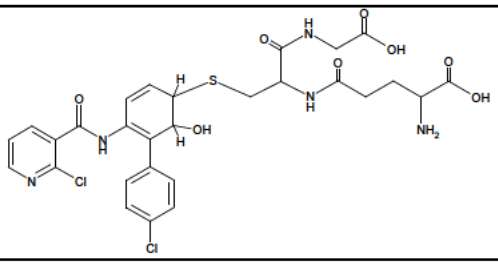
Substance Code	Study	Occurrence (Compartment)	Chemical structure
M510F39	Rat	Urine	
M510F40	Rat	Urine	
M510F41	Rat	Urine	
M510F42	Rat	Urine, feces kidney, liver	
M510F43	Rat	Liver	
M510F45	Rat	Liver	
M510F46 or isomer	Rat	Liver	

Table 5.1.1-7: Overview of all metabolites identified in rat metabolism studies

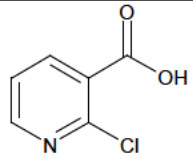
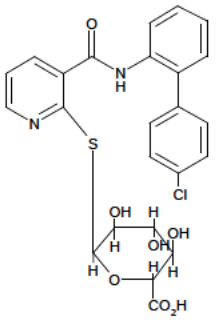
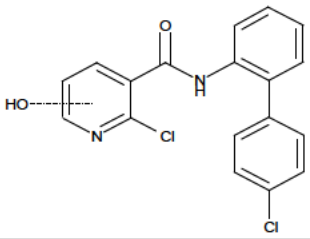
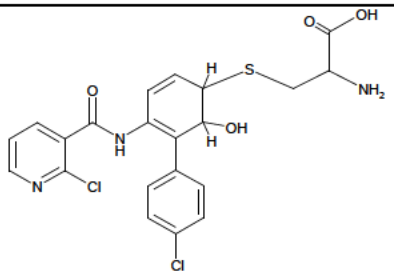
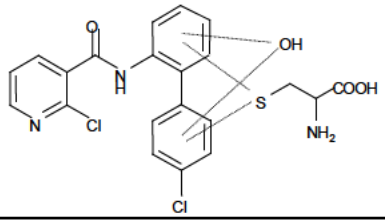
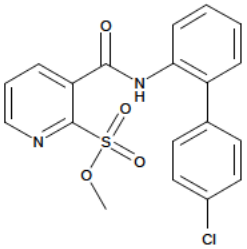
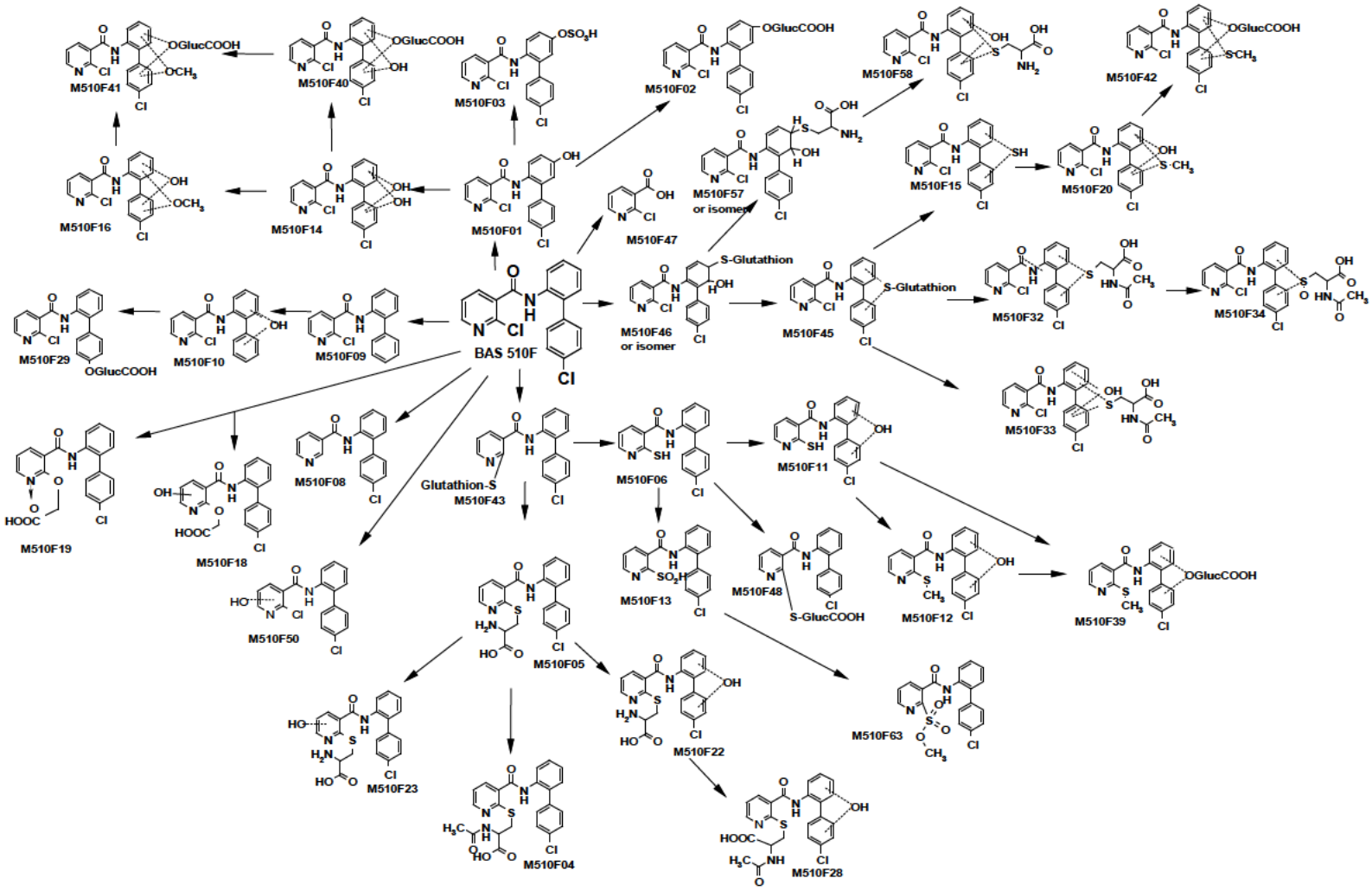
Substance Code	Study	Occurrence (Compartment)	Chemical structure
M510F47	Rat	Urine, liver	
M510F48	Rat	Urine, feces, plasma, kidney	
M510F50	Rat	Bile	
M510F57 or isomer	Rat	Bile	
M510F58	Rat	Bile	
M510F63	Rat	Feces	

Figure 5.1.1-1: Proposed metabolic pathway of boscalid in rats



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., page 22).
In the absence of validated test method or guidance documents, this data requirement is waived in accordance to SANCO Guidance Document SANCO/10181/2013-rev 2.1 (13 May 2013).

Overall conclusion

Boscalid has been extensively studied for absorption, distribution, metabolism and excretion. Taking all studies into consideration, the following general conclusions can be drawn:

- Boscalid is rapidly excreted via urine and feces.
- The majority of the radioactivity was excreted via feces (85% of the dose) and smaller amounts via urine (15% of the dose). The biliary excretion was more pronounced in the low dose (50 mg/kg bw) animals (40%) compared to high dose (500 mg/kg bw) animals (11%). The bioavailability was estimated to be 55%.
- There is no evidence of any cumulative potential of boscalid. Throughout the time course of the experiments, only very low concentrations, if any, were found in tissues and organs.
- The metabolite patterns in tissues and excreta were largely comparable for both sexes and for all dose groups investigated.
- In total the following transformation steps were observed in rats:

    - Oxidation of the diphenyl ring system at the 4 position of the phenyl ring (Phase I) and
    - Subsequent transformation via substitution of the Cl of the 2-chloropyridine moiety against SH by conjugation with glutathione
    - Cleavage to the cysteine conjugate and further to the SH-compound
    - Subsequent Conjugation with glucuronic acid or sulfate or methylation (Phase II).

Taking into account all available data, the following endpoints, adopted to the new format of the list of endpoints, are proposed:

Absorption, distribution, metabolism and excretion (toxicokinetics) (Regulation (EU) N° 283/2013, Annex Part A, point 5.1)

Rate and extent of oral absorption/systemic bioavailability	Approx. 44 % (based on bile excretion within 48 h and urinary excretion within 6 h, low dose)		
Toxicokinetics	Biphasic uptake and elimination		
		Low dose (50 mg/kg bw)	High dose (500 mg/kg bw)
	1^{st} C_{max} (µg eq/g)	0.99 to 1.40	2.61 to 3.52
	1^{st} T_{max} (h)	0.5 to 1.0	0.5 to 1.0
	Initial T1/2 (h)	7.2 to 8.2	8.0 to 9.1
	2^{nd} C_{max} (µg eq/g)	ca 1.56	3.77 to 4.46
	2^{nd} T_{max} (h)	ca 8	ca 8
	Terminal T1/2 (h)	30.1 to 41.7	20.2 to 27.4
Distribution	Widely distributed. Highest residues in liver and adipose tissue (8 h, low dose) In high-dose females, highest residues were observed in thyroid and kidney		
Potential for bioaccumulation	No evidence for accumulation		
Rate and extent of excretion	Complete excretion of low dose within 48 h (approx. 20% via urine and 80% via feces)		
Metabolism in animals	Extensive (<1% of absorbed dose excreted as parent via urine or bile), 38 metabolites identified in rat matrices. Major pathway was hydroxylation at the diphenyl moiety and subsequent O-glucuronidation		
In vitro metabolism	This data requirement is waived in accordance to SANCO Guidance Document SANCO/10181/2013-rev 2.1 (13 May 2013).		
Toxicologically relevant compounds (animals and plants)	Boscalid (parent)		
Toxicologically relevant compounds (environment)	Boscalid (parent)		

CA 5.2 Acute Toxicity

Most studies in M-CA 5.2 have already been peer-reviewed by the Rapporteur Member State Germany and national competent authorities following submission of the original dossier for Annex I inclusion (2000):
One new *in vivo* study was performed. The new maximization test was performed on special request of a non-EU regulatory authority after the time of dossier submission for Annex I inclusion of Boscalid in 2000. The new results were confirming the conclusions of the previous study. All other information available on in vivo studies is presented in the original dossier (2000) and has been evaluated by European competent authorities and Germany as the Rapporteur Member State and the endpoints were fixed in the European Commission Review Report for the active substance Boscalid (SANCO/3919/2007 –Rev 5, 21 January 2008).
For the convenience of the reviewer brief summaries of the acute toxicity studies as extracted from the Monograph (2002) have been provided under the respective chapters CA 5.2.1 – CA 5.2.6.

Boscalid technical active substance was tested in accordance with EU requirements for acute toxicity tests via different routes of administration. These GLP studies have been evaluated and peer reviewed during Annex I inclusion process and are complying with today's scientific standards. Boscalid is considered to be of low acute toxicity by the oral, dermal, and inhalation routes of exposure. In the rabbit, Boscalid was found to be non-irritating to the skin and to the eye. Skin sensitisation studies in guinea pigs conducted according to the method of Magnusson and Kligman (maximization test, M&K test) showed that the compound is not a skin sensitiser.

Submission of not yet peer-reviewed studies in this supplementary dossier:
In accordance with the data requirements for active substances of Commission Regulation (EU) No 283/2013 of 1 March 2013, an *in vitro* NRU phototoxicity study was conducted with Boscalid in Balb/c 3T3 cells and a detailed summary is given in chapter CA 5.2.7. Boscalid was not phototoxic *in vitro* at the concentrations tested up to the limit dose.

An overview of all mandatory toxicity studies of M-CA 5.2 is given in Table 5.2-1. For the already peer-reviewed studies, the respective EU agreed endpoints are marked in bold. Based on the available studies and according to CLP Reg. (EC) 1272/2008 no classification is warranted for Boscalid as to acute toxicity, skin and eye irritation and dermal sensitization.

Table 5.2-1: Summary of acute toxicity studies with Boscalid (BAS 510 F)

Study type	Species (Strain)/ Test system	Result	Classification	Reference** BASF DocID
Acute oral LD_{50}	Rat (Wistar)	**> 5000 mg/kg bw**	None	[redacted] 1998(c) 1998/10643
Acute dermal LD_{50}	Rat (Wistar)	**> 2000 mg/kg bw**	None	[redacted] 1998(c) 1998/10642 (2000/1018711)
Acute inhalation LC_{50}	Rat (Wistar)	**> 6.7 mg/L**	None	[redacted] D. 1998 1998/10803
Skin irritation	Rabbit (New Zealand White)	**Not irritating**	None	[redacted] 1998(d) 1998/10640 (2000/1018712)
Eye irritation	Rabbit (New Zealand White)	**Not irritating**	None	[redacted] 1998(e) 1998/10641 (2000/1018713)
Skin Sensitisation (Maximization Test)*	Guinea pig (Dunkin Hartley)	Not sensitising	None	[redacted] 2003 2003/1022252*
Skin Sensitisation (Maximization Test)	Guinea pig (Dunkin Hartley)	**Not sensitising**	None	[redacted] 1998(a) 1998/10638
In vitro 3T3 NRU Phototoxicity Test*****	Balb/c3T3 cells	Not phototoxic at concentrations up to 1000 µg/mL	None	Cetto, V., Landsiedel, R. 2012 2013/1259488*

EU agreed endpoints are marked in bold
***** New study, not yet peer-reviewed
** Study amendments are indicated in brackets

Based on the available studies, the conclusion for relevant endpoints adopted to the new list of endpoint format for the current renewal of approval remains as follows:

Acute toxicity (Regulation (EU) No 283/2013, Annex Part A, point 5.2)

Rat LD_{50} oral	> 5000 mg/kg bw	
Rat LD_{50} dermal	> 2000 mg/kg bw	
Rat LC_{50} inhalation	> 6.7 mg/L	
Skin irritation	Non-irritant	
Eye irritation	Non-irritant	
Skin sensitisation	Not sensitising	
Phototoxicity	Not phototoxic	

CA 5.2.1 Oral

[redacted] 1998(c): BAS 510 F: Acute oral toxicity in rats; BASF DocID 1998/10643
[redacted] 2000: Amendment BAS 510 F: Acute oral toxicity in rats (Re-analysis of test substance's stability), BASF DocID 2000/1018715

Note: This study report has been part of the documentation for the first Annex I inclusion process and a short summary is therefore presented here

Groups of 5 male and 5 female Wistar rats (Chbb: thom, SPF) were administered 2000 mg/kg bw and 5000 mg/kg bw Boscalid (95.3% purity, batch N 26) in a 0.5% aqueous CMC preparation (dose volume of 10 mL/kg bw (low dose) and 20 mL/kg bw (high dose)) by gavage and were observed for 14 days. No mortality occurred during the study period. In the high dose, clinical signs comprised impaired general state, dyspnoea, staggering, excitation, erythema and piloerection observed in two males and one female on the day of application. All animals appeared normal within two days after application. No signs of toxicity were observed in the dose group of 2000 mg/kg bw. Body weight gain was unaffected by the test item. At necropsy, no pathological changes were observed that could be attributed to administration of the test material.
The oral LD_{50} of the test substance was greater than 5000 mg/kg bw in male and female rats. According to CLP Reg. (EC) 1272/2008 classification criteria, no classification is warranted as to acute oral toxicity for Boscalid.

CA 5.2.2 Dermal

[redacted] 1998(b)**:** BAS 510 F: Acute dermal toxicity in rats; BASF DocID 1998/10642
[redacted]: Amendment BAS 510 F: Acute dermal toxicity in rats (Re-analysis of test substance's stability), BASF DocID 2000/1018711

Note: This study report has been part of the documentation for the first Annex I inclusion process and a short summary is therefore presented here

Groups of 5 male and 5 female Wistar rats (Chbb: thom, SPF) were administered 2000 mg/kg bw Boscalid (95.3% purity, batch N 26) suspended in a 0.5% aqueous CMC preparation to the clipped trunk skin (~ 50 cm^2) for 24 hours under semi-occlusive conditions. Thereafter, test item was removed with warm water and animals were observed for 14 days. No mortality occurred during the study period. No systemic signs of toxicity were observed. One female showed well-defined erythema 1 day after application that was reversible within 7 days. Body weight gain was unaffected by the test item. At necropsy, no pathological changes were observed that could be attributed to administration of the test material.
The dermal LD_{50} of the test substance was greater than 2000 mg/kg bw in male and female rats. According to CLP Reg. (EC) 1272/2008 classification criteria, no classification is warranted as to acute dermal toxicity for Boscalid.

CA 5.2.3 Inhalation

[redacted] 1998: BAS 510 F – Acute inhalation study in Wistar rats – 4-hour dust exposure; BASF DocID 1998/10803

Note: This study report has been part of the documentation for the first Annex I inclusion process and a short summary is therefore presented here

Groups of 5 male and 5 female Wistar rats (Chbb: thom, SPF) were exposed with 6.7 mg/L Boscalid (95.3% purity, batch N 26) as a dust aerosol for 4 hours in a head-nose inhalation system and observed for 14 days. The particle size distribution revealed a mass median aerodynamic diameter (MMAD) of 3.4 µm with a geometrical standard deviation (GSD) of 3.4, which is within the respirable range. No mortality occurred during the study period. Clinical examination revealed attempts to escape, irregular and dragging respiration, respiratory sounds as well as urine smeared fur, piloerection and squatting posture. All clinical signs were reversible within 3 days. Body weight gain was unaffected by the test item. At necropsy, no pathological changes were observed that could be attributed to administration of the test material.
The inhalation LC_{50} of the test substance was greater than 6.7 mg/L in male and female rats. According to CLP Reg. (EC) 1272/2008 classification criteria, no classification is warranted as to acute inhalation toxicity for Boscalid.

CA 5.2.4 Skin irritation

[redacted] 1998(d)**:** Study on the acute dermal irritation/corrosion of BAS 510 F in the rabbit (Re-analysis of test substance's stability); BASF DocID 1998/10640
[redacted] 2000: Amendment BAS 510 F – Acute dermal irritation / corrosion in the rabbit; BASF DocID 2000/1018712

Note: This study report has been part of the documentation for the first Annex I inclusion process and a short summary is therefore presented here

Two male and 4 female White New Zealand rabbits (SPF) were exposed with 0.5 g Boscalid (95.3% purity, batch N 26) on the clipped trunk skin (patch size 6.25 cm²) for 4 hours under semi-occlusive conditions. Thereafter, test item was removed with Lutrol® E 400 and Lutrol® E 400/water (1:1) and animals were observed for 72 hours. Skin readings were performed at 1, 24, 48 and 72 hours after removal of the test item. No mortalities occurred during the study period. No systemic signs of toxicity were observed. The average irritation score (24 – 72 hours) was 0.2 and 0.0 for erythema and oedema, respectively. Since all skin reactions were reversible within 48 hours after patch removal, the study was terminated after 72 hours.

No skin irritation potential of the test substance was observed in male and female rabbits. According to CLP Reg. (EC) 1272/2008 classification criteria, no classification is warranted as to skin irritation for Boscalid.

CA 5.2.5 Eye irritation

[redacted] 1998(e)**:** Study on the acute eye irritation of BAS 510 F in the rabbit; BASF DocID 1998/10641
[redacted] 2000: Amendment BAS 510 F – Acute eye irritation in the rabbit (Re-analysis of test substance's stability); BASF DocID 2000/1018713

Note: This study report has been part of the documentation for the first Annex I inclusion process and a short summary is therefore presented here

One eye of 2 male and 4 female White New Zealand rabbits (SPF) was instilled with 0.1 mL bulk volume (~ 21 mg) of Boscalid (95.3% purity, batch N 26) for 24 hours, whereas the other eye served as control. Thereafter, the test item was removed with tap water and animals were observed for 72 hours. Eye readings were performed at 1, 24, 48 and 72 hours after removal of the test item. No mortality occurred during the study period. No signs of toxicity were observed. The average irritation score (24 – 72 hours) was 0.0 for corneal opacity, iris and chemosis, and 0.4 for conjunctivae redness with maximum grade of 1 in individual animals. Since all eye findings were reversible within 72 hours, the study was terminated after the 72 hour reading.

No eye irritation potential of the test substance was observed in male and female rabbits. According to CLP Reg. (EC) 1272/2008 classification criteria, no classification is warranted as to eye irritation for Boscalid.

CA 5.2.6 Skin sensitisation

[redacted] 1998(a): BAS 510 – Maximization test in guinea pigs; BASF DocID 1998/10638
[redacted] 2000: Amendment BAS 510 F – Acute dermal irritation / corrosion in the rabbit (Re-analysis of test substance's stability); BASF DocID 2000/1018714

Note: This study report has been part of the documentation for the first Annex I inclusion process and a short summary is therefore presented here

Boscalid (95.3% purity, batch N 26) was tested in a maximization test in a group of 20 female Dunkin Hartley guinea pigs (Crl: (HA) BR (SPF)) according to Magnusson and Kligman. Intradermal induction (using the adjUV/visnt technique, day 0) and the challenge (day 14) were performed with a 5% test item preparation in 1% CMC-aqueous solution, and 25% test item preparation in 1% CMC- solution was used for the dermal (percutaneous) induction (day 7). For the dermal applications, the test item was incubated 48 and 24 hours under occlusive conditions for the dermal induction and the challenge, respectively. Skin readings were performed 24 and 48 hours after intradermal and dermal inductions, respectively, and 24 and 48 hours after the patch removal after the challenge application. A control group of 20 females was treated with the vehicle in the induction phase, and was treated in the same manner as the test group animals in the challenge phase.

A concurrent positive control was not included in this study, but a reliability check with Alpha-Hexylcinnamaldehyde (techn. 85%) was performed twice a year in the test institute, showing the ability of the test system to detect sensitising substances under the laboratory conditions chosen and this guinea pig strain.
Intradermal induction induced well-defined erythema and slight oedema at the injection sites of the test substance preparation in all test group animals. After the dermal induction, partially open incrustation (caused by the intradermal induction) could be observed in addition to moderate and confluent erythema and swelling in all test group animals. The challenge resulted in very slight erythema in 3 and 4 out of 19 animals 24 and 48 hours after patch removal, respectively. Treatment with the vehicle alone caused no dermal irritation.
1 animal of the control group and the test group died 10 or 11 days after study begin, respectively. Macroscopic examination revealed that the animals suffered from pneumonia, and thus the cause of death was not considered substance-related. Body weights were not affected by the test item.

No skin sensitisation potential of the test substance was observed in guinea pigs. According to CLP Reg. (EC) 1272/2008 classification criteria, no classification is warranted as to skin sensitisation for Boscalid.

Report: CA 5.2.6/1
[redacted], 2003a
BAS 510 F - Maximization test in guinea pigs
2003/1022252
Guidelines: EEC 96/54 B 6, OECD 406, EPA 870.2600, JMAFF No 12 Nosan No 8147
GLP: yes
(certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Note: This study report has **not** been part of the documentation for the first Annex I inclusion process and a full evaluation is therefore presented here

Executive Summary

Boscalid (94.3% purity, batch N46) was tested in a maximization test in Dunkin Hartley guinea pigs (HsdPoc: DH (SPF)) according to Magnusson and Kligman. Based on the results of a pre-test, the intradermal induction was performed with a 5% test item preparation in 1% CMC-aqueous preparation into the neck region of the animals. The percutaneous induction (7 days after intradermal induction) was performed with a 25% test item preparation in 1% CMC-aqueous preparation and two challenge exposures (14 and 21 days after percutaneous induction) was performed with a 5% test item preparation in 1% CMC aqueous preparation. The study was performed in 10 control and 20 test group animals. Readings were performed 24 hours after the intradermal injection and 24 hours after removal of the patch with regard to percutaneous induction. The challenges were carried out with the test substance preparation applied for 24 hours to the intact skin of the flank under occlusive conditions. 24 and 48 hours after removal of the patch, skin readings were performed. A positive control with a known sensitiser was not included into the study. However, studies with Alpha-Hexylcinnamaldehyde (techn. 85%) are regularly performed as reliability check in the test institute and showed that the test system is able to detect sensitising substances under the laboratory conditions chosen (sensitisation rate of 100% in the guinea pig strain in the relevant period).
The intradermal induction caused moderate and confluent to intense erythema and swelling at the injection sites of the test substance preparation in all test group animals. After the percutaneous induction, partially open incrustation (caused by the intradermal induction) could be observed in addition to moderate and confluent erythema and swelling in all test group animals. The 1st challenge resulted in discrete or patchy erythema in 3/20 animals 24 hours after patch removal that were completely reversible until the 48-hour reading time point. After the 2nd challenge, discrete or patchy erythema were noted in 2/20 animals 24 hours after patch removal which were reversible in one of these animals and persisted in another until the 48-hour reading time point.
The skin reactions observed in few animals of the test group after the challenges are considered to represent signs of slight irritation. This interpretation is supported by the absence of an increased incidence in skin findings after the second challenge.
No mortality occurred in this study, and body weight was not affected by the test item.

Based on the results of this study and applying the evaluation criteria, it was concluded that Boscalid **does not have a sensitising effect** on the skin of the guinea pig in the Maximization Test under the test conditions chosen.

(DocID 2003/1022252)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: BAS 510 F

Description:	Solid /white
Lot/Batch #:	N46
Purity:	94.3%
Stability of test compound:	the stability under storage conditions (room temperature) over the study period was guaranteed (see Certificate of Analysis 99413 1). The homogeneity of the test substance was confirmed by the analysis.

2. Vehicle / Positive control: Vehicle:
1% CMC (cleaned sodium carboxymethylcellulose) in doubly distilled water
Positive control: Alpha-Hexylcinnamaldehyde, techn. 85%

3. Test animals:

Species:	Guinea Pig
Strain:	Dunkin Hartley, Hsd Poc: DH (SPF)
Sex:	females (main test) males and females (pre-test)
Age:	approximately 7 weeks
Weight at dosing (mean):	324 - 387 g
Source:	Harlan Winkelmann, Borchen, Germany
Acclimation period:	7 days
Diet:	Kliba Labordiät (Kaninchen/ Meerschweinchen-Haltungsdiät; Provimi Kliba SA, Kaiseraugst, Switzerland) ad libitum
Water:	Tap water, ad libitum
Housing:	Groups of 5 animals were housed in stainless steel wire mesh cages with plastic-coated grating, minimum floor area: 2000 cm²

Environmental conditions:

Temperature:	20 - 24 °C
Humidity:	30 - 70 %
Air changes:	Central air-conditioning
Photo period:	Alternating 12-hour light and dark cycles, artificial light from 6 am to 6 pm

B. STUDY DESIGN AND METHODS

1. Dates of work: 19-Sep-2003 - 28-Nov-2003

2. Animal assignment and treatment:
The skin sensitising potential of BAS 510 F (Boscalid technical material) was assessed using the Maximization Test based on the method of Magnusson and Kligman. For this, female guinea pigs were randomly allocated to groups. Ten animals were used as control group animals and 20 animals in the test group.
The test substance preparations were produced on a weight by weight basis shortly before the application by stirring with a high speed homogenizer (Ultra-Turrax) and a magnetic stirrer. The stability and the concentration control analysis of the test substance in the vehicle were determined indirectly by the homogeneity analysis. The homogeneity of the test substance preparation during application was provided by stirring with a magnetic stirrer. Furthermore, the homogeneity of the test substance preparation used for the challenge application was verified by analysis.
The fur of the animals was clipped at least 2 hours before each test item application at the appropriate application sites. If necessary, the fur was additionally clipped at least 2 hours before evaluation of the skin reactions.

3. Clinical observation:
Mortality was checked twice daily on working days and once daily on weekends and public holidays. No detailed clinical examination of the individual animals was performed but any obvious signs of systemic toxicity and/or local inflammation at the application sites were recorded.

4. Body weights:
Individual body weights were determined on day 0, after the 1st challenge and after the 2nd challenge (at the last day of observation).

5. Pre-test:
Intradermal pre-test: A 5% test substance preparation (in 1% CMC-solution in doubly distilled water) was intra-dermally injected to one female animal. Six intradermal injections were applied at the neck region: front row: 2 injections each of 0.1 mL Freund´s complete adjUV/visnt without test item emulsified with 0.9 % aqueous NaCl-solution in a ratio of 1:1; middle row: 2 injections each of 0.1 mL of 5% test item preparation in 1% aqueous CMC preparation; back row: 2 injections each of 0.1 mL Freund's complete adjUV/visnt / vehicle (1:1) with 5% test item. Skin reactions were assessed 24 hours after the beginning of the application.

Percutaneous pre-test:
Gauze patches of 2 cm x 2 cm (6 layers surgical gauze Ph. Eur. from Lohmann GmbH & Co. KG) containing 0.5 mL of test substance preparation were applied to the flanks of test groups of 3 animals each with either 25 and 50% test item or 5 and 10% test item prepared in 1% CMC-solution in doubly distilled water.
Gauze patches were fixed by means of occlusive dressing. The dressing consisted of rubberized linen patches (4 x 4 cm from Russka), patches of Idealbinde (5 x 5 cm from Pfälzische Verbandstoff-Fabrik) and Fixomull® Stretch (adhesive fleece) from Beiersdorf AG.
The animals were exposed for 24 hours and skin readings were performed 1, 24 and 48 h after removal of the patch.

6. Main study – intradermal induction:
Based on the results of the pre-test, intradermal induction was performed with 5% test substance preparations in 1% CMC-solution. Control group animals received the same injections but with the test substance preparation being replaced by the vehicle.

7. Main study – percutaneous induction:
One week after intradermal induction, 0.5 mL of the 25% test item preparation was applied to each test group animal for 48 hours under the same conditions as described in the percutaneous pre-test. The control animals were not treated since the 1% CMC-solution in doubly distilled water used as formulating agent was not expected to influence the result of the study.

8. Main study - challenges:
The 1st challenge was carried out 14 days after the percutaneous induction, and the 2nd challenge one week after the first challenge. 0.5 mL of the 5% test item preparation was applied to the intact flank of the test and control group animals. The animals were exposed under occlusive conditions as described above for 24 hours and skin readings were performed 24 and 48 hours after removal of the patch.

9. Evaluation of results
The number of animals with skin findings at 24 and/or 48 hours after the removal of the patch was taken into account for the determination of the sensitisation rate. The evaluation "sensitising" results if at least 30% of the test animals exhibit skin reactions.

10. Positive controls
A positive control (reliability check) with a known sensitizer was not included in this study. However, a separate study with the positive control Alpha-Hexylcinnamaldehyde (techn. 85%) is regularly performed in the laboratory.

II. RESULTS AND DISCUSSION

Test substance concentrations and homogeneity of the preparations were confirmed by analysis.

Results of the pre-test and the main study are as summarized below.

A. PRE-TEST

In the **intradermal pre-test** intense erythema and swelling was observed following injection of Freund's adjUV/visnt or the 5% test substance preparation in Freund's adjUV/visnt. The 5% test substance preparation in aqueous CMC caused moderate and confluent erythema and swelling. In the **percutaneous pre-test** applications of 50, 25, 10 and 5% test substance preparations in aqueous 1% CMC resulted were investigated at 1, 24 and 48 hours readings. Results are summarized in Table 5.2.6-1 below. The maximum non-irritant concentration was found to be a 5% test substance preparation in 1% CMC-solution in doubly distilled water, and thus this concentration was chosen for both challenges.

Table 5.2.6-1: BAS 510 F - Skin reactions in the percutaneous pre-test

Animal #	Readings at 1 hour				Readings at 24 hours				Readings at 48 hours			
	% test substance concentrations in 1% CMC-solution in doubly distilled water											
	50%	25%	10%	5%	50%	25%	10%	5%	50%	25%	10%	5%
73	2	2	-	-	2	2	-	-	0	0	-	-
74	1	1	-	-	0	0	-	-	0	0	-	-
75	2	2	-	-	2	1	-	-	0	0	-	-
1	-	-	0	0	-	-	1	0	-	-	0	0
2	-	-	2	0	-	-	1	0	-	-	0	0
3	-	-	0	0	-	-	0	0	-	-	0	0
4	-	2	1	-	-	0	0	-	-	0	0	-
5	-	2E	1	-	-	1	1	-	-	1	0	-
6	-	1	0	-	-	0	0	-	-	0	0	-

Scoring
0 = no visible change, 1 = discrete or patch erythema, 2 = moderate and confluent erythema, 3 = intense erythema and swelling
E = swelling, K = incrustation, partially open

B. OBSERVATIONS

No abnormalities were observed during general observation.

C. BODY WEIGHTS

Body weight gain was not adversely affected during the course of the study.

D. SKIN REACTIONS AFTER INTRADERMAL INDUCTION

Injections of a 5% test item preparation in 1% CMC-solution in doubly distilled water caused moderate and confluent to intense erythema and swelling at the injection sites of the test substance preparation in all test group animals (see Table 5.2.6-2).

Table 5.2.6-2: BAS 510 F - Skin reactions after intradermal induction in test group animals

Position of injection: neck region				
Form of application:				
Findings 24 hours after the beginning of application				
Animal #	**Application site**	**A) Freund's complete adjUV/visnt / 0.9% aqueous NaCl Solution (1:1)**	**B) Test item 5 % in 1% CMC-solution in doubly distilled water**	**C) Test item 5 % in A)**
311	right	3	2 E	3
	left	3	2 E	3
312	right	3	2 E	3
	left	3	2 E	3
313	right	3	2 E	3
	left	3	2 E	3
314	right	3	2 E	3
	left	3	2 E	3
315	right	3	2 E	3
	left	3	2 E	3
316	right	3	2 E	3
	left	3	2 E	3
317	right	3	2 E	3
	left	3	2 E	3
318	right	3	2 E	3
	left	3	2 E	3
319	right	3	2 E	3
	left	3	2 E	3
320	right	3	2 E	3
	left	3	2 E	3
321	right	3	2 E	3
	left	3	2 E	3
322	right	3	2 E	3
	left	3	2 E	3
323	right	3	2 E	3
	left	3	2 E	3
324	right	3	2 E	3
	left	3	2 E	3
325	right	3	2 E	3
	left	3	2 E	3
327	right	3	2 E	3
	left	3	2 E	3
328	right	3	2 E	3
	left	3	2 E	3
328	right	3	2 E	3
	left	3	2 E	3
329	right	3	2 E	3
	left	3	2 E	3
330	right	3	2 E	3
	left	3	2 E	3

Scoring
0 = no visible change, 1 = discrete or patch erythema, 2 = moderate and confluent erythema, 3 = intense erythema and swelling
E = swelling, K = incrustation, partially open

E. SKIN REACTIONS AFTER PERCUTANEOUS INDUCTION

The percutaneous induction with a 25% test substance preparation in 1% CMC-solution in doubly distilled water led to a partially open incrustation (caused by the intradermal induction) and moderate and confluent erythema in all test group animals (see Table 5.2.6-3).

Table 5.2.6-3: BAS 510 F - Skin reactions 48 hours after percutaneous induction

Animal #	Test item 25 % in 1% CMC-solution in doubly distilled water
311	2 E K
312	2 E K
313	2 E K
314	2 E K
315	2 E K
316	2 E K
317	2 E K
318	2 E K
319	2 E K
320	2 E K
321	2 E K
322	2 E K
323	2 E K
324	2 E K
325	2 E K
326	2 E K
327	2 E K
328	2 E K
329	2 E K
330	2 E K
Scoring 0 = no visible change, 1 = discrete or patch erythema, 2 = moderate and confluent erythema, 3 = intense erythema and swelling E = swelling, K = incrustation, partially open	

F. SKIN REACTIONS AFTER CHALLENGE

The both challenges with a 5% test substance preparation in 1% CMC-solution in doubly distilled water did not cause any skin reactions in animals of the control group 24 and 48 hours after removal of the patch. In the test group animals, the 1st challenge resulted in discrete or patchy erythema in 3/20 animals 24 hours after patch removal that were completely reversible until 48-hour reading time point. After the 2nd challenge, discrete or patchy erythema was noted in 2/20 animals of the test group 24 hours after patch removal that were reversible only in one of these animals and persisted in another until the 48-hour reading time point (see Table 5.2.6-4).

Table 5.2.6-4: BAS 510 F - Skin reactions after challenge

Skin findings	Control group		Test group	
	24 h	48 h	24 h	48 h
	1st challenge			
Grade 0	10/10[#]	10/10	17/20	20/20
Grade 1	-	-	3/20	-
	2nd challenge			
Grade 0	10/10	10/10	18/20	19/20
Grade 1	-	-	2/20	1/20

[#] x/y = number of findings / number of animals tested

The skin reactions observed in single animals of the test group after the challenges are considered to represent signs of slight irritation. This interpretation is supported by the absence of an increased incidence in skin findings after the second challenge, with animals eventually having acquired increased sensitivity by the first challenge.

G. POSITIVE CONTROL

The positive control Alpha-Hexylcinnamaldehyde was applied in concentration of 5% in paraffin oil for the intradermal induction. Then 10% and 5% in Lutrol® E 400 were used for the percutaneous induction and for both challenges, respectively. The results of the latest study conducted with the positive control are presented in Table 5.2.6-5.

Table 5.2.6-5: Skin reactions after challenges with the positive control

Skin findings	5% test substance in Lutrol® E 400			Vehicle control Lutrol® E 400		
	24 h	48 h	Total	24 h	48 h	Total
	1st challenge					
Control group 1	3/5[#]	1/5	3/5	3/5	1/5	3/5
Control group 2	no application of test substance			3/5	0/5	3/5
Test group	10/10	7/10	10/10	8/10	0/10	8/10
	2nd challenge					
Control group 1	4/5	4/5	4/5	2/5	0/5	2/5
Control group 2	2/5	0/5	2/5	2/5	0/5	2/5
Test group	10/10	7/10	10/10	1/10	0/10	1/10

[#] x/y = number of positive reactions/number of animals tested (reading at 24 h and/or 48 h after the removal of the patch)

Based on the results of this study and applying the evaluation criteria for this test, it was concluded that Alpha-Hexylcinnamaldehyde, techn. 85% has a sensitising effect on the skin of the guinea pig in the maximization test under the test conditions chosen.
The skin reactions in the control group and test group induced by Lutrol® E 400 are interpreted as primary irritation because they were reversible by large during the 48 hours skin reading period. Such findings are observed at times with this vehicle. An explanation for the effect is lacking but is not considered to compromise the validity of the study. From the results of the test group, showing high incidence of persistent skin reactions as compared to lower reversible incidence in vehicle treated application sites, the skin sensitising effect is clearly shown and thus provides adequate evidence that the animal strain used in the test facility is able to detect skin sensitizing substances.

III. CONCLUSION

Based on the results of this study, it is concluded that BAS 510 F (Boscalid technical material) does not have sensitising properties in the guinea pig maximization test under the test conditions chosen.

CA 5.2.7 Phototoxicity

Report: CA 5.2.7/1
Cetto V.,Landsiedel R., 2013a
BAS 510 F (Boscalid) - In vitro 3T3 NRU phototoxicity test
DocID 2013/1259488

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)

Note: This study report has **not** been part of the documentation for the first Annex I inclusion process and a full evaluation is therefore presented here

Executive Summary

Boscalid (94.4% purity, batch N37) was tested for its ability to induce phototoxic effects in Balb/c 3T3 cells *in vitro*. The photo-cytotoxicity was estimated by means of the Neutral Red Uptake (NRU) method. Two independent experiments were carried out, both with and without irradiation with a solar simulator emitting light in the UV/vis spectrum. However, the 1st experiment was judged invalid due to lacking the predefined criteria of acceptance. In the 2nd experiment, vehicle and positive controls clearly fulfilled the acceptance criteria, and the experiment was assessed valid.
Based on an initial range-finding phototoxicity test for the determination of the experimental concentrations, the following concentrations were tested in this study with and without UV/vis irradiation: 0, 4.6, 10.0, 21.5, 46.4, 100.0, 215.4, 464.2 and 1000 µg/mL.
In this study, in the absence and the presence of UV/vis irradiation, no cytotoxicity was observed up to the highest concentration required. Precipitation at 46.4 µg/mL and above was observed with and without UV/vis irradiation. The test substance was predicted to have no phototoxic potential as indicated by Photo-Irritancy-Factor (PIF) values of *1. Due to the clearly negative results both in the 2nd experiment and the pre-test no additional testing of the test substance was required. The positive control chlorpromazine led to the expected increased cytotoxicity with UV/vis irradiation as indicated by PIF values of 33.7.
Thus, under the experimental conditions of this study, Boscalid is not considered to be a phototoxic substance in the *in vitro* 3T3 NRU Phototoxicity Test using Balb/c 3T3 cells.
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(DocID 2013/1259488)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material	BAS 510 F (Boscalid)
Description:	Solid, white
Lot/Batch #:	N37
Purity:	94.4% (tolerance ± 1.0%)
Stability of test compound:	Expiry date: 30 June 2018 The homogeneity of the test substance was ensured by mixing before preparation of the test substance solution. The stability of a comparable batch (N26) in the vehicle DMSO and in water was determined analytically (Project No.: 08B0179/976024 and 08B0179/976022).
Solvent used:	Dimethyl sulfoxide (DMSO)
2. Control Materials:	
Vehicle control:	DMSO 1% (v/v) in phosphate buffered saline (PBS)
Positive control compounds:	Chlorpromazine (CPZ, source: Sigma, cat. No. C8138) was dissolved in DMSO; 8 concentrations tested - 1.9 to 180 µg/mL without radiation, 0.03 to 3.2 µg/mL with radiation
3. Test organisms:	Balb/c 3T3, clone A31: fibroblast cell line isolated from the muscle tissue of a mouse embryo. 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 on 09 Aug 2006 and is stored at -196°C (liquid nitrogen).

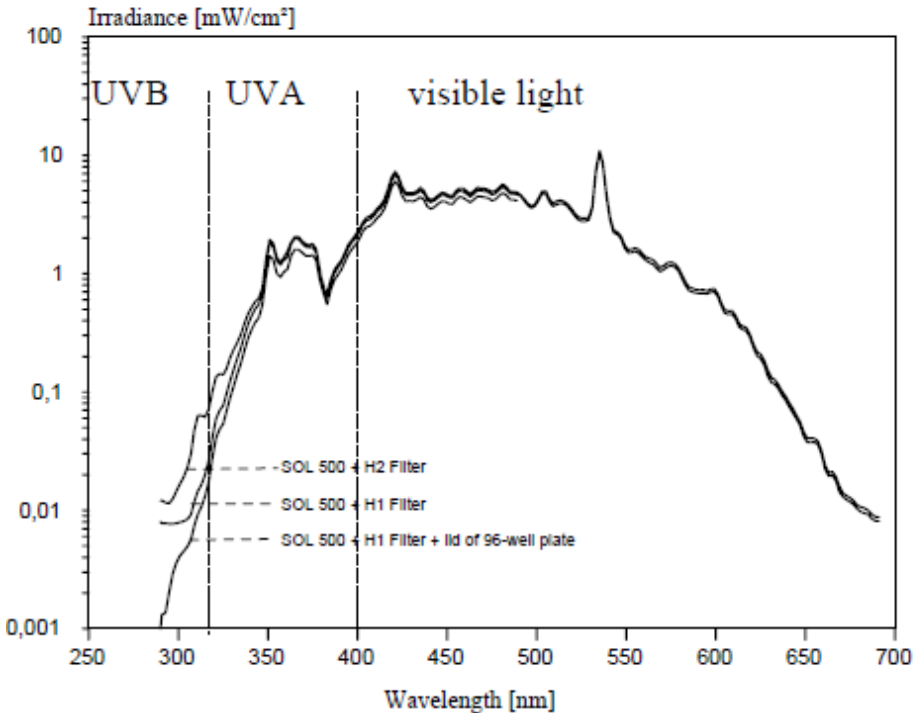
4. Culture media and reagents:

Culture medium:	Dulbecco's Modified Eagle's Medium (DMEM) supplemented with - 10% (v/v) new-born 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 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 UV/vis 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 432). However, the experimental setting was shown to be sufficient to detect phototoxic effects also of chemicals typically absorbing in the UVB range, e.g. the concurrent positive control Chlorpromazin (absorption peak at 309 nm) or Amiodarone. The exposure rates were determined with UV-meter RM-21 (Dr. Gröbel GmbH, 76275 Ettlingen, Germany).

Note: in the study report the irradiation was described as UVB (>320 nm) only. Based on the known emission spectrum of the SOL500 with H1 filter this description is misleading and the irradiation conditions are fully in line with the OECD 432.

6. Test concentrations:

Pre-test:	Up to 1000 µg/mL with and without irradiation. No cytotoxicity was observed with and without UV/vis irradiation.
Main NRU test:	Based on the results of the pre-test the following concentrations were used in the main study: Without UV/vis: 0, 4.6, 10.0, 21.5, 46.4, 100.0, 215.4, 464.2 and 1000 µg/mL With UV/vis: 0, 4.6, 10.0, 21.5, 46.4, 100.0, 215.4, 464.2 and 1000 µg/mL

B. STUDY DESIGN AND METHODS

1. Dates of experimental work: 05-Jun-2013 to 17-Jul-2013

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 10^4 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. After pre-incubation for 1 hour in the dark (5% (v/v) CO_2, ≥ 90% humidity; 37°C) one 96 well-plate per substance was irradiated for 50 minutes with UV/vis (UV intensity underneath the lid 1.5 - 2.1 mW/cm^2 = 5 J/cm^2) whereas the respective reference plate was kept in the dark for the same period. Thereafter the test substance was removed and the cells washed at least once with 100 µL PBS. After replenishing the wells with culture medium the cells were incubated overnight under the conditions indicated above. The medium was removed 24 hours after the start of treatment and after washing with 100 µL PBS the wells were filled with 100 µL medium containing 50 µg/mL Neutral Red. Subsequently the plates were incubated for another 3 hours. Each step was performed under light protected conditions in the lab to prevent uncontrolled photo activation. Finally, the cells were washed again with 100 µL PBS and the dye was extracted by 100 µL Neutral Red desorb solution. Cytotoxicity was determined by measuring the Neutral Red Uptake by means of a microplate reader (Perkin Elmer, Waltham, Massachusetts, US; Wallac 1420 multi-label 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 choice of the adequate model depends on the test conditions. Since the absolute values shown were rounded but the calculations were made using the unedited values, there may be deviations in the given relative values.

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%.

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

§ The authors of the study denominate the above quotient as ‘cytotoxicity’, which may be not completely accurate. Thus, in this summary the appropriate term ‘viability’ is used. This applies also to Table 5.2.7-1 to Table 5.2.7-2.

In case of cytotoxicity, an EC_{50} value (concentration at which the viability is reduced by 50% relative to the respective vehicle control) was calculated by a linear interpolation method (linear dose-response curve). Therefore two viability values were needed: one between 100% and 50% and one between 50% and 0%. From these two points the concentration that inhibits the Neutral Red uptake by 50% of the respective control was calculated.

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

- The Photo-Irritancy-Factor (PIF) 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 two special cases: Case 1 accounts for situations in which an EC_{50} can only be calculated in the presence of UV/vis irradiation. Case 2 accounts for situations where an EC_{50} cannot be calculated in absence and presence of irradiation. These special cases do not apply to this study. Even though described in the report these cases are not described in this summary.
- The Mean Photo Effect prediction model which is used if no EC_{50} was obtained in the absence and presence of UV light. This is not the case in this study. Even though described in the report this prediction model is not described in this summary.

3.2 Photo-Irritancy-Factor (PIF)

If no cytotoxicity occurs 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 rules:

PIF = *1:	no phototoxic potential predicted

3.3 Other parameters

pH value:
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 top dose 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.

5. Acceptance criteria:

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

- The vehicle control needs to fulfil the following criteria:
  - The mean OD_{550} 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 fulfil 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
  - PIF ≥ 6.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

Osmolarity and pH values were not influenced by test substance treatment. In this study, in the absence and presence of UV/vis irradiation, test substance precipitation in culture medium at the end of treatment was observed at 46.4 µg/mL and above in the pre-test and 2nd experiment.
In addition, changes in cell morphology were observed at the end of exposure period at 464.2 µg/mL and above 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 2nd experiment in the absence and the presence of UV/vis irradiation. Therefore, no EC_{50} values could be calculated. The cell densities were not reduced.
Based on these observations a formal PIF = *1 has to be used to characterize the result. (see Table 5.2.7-1).

Table 5.2.7-1: Mean relative viability of Boscalid with (+) and without (-)UV/vis irradiation in Balb 3T3 cells

Test group	UV/vis irradiation *	Precipitation**	Mean $OD_{corr.}$ ***	Relative viability [% of control]	
				Mean	SD
Vehicle control 1	-		0.575	-	10.4
Vehicle control 2	-		0.638	-	2.8
Vehicle mean	-		0.607	100	8.8
Boscalid					
4.6 µg/mL	-	-	0.638	105.2	0.8
10.0 µg/mL	-	-	0.651	107.3	0.9
21.5 µg/mL	-	-	0.637	104.9	1.7
46.4 µg/mL	-	+	0.699	115.2	6.7
100.0 µg/mL	-	+	0.724	119.3	2.2
215.4 µg/mL	-	+	0.728	120.0	3.5
464.2 µg/mL	-	+	0.711	117.1	7.4
1000.0 µg/mL	-	+	0.684	112.7	4.3
Vehicle control 1	+		0.624	-	2.4
Vehicle control 2	+		0.673	-	1.3
Vehicle mean	+		0.649	100	4.3
Boscalid					
4.6 µg/mL	+	-	0.602	92.7	1.3
10.0 µg/mL	+	-	0.618	95.2	4.0
21.5 µg/mL	+	-	0.587	90.5	2.8
46.4 µg/mL	+	+	0.610	94.1	6.3
100.0 µg/mL	+	+	0.629	96.9	3.7
215.4 µg/mL	+	+	0.615	94.7	2.0
464.2 µg/mL	+	+	0.588	90.6	2.4
1000.0 µg/mL	+	+	0.628	96.9	2.6

*: Irradiation with Sol 500 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)

Due to the clearly negative results both in the 2nd experiment and the pre-test no additional testing of the test substance was required.

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 2nd experiment in the absence and the presence of UV/vis irradiation (see Table 5.2.7-2).
In the experiment without UV/vis irradiation, there was a decrease in viability at ≥ 30.0 µg/mL (EC_{50}: 22.4 µg/mL). With UV/vis irradiation, there was a decrease in viability at ≥ 0.8 µg/mL (EC_{50}: 0.7 µg/mL).

Based on the EC_{50} values, PIF's of 33.7 was obtained in the 2nd experiment, indicating a strong phototoxic potential, and thus confirming the sensitivity of the test system.

Table 5.2.7-2: Mean relative viability of Chlorpromazine with (+) and without (-)UV/vis irradiation in Balb/c 3T3 cells

Test group	UV/vis irradiation*	Mean $OD_{corr.}$ **	Relative viability [% of control]	
			Mean	SD
Vehicle control 1	-	0.638	-	2.1
Vehicle control 2	-	0.680	-	1.1
Vehicle mean	-	0.659	100.0	3.7
Chlorpromazine				
1.9 µg/mL	-	0.670	101.7	1.9
3.8 µg/mL	-	0.691	104.9	1.8
7.5 µg/mL	-	0.660	100.2	4.0
15.0 µg/mL	-	0.562	85.3	3.3
30.0 µg/mL	-	0.091	13.8	2.3
60.0 µg/mL	-	0.000	-0.1	0.1
90.0 µg/mL	-	0.001	0.1	0.2
180.0 µg/mL	-	0.002	0.3	0.2
Vehicle control 1	+	0.644	-	2.2
Vehicle control 2	+	0.657	-	4.5
Vehicle mean	+	0.650	100.0	3.6
Chlorpromazine				
0.03 µg/mL	+	0.635	97.7	1.2
0.05 µg/mL	+	0.632	97.1	2.2
0.10 µg/mL	+	0.619	95.2	2.8
0.20 µg/mL	+	0.607	93.3	6.6
0.40 µg/mL	+	0.621	95.5	2.6
0.80 µg/mL	+	0.174	26.8	8.1
1.60 µg/mL	+	0.002	0.2	0.3
3.20 µg/mL	+	0.005	0.8	0.1

*: Irradiation with Sol 500 solar simulator for 50 minutes (approx. 5 J/cm²)
**: Mean OD corrected: mean absorbance (test group) minus mean absorbance (blank)

III. CONCLUSION

According to the results of the present study, Boscalid is not considered to be a phototoxic substance in the *in vitro* 3T3 NRU Phototoxicity Test.

CA 5.3 Short-Term Toxicity

Short-term toxicity (90-day) studies with oral administration in three different species (rats, mice, dogs) and a 1-year dog study as well as 28-day dermal toxicity study in rats have been peer-reviewed by the Rapporteur Member State Germany and national competent authorities following submission of the original dossier for Annex I inclusion (2000) and were considered to be acceptable. The endpoints were fixed in the European Commission Review Report for the active substance Boscalid (SANCO/3919/2007 –Rev 5, 21 January 2008).

For the reviewer's convenience, these studies are summarized in respective chapters CA 5.3.1 - CA 5.3.3 as extracted from the Monograph (2002). A tabulated summary is provided in Table 5.3-1 below.

Table 5.3-1: Summary of short-term toxicity studies with Boscalid (BAS 510 F)

Study	NOAEL males/females mg/kg bw/day	LOAEL males/females mg/kg bw/day	Effects	Reference BASF DocID
Rat: 90-day oral 0, 100, 500, 2000, 5000 & 15000 ppm	34/40 (500 ppm)	137/159 (2000 ppm)	**≥2000 ppm**: Altered clinical chemistry & haematological parameters, increased thyroid weight associated with follicular cell hypertrophy & hyperplasia (males) **≥5000 ppm**: Increased thyroid weight (both sexes) Increased liver weight & centrilobular hypertrophy	[redacted] 2000/1012190
Mouse: 90-day oral 0, 150, 1000, 4000 & 8000 ppm	29/42 (150 ppm)	197/277 (1000 ppm)	**≥1000 ppm**: Altered clinical chemistry: Decreased cholesterol (males) Increased liver weight (both sexes) **≥4000 ppm**: Altered clinical chemistry (total protein, albumin, globulins decreased in males, increase in alanine aminotransferase in females) Fatty liver change grade 4	[redacted] 2000/1000188
Dog: 90-day oral 0, 250, 2500 & 25000 ppm	7.6 /8.1 (250 ppm)	78 /82 (2500 ppm)	**≥2500 ppm**: Altered clinical chemistry: Increased alkaline phosphatase Increased liver weight (absolute and relative, both sexes) **25000 ppm**: Initial body weight loss (both sexes), followed by reduced body weight gain (females) Haematology: Reduction in haemoglobin and erythrocytes (females only) Increased thyroid weight (females only, absolute weight without statistical significance and relative weight statistically identified)	[redacted] et al 2000/1012306

Study	NOAEL males/females mg/kg bw/day	LOAEL males/females mg/kg bw/day	Effects	Reference BASF DocID
Dog: 12-month oral 0, 200, 800, 2000 & 20000 ppm	22/22 (800 ppm)	57/58 (2000 ppm)	**≥2000 ppm**: Reduced body weight gain (females) Altered clinical chemistry: Increased alkaline phosphatase (both sexes) Increased liver weight (absolute and relative, females only), increased thyroid weight (both sexes). Without histopathological correlate in both, liver and thyroids. **20000 ppm**: Initial body weight loss, followed by reduced body weight gain (females) Altered clinical chemistry: Increased triglycerides and chloride (both sexes), cholesterol, total protein, globulins (females only, sampling at 3 months)	[redacted] et al 2000/1016881
Rat: 28-day dermal 0, 100, 250 & 1000 mg/kg bw/day	1000	-	Absence of adverse systemic effects (marginal reduction of bilirubin (males only)	[redacted] 2000/1013240

Boscalid has been shown to be of low toxicity as demonstrated by the high dose levels chosen, which were at the limit dose of 1000 mg/kg bw in feeding studies in rats, mice and above 500 mg/kg bw/day in dogs. Even at this dose level, clinical signs of toxicity or adverse effects on feed consumption and or body weight gain were observed rarely. The signs of toxicity observed in the three species tested (rat, mouse, dog) were overall similar and consisted mainly of alterations in clinical chemistry.
Main target organ was the liver. Weight increases of the liver were observed in all three species. Histopathological changes mainly consisted of hypertrophy and fatty change in rodents and suggest an adaptation of this organ to increased functional demand.

In rats and dogs the thyroids were identified as a second target organ as evidenced by weight increases (rats and dogs) and histopathologically by follicular cell hypertrophy/hyperplasia (rats only).

In a 28-day dermal toxicity study in rats, no substance-related systemic adverse effects were detected up to the highest dose of 1000 mg/kg bw tested. There were no signs of local irritation in this study.

A particular species sensitivity was not clearly evident from comparison of LOAELs and NOAELs obtained in oral short-term toxicity studies performed in rats, mice and dogs. The fact that the studies conducted with dogs yielded the lowest NOAEL and LOAEL values might just have resulted from the choice of different dose levels tested in rats, mice and dogs, respectively.

The 1-year oral dog feeding study was identified as the most relevant short-term toxicity study regarding setting of the reference value for non-dietary risk assessment purposes (i.e. AOEL setting). In this study the NOAEL identified (22 mg/kg bw/d) is above the NOAEL of the 90-day dog study (7.6 /8.1 mg/kg bw/d) and below the LOAEL of the 90-day dog study (78 /82mg/kg bw/d). The fact that the NOAEL of the 90-day study was below that of the 1-year dog study is due to the dose levels chosen rather than an altered sensitivity of test animals.

Studies submitted in this supplementary dossier (not yet peer-reviewed):
No additional data on short-term toxicity of Boscalid were generated by the applicant.

Based on the available data on the short-term toxicity, classification of Boscalid for this endpoint is not required according to the criteria laid down in Regulation (EU) No. 1272/2008 (CLP). Moreover, the data on short-term toxicity of Boscalid have already been evaluated within the Annex I inclusion process, coming to the conclusion that Boscalid does not need to be classified for human health (SANCO/3919/2007-rev. 5, 21 January 2008).

Thus, the conclusion for relevant endpoints adopted to the new list of endpoint format for the current re-registration is presented as follows:

Short-term toxicity (Regulation (EU) N° 283/2013, Annex Part A, point 5.3)

Target organ / critical effect	**Rat:** Liver and thyroids: increased organ weights and histological findings, alterations of clinical chemistry and haematological parameters. **Mouse:** Liver: increased organ weights and altered clinical chemistry parameters. **Dog:** Liver and thyroids: increased organ weights, alterations of clinical chemistry parameters.	
Relevant oral NOAEL	90-day, rat: 34 mg/kg bw/day (500 ppm) 90-day, mouse: 29 mg/kg bw/day (150 ppm) 90-day dog: 7.6 mg/kg bw/day (250 ppm) 1-year dog: 22 mg/kg bw/day (800 ppm) Overall NOAEL dog: 22 mg/kg bw/day	
Relevant dermal NOAEL	28-day, rat: 1000 mg/kg bw/day	
Relevant inhalation NOAEL	No data - not required	

CA 5.3.1 Oral 28-day study

No EU data requirement. The short-term toxicity of Boscalid by the oral route of application was investigated based on studies started with 90-day up to 1-year exposure periods.

CA 5.3.2 Oral 90-day study

Boscalid - Subchronic oral toxicity study in Wistar rats - Administration in the diet for 3 months [redacted] et al., 2000a)
Doc ID 2000/1012190

Guidelines: According to OECD 408 (1981), EEC 87/302, EPA 82-1 and JMAFF
Deviations: None
GLP: Yes
Acceptance: The study has been evaluated and considered acceptable in the EU registration process for the first Annex I inclusion.

Note: This study report has been part of the documentation for the first Annex I inclusion process but a more detailed summary is presented here to account for requirements of Regulation (EC) No. 1107/2009

Boscalid (95.3% purity, batch N 26) was administered to groups of 10 male and 10 female Wistar rats (Chbb:THOM (SPF), source: Boehringer Ingelheim Pharma KG, Germany) at dietary levels of 0, 100, 500, 2000, 5000 and 15000 ppm for a period of 3 months. These concentrations were equivalent to the substance intake of 0, 7, 34, 137, 347 and 1055 mg/kg bw/day in males, and 0, 8, 40, 159, 395 and 1225 mg/kg bw/day in females.

Findings
The stability of the test substance during the study period was analytically proven. The stability and homogeneity of the test substance in the diet were confirmed by analysis. The correctness of the concentrations was analytically confirmed.
There were no mortalities and clinical signs of toxicity in any of the dose groups chosen. Body weight gain and feed consumption did not show treatment related differences. Following the 90-day dietary administration of Boscalid to Wistar rats, adverse effects were observed in animals of dose group ≥ 2000 ppm.
Findings consisted of decreased triglycerides and increased protein and albumin in both sexes, decreased prothrombin time and increased globulin and cholesterol in females, and decreased bilirubin and absolute as well as relative spleen weights (15000 ppm) and increased number of red blood cells, levels of haemoglobin, haematocrit and calcium in males as shown in Table 5.3.2-1 below.

Table 5.3.2-1: 90-day feeding rat-summary of clinical chemical and haematological findings

Parameter	Sex	Dose Level (ppm)					
		0	**100**	**500**	**2000**	**5000**	**15000**
Prothrombin time (sec)	M	33.3	31.2	32.9	34.0	33.7	33.5
	F	30.2	30.5	29.0	28.8	29.0	**27.4****
Red blood cells (tera/L)	M	8.1	8.39	8.47	**8.52****	**8.87*****	**8.77****
	F	8.17	8.12	8.10	8.03	8.08	8.13
Hemoglobin (mmol/L)	M	9.2	9.3	9.4	9.5	**9.9****	**9.7***
	F	9.5	9.4	9.4	9.3	9.4	9.3
Hematocrit (I/L)	M	0.412	0.421	0.422	**0.430***	**0.450*****	**0.441****
	F	0.417	0.414	0.414	0.411	0.412	0.410
Alkaline phosphatase (µkat/l)	M	5.24	4.79	4.34	5.02	4.77	4.72
	F	4.05	**4.70***	**3.53****	**3.06****	**3.19****	**2.99****
γ-Glutamyl-transferase (nkat/l)	M	12(8)	10(7)	11(7)	**30***(25)	**28****(10)	**47***(15)
	F	14(7)	14(6)	18(9)	21(10)	**37***(12)	**42***(10)
Total protein (g/l)	M	62.85	61.92	62.14	62.05	**67.13****	**67.51***
	F	65.45	64.52	64.07	66.73	65.69	70.29*
Albumin (g/l)	M	33.02	33.30	33.75	33.24	**35.37*****	**35.39****
	F	36.96	37.22	37.37	37.94	36.31	39.02
Triglycerides (mmol/l)	M	3.09	2.62	3.34	2.96	2.93	**2.21***
	F	3.07	3.43	3.42	3.17	2.71	1.97
Globulin (g/l)	M	29.83	28.62	28.39	28.81	31.76	32.11
	F	28.49	27.30	**26.70***	28.79	29.37	**31.27****
Cholesterol (mmol/l)	M	2.03	2.21	1.95	1.99	2.01	2.30
	F	2.22	2.31	2.09	2.09	2.46	**2.88****
Bilirubin (µmol/l)	M	2.48	2.73	2.15	**1.74****	**1.61****	**1.66****
	F	2.24	2.21	2.19	1.91	1.74	2.33
Calcium (mmol/l)	M	2.70	**2.79***	2.74	2.76	**2.86*****	**2.86*****
	F	2.66	2.73	2.70	2.73	2.73	2.76

*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$ (Kruskal-Wallis + Mann-Whitney u-test, two sided) Standard deviation in brackets

Treatment-related findings included increased liver weights (≥ 5000 ppm) with correlating histopathological changes (centrilobular hypertrophy), supported by alterations of clinical chemistry (increased gamma-glutamyltransferase) in both sexes. Increased thyroid weights (≥ 2000 ppm) with correlating histopathological changes (follicular cell hypertrophy /hyperplasia) were observed in males. Minimal up to slight hypertrophy of the follicular epithelium and slight diffuse hyperplasia of follicular cells were observed with increased incidence in dose groups of 2000 ppm onwards, albeit without a clear dose response. In female rats statistically significantly increased thyroid weights were observed at dose levels of 5000 ppm and 15000 ppm. Although no corresponding histomorphological changes were seen at these dose levels (see table 5.3.2-4) the organ weight changes suggest a dose related response in female rats.

In male rats spleen weights (absolute and relative) were statistically significantly decreased at 15000 ppm and adrenal weights (absolute and relative) were decreased at 5000 ppm and 15000 ppm dose levels. However, there was no histological finding correlated to the decreased weights observed in both organs as evidenced by histopathological investigation of the control and top dose group. There were no findings in other organs including the endocrine system which suggest adverse findings attributable to the administration of the test substance.
Details of findings are presented in Table 5.3.2-2 below.

Table 5.3.2-2: 90-day feeding rat - summary of target organ weights

Parameter	Sex	Dose Level (ppm)					
		0	100	500	2000	5000	15000
Organ weights							
Absolute liver weight (g)	M	13.95	14.66	14.03	14.33	15.03	**16.64****
	F	7.51	7.59	7.84	7.66	**8.19***	**9.24****
Relative liver weight	M	3.17	3.25	3.23	3.32	**3.54****	**3.76****
	F	2.99	2.99	3.15	3.18	**3.35****	**3.73****
Absolute thyroid weight (mg)	M	23.4	23.2	23.7	**28.3****	24.2	**31.4****
	F	17.3	17.1	17.2	18.5	**20.3***	**22.6****
Relative thyroid weight	M	0.005	0.005	0.005	**0.007****	0.006	**0.007****
	F	0.007	0.007	0.007	0.008	**0.008****	**0.009****
Absolute spleen weight (g)	M	0.961	0.976	0.796	0.787	0.749	**0.731***
	F	0.533	0.545	0.546	0.534	0.502	0.542
Relative spleen weight	M	0.214	0.217	0.184	0.182	0.176	**0.165****
	F	0.212	0.215	0.219	0.222	0.205	0.219
Absolute adrenal weight (mg)	M	104.4	86.4	95.8	92.8	**70.1****	**83.6***
	F	102.3	106.4	101.5	101.8	102.7	91.2
Relative adrenal weight	M	0.023	0.019	0.022	0.022	**0.017****	**0.019***
	F	0.041	0.042	0.041	0.042	0.042	0.037

*: $p < 0.05$, **: $p < 0.01$ (Kruskal-Wallis + Wilcoxon-test, two sided)

Macroscopic and microscopic findings in target organs and those related to the endocrine system are presented in the Table 5.3.2-3 below. No other findings than isolated incidence of a cyst in the ovary of one female of the 15000 ppm dose level and dilated uterus in one female at the 500 ppm dose level were made which were not considered to be attributable to the test substance.

Table 5.3.2-3: 90-day feeding rat - summary of macroscopic and microscopic findings in target organs and organs of the endocrine system

Parameter	Sex	Dose Level (ppm)					
		0	100	500	2000	5000	15000
Macroscopic findings							
Liver	M	1 / 10	0 / 10	0 / 10	0 / 10	0 / 10	3 / 10
Focus	F	0 / 10	0 / 10	1 / 10	2 / 10	1 / 10	1 / 10
Thyroid	M	0 / 10	-	-	-	-	0 / 10
Size reduced	F	1 / 10	-	-	-	-	0 / 10
Spleen	M	0 / 10	-	-	-	-	0 / 10
	F	0 / 10	-	-	-	-	0 / 10
Adrenal cortex	M	1 / 10	-	-	-	-	0 / 10
Enlarged	F	0 / 10	-	-	-	-	0 / 10
Ovaries							
Cyst	F	0 / 10	0 / 10	0 / 10	0 / 10	0 / 10	1 / 10
Uterus							
Dilation	F	0 / 10	0 / 10	1 / 10	0 / 10	0 / 10	0 / 10
Microscopic findings							
Liver							
Granuloma	M	10 / 10	10 / 10	10 / 10	10 / 10	10 / 10	10 / 10
Kupffer cells	F	10 / 10	10 / 10	10 / 10	10 / 10	10 / 10	10 / 10
Hypertrophy	M	0 / 10	0 / 10	0 / 10	0 / 10	8 / 10	10 / 10
central	F	0 / 10	0 / 10	0 / 10	0 / 10	2 / 10	7 / 10
Fatty change	M	1 / 10	0 / 10	0 / 10	0 / 10	1 / 10	2 / 10
central	F	0 / 10	0 / 10	0 / 10	0 / 10	0 / 10	0 / 10
Fatty change	M	0 / 10	2 / 10	0 / 10	0 / 10	2 / 10	5 / 10
portal	F	0 / 10	0 / 10	0 / 10	0 / 10	0 / 10	0 / 10
Fatty change	M	9 / 10	8 / 10	7 / 10	9 / 10	4 / 10	0 / 10
midzonal	F	7 / 10	9 / 10	9 / 10	6 / 10	4 / 10	2 / 10
Fatty change	M	0 / 10	0 / 10	0 / 10	0 / 10	0 / 10	0 / 10
diffuse	F	0 / 10	0 / 10	0 / 10	0 / 10	0 / 10	0 / 10
Fatty change	M	1 / 10	0 / 10	0 / 10	0 / 10	0 / 10	2 / 10
focal	F	0 / 10	0 / 10	1 / 10	2 / 10	1 / 10	0 / 10
Pigmentation	M	0 / 10	0 / 10	0 / 10	0 / 10	0 / 10	0 / 10
portal	F	0 / 10	1 / 10	0 / 10	0 / 10	0 / 10	0 / 10
Necrosis	M	1 / 10	0 / 10	0 / 10	0 / 10	0 / 10	0 / 10
focal/mid focal	F	0 / 10	0 / 10	0 / 10	0 / 10	0 / 10	0 / 10

Parameter	Sex	Dose Level (ppm)					
		0	100	500	2000	5000	15000
Thyroid follicular cell							
Hypertrophy	M	1 / 10	2 / 10	3 / 10	7 / 10	7 / 10	8 / 10
	F	0 / 10	0 / 10	0 / 10	0 / 10	0 / 10	0 / 10
Hyperplasia	M	1 / 10	2 / 10	3 / 10	7 / 10	7 / 10	8 / 10
	F	0 / 10	0 / 10	0 / 10	0 / 10	0 / 10	0 / 10
Cyst	M	0 / 10	0 / 10	0 / 10	0 / 10	0 / 10	0 / 10
	F	0 / 10	1 / 10	0 / 10	0 / 10	0 / 10	0 / 10
Spleen	M	0 / 10	-	-	-	-	0 / 10
	F	0 / 10	-	-	-	-	0 / 10
Adrenal cortex	M	0 / 10	-	-	-	-	0 / 10
	F	0 / 10	-	-	-	-	0 / 10
Adrenal medulla	M	0 / 10	-	-	-	-	0 / 10
	F	0 / 10	-	-	-	-	0 / 10
Pituitary gland	M	2 / 10	-	-	-	-	0 / 10
Cyst	F	0 / 10	-	-	-	-	2 / 10
Ovaries							
Cyst	F	0 / 10	0 / 10	0 / 10	0 / 10	0 / 10	1 / 10
Uterus							
Dilation	F	0 / 10	0 / 10	1 / 10	0 / 10	0 / 10	0 / 10

Table 5.3.2-4: 90-day feeding rat – incidence and grading of findings in liver and thyroids

	Male						Female					
Dose groups (ppm)	**0**	**100**	**500**	**2000**	**5000**	**15000**	**0**	**100**	**500**	**2000**	**5000**	**15000**
Animals (n)	10	10	10	10	10	10	10	10	10	10	10	10
Liver												
Granuloma Kupff.												
Grade 1		1		3	6	1	7	7	5	5	8	5
Grade 2	7	6	8	7	2	8	3	3	5	5	2	5
Grade 3	3	3	2		2	1						
Hypertrophy, central												
Grade 1					5	1					2	4
Grade 2					3	6						3
Grade 3						3						
Fatty change central												
Grade 2	1				1	2						
Fatty change portal												
Grade 1		1				1						
Grade 2						2						
Grade 3		1				2						
Fatty change midzon												
Grade 1	3		4	4	3		3	6	4	5	2	2
Grade 2	3	4	1	3	1		4	3	4		2	
Grade 3	3	3	2	2					1	1		
Grade 4		1										
Thyroids												
Hypertrophy follicul												
Grade 1		1				2						
Grade 2	1	1	3	7	7	6						
Hyperplasia diffuse												
Grade 1		1				2						
Grade 2	1	1	3	7	7	6						

Conclusion

Based on the results of this 90-day dietary study, the short-term NOAEL in the rat can be established at 500 ppm (equivalent to 34 mg/kg bw/day in males and 40 mg/kg bw/day in females).

Boscalid - Subchronic oral toxicity study in C57BL mice - Administration in the diet for 3 months (et al., 2000b)
Doc ID 2000/1000188

Guidelines: According to OECD 408 (1981), EEC 87/302, EPA 82-1 and JMAFF
Deviations: None
GLP: Yes
Acceptance: The study has been evaluated and considered acceptable in the EU registration process for the first Annex I inclusion.

Note: This study report has been part of the documentation for the first Annex I inclusion process but a more detailed summary is presented here to account for requirements of Regulation (EC) No. 1107/2009

Boscalid (95.3% purity, batch N 26) was administered to groups of 10 male and 10 female C57BL mice (source: Centre d'Elevage R. Janvier, France) at dietary levels of 0, 150, 1000, 4000 and 8000 ppm for a period of 3 months. These concentrations were equivalent to the substance intake of 0, 29, 197, 788 and 1518 mg/kg bw/day in males, and 0, 42, 277, 1184 and 2209 mg/kg bw/day in females.

Findings
The stability of the test substance during the study period was analytically confirmed. The stability and homogeneity of the test substance in the diet were confirmed by analysis. The correctness of the concentrations was demonstrated.
There were no mortalities and clinical signs of toxicity in any of the dose groups up to the top dose level. No adverse effects on body weight and body weight gain and feed consumption were observed, nor was so for haematology. Following the 90-day dietary administration of Boscalid to C57BL mice, adverse effects were observed in animals of dose groups at ≥ 1000 ppm.
Treatment-related findings included increased absolute and relative liver weights with correlating alterations of clinical chemistry parameters, like decreased protein, albumin, globulin and slightly increased alanine aminotransferase (females) at ≥ 4000 ppm. The only test substance related adverse effect in clinical chemistry at 1000 ppm was decrease of cholesterol in males.

Table 5.3.2-5: 90-day feeding mouse-summary of clinical chemical findings

Parameter	Sex	Dose Groups (ppm)				
		0	150	1000	4000	8000
Total protein (g/l)	M	62.49	62.46	61.13	**59.90****	**59.21****
	F	60.33	58.17	58.75	58.54	57.30
Albumin (g/l)	M	38.77	38.81	37.92	**37.53****	**36.82****
	F	39.15	36.94	38.56	38.53	37.63
Globulin (g/l)	M	23.72	23.65	22.76	**22.39****	**22.06****
	F	21.18	21.05	20.51	19.92	19.67
Cholesterol (mmol/l)	M	2.57	2.53	**2.26****	**1.91*****	**1.84*****
	F	1.76	1.96	1.66	1.59	1.57
Alanine amino-transferase (µkat/l)	M	0.87	0.75	0.91	0.96	0.95
	F	1.03	0.98	1.13	1.23*	1.21*

*: $p < 0.05$, **: $p < 0.01$ (Kruskal-Wallis + Wilcoxon-test, two sided)

Concomitant to increased liver weights, fatty liver change (diffuse infiltration, grade 4) was observed in males of dose group ≥ 4000 ppm as detailed in Table 5.3.2-6. Absolute liver weight in females was statistically significantly increased in females at the dose level of 150ppm and in males at the dose level of 1000 ppm. The finding in females has been concluded to be not related to the test substance for some reasons. The absolute liver weights in the control mice were below the lowest single control values in the historical control data base as shown in Table 5.3.2-7. Relative liver weights were not affected by the treatment and the incidence of fatty changes gave no difference to control animals. There were no findings in other organs including the endocrine system which suggest adverse findings attributable to the administration of the test substance.

Table 5.3.2-6: 90-day feeding mouse - summary of target organ weights and pathological changes

Parameter	Sex	Dose Groups (ppm)				
		0	150	1000	4000	8000
Organ weight						
Absolute liver weight	M	1096.8	1113.5	1228.2*	1220.0**	1396.5**
(g)	F	886.9	955.6*	946.6	995.2*	1085.0**
Relative liver weight	M	4.39	4.51	5.01**	4.97**	5.66**
	F	4.72	4.87	5.09*	5.23*	5.70**
Macroscopic findings						
Liver						
Discoloration	F	0 / 10	1 / 10	0 / 10	0 / 10	0 / 10
Necrosis	F	0 / 10	0 / 10	1 / 10	1 / 10	0 / 10
Ovaries						
Cyst	F	2 / 10	0 / 10	0 / 10	0 / 10	0 / 10
Uterus						
Dilation	F	0 / 10	1 / 10	0 / 10	0 / 10	0 / 10
Discoloration	F	0 / 10	0 / 10	0 / 10	0 / 10	1 / 10
Mass	F	0 / 10	0 / 10	0 / 10	0 / 10	1 / 10
Microscopic findings						
Diffuse fatty change in liver	M	0/10	0/10	0/10	3/10	5/10
	F	0/10	0/10	1/10	0/10	1/10
Fatty change liver centrilobular / diffuse		N=10	N=10	N=10	N=10	N=10
Grade 1	M	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0
Grade 2		0 / 5	0 / 3	1 / 6	0 / 1	0 / 1
Grade 3		0 / 5	0 / 7	0 / 3	0 / 6	0 / 4
Grade 4		0 / 0	0 / 0	0 / 0	0 / 3	0 / 5
Grade 1	F	0 / 2	0 / 1	0 / 0	0 / 4	0 / 2
Grade 2		0 / 2	0 / 3	0 / 2	0 / 3	0 / 4
Grade 3		1 / 5	0 / 4	0 / 7	1 / 1	0 / 2
Grade 4		0 / 0	1 / 0	0 / 1	1 / 0	1 / 1
Ovaries						
Cyst	F	2 / 10	-	-	-	0 / 10

Parameter	Sex	Dose Groups (ppm)				
		0	150	1000	4000	8000
Uterus						
Deciduoma	F	0 / 10	-	-	-	1 / 10
Dilation	F	0 / 10	1	-		0 / 10
Endometritis, pur.	F	0 / 10	1	-	-	0 / 10
Hyperplasisa, endometrial	F					
Grade 2		2 / 10	-	-	-	2 / 10
Grade 3		-	-	-	-	1 / 10
Adrenal cortex						
Accessory adrenalcortical tissue	F	-	-	-	-	2 / 10

*: $p < 0.05$, **: $p < 0.01$ (Kruskal-Wallis + Wilcoxon-test, two sided)

Table 5.3.2-7: 90-day feeding mouse-historical control values in C57Bl mice

Study period	Male				Female			
	Absolute liver weight (mg)		Relative liver weight		Absolute liver weight (mg)		Relative liver weight	
	Individual	Mean	Individual	Mean	Individual	Mean	Individual	Mean
10.88-01.89	1182	1208	4.321	4.352	916	970	4.962	4.951
18.99-03.89	1215		4.282		1053		5.071	
10.90-01.91	1218		4.184		965		4.944	
11.90-02.91	1141		4.396		953		5.026	
12.91-03.92	1172		4.581		1026		5.100	
03.92-06.92	1198		4.375		957		5.064	
09.93-13.93	1443		4.283		1001		4.719	
11.97-01.98*	1097		4.392		887		4.723	

*Data from current study

Conclusion

Based on the results of this 90-day dietary study, the short-term NOAEL in the mouse can be established at 150 ppm (equivalent to 29 mg/kg bw/day in males and 42 mg/kg bw/day in females).

Boscalid - Subchronic oral toxicity study in Beagle dogs - Administration in the diet for 3 months (et al., 2000)
Doc ID 2000/1012306

Guidelines: According to OECD 409 (1981), EEC 87/302, EPA 82-1 and JMAFF
Deviations: None
GLP: Yes
Acceptance: The study has been evaluated and considered acceptable in the EU registration process for the first Annex I inclusion.

Note: This study report has been part of the documentation for the first Annex I inclusion process but a more detailed summary is presented here to account for requirements of Regulation (EC) No. 1107/2009

Boscalid (94.4% purity, batch N 37) was administered to groups of 5 male and 5 female Beagle dogs (source: BASF's own Beagle breed) at dietary levels of 0, 250, 2500 and 25000 ppm for a period of 3 months. These concentrations were equivalent to the substance intake of 0, 7.6, 78 and 729 mg/kg bw/day in males, and 0, 8.1, 82 and 825 mg/kg bw/day in females.

Findings
The stability of the test substance during the study period was analytically confirmed. The stability and homogeneity of the test substance in the diet were confirmed by analysis. The correctness of the test substance concentrations in the diet was demonstrated.
There was no mortality. No clinical signs of toxicity were observed in the study except light brown discoloured faeces, partly with soft consistency in all animals of the 25000 ppm dose group and transiently in 3 males and 3 females of the 2500 ppm dose group. At the high dose level an initial slight body weight loss and retarded body weight gain were observed in both sexes. Retarded body weight gain persisted in females until study termination. At the high dose group feed consumption was reduced reaching 96% of the administered amount in both sexes (Table 5.3.2-8). There were no ophthalmological findings.

Table 5.3.2-8: 90-day feeding dog-feed consumption and body weight change

Sex	**Male**				**Female**			
Dose (ppm)	**0**	**250**	**2500**	**25000**	**0**	**250**	**2500**	**25000**
Feed consumption (% of administered amount)	100	100	100	96	98	99	99	96
Body weight change (kg)	+0.7	+1.2	+0.5	+0.8	+1.0	+0.6	+0.7	+0.2

Following a 90-day dietary administration of Boscalid to Beagle dogs, adverse effects were observed in animals of dose group ≥ 2500 ppm. Hematological findings comprised reduced red blood cells ((RBC, day 90) and reduced haemoglobin (Hb, day 44 & 90) in females at the 25000 ppm dose level.

Table 5.3.2-9: 90-day feeding dog-haematology and clinical chemistry findings

Sex	Male				Female			
Dose (ppm)	**0**	**250**	**2500**	**25000**	**0**	**250**	**2500**	**25000**
Hematology								
RBC (tera/l)								
day -4/-1	7.04	6.56	6.67	6.77	6.88	7.33	7.36	6.79
day 43/44	7.09	6.98	6.60	6.84	7.32	7.47	7.71	6.79
day 87/90	7.19	7.30	6.78	6.69	7.35	7.27	7.59	**6.56***
Hb (mmol/l)								
day -4/-1	9.8	9.4	9.3	9.5	9.8	10.2	10.3	9.6
day 43/44	9.9	10.0	9.3	9.7	10.7	10.6	10.9	**9.9***
day 87/90	10.0	10.4	9.4	9.5	10.6	10.2	10.8	**9.5***
Clinical chemistry								
ALP (µkat/L)								
day -4/-1	4.82	4.93	4.22	4.83	4.59	4.63	4.89	4.21
day 43/44	4.57	5.56	5.50	**12.95****	3.85	4.39	6.44	8.133
day 87/90	3.60	4.97	5.17	**9.99****	3.05	4.24	**5.70****	**8.80****
ALT (µkat/L)								
day -4/-1	0.58	0.46	0.55	0.49	0.63	0.53	0.55	0.61
day 43/44	0.59	0.53	0.47	0.33	0.74	0.63	**0.42****	**0.40****
day 87	0.69	0.57	0.58	**0.35****	0.98	0.66	**0.43****	**0.47****
AST (µkat/L)								
day -4/-1	0.51	0.40	0.45	0.49	0.51	0.44	0.44	0.44
day 43/44	0.49	0.46	0.48	0.39	0.4	0.51	0.57	0.41
day 87/90	0.52	0.48	0.44	**0.38****	0.69	0.47	0.38	0.37
TRIG (mmol/l)								
day -4/-1	0.45	0.48	0.37	0.44	0.38	0.38	0.38	0.38
day 43/44	0.38	0.41	0.52	**0.81****	0.39	0.43	0.51	**0.71****
day 87/90	0.30	**0.40***	**0.44***	**0.60****	0.33	0.44	**0.49***	**0.66****

*: $p < 0.05$, **: $p < 0.01$ (Kruskal-Wallis + Mann-Whitney u-test)

Treatment-related findings included increased liver weights (≥ 2500 ppm) with correlating alterations of clinical chemistry parameters such as increased alkaline phosphatase (ALP) in both sexes. Alanine aminotransferase (ALT) was statistically significantly decreased in both sexes at the 25000 ppm dose level and in females at the 2500 ppm dose level. In addition aspartate aminotransferase (AST) was decreased in males at the 25000 ppm dose level. However, the decrease of both blood enzymes has not been considered as a toxicologically adverse effect.
Additional findings were increased triglycerides seen in both sexes at ≥ 2500 ppm dose levels and at the 250 ppm dose level (day 87, males only). However, the statistical significance gained at the 250 ppm & 2500 ppm dose levels were due to unusually low control values. The values of triglycerides in the dose groups of 250 ppm and 2500 ppm were within the historical range (mean of males: 0.38 mmol/L, minimum 0.24 mmol/L, maximum 0.45 mmol/L and mean of females: 0.45 mmol/L, minimum 0.38 mmol/L, maximum 0.51 mmol/L) and the numerical differences which attained statistical significance were due to unusually low control values. Thus, increased triglyceride levels in these dose groups were considered not to be adverse effects in these dose groups.
Increased thyroid weights were observed in females at the top dose level with gaining statistical significance for relative organ weights (Table 5.3.2-10).

The effects in these organs were, however, not supported by gross and histopathological changes.
There were no findings in other organs including the endocrine system which suggest adverse findings attributable to the administration of the test substance.

Table 5.3.2-10: 90-day feeding dog - summary of target organ weight findings

Parameter	Sex	Dose level (ppm)			
		0	250	2500	25000
Organ weight					
Absolute liver weight (g)	M	357.2	401.4	**420.8***	**506.6****
	F	321.8	332.2	**381.9****	**479.7****
Relative liver weight	M	3.26	3.30	3.65	**4.15***
	F	2.85	3.01	**3.45***	**4.65****
Absolute thyroid weight (g)	M	0.830	0.964	0.924	0.954
	F	0.794	0.798	0.862	1.084
Relative thyroid weight	M	0.008	0.008	0.008	0.008
	F	0.007	0.007	0.008	**0.01****
Absolute kidney weight (g)	M	52.8	53.1	50.9	47.0
	F	43.9	40.3	44.1	44.2
Relative kidney weight	M	0.48	0.44	0.44	**0.39****
	F	0.39	0.37	0.40	0.43

*: $p < 0.05$, **: $p < 0.01$ (Kruskal-Wallis + Wilcoxon-test, two sided)

Table 5.3.2-11: 90-day feeding dog - macroscopic findings in target organs and organs of the endocrine system

Sex	Male				Female			
Dose (ppm)	**0**	**250**	**2500**	**25000**	**0**	**250**	**2500**	**25000**
Kidneys								
Cyst	-	-	-	-	-	-	-	1 / 5
Ovaries								
Cyst					-	1 / 5	-	-
Pituitary glands								
Cyst	-	-	-	-	1 / 5	-	-	-

Table 5.3.2-12: 90-day feeding dog - microscopic findings in target organs and organs of the endocrine system

Sex	Male				Female			
Dose (ppm)	**0**	**250**	**2500**	**25000**	**0**	**250**	**2500**	**25000**
Liver								
Granuloma, Kupffer cells	5 / 5	5 / 5	5 / 5	5 / 5	4 / 5	5 / 5	5 / 5	4 / 5
Fat storage	-	-	-	-	-	-	-	1 / 5
Kidneys								
Lithiasis, tubular	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5
Prostrate								
Lymphoid cell infiltr.	-	1 / 5	-	-				
Thyroid glands								
C-cell hyperplasia	-	1 / 5	1 / 5	-	1 / 5	1 / 5	1 / 5	1 / 5
Inflammation, focal	-	-	-	1 / 5	-	-	-	-
Ectopic thymus	-	-	-	-	-	-	1 / 5	-
Ovaries								
Cysts					1 / 5	1 / 5	1 / 5	-
Pituitary glands								
Cyst	-	-	-	-	1 / 5	-	-	-
Adrenal cortex								
Accessory adrenal	-	-	-	-	-	-	-	1 / 5

Conclusions

Based on the results of this 90-day dietary study, the short-term NOAEL in dogs can be established at 250 ppm (equivalent to 7.6 mg/kg bw/day in males and 8.1 mg/kg bw/day in females).

Boscalid - chronic oral toxicity study in Beagle dogs - Administration in the diet for 12 months (et al., 2000)
Doc ID 2000/1016881

Guidelines: According to OECD 452 (1981), EEC 87/302, EPA 870.4100 and JMAFF
Deviations: None
GLP: Yes
Acceptance: The study has been evaluated and considered acceptable in the EU registration process for the first Annex I inclusion.

Note: This study report has been part of the documentation for the first Annex I inclusion process but a more detailed summary is presented here to account for requirements of Regulation (EC) No. 1107/2009. The 12-months feeding study in dogs was also identified as relevant study for setting of the Acceptable Operator Exposure Level reference dose. For this reason this study is in detail evaluated here following the OECD format.

Executive summary
Boscalid (94.4% purity, batch N 37) was administered to groups of 5 male and 5 female Beagle dogs (source: BASF's own Beagle breed) at dietary levels of 0, 200, 800, 2000 and 20000 ppm for a period of 12 months. These concentrations were equivalent to the substance intake of 0, 5.5, 21.8, 57.4 and 544 mg/kg bw/day in males, and 0, 5.8, 22.1, 58.3 and 592.9 mg/kg bw/day in females. Initial reduced body weight gain or body weight loss was observed in animals of both sexes treated with $\geq$ 2000 ppm. The mean body weight gain of females treated with 20000 ppm was decreased until the end of the administration period, albeit without statistical significance.

Clinical signs consisted of discoloured faeces of soft consistency in both sexes (20000 ppm) and vomiting in one female (20000 ppm). Following a 1-year dietary administration of Boscalid to Beagle dogs, substance-induced effects were observed in animals of dose group $\geq$ 2000 ppm. Treatment-related findings included increased liver weights with correlating alterations of clinical chemistry parameters (increased alkaline phosphatase in both sexes), and increased thyroid weights in both sexes. The effects on both target organs were however, not supported by histopathology. Additional findings consisted of increased chloride and triglycerides in both sexes, and increased cholesterol, protein and globulins in females. The most clinical chemistry parameters alterations were no longer observable or not statistically significant at the end of the study period, indicating an adaptation of the liver to increased functional demand.

(DocID 2000/1016881)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:	Boscalid (BAS 510 F; Reg.No. 300 355)
Description:	solid / white
Lot/Batch #:	N37
Purity:	94.4%
Stability of test compound:	The stability of the test substance was verified by re-analysis after the in-life phase of the study. The re-analysed purity was still 94.4% on 01 Dec. 1999.
2. Vehicle and/or positive control:	None
3. Test animals:	
Species:	Dog
Strain:	Purebred Beagle Dog
Sex:	Male and female
Age:	about 7 - 8 month (at start of administration)
Weight at dosing (mean):	Males: 11.9 (10.0 - 14.5) kg Females: 10.8 (8.8 - 12.7) kg
Source:	BASF's own Beagle breed
Acclimation period:	7 days
Diet:	Dog maintenance KLIBA laboratory diet (Provimi Kliba SA, Kaiseraugst, Switzerland). Animals received daily feed ration of 350 g of powdered feed that was made into a paste with 350 mL drinking water in a feed bowl immediately before administration to each dog.
Water:	In kennel (automatic watering device): blended water (demineralized water and tap water), ad libitum In metabolism cage: approx. 500 mL drinking water
Housing:	Animals were housed in individual inner kennels, and had always access (day and night) to the outer kennels: up to study day 35: inner kennel: ~ 1.5 m², outer kennel: ~ 4.5 m² Study day 35 - 373: inner kennel: ~ 2.7 m², outer kennel: ~ 2.7 m²
Environmental conditions:	
Temperature:	Additional heating of the air by the forced ventilation system was supplied in winter
Humidity:	Not specified
Air changes:	Animal rooms were ventilated by a forced ventilation system
Photo period:	Natural day/night rhythm, with additional artificial light as required during working hours

B. STUDY DESIGN AND METHODS

1. Dates of experimental work: 01-Oct-1998 - 29-Aug-2000
(In-life dates: 13-Oct-1998 (start of administration) to 13/21-Oct-1999 (necropsy))

2. Animal assignment and treatment:
Boscalid was administered to groups of 5 male and 5 female dogs at dietary concentrations of 0, 200, 800, 2000 and 20000 ppm daily for 12 months. The diet was offered for a maximum period of 2 hours each.

3. Test substance preparation and analysis:
For each concentration, the test substance was weighed out and thoroughly mixed with a small amount of feed. Then corresponding amounts of feed, depending on the dose group, were added to this premix in order to obtain the desired concentrations and mixed in a laboratory mixer of Fa. RUBERG for about 10 minutes. The mixtures of test substance and feed were prepared at about two-weekly intervals and stored at room temperature. Preparation of the diet by mixing 350 g feed with about 350 mL drinking water was done in the late morning shortly before the feed was offered to the animals.
The stability of the test substance in the diet, the stability of the test substance in dog feed made into a paste (mixing ratio: 1 part ground feed/test substance mixture and 1 part drinking water) over a period of 24 hours, the homogeneity of the test substance preparations were analytically determined and concentration control analyses were carried out via HPLC/UV. Homogeneity analysis of the diet preparations was performed at the beginning of the administration period by taking 3 samples from the top, middle and bottom of the beaker for the low (200 ppm) and high dose level (20000 ppm). Concentration control analyses were performed on samples of all concentrations that were drawn at the start of the administration period as well as in intervals of about 3 months thereafter.

4. Statistics:

Means and standard deviations of each test group were calculated for several parameters. Further statistical analyses were performed according to following tables:

Statistics of clinical examinations

Parameter	Statistical test
body weight data	Parametric one-way analysis using the F-test (ANOVA, two-sided). If the resulting p-value was equal or less than 0.05, a comparison of each group with the control group was performed using DUNNETT's test (two-sided) for the hypothesis of equal means

Statistics of clinical pathology

Parameter	Statistical test
Clinical pathology parameters, except differential blood count	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 MANN-WHITNEY U-test (two-sided) for the equal medians
Urinalysis, except volume, colour, turbidity and specific gravity	Pair-wise comparison of each dose group with the control group using FISHER's exact test for the hypothesis of equal proportions

Statistics of pathology

Parameter	Statistical test
Organ weights	Non-parametric one-way analysis using KRUSKAL-WALLIS test (two-sided). If the resulting p-value was equal or less than 0.05, a pairwise 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:

A check for any moribund or dead animals was made twice a day on working days and once a day on weekends and public holidays.
The animals were examined for clinical signs of toxicity at least once each working day, if signs occurred, several times daily. The chosen housing conditions with kennels of appropriate size including areas inside and outside the building allowed the animals to move freely. Therefore deviations in motor activity and altered behaviour could be determined if evident.
Clinical examination included but was not limited to the following parameters:

- General state
- Body position and body posture
- Activity and behaviour
- Skin and fur
- Mucosal membranes
- Eyes and nose
- Reflexes
- Respiration
- Visible swelling masses
- Stool and urine

2. Body weight:

Body weight was determined on day -7 (beginning of the adaptation period) and thereafter in weekly intervals.
The difference between the body weight on the respective day of weighing and the body weight on day 0 was calculated as body weight gain.

3. Feed consumption, feed efficiency and compound intake:

Feed consumption was determined daily, staring on day -7 (beginning of the adaptation period).
Feed efficiency (group means) was calculated based upon individual values for body weight and feed consumption:

$$\text{Food efficiency for day x} = \frac{BW_x - BW_Y}{FC_{y\ to\ x}}\ x\,100$$

BW_x and BW_y = body weight [g] at day x and day y (last weighing date before day x)
$FC_{y\ to\ x}$ = the mean feed consumption from day y to x calculated as mean daily feed consumption [g] on day x, multiplied by the number of days from day y to day x, divided by 2 (since ½ of the feed ratio consisted of powdery diet and the other half of water

The mean daily intake of test substance (group means) was calculated based upon individual values for body weight and feed consumption.

$$\text{Substance intake for day x} = \frac{FC_x \times D}{BW_x}$$

BW_x = body weight [g] at day x
FC_x = the mean feed consumption [g] for day x, divided by 2 (since ½ of the feed ratio consisted of powdery diet and the other half of water
D = dose in ppm

4. Haematology and clinical chemistry:
Blood samples were taken from the vena cephalica antebrachii in the morning from fasted, un-anaesthetised animals.
The assays of blood and serum parameters were performed under internal laboratory quality control conditions with commercial reference controls to assure reliable test results that were expressed in units of the International System (SI).
The following haematological and clinical chemistry parameters were determined for all animals:

Haematology:					
Red blood cells		*White blood cells*		*Clotting Potential*	
✓	Erythrocyte count (RBC)	✓	Total leukocyte count (WBC)	✓	activated partial thrombo-plastin time (PTT)
✓	Haemoglobin (HGB)	✓	Differential blood count	✓	Prothrombin time (Quick's test (QT)
✓	Haematocrit (Hct)	✓	Platelet count (PLT)		
✓	Mean corp. volume (MCV)		Platelet-crit		
✓	Mean corp. haemoglobin (MCH)		Mean platelet volume		
✓	Mean corp. Hb. conc. (MCHC)		Platelet distribution width		
	Reticulocytes (RET)				
	Red cell distribution width				

Clinical chemistry:					
Electrolytes		*Metabolites and Proteins*		*Enzymes:*	
✓	Calcium	✓	Albumin	✓	Alanine aminotransferase (ALT)
✓	Chloride	✓	Globulin	✓	Aspartate aminotransferase (AST)
✓	Phosphorus (inorganic)		Bile acids	✓	Alkaline phosphatase (ALP)
✓	Potassium	✓	Bilirubin (total)	✓	γ-glutamyltransferase (γ-GT)
✓	Sodium	✓	Cholesterol		
✓	Magnesium	✓	Creatinine		
		✓	Glucose		
		✓	Protein (total)		
		✓	Triglycerides		
		✓	Urea		

5. Urinalysis:

For urinalysis the individual animals were transferred to metabolism cages and urine was collected overnight. No feed but about 500 mL drinking water was supplied during urine collection.

The following quantitative or semi-quantitative parameters were determined for all animals:

Urinalysis					
Quantitative parameters:		*Semi quantitative parameters*			
✓	Urine volume	✓	Bilirubin	✓	Nitrite
	Osmolality	✓	Blood	✓	Protein
✓	Specific gravity	✓	Colour and turbidity	✓	pH-value
		✓	Glucose	✓	Urobilinogen
		✓	Ketones	✓	Sediment (microscopical exam.)

6. Ophthalmoscopy:

Before the beginning of the administration period (day -7), and at the end of the administration period all dogs were examined for changes of their eyes using a KOWA-RC 2 fundus camera.

7. Sacrifice and pathology:

The test animals were sacrificed by exsanguination from the cervical and brachial vessels under anaesthesia. The exsanguinated animals were necropsied and assessed by pathology.

The following organs were sampled, weighed and examined histopathologically after haematoxylin-eosin staining:

Pathology:											
The following organs were collected (column C), weighed (W) and examined histopathologically (H, ✓: all animals per group and sex, #: all affected animals per group and sex).											
C	W	H		C	W	H		C	W	H	
✓	✓	✓	adrenals[&]	✓	✓	✓	liver	✓		✓	stomach
✓		✓	aorta	✓		✓	lung	✓	✓	✓	testes
			bone	✓		✓	lymph nodes[#]	✓		✓	thymus
✓	✓	✓	brain	✓		✓	mammary gland (♀)				tongue
✓		✓	caecum				nose/nasal cavity	✓	✓	✓	thyroid/parathyroid
			cervix	✓	✓	✓	ovaries with oviduct*	✓		✓	trachea
✓		✓	colon	✓		✓	pancreas	✓		✓	urinary bladder
✓		✓	duodenum				pharynx	✓		✓	uterus
✓	✓	✓	epididymides	✓		✓	pituitary gland	✓		✓	vagina
✓		✓	esophagus	✓		✓	prostate				
✓		✓	eyes	✓		✓	rectum		✓		body (anaesthetised animals)
✓		✓	femur’	✓		✓	salivary gland[$]				
✓		✓	gall bladder	✓		✓	Sciatic nerve				
✓		#	gross lesions				seminal vesicles				
			Harderian glands	✓		✓	skeletal muscle				
✓		✓	heart	✓		✓	skin				
✓		✓	ileum	✓		✓	spinal cord[§]				
✓		✓	jejunum	✓		✓	spleen				
✓	✓	✓	kidneys	✓		✓	sternum with marrow				
[&] histopathology of cortex and medulla; ’ with knee joint and marrow - bone marrow histopathologically examined; [#]mesenteric and axillary; * oviduct not weighed; [$] mandibular and paroid; [§] cervical, thoracic and lumbar cord											

II. RESULTS AND DISCUSSION

A. TEST SUBSTANCE ANALYSES

The stability of the test substance in the diet for a period of 32 days at room temperature and the stability of the test substance in wetted dog feed (mixing ratio: 1 part ground diet/test substance mixture and 1 part drinking water) over a period of 24 hours were verified analytically. As the diet/test substance mixtures were stored no longer than about 2 weeks and the wetted mixture were offered no longer than 2 hours, the stability was guaranteed.

The results of homogeneity and concentration analysis are summarised in Table 5.3.2-13 below:

Table 5.3.2-13: Analysis of diet preparations for homogeneity and test-item content

Dose level (ppm)	Sampling	Analysis	Concentration (ppm) Mean ± SD[§]	Relative standard deviation (%)	Mean % of nominal concentration[§]
200 ppm	08.10.98	09.10.98	200 ± 7[#]	3.4[#]	99.8
	15.01.99	15.01.99	210		105.0
	08.04.99	12.04.99	231		115.5
	29.06.99	01.07.99	209		104.5
	09.09.99	13.09.99	204		102.0
average			**211 ± 12**		**105.4 ± 6.0**
800 ppm	08.10.98	09.10.98	785		98.1
	15.01.99	15.01.99	772		96.5
	08.04.99	12.04.99	815		101.9
	29.06.99	01.07.99	813		101.6
	09.09.99	13.09.99	726		90.8
average			**782 ± 36**		**97.8 ± 4.5**
2000 ppm	08.10.98	09.10.98	1939		97.0
	15.01.99	15.01.99	1844		92.2
	08.04.99	12.04.99	2014		100.7
	29.06.99	01.07.99	2032		101.6
	09.09.99	13.09.99	1947		97.4
average			**1955 ± 74**		**97.8 ± 3.7**
20000 ppm	08.10.98	09.10.98	20182 ± 433[#]	2.2[#]	100.9
	15.01.99	15.01.99	18950		94.8
	08.04.99	12.04.99	20110		100.6
	29.06.99	01.07.99	20166		100.8
	09.09.99	13.09.99	18632		93.2
average			**19608 ± 755**		**98.0 ± 3.8**

[#] based on mean values of the three individual samples (homogeneity samples)
[§] Values may not calculate exactly due to rounding of figures

The homogeneity of the mixtures was verified at concentrations of 200 and 20000 ppm, as the relative standard deviation was 3.4 and 2.2%, respectively.
The concentration control analysis revealed values in the range from 92.2% to 105.5% of the nominal concentration for all dose levels. No test substance was determined in control diets.

B. OBSERVATIONS

1. Clinical signs of toxicity

No treatment-related clinical signs of toxicity were observed in animals of both sexes received 200, 800 and 2000 ppm.

At 20000 ppm, animals showed light-brown discoloured faeces of soft consistency from the beginning of the study until study day 350. Thereafter until the end of the administration period this finding was observed occasionally in 4/5 males and females of this dose group, while the 1/5 male and female showed this finding constantly until the end of the study period. Although no concurred findings were noted in clinical pathology or histopathology, this observations was considered treatment-related due to high incidence and duration of occurrence.
In 1/5 female treated with 20000 ppm, almost daily vomiting was noticed between study day 266 and 371. This finding was equivocal being either treatment-related, or related to incidental aversion to the diet of that particular animal.

2. Mortality

No animal died during the study.

C. BODY WEIGHT AND BODY WEIGHT GAIN

Overall, body weight of the animals did not statistically significantly differ from the animals of the control group at any time. Body weight gain of the animals of both sexes in doses of 200 and 800 ppm did not statistically significantly differ from the animals of the control group at any time.

Males administered 2000 and 20000 ppm showed statistically significant reduction of the body weight gain on study day 14. The mean body weight gain of males treated with 2000 ppm was less until the end of the study period without gaining statistical significance. Males of the high dose group did not show this effect. Thus, due to missing dose-relationship, the statistically significant deviation on day 14 has been considered to be incidental and not related to the test-substance application.

In females treated with 2000 ppm, reduced body weight gain was observed from study day 126 up to study end, albeit gaining statistical significance. At 20000 ppm, statistically significant decrease was observed on study days 7 and 14 in females. In the further course of the study, the mean body weight gain of females treated with 20000 ppm was decreased until the end of the administration period, however not statistically significant. Although the reduced body weight gain in females administered 2000 and 20000 ppm remained without statistical significance at the end of the study period, this effect was assessed as substance-related.

Table 5.3.2-14: 52 weeks feeding dog - mean body weight and body weight gain

Date	Parameter	Dose level (ppm)									
		Males					Females				
		0	**200**	**800**	**2000**	**20000**	**0**	**200**	**800**	**2000**	**20000**
Week 0 (day 0)	BW (kg)	12.1	11.8	11.9	11.7	11.9	11.0	10.5	10.8	10.7	10.8
	SD	± 1.7	± 1.3	± 1.1	± 1.1	± 1.4	± 1.1	± 1.1	± 1.0	± 0.7	± 0.8
Week 1 (day 7)	BW (kg)	12.5	12.0	12.1	11.9	12.0	11.2	10.6	11.1	10.9	10.7
	SD	± 1.7	± 1.2	± 1.1	± 1.1	± 1.4	± 1.1	± 0.9	± 0.9	± 0.7	± 0.9
	Δ (kg)	0.4	0.2	0.2	0.1	0.1	0.2	0.2	0.2	0.2	**0.0***
	SD	± 0.2	± 0.1	± 0.1	± 0.2	± 0.1	± 0.0	± 0.2	± 0.1	± 0.1	± 0.1
Week 2 (day 14)	BW (kg)	12.7	12.1	12.3	11.9	12.0	11.4	10.7	11.1	10.9	10.6
	SD	± 1.7	± 1.1	± 1.0	± 1.0	± 1.4	± 1.0	± 1.0	± 0.8	± 0.7	± 1.0
	Δ (kg)	0.5	0.3	0.3	**0.1****	**0.1****	0.3	0.2	0.3	0.1	**-0.1****
	SD	± 0.2	± 0.2	± 0.2	± 0.2	± 0.1	± 0.1	± 0.2	± 0.2	± 0.1	± 0.2
Week 26 (day 182)	BW (kg)	13.4	13.0	12.8	12.4	13.1	13.2	12.0	12.6	12.2	11.7
	SD	± 1.6	± 1.1	± 0.9	± 1.3	± 1.0	± 0.8	± 0.8	± 0.8	± 0.8	±1.4
	Δ (kg)	1.4	1.2	0.9	0.7	1.2	2.1	1.6	1.8	1.5	0.9
	SD	± 0.3	± 1.1	± 0.7	± 1.1	± 0.8	± 0.4	± 0.7	± 1.0	± 0.5	± 0.9
Week 56 (day 364)	BW (kg)	13.8	13.3	13.5	12.6	13.5	13.5	12.6	13.3	12.0	12.1
	SD	± 1.5	± 0.9	± 1.0	± 1.4	± 0.8	± 1.6	± 1.3	± 0.9	± 0.8	± 1.5
	Δ (kg)	1.7	1.5	1.5	0.9	1.5	2.4	2.2	2.5	1.3	1.4
	SD	± 0.6	± 1.1	± 0.4	± 1.2	± 0.9	± 0.8	± 1.1	± 1.4	± 0.3	± 1.1

BW = body weight; Δ = body weight gain

*: $p \leq 0.05$; **: $p \leq 0.01$ (ANOVA + Dunnett's test, two-sided)

D. FEED CONSUMPTION, FEED EFFICIENCY AND COMPOUND INTAKE

Occasionally reduced feed consumption (not statistically significant) was observed in animals during the entire study period. However, this finding was lacking either consequent frequency or a dose-response relationship and was also observed in 4/5 control females. Based on this observation, it was concluded that the test substance had no effect on the feed consumption of the animals of both sexes.

The mean feed consumption during the administration period was 100% for males and in the range of 96 - 98% and females, respectively, as compared with the control animals.

A temporary reduction of feed efficiency in the mean values of females administered 2000 and 20000 ppm, albeit lacking statistically significance, was reflecting the observed impairment of the body weight development, and thus also assessed as being substance-related.

The approximate, mean daily test substance intake during the administration period is summarised in Table 5.3.2-15 below:

Table 5.3.2-15: 52 weeks feeding dog - mean substance intake

Substance intake	Dose level (ppm)									
	Males					Females				
	0	200	800	2000	20000	0	200	800	2000	20000
(mg/kg bw/day)	-	5.5	21.8	57.4	544.0	-	5.8	22.1	58.3	592.9

E. CLINICAL PATHOLOGY

1. Haematology

There were no treatment-related alterations of haematology parameters measured in any animal administered Boscalid.

2. Clinical chemistry findings

Males at the dose level of 2000 and 20000 ppm and females at the dose level of 20000 ppm showed statistically significantly decreased alanine aminotransferase (ALT), aspartate aminotransferase (AST) was statistically significantly decreased in females at 20000 ppm. The decrease of both blood enzymes has not been considered as a toxicologically adverse effect.

Increased alkaline phosphatase (ALP) activities were observed throughout the study in males at the dose level of 2000 and 20000 ppm and females at the dose level of 20000 ppm.

Table 5.3.2-16: 52 weeks feeding dog - clinical chemistry findings

Sex	Male					Female				
Dose (ppm)	**0**	**200**	**800**	**2000**	**20000**	**0**	**200**	**800**	**2000**	**20000**
Enzymes										
ALT (µkat/L)										
day -4/-1	0.78	0.69	0.81	0.53	0.66	0.55	0.63	0.69	0.56	0.55
day 90/91	0.72	0.59	0.58	**0.39****	**0.34****	0.53	0.49	0.51	0.39	**0.26****
day 185/188	1.16	0.75	0.76	**0.49****	**0.49****	0.47	0.53	**0.75***	0.47	0.37
day 360/360	0.88	0.74	0.77	**0.50****	**0.46****	0.51	0.46	0.51	0.45	0.38
AST (µkat/L)										
day -4/-1	0.54	0.50	0.53	0.52	0.49	0.51	0.55	0.73	0.49	0.65
day 90/91	0.59	0.47	0.50	0.47	0.42	0.48	0.47	0.51	0.44	**0.32***
day 185/188	0.50	0.45	0.43	0.43	0.37	0.37	0.40	0.42	0.38	0.32
day 360/360	0.51	0.49	0.48	0.49	0.39	0.42	0.40	0.48	0.43	0.37
ALP (µkat/L)										
day -4/-1	4.67	3.86	4.06	4.78	4.42	4.56	4.17	4.35	4.01	4.22
day 90/91	3.63	3.33	4.74	**6.67****	**8.75****	3.78	4.76	4.89	5.92	**10.45****
day 185/188	3.15	2.70	4.31	**6.27****	**8.43****	3.84	4.71	5.74	5.34	**11.36****
day 360/360	2.95	2.63	3.53	5.59	**7.02****	3.49	4.08	5.01	5.77	**11.70****

Sex	Male					Female				
Dose (ppm)	**0**	**200**	**800**	**2000**	**20000**	**0**	**200**	**800**	**2000**	**20000**
Blood chemistry										
Total Protein (g/L)										
day -4/-1	60.34	58.88	58.48	57.63	57.73	58.28	58.41	61.27	57.94	60.68
day 90/91	58.19	59.96	58.20	57.67	58.94	60.63	60.05	63.44	62.82	**65.70****
day 185/188	61.45	60.04	60.41	61.05	63.30	60.38	60.69	61.20	60.82	**65.44****
day 360/360	60.38	60.68	60.80	60.85	61.43	61.66	60.26	64.53	62.73	65.04
Globulin (g/L)										
day -4/-1	29.62	28.46	27.73	27.94	27.48	26.47	26.94	28.62	26.30	28.56
day 90/91	27.41	29.07	27.56	27.54	28.26	28.65	28.92	30.93	30.43	**33.30****
day 185/188	29.80	28.58	29.20	29.47	30.98	27.44	28.89	29.15	28.12	31.54
day 360/360	29.06	29.34	29.31	29.60	30.53	28.96	28.23	31.91	29.52	32.66
Triglycerides (mmol/L)										
day -4/-1	0.43	0.48	0.45	0.46	0.42	0.32	0.37	**0.43****	0.30	**0.42***
day 90/91	0.36	0.39	0.45	0.45	**0.65****	0.39	0.48	**0.52***	0.53	**0.69****
day 185/188	0.40	0.43	0.48	**0.60***	**0.66****	0.43	0.48	0.52	0.40	0.53
day 360/360	0.40	0.37	0.47	0.48	**0.60****	0.50	0.46	0.71	0.48	0.58
Chol. (mmol/L)										
day -4/-1	5.22	4.93	4.65	4.62	4.82	3.96	4.17	4.82	4.01	4.81
day 90/91	4.93	5.03	4.86	5.16	6.02	4.12	4.49	**5.23****	4.84	**6.59****
day 185/188	4.89	4.63	4.90	5.04	6.15	5.18	5.37	6.99	5.29	6.01
day 360/360	4.95	4.76	5.05	5.22	5.75	5.67	4.98	7.19	5.29	6.98
Cl (mmol/L)										
day -4/-1	111.9	112.9	113.2	114.0	115.3	113.1	113.7	113.3	113.2	111.9
day 90/91	114.2	114.4	112.4	114.1	**112.1****	114.3	114.1	112.6	113.5	**110.3****
day 185/188	116.0	115.6	115.2	115.3	114.8	114.5	115.6	114.8	114.8	113.3
day 360/360	114.7	115.7	114.5	114.1	113.0	113.3	114.8	112.2	112.9	111.9

*: $p \leq 0.05$; **: $p \leq 0.02$; (Kruskal-Wallis & Mann-Whitney u-test, two-sided)

Additionally, statistical significant increase in total protein and globulin was observed in females of the 20000 ppm dose level, mainly after 3 months of test substance administration. Triglyceride levels were statistically significantly increased in males (2000 and 20000 ppm dose levels) and in females (800 and 20000 ppm dose levels). In females of the same dose levels (i.e. 800 and 20000 ppm) cholesterol was in addition increased. These findings of the 800 ppm dose level have been considered to be not test substance related, as in part already occurring prior to test substance administration (triglycerides) and lacking of a dose-response relationship throughout the study period over all blood sampling events.

Furthermore, at 20000 ppm male dogs revealed decreased chloride (Cl) concentrations on day 90.

F. URINALYSIS

No treatment-related changes were observed in urinalysis evaluations.

G. OPHTHALMOSCOPY

There were no ocular changes or abnormalities that could be attributed to treatment.

H. NECROPSY

1. Organ weight

Target organs identified were the liver and the thyroids. Male animals administered 2000 and 20000 ppm Boscalid had a statistically significant increase in absolute and relative thyroid weights. Females showed a statistically significant increase in relative liver and thyroid weights at the 2000 ppm and 20000 ppm dose levels. Females in addition showed a statistically significant increase of the relative thyroid weights at the 20000 ppm dose level. Details of target organ weights are presented in the table below.

Table 5.3.2-17: 52 weeks feeding dog - summary of absolute (g) and relative (% of terminal body weight) organ weights

Parameter	Sex	Dose level (ppm)				
		0	200	800	2000	20000
Terminal body weight (kg)	M	13.86	13.36	13.56	12.72	13.56
	F	13.50	12.58	13.26	12.06	12.26
Absolute liver weight (g)	M	406.22	381.76	420.31	481.10	527.45
	F	384.67	366.60	409.81	420.98	**544.61***
Relative liver weight	M	2.96	2.86	3.09	3.82	3.89
	F	2.85	2.92	3.09	**3.51***	**4.43****
Absolute thyroid weight (g)	M	0.95	1.23	0.90	**1.32***	**1.47***
	F	1.16	1.14	1.03	1.16	1.65
Relative thyroid weight	M	0.007	0.009	0.007	**0.010***	**0.011***
	F	0.009	0.009	0.008	0.010	**0.013***

*: $p \leq 0.05$; **: $p \leq 0.01$; (Kruskal-Wallis, one sided and Wilcoxon-test, two-sided)

2. Gross lesions

All recorded gross lesions were of incidental or spontaneous nature and not treatment-related. No gross lesions were found in the target organ liver. Nevertheless, paying particular attention to the organs of the endocrine system, the single incidences noticed are summarised below.

Table 5.3.2-18: 52 weeks feeding dog - macroscopic findings in target organs and organs of the endocrine system

Sex	Male					Female				
Dose (ppm)	**0**	**200**	**800**	**2000**	**20000**	**0**	**200**	**800**	**2000**	**20000**
Testes - reduced size	- -	- -	1 / 5 -	- -	- -					
Epididymides - thickened	1 / 5 -	- -	- -	- -	- -					
Prostate - reduced size	- -	- -	1 / 5 -	1 / 5 -	- -					
Ovaries - enlarged						- -	- -	1 / 5 -	- -	- -
Uterus - cyst						- -	- -	1 / 5 -	- -	- -
Adrenal cortex - focus	- -	- -	- -	- -	1 / 5 -	- -	- -	- -	- -	- -
Thyroid glands - cyst - enlarged	- -	- -	- -	1 / 5 -	- -	- -	- -	- -	- -	- 1 / 5
Pituitary gland - cyst	1 / 5 -	- -	1 / 5 -	- -	1 / 5 -	- -	- -	- -	- -	- -

3. Histopathology

Histopathological evaluation gave no evidence for treatment related findings (Table 5.3.2-19). Microscopic changes seen in the liver could not be correlated with the weight increase as seen at the dose levels of 2000 and 20000ppm, were comparable with the incidence of findings in the control animals and did not follow a dose response. They have thus been considered as incidental in nature. The recorded histopathological findings in the thyroid gland occurred in control and all dose groups as single findings, distributed equally among the control and all dose groups and gave no indication of being correlated with the treatment. All other morphology changes detected did show either a single occurrence or no dose-response relationship and were seen to be of incidental or spontaneous nature and were thus not considered as being induced by the test substance. This has been considered to also apply to the organs of the endocrine system, the single incidences noticed are summarised below.

Table 5.3.2-19: Microscopic findings in target organs and organs of the endocrine system

Sex	Male					Female				
Dose (ppm)	**0**	**200**	**800**	**2000**	**20000**	**0**	**200**	**800**	**2000**	**20000**
Liver										
- granuloma, Kupffer cells	4 / 5	5 / 5	4 / 5	3 / 5	4 / 5	5 / 5	5 / 5	4 / 5	5 / 5	5 / 5
- single cell necrosis	3 / 5	2 / 5	1 / 5	-	2 / 5	1 / 5	1 / 5	-	-	1 / 5
- haemosiderosis, Kupffer cells	3 / 5	1 / 5	3 / 5	3 / 5	2 / 5	1 / 5	2 / 5	2 / 5	4 / 5	-
- fatty infiltration	-	1 / 5	-	-	-	-	-	-	-	-
- pigmentation, bile duct	2 / 5	-	4 / 5	3 / 5	2 / 5	-	-	2 / 5	1 / 5	-
- infiltrates, monocytes	1 / 5	1 / 5	2 / 5	1 / 5	3 / 5	2 / 5	1 / 5	-	-	1 / 5
- bile duct proliferation	-	-	-	-	-	-	1 / 5	-	-	1 / 5
Testes										
- inflammatory cells	1 / 5	-	-	-	-					
- tubular atrophy	-	-	1 / 5	-	-					
- giant cells	-	-	1 / 5	-	-					
Epididymides	-	-	-	-	-					
Prostate										
- reduced activity	-	-	1 / 5	1 / 5	-					
Ovaries						-	-	-	-	-
Uterus										
- cyst						-	-	-	1 / 5	-
Adrenal cortex										
- accessory adrenal	-	-	1 / 5	-	-	-	1 / 5	-	-	-
Thyroid glands										
- hyperplasia, c-cell, diffuse	-	4 / 5	1 / 5	-	2 / 5	1 / 5	1 / 5	1 / 5	-	1 / 5
- hyperplasia, c-cell, focal	1 / 5	-	-	-	-	-	-	3 / 5	1 / 5	1 / 5
- thyreoiditis	-	-	1 / 5	-	1 / 5	1 / 5	2 / 5	-	-	1 / 5
- cysts	-	-	-	2 / 5	-	-	-	-	-	-
- hypertrophy, follicular epithelium.	-	-	-	-	-	-	-	-	1 / 5	-
Pituitary gland										
- cyst	2 / 5	3 / 5	1 / 5	1 / 5	1 / 5	1 / 5	1 / 5	-	2 / 5	2 / 5

III. CONCLUSIONS

Dose-dependent effects were observed in dogs administered Boscalid in doses of 2000 and 20000 ppm, as evident by initial body weight losses, retarded body weight gain in females and corresponding reduced feed efficiency, more prominent in females but also occurred in males. Increase in liver and thyroids weights were observed at these dose levels albeit not corroborated by dose dependent macro- and microscopic findings. However, the increased liver weights were considered to be associated with clinic-chemical alterations that might be related to induction of the hepatic microsomal enzyme system such as increased alkaline phosphatase.

The dose levels of 200 and 800 ppm were tolerated by the male and female dogs without any changes that could be attributed to the test substance.

Based on these findings, the no observed adverse effect level (NOAEL) for Beagle dogs under the conditions of the study was 800 ppm, that is equivalent to the substance intake of 21.8 and 22.1 mg/kg bw/day for males and females, respectively.

CA 5.3.3 Other routes

Boscalid - Repeated dose dermal toxicity study in Wistar rats - Administration for 4 weeks ([redacted] et al., 2000c)
Doc ID 2000/1013240

Guidelines: According to OECD 410 (1981), EEC 92/69 and EPA 870.3200
Deviations: None
GLP: Yes
Acceptance: The study has been evaluated and considered acceptable in the EU registration process for the first Annex I inclusion.

Groups of 10 male and 10 female Wistar rats (Chbb:THOM (SPF), source: Boehringer Ingelheim Pharma KG, Germany) were exposed to Boscalid (96.3% purity, batch N 46) by the dermal route at concentrations of 0 (vehicle), 100, 250 and 1000 mg/kg bw for 4 weeks (6 h/day; 5 days/week) under semi-occlusive conditions. The vehicle was 0.5% aqueous carboxymethyl cellulose (CMC) solution with 0.5% Cremophor EL.
One female of the high dose spontaneously died on day 13 of the test substance administration because of septicemia. The main findings related to the death were localized in the uterus (pyometra) and urinary tract (papillary necrosis and severe, suppurative pyelonephritis of the kidneys, dilation and inflammation of the urinary bladder and the ureter). One male of the 100 mg/kg bw dose group showed piloerection on day 22. Based on the isolated occurrence and the lack of a dose-response relationship this finding was considered incidental and not treatment related.
Following a 4-week dermal application of Boscalid to the clipped dorsal skin of Wistar rats, no adverse effects were observed in animals treated with up to 1000 mg/kg bw test item. The only finding was a slight, but significant bilirubin reduction in males of the top dose group that might be related to induction of hepatic enzymes (especially phase II) shown in other studies (See M-CA 5.8.2), and was interpreted as a sign of an adaptation rather than toxicologically relevant adverse effect. No signs of dermal irritation were observed during the course of this study.

Based on the results of this 28-day repeated dose dermal toxicity study, the short-term NOAEL after dermal exposure in the rat can be established at 1000 mg/kg bw for both sexes.

CA 5.4 Genotoxicity Testing

All studies in M-CA 5.4 have already been peer-reviewed by the Rapporteur Member State Germany and national competent authorities following submission of the original dossier for Annex I inclusion (2000):

An adequate data-package of in vitro genotoxicity studies in bacterial and mammalian cell systems and of in vivo genotoxicity has been evaluated and has been considered acceptable. For the reviewer's convenience, these studies are summarized under the respective chapters CA 5.4.1 - CA 5.4.3 as extracted from the Monograph (2002). Tabulated summaries and conclusions are provided in Table 5.4-1.

No additional data on genotoxicity according to EU requirements was generated by the applicant. Published literature information has in addition been evaluated in this document which overall is not considered to change the conclusions as drawn in the evaluation process for the first Annex I inclusion.

Table 5.4-1: Mutagenicity studies conducted with Boscalid as evaluated in the Monograph (2002)

Study type & strains (species)	Test conditions	Result	Reference (BASF DocID)
In vitro studies			
Bacterial reverse mutation assay (Ames test) *Salmonella typhimurium* (TA 1535, 100, 1537, 98); *Escherichia coli (WP2uvrA)*	Test substance purity: 95.3% With and without S-9 mix Test concentrations: Standard plate test up to 5500 µg/plate Pre-incubation test up to 5000 µg/plate	 Negative Negative	Engelhardt G., Hoffmann H.D. 1998/11440
Mammalian cell forward mutation assay HPRT Chinese hamster ovary cells (CHO)	Test substance purity: 94.4% With and without S-9 mix Test concentrations: With S-9 mix up to 1000 µg/mL Without S-9 mix up to 500 µg/mL	 Negative Negative	Engelhardt G., Hoffmann H.D. 2000/1000180
Mammalian chromosome aberration test Chinese hamster V 79 cells	Test substance purity: 94.4% Test concentrations: With S-9 mix up to 500 µg/mL Without S-9 mix up to 500 µg/mL	 Negative Negative	Engelhardt G., Hoffmann H.D. 1999/10978
Unscheduled DNA synthesis – UDS assay Wistar rat derived primary hepatocytes	Test substance purity: 94.4% Test concentrations: up to 50 µg/mL	 Negative	Engelhardt G., Hoffmann H.D. 2000/1011413
In vivo studies			
In vivo cytogenicity Mouse micronucleus test NMRI mouse	Test substance purity: 94.4% Dosing: Two intraperitoneal injections up to 2000 mg/kg bw	 Negative	[redacted] 1999/11048

Boscalid was tested in a battery of in vitro and one in vivo mutagenicity assays using test material with 94.4% to 95.3% purity.
Results from these studies showed that Boscalid does not induce base-pair or frame-shift mutation in any of the bacterial tester strains (*S. typhimurium*, *E.coli*), or gene mutation in mammalian cells in vitro (CHO-HRPT assay). No indication of clastogenicity was observed in the in vitro chromosome aberration assay in Chinese Hamster V79 cells, or in the in vivo mouse micronucleus assay. No induction of DNA repair was observed in the in vitro UDS assay in primary rat hepatocytes.
In conclusion, there is no evidence from the available information regarding any mutagenic or clastogenic effect of the active substance Boscalid.

Boscalid was not phototoxic in the 3T3 NRU Phototoxicity Test and thus did not trigger the need for further investigations on photomutagenicity. This is in addition supported by the absence of validated and agreed test methods being in place. In accordance with the 'guidance for applicants on preparing dossiers' as laid down in SANCO/10181/2013-rev. 3 waiving of particular data requirements is acceptable if agreed test methods or guidance documents are not available.

Based on the available data on the genotoxicity, classification of Boscalid for this endpoint is not required according to the criteria laid down in Regulation (EU) No. 1272/2008 (CLP). Moreover, the data on genotoxicity of Boscalid have already been evaluated within the previous Annex I inclusion process, coming to the conclusion that Boscalid does not need to be classified for human health (SANCO/3919/2007-rev. 5, 21 January 2008).

Based on the available studies, the conclusion for relevant endpoints adopted to the new list of endpoint format for the current re-registration is as follows:

Genotoxicity (Regulation (EU) N° 283/2013, Annex Part A, point 5.4)

In vitro studies	Ames test: CHO-HRPT assay: Chromosome aberration assay in Chinese hamster V79 cells: UDS in primary Wistar rat hepatocytes:	Negative Negative Negative Negative	
In vivo studies	NMRI mouse micronucleus assay:	Negative	
Photomutagenicity	Not required		
Potential for genotoxicity	No evidence for genotoxic potential		

Comparison with CLP criteria

According to the criteria of the CLP (Regulation 1272/2008/EC), a mutation means a permanent change in the amount or structure of the genetic material in a cell. The term ‘mutation’ applies both to heritable genetic changes that may be manifested at the phenotypic level and to the underlying DNA modifications when known (including specific base pair changes and chromosomal translocations). The term ‘mutagenic’ and ‘mutagen’ will be used for agents giving rise to an increased occurrence of mutations in populations of cells and/or organisms.

In a series of *in vitro* and *in vivo* tests with Boscalid several endpoints of potential genotoxicity were measured such as gene mutation in bacterial and mammalian cells, chromosomal aberration and DNA damage/repair. In all assays Boscalid was shown to be devoid of mutagenic activity.

Conclusion on classification and labelling

Overall, based on the available studies Boscalid was evaluated to have no genotoxic potential. In conclusion, in comparison with the criteria on classification and labelling for mutagenicity, Boscalid does not qualify for classification as “mutagenic” according to Regulation 1272/2008/EC.

CA 5.4.1 In vitro studies

Salmonella typhimurium/Escherichia coli reverse mutation assay (standard plate test and preincubation test) with Boscalid (Engelhardt and Hoffmann, 1998)
DocID 1998/11440

Guidelines: According to OECD 4471 (1997), EEC 92/69 B 14, EEC 92/69 B 13
Deviations: None
GLP: Yes
Acceptance: The study has been evaluated and considered acceptable in the EU registration process for the first Annex I inclusion.

Note: This study report has been part of the documentation for the first Annex I inclusion process and a short summary is therefore presented here

S. typhimurium strains TA 98, TA 100, TA 1535 and TA 1537, and a strain of *E. coli* WP2 uvrA were exposed with Boscalid (95.3% purity, batch N 26) using DMSO as a solvent in the presence and absence of metabolic activation (liver S-9 mix of Aroclor 1254-induced Sprague Dawley rats) for 48 hours. Vehicle and positive controls were included in each experiment. The test item was tested via standard plate test (SPT) and the pre-incubation test (PIT) in concentrations ranged from 22 to 5500 µg/plate and from 20 to 5000 µg/plate, respectively.
Precipitation and weak bacteriotoxicity of the test substance was observed at concentrations ≥ 500 µg/plate. An increase in the number of his^+ and trp^+ revertants was not observed in SPT and PIT either without S-9 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.
According to the results of the study, the test substance Boscalid is not mutagenic in the Ames reverse mutation assay under the experimental conditions chosen.

In vitro gene mutation test with Boscalid in CHO cells (HPRT locus assay), (Engelhardt and Hoffmann, 2000)
DocID 2000/1000180

Guidelines: According to OECD 476 (1997), EEC 87/302
Deviations: None that compromised the validity of the study
GLP: Yes
Acceptance: The study has been evaluated and considered acceptable in the EU registration process for the first Annex I inclusion

Note: This study report has been part of the documentation for the first Annex I inclusion process and a short summary is therefore presented here

Boscalid (94.4% purity, batch N 37) was examined in vitro for mutagenic activity (using the *hprt*-locus) by assaying for the induction of 6-thioguanine resistant mutants in Chinese hamster ovary (CHO) cells. The test was performed with and without metabolic activation (liver S-9 mix from Aroclor 1254-induced Sprague Dawley rats). Concentrations tested ranged from 3.125 to 500 µg/mL without S-9 mix and from 10.24 to 1000 µg/mL with S-9 mix. The solvent DMSO was used as vehicle control, while 3-methylcholanthrene and ethyl methane sulfonate were used as positive controls with and without S-9-mix activation, respectively.
Cytotoxicity, observed as reduced cell density, was recorded at concentrations ≥ 64 µg/mL in absence of S-9 mix. Precipitation was noted at concentrations ≥ 31.25 µg/mL in absence and presence of S-9 mix. No significant increases in mutant frequency as compared with the vehicle control were observed with and without S-9 mix. The vehicle control values were within the range of historical control data. The positive controls demonstrated that the system was able to detect known mutagens.
Based on the results of this study, Boscalid did not induce gene mutations at the *hprt*-locus in the in vitro mammalian cell test under the experimental conditions chosen.

In vitro chromosome aberration assay with Boscalid in V79 cells, (Engelhardt and Hoffmann, 1999)
DocID 1999/10978

Guidelines: According to OECD 473 (1997), EEC 92/69 B 10
Deviations: None that compromised the validity of the study
GLP: Yes
Acceptance: The study has been evaluated and considered acceptable in the EU registration process for the first Annex I inclusion.

Note: This study report has been part of the documentation for the first Annex I inclusion process and a short summary is therefore presented here

Boscalid (94.4% purity, batch N 37) was tested in vitro for its ability to induce structural chromosome aberrations in Chinese Hamster V79 cells. Based on previously assessed cytotoxicity (mitotic index), Boscalid was tested at concentrations ranging from 20 to 500 µg/mL with and without metabolic activation (liver S-9 mix from Aroclor 1254-induced Sprague Dawley rats). Without metabolic activation experiments with 4 h and 18 h exposure/18 h sampling times, and 28 h exposure/ 28 h sampling times were performed, while in the presence of S-9 mix 4 h exposure/18 h and 28 h sampling times were applied. For each independent experiment, vehicle (DMSO) and positive controls (cyclophosphamide (CPA) and ethyl methane sulfonate (EMS) for the experiments with and without metabolic activation, respectively) were included to demonstrate the sensitivity of the test system. Metaphase arrest was induced by colcemid treatment 2-3 hours prior cell harvest. Two hundred metaphases per dose group and vehicle control, and 50 metaphases per positive control group were analysed for chromosomal aberrations.
No cytotoxicity was indicated by the mitotic index at any dose tested and any experimental condition. Precipitation was noted at concentrations ≥ 62.50 µg/mL in absence and presence of S-9 mix. No significant increase in the number of structural chromosomal aberrations inclusive and exclusive gaps neither without nor with S-9 mix were induced by the test item compared with the vehicle control at any exposure and sampling times. The frequencies of aberration in the concurrent vehicle control were within the range of historical control data. The positive controls EMS and CPA induced substantial chromosome damage in any experiment that were within the historical range, demonstrating the ability of the system to detect known clastogens.
Based on the results of this study, Boscalid has no chromosome-damaging (clastogenic) properties in V79 cells under the in vitro conditions chosen.

In vitro unscheduled DNA synthesis (UDS) assay with Boscalid in primary rat hepatocytes, (Engelhardt and Hoffmann, 2000)
Doc ID 2000/1011413

Guidelines: According to OECD 482 (1986), EEC 87/302
Deviations: None
GLP: Yes
Acceptance: The study has been evaluated and considered acceptable in the EU registration process for the first Annex I inclusion.

Note: This study report has been part of the documentation for the first Annex I inclusion process and a short summary is therefore presented here

Boscalid (94.4% purity, batch N 37) was tested for its ability to induce DNA repair synthesis (unscheduled DNA synthesis, UDS) in vitro in primary hepatocytes of male Wistar rats (ChBB; Thom, SPF source: Boehringer Ingelheim Pharma KG, Germany). Based on previously assessed cytotoxicity (LDH release into the medium and lactate concentration), Boscalid was tested at concentrations ranging from 1 to 50 µg/mL in two independent experiments. For each experiment, negative (culture medium), vehicle (DMSO) and positive (2-Acetylaminofluorene, 2-AAF) controls were included to demonstrate the validity of the assay and the sensitivity of the test system. Both substance exposure and labelling with ^{3}H-thymidine lasted for about 18-20 hours, radiography was performed according to the Butterworth method and 100 cells/treatment group were evaluated.
Cytotoxicity was observed depending on the experiment and the parameter tested from about 10 µg/mL - 25 µg/mL onward. Precipitation was noted at concentrations ≥ 50 µg/mL. Both UDS parameter, the mean number of net nuclear grain counts and % of cells in repair, induced by the test item at any concentration tested was comparable to the concurrent negative and vehicle controls. The results of the test item, negative and vehicle controls were all within the range of historical control data. The substantial induction of DNA repair by the positive control 2-AAF was within the historical data range, demonstrating the sensitivity of the test method applied.
Based on the results of this study, Boscalid did not cause an increase in unscheduled DNA synthesis and was negative in the in vitro assay using primary rat hepatocytes.

Literature information

Report: CA 5.4.1/1
Knight A.W. et al., 2009 a
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 analyse 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 tumours 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.
The endpoints determined and methods applied in the 3 HTS-assays were growth arrest and DNA damage, recorded by p53 regulated induction of GFP protein (GreenScreen HC GADD45a-GFP), DNA damage induced p53 activation analysed by immunostaining (CellCiphr p53), and p53-controlled beta lactamase activation analysed by technology based on fluorescence resonance energy transfer.

Negative result were obtained with Boscalid in the GreenScreen HC GADD45a-GFP, CellCiphr p53 and CellSensor p53RE-bla assays tested up to 100 µM, indicating that Boscalid has no genotoxic properties supporting available in vitro and in vivo data.

Conclusion: Supplementary screening information

Report: CA 5.4.1/2
Cayir A. et al., 2012 a
Micronuclei, nucleoplasmic bridges, and nuclear buds induced in human lymphocytes by the fungicide Signum and its active ingredients (Boscalid and Pyraclostrobin)
2012/1366624
Guidelines: none
GLP: no

Executive Summary of the Literature

Boscalid and the Boscalid containing formulation Signum as well as the active substance pyraclostrobin were tested for their ability to induce micronuclei, nucleoplasmic bridges, and nuclear buds in human lymphocytes in vitro. The method used was similar to OECD guideline 487, but used the additional endpoints nucleoplasmic bridges, and nuclear buds, that are also indicative for a genotoxic effect. Both active ingredients used for the assays were of >99% purity and the formulation Signum was a commercial formulation containing 26.7% w/w Boscalid and 6.7 % w/w Pyraclostrobin.
Heparinized blood was obtained from 2 young donors that reported to be healthy but were not tested for their health status. Two parallel cultures were tested for each test concentration and culture condition in the absence of metabolic activation only. In a G_0 phase protocol blood was treated with the test concentrations for 24 hours without phytohemagglutinin (PHA), then the test item was removed and PHA was added to the culture medium. After 44 hours cytochalasin B was added and cultures were harvested 72 hours after initiation. In the second protocol proliferating lymphocytes were treated with PHA for 44 hours before the test items were added. Cytochalasin B was added 48 hours after initiation and cells were harvested 72 hours after start. Signum and Boscalid were tested in 8 concentrations in both G_0 Phase and proliferating lymphocytes while Pyraclostrobin was tested in 7 concentrations in G_0 Phase lymphocytes and only 3 concentrations in proliferating lymphocytes. Cells were fixed and slides stained with Giemsa before microscopic evaluation. 1000 binucleated cells were scored for each culture resulting in a total of 4000 binucleated cells per concentration. Parameters evaluated were micronuclei, nuclear buds and nucleoplasmic bridges as a measure of genotoxicity and cytokinesis-block proliferation index (CBPI) and % cytostasis as a measure of cytotoxicity. Mitomycin C served as a positive control and yielded a statistically significant increase in micronuclei in all experiments.

For all 3 test items an increase in micronuclei compared to the concurrent vehcile control was observed both in G_0 and proliferating cells. This increase was not dose dependent for both Boscalid and Signum with only single concentrations reaching statistical significance. The study authors attribute the missing dose response for micronucleus induction to the increasing level of % cytostasis, nevertheless all concentrations tested were well below the cytostasis limit of 55%. For Pyraclostrobin a more pronounced effect on miconucleus formation was noted that was dose-related even though levels of cytostasis were enhanced compared to Signum and Boscalid. The authors do not indicate their historical control data for the study design employed. On this basis it is difficult to assess the biological relevance of the effects observed. Nevertheless, it can be concluded that under the conditions described, Boscalid, Pyraclostrobin and the formulated product Signum induce clastogenic or aneugenic effects in vitro.

This is not considered relevant information since for both Boscalid and Pyraclostrobin comprehensive in vitro and in vivo data for genotoxicity are already available and have been evaluated for Annex I inclusion. The relevant follow up assay for a positive in vitro micronucleus assay in either mammalian cell lines or human lymphocytes is a rodent bone marrow micronucleus assay addressing both aneugenic and clastogenic effects. This study has been performed for Boscalid and was clearly negative (Boscalid mouse micronucleus after intraperitoneal administration (BASF DocID 1999/11048), see section 5.4.2). For Pyraclostrobin a chromosomal aberration assay in mice was likewise negative. The author`s recommendation to perform a Comet assay in vivo is not considered useful since this assay addresses clastogenic and mutagenic but not aneugenic effects and would not lead to a better understanding of the genotoxic profile.

Genotoxicity testing of formulated products is not a data requirement in the EU and is in general not considered useful since testing of the undiluted active substance for a genotoxic hazard is considered more appropriate. Therefore, no in vivo studies on the formulation Signum are available. Nevertheless, in vivo micronucleus tests in mouse bone marrow have been performed with a number of different formulations containing Pyraclostrobin and with one formulation containing Boscalid for registration in Brazil. All these studies were negative, thus proving that the presence of formulation auxiliaries does not alter the genotoxic profile of the active substances. Table 5.4.1-1 shows a list of in vivo micronucleus studies available for formulated products containing Boscalid. Study reports are available on request.

Table 5.4.1-1: *In vivo* micronucleus assays with formulated products containing Boscalid

Study type	Test System	Formulation tested	Result	Reference*
In vivo Cytogenicity	Mouse Micronucleus test (0, 93.8 -750 mg/kg bw); Oral gavage	SC-type 200 g/L Boscalid	Negative	2008/1037578
In vivo Cytogenicity	Mouse Micronucleus test (0, 75 - 700 mg/kg bw); intraperitoneal administration	WG-type 500 g/kg Boscalid	Negative	2001/1015066

*study reports available on request

Based on negative genotoxicity datasets for both Boscalid and Pyraclostrobin and a set of negative *in vivo* micronucleus assays in mice with different formulated products, the *in vitro* findings reported by Cayir et al. are not considered to have any relevance *in vivo*.

Conclusion: Supplementary information

CA 5.4.2 In vivo studies in somatic cells

Cytogenetic study in vivo with Boscalid in the mouse micronucleus test after two intraperitoneal administrations, ([redacted], 1999)
DocID 1999/11048

Guidelines: According to OECD 474 (1997), EEC 92/69, method B. 12
Deviations: None that compromised the validity of the study
GLP: Yes
Acceptance: The study has been evaluated and considered acceptable in the EU registration process for the first Annex I inclusion.

Note: This study report has been part of the documentation for the first Annex I inclusion process and a short summary is therefore presented here

Boscalid (94.4% purity, batch N 37) was tested for clastogenicity and aneugenicity using the in vivo bone marrow micronucleus test method. Groups of 5 male NMRI mice (source: Charles River Deutschland GmbH, Germany) were administered doses of 0 (vehicle: 0.5% CMC formulation), 500, 1000 and 2000 mg/kg bw by intraperitoneal injection twice, with an application volume of 2 mL/100 g bw and an interval of 24 hours between both applications. Cyclophosphamide (CPA) and vincristine were used as the positive controls for clastogenic and aneugenic effect, respectively, and were administered by the intraperitoneal route (20 mg/kg bw CPA or 0.15 mg/kg bw vincristine) with an application volume of 1 mL/100 g bw, once. Animals were sacrificed 24 hours after the last application, bone marrow smear slides were prepared from 2 femora and 2000 polychromatic erythrocytes (PCE) per animal were analysed for micronuclei. The ratio of PCE to normochromatic erythrocytes (NCE) was determined to assess inhibition of erythropoiesis.
Clinical signs of toxicity consisted of piloerection and squatting posture were observed in all animals treated with the test substance at all dose levels within 24 hours after each injection. No clinical signs of toxicity were observed in animals of vehicle and positive control groups. No inhibition of erythropoiesis induced by the test item was detected, as the PCE/NCE ratio was not affected. No micronuclei induction by the test item was detected at any dose tested, as the rate of the micronuclei determined was comparable with the vehicle control that in turn was within the range of historical control data. Both positive controls for clastogenic and aneugenic effects (CPA and vincristine, respectively) led to the expected increase in the rate of PCE containing small or large micronuclei, respectively.
Based on the result of this study, Boscalid did not induce structural chromosome damage (clastogenic effects) in the in vivo micronucleus test in mice, and showed no indication for any impairment of chromosome distribution in the course of the mitosis (aneugenic effects).

CA 5.4.3 In vivo studies in germ cells

The results of the in vitro as well as the in vivo studies demonstrated that Boscalid has no mutagenic or genotoxic potential. Therefore, there was no necessity to evaluate the test substance in an in vivo study using germ cells.

CA 5.5 Long-Term Toxicity and Carcinogenicity

All studies in M-CA 5.5 have already been peer-reviewed by the Rapporteur Member State Germany and national competent authorities following submission of the original dossier for Annex I inclusion (2000):

An adequate set of long-term toxicity and carcinogenicity studies has been evaluated and has been considered acceptable. The endpoints were fixed in the European Commission Review Report for the active substance Boscalid (SANCO/3919/2007 –Rev 5, 21 January 2008).
For the reviewer's convenience, these studies are summarized below as extracted from the Monograph (2002), and the tabulated summary is provided in Table 5.5-1.

Table 5.5-1: Summary of long-term toxicity/carcinogenicity studies performed with Boscalid

Study	NOAEL Males/females mg/kg bw/day	LOAEL Males/females mg/kg bw/day	Main adverse effect	Reference BASF DocID
24-month oral chronic toxicity in Wistar rats 0, 100, 500, 2500, 15000* ppm	4.4/5.9 (100 ppm)	21.9/30.0 (500 ppm)	**≥500 ppm**: Altered clinical chemistry & haematology, increased incidence of eosinophilic focal cellular alteration of liver (males) **2500 ppm**: Increased absolute thyroid weight (males), increased relative liver weight (females). Increase in thyroid follicular hypertrophy and centrilobular liver cell hypertrophy (both sexes)	[redacted] et al., 2001 2001/1000114 (2002/1004026)
24-month oral carcinogenicity study in Wistar rats 0, 100, 500, 2500, 15000* ppm	4.6/6.0 (100 ppm)	23.0/29.7 (500 ppm)	**≥500 ppm**: Liver centrilobular hypertrophy **2500 ppm**: Increase in absolute and relative thyroid weight (males), increased incidence of thyroid follicular cell adenomas (not considered as relevant to human)	[redacted] et al., 2001 2001/1000115
18-month carcinogenicity C57BL mouse 0, 80, 400, 2000, 8000 ppm	13/18 (80 ppm)	65/90 (400 ppm)	**≥400 ppm**: Reduced body weight, increase in relative liver weight (males) **≥2000 ppm**: Increase in absolute and relative liver weight & increased incidence of periportal hepatocellular hypertrophy (females) **8000 ppm**: Increase in absolute liver weight & increased incidence of periportal hepatocellular hypertrophy (males)	[redacted] et al., 2001 2001/1000116

For Boscalid, a 24-month chronic feeding study and a carcinogenicity study were performed in Wistar rats. In addition, a carcinogenicity study was performed in C57BL mice.
In rats the target organs identified were the liver and the thyroids. In the liver pathological changes comprised relative organ weight increase and liver centrilobular hypertrophy in females at the 2500 ppm dose level. Liver centrilobular hypertrophy was also seen in males from the 500 ppm dose level onwards. The incidence of eosinophilic foci was numerically increased in males from dose levels of 500 ppm onwards with concomitant increase in size at 2500 ppm being considered as test substance related. There were concomitant changes of clinical chemistry indicative of liver toxicity.
In the thyroids there was an increase in absolute and relative thyroid weight at the dose level of 2500 ppm combined with slightly increased incidence of thyroid follicular cell adenoma.
In regard to the mechanism related to tumor formation in thyroids, effects of Boscalid on hepatic enzymes were investigated. Boscalid mediated enhanced hepatic metabolism, suggests increased hepatic clearance of thyroid hormones followed by thyroid hormone imbalance and thus chronic stimulation of the organ.

The NOAEL for chronic toxicity and carcinogenicity in the rat has been set at the dose level of 100 ppm, equivalent to 4.4 mg/kg bw/day.

In C57BL mice, no treatment related tumors could be identified up to the maximum dose level of 8000 ppm of Boscalid in the food. Reduced body weight was observed at the dose level of 400 ppm onwards in males and at 8000 ppm in females. Predominant finding was increase in liver weight in males from the dose level of 400 ppm onwards and in females from 2000 ppm onwards combined with periportal hepatocellular hypertrophy. The latter finding was also present in males at the 8000 ppm dose level.
The NOAEL for this study was set at 80 ppm corresponding to 13 mg/kg bw/day.

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

No additional data on long-term toxicity and carcinogenicity of Boscalid were generated by the applicant.

Based on the available data regarding chronic toxicity and carcinogenicity, classification of Boscalid for this endpoint is not justified according to the criteria laid down in Regulation (EU) No. 1272/2008 (CLP).

Thus, the conclusion for relevant endpoints adopted to the new list of endpoint format for the current re-registration is presented as follows:

Long-term toxicity and carcinogenicity (Regulation (EU) N°283/2013, Annex Part A, point 5.5)

Long-term effects (target organ/critical effect)	**Rat**: Liver / centrilobular hypertrophy, eosinophilic foci Thyroid / follicular cell adenoma **Mouse:** Liver / periportal hepatocellular hypertrophy	
Relevant long-term NOAEL	**Rat: 4.4 mg/kg bw/day (100 ppm)** Mouse: 13 mg/kg bw/day (80 ppm)	
Carcinogenicity (target organ, tumour type)	Rat: Slight increase of thyroid follicular cell adenoma; not relevant to man. No classification and labelling necessary	
Relevant NOAEL for carcinogenicity	**Rat: 4.4 mg/kg bw/day (100 ppm)** Mouse: Not applicable	

Comparison with CLP Criteria

According to the criteria of the CLP (Regulation 1272/2008/EC), the term “carcinogen” means a substance or a mixture of substances which induce cancer or increase its incidence. Substances which have induced benign and malignant tumours in well performed experimental studies on animals are considered also to be presumed or suspected human carcinogens unless there is strong evidence that the mechanism of tumour formation is not relevant for humans.

Chronic toxicity/carcinogenicity studies were performed with Boscalid in two rodent species: rats and mice. There was no relevant treatment related increase in tumour formation in rats or mice. The slight increase in thyroid follicular cell adenoma observed in the rat was due to the higher metabolic activity of hepatocytes with concomitant increased hepatic clearance of T3/T4 hormones and as a consequence to a secondary functional stimulation of the thyroid glands by a feedback mechanism that is evident to induce the slight increase in incidences of follicular cell adenomas in the mid and high dose groups. This mode of action is considered specific to rodents (rats) and does not apply to humans.
In conclusion, based on the assessment of all available data Boscalid is not subject to classification for carcinogenicity according to Regulation 1272/2008/EC.

Conclusion on classification and labelling

The available data on carcinogenicity of Boscalid do not meet the criteria for classification according to Regulation (EC) 1272/2008 or Directive 67/548/EEC.

Boscalid - Chronic toxicity study in Wistar rats - Administration in the diet for 24 months ([redacted] et al., 2001)
DocID 2001/1000114

Guidelines: According to OECD 452 (1981), EEC 87/302, EPA/FIFRA (Subdivision F; para. 83-1) and JMAFF
Deviations: None
GLP: Yes
Acceptance: The study has been evaluated and considered acceptable in the EU registration process for the first Annex I inclusion.

Note: The chronic feeding study in rats was identified as relevant study for setting of the Acceptable Daily Intake (ADI) reference dose. For this reason this study is in detail evaluated here following the OECD format.

Executive summary

Boscalid (94.4% purity, batch N 37) was administered to groups of 20 male and 20 female Wistar rats (source: Boehringer Ingelheim Pharma KG, Germany) at dietary levels of 0, 100, 500, and 2500 ppm for a period of 24 months. These concentrations were equivalent to the substance intake of 0, 4.4, 22 and 110 mg/kg bw/day in males, and 0, 5.9, 30 and 150 mg/kg bw/day in females.
Due to excessive toxicity (severe effects on body weights), the initial 15000 ppm top dose groups were discontinued after approximately 17 months of test substance administration.
Following the 24-month dietary administration of Boscalid to Wistar rats, adverse effects were observed in animals of the dose group ≥ 500 ppm. Treatment-related findings included increased relative liver weights (2500 ppm, females) with correlating histopathological changes (centrilobular hypertrophy in both sexes at 2500 ppm and increased incidence of eosinophilic foci of cellular alteration in males ≥ 500 ppm), supported by alterations of clinical chemistry (indicative of hepatic microsomal enzyme induction in both sexes). Increased absolute thyroid weights (2500 ppm, males) with correlating histopathological changes (diffuse follicular cell hypertrophy and focal follicular cell hyperplasia in both sexes at 2500 ppm) were seen. Mild anaemic process (decreased haemoglobin, haematocrit, mean corpuscular volume and mean corpuscular haemoglobin) in females at 2500 ppm and in part in males at 500 ppm were observed.
Based on the results of this 24-month dietary study, the long-term NOAEL in the rat can be established at 100 ppm (equivalent to 4.4 mg/kg bw/day in males and 5.9 mg/kg bw/day in females).

(DocID 2001/1000114)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: Boscalid (BAS 510 F; Reg.No. 300 355)

Description:	solid / white
Lot/Batch #:	N37 (Tox-batch III)
Purity:	94.4%
Stability of test compound:	The stability of the test substance was verified by re-analysis via HPLC after the in-life phase of the study. The re-analyzed purity was still 94.4% on 27 Oct. 2000, the expiration date was Oct. 2003.

2. Vehicle and/or positive control: None

3. Test animals:

Species:	Rat
Strain:	Wistar (Chbb:THOM (SPF))
Sex:	Male and female
Age:	42 days (at start of administration)
Weight at dosing (mean):	Males: 181.5 (163.5 - 206.2) g Females: 139.2 (114.4 - 156.9) g
Source:	Boehringer Ingelheim Pharma KG, Biberach/Riss, Germany
Acclimation period:	8 days
Diet:	Kliba maintenance diet rat/mouse/hamster, meal (Provimi Kliba SA, Kaiseraugst, Switzerland), ad libitum
Water:	drinking water from bottles, ad libitum
Housing:	Animals were housed individually in type DK III stainless steel wire mesh cages (Becker & Co., Castrop-Rauxel, Germany) with a floor area of about 800 cm². Underneath the cages, waste trays were fixed containing absorbent material (type ¾ dust free embedding, supplied by SSNIFF, Soest, Germany).
Environmental conditions:	
Temperature:	20 - 24°C
Humidity:	30 - 70%
Air changes:	animals were housed in a fully air-conditioned room
Photo period:	12 hours / 12 hours (Light: from 06.00 a. m. - 06.00 p. m; dark: from 06.00 p. m. - 06.00 a. m.)

B. STUDY DESIGN AND METHODS

1. Dates of experimental work: 19-Jan-1998 - 28-Feb-2001
(In-life dates: 27-Jan-1998 (start of administration) to 26-Jan/08-Feb-2000 (necropsy))

2. Animal assignment and treatment:
Boscalid was administered to groups of 20 male and 20 female Wistar rats at dietary concentrations of 0, 100, 500, 2500 ppm daily for 24 months. Due to severe toxicity at the initial top dose level of 15000 ppm (i.e. severe effects on body weight), the treatment was discontinued for both sexes and animals sacrificed after about 17 month test item administration.

3. Test substance preparation and analysis:
For the high concentration, the respective amount of the test substance was directly added to the food in the laboratory mixer. For the remaining test substance concentrations, food of the top dose level was weighed out and mixed with the corresponding amounts of untreated food, depending on dose group in order to obtain the desired concentrations and mixed for 10 minutes in a laboratory mixer. Usually, the test substance preparations were mixed weekly.
The stability of the test substance in the diet over a period of 32 days at room temperature was verified before the start of the study.
Homogeneity and/or concentration control analyses for the current study and for the parallel carcinogenicity study were performed at the start of the study-period as well as after 3, 6, 9, 12, 15 and 18 month via HPLC/UV. No concentration control analyses were performed toward the end of the study. However, as all analyses up to 18 months were found to be consistent with the nominal test substance concentrations and procedures of test substance preparation were not changed for the remainder of the study it has been considered reasonable to expect that test substance concentrations were correct over the whole study. Homogeneity was analysed by taking 3 samples from the top, middle and bottom of the beaker for the low (100 ppm) and high dose level (15000 ppm).

4. Statistics:

Means and standard deviations of each test group were calculated for several parameters. Further statistical analyses were performed according to following tables:

Statistics of clinical examinations

Parameter	Statistical test
Food consumption, body weight, body weight gain, food efficiency	Parametric one-way analysis using the F-test (ANOVA, two-sided). If the resulting p-value was equal or less than 0.05, a comparison of each group with the control group was performed using DUNNETT's test (two-sided) for the hypothesis of equal means

Statistics of clinical pathology

Parameter	Statistical test
Clinical pathology parameters, except differential blood count and reticulocytes	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 MANN-WHITNEY U-test (two-sided) for the equal medians
Urinalysis, except volume, colour, turbidity and specific gravity	Pair-wise comparison of each dose group with the control group using FISHER's exact test for the hypothesis of equal proportions

Statistics of pathology

Parameter	Statistical test
Organ weights	Non-parametric one-way analysis using KRUSKAL-WALLIS test (two-sided). If the resulting p-value was equal or less than 0.05, a pairwise 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:

A check for any moribund or dead animals as well as for clinical signs of toxicity was made twice a day (in the morning and in the late afternoon) on working days and once a day (in the morning) on weekends and public holidays. Once a week, an additional comprehensive clinical examination (including palpation) was carried out.

2. Body weight:

Body weight was determined before the start of the administration period in order to randomize the animals. During the study period, body weight was determined on day 0, once a week during the first 13 weeks of the administration period, thereafter at 4-week intervals, and prior to start of necropsy.
The difference between the body weight on the respective day of weighing and the body weight on day 0 was calculated as body weight gain.

3. Food consumption, food efficiency and compound intake:

Food consumption was determined once a week over a period of 7 days during the first 13 weeks of the administration period, thereafter at 4-week intervals, and prior to start of necropsy. The values were calculated as food consumption in grams per animal and day.
Food efficiency (group means) was calculated based upon individual values for body weight and food consumption:

$$\text{Food efficiency for day x} = \frac{BW_x - BW_Y}{FC_{y\ to\ x}}\, x\, 100$$

BW_x and BW_y = body weight [g] at day x and day y (last weighing date before day x)
$FC_{y\ to\ x}$ = the mean food consumption from day y to x calculated as mean daily food consumption [g] on day x, multiplied by the number of days from day y to day x

The mean daily intake of test substance (group means) was calculated based upon individual values for body weight and food consumption.

$$\text{Substance intake for day x} = \frac{FC_x \times D}{BW_x}$$

BW_x = body weight [g] at day x
FC_x = the mean food consumption [g] for day x
D = dietary test substance concentration in ppm

4. Haematology and clinical chemistry:

Blood samples were taken from the retro-orbital venous plexus in the morning from non-fasted, un-anaesthetised animals or (from nominal day 395/400 onwards) from fasted animals without anaesthesia.

The assays of blood and serum parameters were performed under internal laboratory quality control conditions with commercial reference controls to assure reliable test results that were expressed in units of the International System (SI)

The following haematological and clinical chemistry parameters were determined for all surviving animals:

Haematology:					
Red blood cells		*White blood cells*		*Clotting Potential*	
✓	Erythrocyte count (RBC)	✓	Total leukocyte count (WBC)		activated partial thrombo-plastin time (PTT)
✓	Haemoglobin (Hb)	✓	Differential blood count	✓	Prothrombin time (Quick's test (QT)
✓	Haematocrit (Hct)	✓	Platelet count (PLT)		
✓	Mean corp. volume (MCV)		Platelet-crit.		
✓	Mean corp. haemoglobin (MCH)		Mean platelet volume		
✓	Mean corp. Hb. conc. (MCHC)		Platelet distribution width		
✓	Reticulocytes (RET)				
	Red cell distribution width				

Clinical chemistry:					
Electrolytes		*Metabolites and Proteins*		*Enzymes:*	
✓	Calcium	✓	Albumin	✓	Alanine aminotransferase (ALT)
✓	Chloride	✓	Globulin	✓	Aspartate aminotransferase (AST)
✓	Phosphorus (inorganic)		Bile acids	✓	Alkaline phosphatase (ALP)
✓	Potassium	✓	Bilirubin (total)	✓	γ-glutamyltransferase (γ-GT)
✓	Sodium	✓	Cholesterol		
✓	Magnesium	✓	Creatinine		
		✓	Glucose		
		✓	Protein (total)		
		✓	Triglycerides		
		✓	Urea		

5. Urinalysis:

For urinalysis the individual animals were transferred to metabolism cages and urine was collected overnight.

The following quantitative or semi-quantitative parameters were determined for all animals:

Urinalysis					
Quantitative parameters:		*Semi quantitative parameters*			
✓	Urine volume	✓	Bilirubin	✓	Nitrite
	Osmolality	✓	Blood	✓	Protein
✓	Specific gravity	✓	Colour and turbidity	✓	pH-value
		✓	Glucose	✓	Urobilinogen
		✓	Ketones	✓	Sediment (microscopical exam.)

6. Ophthalmoscopy:
Prior to the start of the administration period, the eyes of all animals were examined after administration of mydriatic (Pharma Stulin GmbH, Germany) using an ophthalmoscope (HEINE OPTOTECHNIK, Herrsching, Germany). Towards the end of the administration period, the eyes of all surviving animals of the control and the highest dose group were examined.

7. Sacrifice and pathology:
At study termination after 24 month, all surviving animals were sacrificed by decapitation under CO_2-anaesthesia. The exsanguinated animals were necropsied and assessed by gross pathology. Gross lesions were examined in all affected animals per dose group and sex.
The following organs were sampled, weighed and examined histopathologically after haematoxylin-eosin staining:

Pathology:
The following organs were collected (column C), weighed (W) and examined histopathologically (H, ✓: all animals per group and sex, #: all control and high dose (2500 ppm) animals)

C	W	H		C	W	H		C	W	H	
✓	✓	#	adrenals	✓			lacrimal gland[%]	✓		#	sternum w. marrow
✓		#	aorta	✓	✓	✓	liver	✓		#	stomach [&]
			bone	✓		✓	lung	✓	✓	#	testes
✓	✓	#	brain	✓		#	lymph nodes[#]	✓		#	thymus
✓		#	caecum	✓		#	mammary gland (♀)				tongue
			cervix				nose/nasal cavity	✓	✓	✓	thyroid/parathyroid[α]
✓		#	colon	✓	✓	#	ovaries with oviduct*	✓		#	trachea
✓		#	duodenum	✓		#	pancreas	✓		#	urinary bladder
✓		#	epididymides				pharynx	✓		#	uterus
✓		#	oesophagus	✓		#	pituitary gland	✓		#	vagina
✓		#	eyes	✓		#	prostate				
✓		#	femur’	✓		#	rectum		✓		body (anaesthetised animals)
			gall bladder	✓		#	salivary gland[$]				
✓		✓	gross lesions	✓		#	Sciatic nerve				
			Harderian glands	✓		#	seminal vesicles				
✓		#	heart	✓		#	skeletal muscle				
✓		#	ileum	✓		#	skin				
✓		#	jejunum	✓		#	spinal cord[§]				
✓	✓	✓	kidneys	✓		#	spleen				

’ with knee joint and marrow; [%] extra-orbital; [#] mesenteric and mandibular; * oviduct not weighed; [$] mandibular and sublingual; [§] cervical, thoracic and lumbar cord; [&] fore-stomach and glandular stomach; [α] parathyroid not weighted and assessed histopathologically only in the control and high dose animals

In all animals that died before study termination or were sacrificed in a moribund state, the scope of histopathology was as indicated for the animals of the control group.

II. RESULTS AND DISCUSSION

Due to excessive toxicity at 15000 ppm that was evident by severe effects on body weight, which were expected to progress in time, both sexes of this dose group were sacrificed after about 17 month of treatment without further examinations.

A. TEST SUBSTANCE ANALYSES

The stability of the test substance in the diet for a period of 32 days at room temperature was verified analytically with a comparable batch as used for the study. As the diet preparations were stored no longer than about 1 week the stability was guaranteed.

The results of homogeneity and concentration analysis are summarised below:

Table 5.5-2: Analysis of diet preparations for homogeneity and test-item content

Dose level [ppm]	Sampling	Analysis	Concentration [ppm] Mean ± SD[§]	Relative standard deviation [%]	Mean % of nominal concentration[§]
100 ppm	22.01.98	02.02.98	100.1 ± 3.8[#]	3.8[#]	100.1
	22.04.98	22.04.98	102.8 ± 1.8[#]	1.8[#]	102.8
	24.07.98	30.07.98	89.9		89.9
	23.10.98	26-28.10.98	96.5		96.5
	19.01.99	20.01.99	102.6		102.6
	20.04.99	22.04.99	102.9		102.9
	15.07.99	19.07.99	98.6		98.6
average			**99.2 ± 4.8**		**99.2 ± 4.8**
500 ppm	22.01.98	02.02.98	492		98.4
	22.04.98	22.04.98	473		94.6
	24.07.98	30.07.98	458		91.6
	23.10.98	26-28.10.98	478		95.6
	19.01.99	20.01.99	469		93.8
	20.04.99	22.04.99	479		95.8
	15.07.99	19.07.99	492		98.4
average			**476 ± 11**		**95.2 ± 2.2**
2500 ppm	22.01.98	02.02.98	2359		94.4
	22.04.98	22.04.98	2613		104.5
	24.07.98	30.07.98	2262		90.5
	23.10.98	26-28.10.98	2322		92.9
	19.01.99	20.01.99	2350		94.0
	20.04.99	22.04.99	2506		100.2
	15.07.99	19.07.99	2452		98.1
average			**2409 ± 121**		**96.4 ± 5.0**
15000 ppm	22.01.98	02.02.98	14432 ± 254[#]	1.7[#]	96.2
	22.04.98	22.04.98	13978 ± 231[#]	1.5[#]	93.2
	24.07.98	30.07.98	13982		93.2
	23.10.98	26-28.10.98	13857		92.4
	19.01.99	20.01.99	14628		97.5
	20.04.99	22.04.99	14835		98.9
	-	-	-		-
average			**14285 ± 403**		**95.2 ± 2.8**

[#] based on mean values of the three individual samples (homogeneity samples)
[§] Values may not calculate exactly due to rounding of figures

The homogeneity of the mixtures was verified at concentrations of 100 and 15000 ppm, as the relative standard deviation was in the range of 1.8 - 3.8 and 1.5 - 1.7%, respectively.
The concentration control analysis gave values in the range from 89.9% to 104.5% of the nominal concentration for all dose levels. No test substance was determined in control diets.

B. OBSERVATIONS

1. Clinical signs of toxicity

No treatment-related findings were observed. All abnormal clinical signs were equally distributed between control and treated animals and/or occurred in single animals, only.

2. Mortality

The number of decedents and mortality rate until day 728 (i.e. 24 month of treatment) is given in the table below.

Table 5.5-3: 24 months feeding rat - decedents number and mortality rate

Sex	Parameter	Dose group [ppm]			
		0	**100**	**500**	**2500**
Both	Animals examined	20	20	20	20
Males	Number	5	4	6	6
	Rate (%)	25	20	30	30
Females	Number	7	3	7	1
	Rate (%)	35	15	35	5

C. BODY WEIGHT AND BODY WEIGHT GAIN

No significant effects on body weight and body weight gain were observed in animals administered 100 - 2500 ppm Boscalid in the food. At the dose group of 15000 ppm statistically significantly reduced body weight and body weight gain from about 200 days of test substance administration onwards were observed in both sexes. The treatment was thus discontinued for both sexes and animals sacrificed after about 17 month test item administration.

D. FOOD CONSUMPTION, FOOD EFFICIENCY AND COMPOUND INTAKE

Food consumption was significantly increased in females administered 500 and 2500 ppm on day 175, and in females treated with 100 - 2500 ppm on day 203. Due to the isolated occurrence, these deviations were assessed as being incidental and not related to the treatment.

Temporary reduction of food efficiency in males administered 100 and 500 ppm and in females treated with 100 - 2500 ppm as well as increase of food efficiency in females of the 500 ppm dose group were observed. Due to the isolated occurrence and the lack of dose-response relationship, these deviations were assessed as being incidental.

The approximate, mean daily test substance intake during the administration period is summarised in Table 5.5-4 below. In order to assure equally spaced intervals for calculation of the mean test substance intake, only the values of days 7, 35, 63, 91 and days 119 to 707 were taken into account.

Table 5.5-4: 24 months feeding rat - substance intake

Substance intake	Dose level [ppm]									
	Males					Females				
	0	100	500	2500	15000	0	100	500	2500	15000
[mg/kg bw/day]	-	4.4	21.9	110.0	*	-	5.9	30.0	150.3	*

* not calculated due to treatment-termination before the end of the study period

E. CLINICAL PATHOLOGY

2. Haematology

Occasionally, in males of the 500 ppm dose group decreases in mean corpuscular volume and mean corpuscular haemoglobin were observed. In females administered 2500 ppm, transient decreases in haematocrit, mean corpuscular volume and mean corpuscular haemoglobin were observed.

Table 5.5-5: 24 months feeding rat - haematology findings

Sex	**Male**				**Female**			
Dose (ppm)	**0**	**100**	**500**	**2500**	**0**	**100**	**500**	**2500**
Haematology examinations								
HCT (L/L)								
day 86/87	0.430	0.436	0.426	0.434	0.430	0.420	0.424	**0.418***
day 176/177	0.418	0.423	0.419	0.424	0.418	0.418	0.411	0.414
day 365/366	0.391	0.396	0.389	0.387	0.384	0.385	0.381	0.382
day 547/548	0.409	0.409	0.406	0.402	0.393	0.393	0.391	0.392
day 720/721	0.387	0.401	0.395	0.404	0.369	0.376	0.371	0.370
MCV (fL)								
day 86/87	51.0	50.9	**49.7****	50.5	52.1	51.6	52.0	**50.9***
day 176/177	50.9	50.8	49.9	50.5	52.0	51.7	51.9	51.1
day 365/366	50.6	50.2	**49.0*****	49.9	50.9	50.8	50.5	**50.0***
day 547/548	53.7	53.4	**52.4****	53.2	54.8	54.2	53.8	**53.3****
day 720/721	54.0	53.6	53.0	53.4	52.1	53.4	52.3	51.6
MCH (fmol)								
day 86/87	1.11	1.11	1.09	1.10	1.15	1.15	1.16	1.13
day 176/177	1.10	1.11	1.09	1.11	1.16	1.17	1.17	1.15
day 365/366	1.13	1.13	**1.11****	1.12	1.16	1.15	1.16	**1.13****
day 547/548	1.12	1.11	**1.08****	1.10	1.16	1.16	1.15	1.13
day 720/721	1.09	1.08	1.06	1.08	1.14	1.15	1.13	1.12
Clotting analysis								
HQT (sec)								
day 86/87	30.1	31.0	30.8	30.7	28.8	29.0	**27.6***	**26.9*****
day 176/177	31.8	31.7	31.8	32.2	28.9	29.1	28.2	28.1
day 365/366	34.0	32.2	32.0	32.6	26.3	26.7	26.9	25.9
day 547/548	30.5	30.6	30.5	30.6	26.4	26.9	25.9	25.7
day 720/721	30.0	30.2	29.8	30.4	25.3	26.0	25.6	25.6

*: $p \leq 0.05$; **: $p \leq 0.02$; ***: $p \leq 0.002$; (Kruskal-Wallis & Mann-Whitney u-test, two-sided)

No changes were observed in reticulocyte counts of either sex.

In females administered 500 and 2500 ppm Boscalid in the food, reduced prothrombin times were observed on day 87. However, since it was an isolated finding, this effect was assessed not to be substance-related. Mild anaemic process in females as evident by decrease of haemoglobin, haematocrit, mean corpuscular volume and mean corpuscular haemoglobin levels were observed. However, no changes in reticulocyte counts and in red blood cell morphology were noticed. The anaemic findings were to some extent more pronounced at the 15000 ppm dose level up to the sampling at day 365/366 which was the last sampling event prior to the premature termination of this dose group (not included in the table above).

2. Clinical chemistry findings

Clinical chemistry examinations were carried out in non-fasted animals at days 86-366, and in fasted animals at days 395 - 721.

Enzyme determinations revealed decreased alkaline phosphatase (ALP) and alanine aminotransferase (ALT) activities in animals of both sexes, and isolated reductions of aspartate aminotransferase (AST) activity in females. Furthermore, gamma glutamyltransferase (γ-GT) activities were increased in males and females [see Table 5.5-6].

At the 15000 ppm dose level findings of statistically significantly reduced ALT, ALP levels and increased γ-GT activities were consistent over all sampling events and for both sexes (not included in the table below).

The enzymes ALT and ALP were statistically significantly decreased at the dose level of 500 ppm in both, males and females at some sampling events. In addition ALT, AST and ALP levels were statistically significantly decreased in females at the 100 ppm dose level at isolated sampling events. The decrease in enzyme activities has been considered to be toxicologically non-adverse.

Changes in γ-GT activities were observed mainly at the 2500 ppm dose level in both males and females as well as in males at the 500 and 100 ppm dose levels. The latter findings have been considered to be incidental in nature and thus not test-substance related.

Table 5.5-6: 24 months feeding rat - clinical chemistry findings

Sex	Male				Female			
Dose (ppm)	**0**	**100**	**500**	**2500**	**0**	**100**	**500**	**2500**
Enzymes								
ALT (µkat/L)								
day 86/87	1.12	1.05	1.08	1.05	0.92	0.90	0.89	**0.78****
day 176/177	1.19	1.17	1.09	**1.02****	0.95	**0.84****	**0.84****	**0.75****
day 365/366	1.13	1.13	1.12	1.00	1.12	**0.91*****	**0.88*****	**0.85*****
day 395/400#	0.91	0.85	0.89	0.91	0.78	0.82	0.80	**0.69****
day 547/548#	0.88	0.75	0.87	**0.67****	0.63	0.58	0.67	0.59
day 720/721#	0.72	0.87	0.71	0.65	0.65	0.68	0.67	0.59
AST (µkat/L)								
day 86/87	2.17	1.93	1.96	2.06	2.03	1.82	2.05	1.92
day 176/177	1.71	1.94	1.70	1.74	1.97	1.67	1.84	**1.45****
day 365/366	1.71	1.73	1.55	1.63	1.83	**1.42***	1.57	1.57
day 395/400#	1.72	2.19	1.78	2.10	1.91	2.36	2.32	1.79
day 547/548#	1.59	1.36	1.79	1.67	1.45	1.37	1.60	1.29
day 720/721#	1.49	2.40	1.64	1.50	2.04	1.76	1.37	1.71
ALP (µkat/L)								
day 86/87	5.72	5.41	**4.98*****	**4.38*****	3.89	3.84	**3.35****	**2.96*****
day 176/177	5.26	5.25	**4.69****	**3.91*****	3.27	3.23	3.01	**2.58*****
day 365/366	5.44	5.41	5.20	**3.87*****	3.23	3.19	2.94	**2.40*****
day 395/400#	3.07	3.20	3.34	**2.65****	1.42	1.44	1.48	**1.23***
day 547/548#	3.33	3.22	3.47	**2.63*****	1.22	1.28	1.21	**1.01****
day 720/721#	3.43	3.76	3.35	**2.46*****	1.27	**1.70****	**1.57****	1.20
γ-GT (µkat/L)								
day 86/87	4	**9****	**11*****	**13****	7	9	6	**18*****
day 176/177	24	25	**29*****	**41*****	0	0	1	0
day 365/366	14	15	**20****	**34*****	12	12	13	**20****
day 395/400#	1	0	1	**18***	7	8	4	10
day 547/548#	19	20	19	**39*****	10	12	7	8
day 720/721#	38	31	44	**54****	37	47	41	43

Sex	Male				Female			
Dose (ppm)	**0**	**100**	**500**	**2500**	**0**	**100**	**500**	**2500**
Blood chemistry								
Total bilirubin (µM)								
day 86/87	1.70	1.65	1.54	1.34	2.55	**2.25****	**1.97*****	**1.80*****
day 176/177	2.10	2.11	1.87	**1.46****	2.47	2.50	2.25	2.19
day 365/366	2.19	2.11	**1.44****	**1.21*****	2.01	1.93	1.81	1.69
day 395/400#	2.43	2.37	2.03	**1.94*****	3.33	**2.92***	**2.65*****	**2.37*****
day 547/548#	2.67	2.37	**2.19***	**2.04****	2.54	2.87	2.57	2.32
day 720/721#	3.06	3.28	2.67	**2.31****	2.49	2.74	2.43	**1.96***
Total protein (g/L)								
day 86/87	65.31	66.38	65.49	**67.91****	66.86	65.63	67.47	**69.24***
day 176/177	67.11	68.64	67.53	**69.45****	71.19	71.12	70.53	72.17
day 365/366	64.90	66.25	65.39	65.40	73.65	73.88	73.29	**77.48***
day 395/400#	67.25	66.62	67.17	**69.29***	75.53	76.15	76.67	**79.97****
day 547/548#	63.24	62.21	62.27	64.00	69.90	68.69	69.84	**74.69****
day 720/721#	63.26	64.37	62.01	64.44	71.19	71.71	71.07	**74.61****
Albumin (g/L)								
day 86/87	35.17	35.58	35.13	35.64	38.44	37.80	38.40	38.76
day 176/177	30.83	31.56	31.16	31.57	35.52	35.12	34.71	35.30
day 365/366	28.45	29.28	28.97	28.51	34.23	34.32	33.60	35.52
day 395/400#	28.89	28.99	28.88	29.41	35.09	35.47	35.55	36.65
day 547/548#	28.77	28.72	28.35	29.45	33.99	33.33	33.74	35.67
day 720/721#	26.06	**27.82****	26.60	**27.70***	32.15	31.32	31.78	33.16
Globulin (g/L)								
day 86/87	30.13	30.80	30.36	**32.27*****	28.42	27.82	29.07	**30.48****
day 176/177	36.28	37.08	36.37	**37.88****	35.66	36.00	35.87	36.87
day 365/366	36.45	36.97	36.41	36.89	39.42	39.56	39.69	**41.96***
day 395/400#	38.36	37.63	38.29	**39.89***	40.44	40.68	41.12	**43.32****
day 547/548#	34.48	33.49	33.93	34.54	35.91	35.35	36.11	**39.01*****
day 720/721#	37.20	36.56	35.42	36.75	39.04	40.40	39.28	41.45
Cholesterol (mM)								
day 86/87	2.01	2.03	2.02	2.09	2.04	2.01	2.13	**2.40*****
day 176/177	2.02	2.06	2.15	2.20	2.13	2.21	2.15	2.22
day 365/366	2.29	2.32	2.45	2.49	2.41	2.55	2.53	**2.87*****
day 395/400#	2.50	2.49	2.72	**2.85****	2.48	2.70	**2.77***	**3.30*****
day 547/548#	2.87	2.47	2.73	2.83	2.90	2.82	2.93	**3.43****
day 720/721#	3.76	3.43	3.78	3.70	3.13	3.48	3.16	3.56

*: $p \leq 0.05$; **: $p \leq 0.02$; ***: $p \leq 0.002$; (Kruskal-Wallis & Mann-Whitney u-test, two-sided)

#: blood samples from fasted animals

Additionally, total protein, globulin and cholesterol levels were increased in animals of both sexes, whereas the increase of albumin concentration was only observed in males at the end of the study period. Furthermore, reduced total bilirubin levels were obtained in animals of both sexes [see Table 5.5-6]. Findings have been associated with liver enzyme induction and de novo synthesis. At the 15000 ppm dose level findings of statistically significantly increased levels of total protein, globulin and cholesterol were consistent over almost all sampling events and for both sexes (not included in the table above).

Some additional statistically significant changes of the clinic-chemical parameters (sporadic increase of calcium level in males treated with 500 ppm and females at the 100 and 2500 ppm dose levels, isolated decrease of inorganic phosphate level in males treated with 500 and 2500 ppm as well as chloride concentration in females at the 2500 ppm dose level) were observed. However, these deviations were marginal, incidental or inconsistent when compared with the other sex or a lack dose-response relationship. Thus, these findings were considered to be of no toxicological relevance.

The most prominent findings observed were associated with hepatic microsomal enzyme induction. Alterations in γ-GT, prothrombin times, total protein, albumin, globulin and cholesterol are considered to be caused by the induction of the de novo synthesis of enzymes, clotting factors, various proteins and cholesterol in the liver. The observed decrease of serum bilirubin has been considered to be the result of an increased excretion due to induction of hepatic phase II enzymes. Thus, this effects has been interpreted as adaptive metabolic changes rather than an adverse effect per se. The decreased activities of ALT, AST and ALP are regarded as treatment-related, however this effect has not been considered to represent a toxicological relevant adverse effect.

F. URINALYSIS

No treatment-related changes were observed in urine parameters measured.

G. OPHTHALMOSCOPY

No substance-related effects were obtained. All findings were spontaneous in nature and equally distributed between treated animals and controls.

H. NECROPSY

1. Organ weight

Organ weight changes were observed in liver, brain and thyroids as shown below:

Table 5.5-7: 24 months feeding rats - summary of absolute (g) and relative (% of terminal body weight) organ weights

Parameter	Sex	Dose level (ppm)			
		0	100	500	2500
Terminal body weight (g)	M	692.21	704.14	698.35	696.29
	F	351.1	354.16	344.05	334.14
Absolute liver weight (g)	M	18.87	19.98	20.78	20.64
	F	10.11	10.44	10.36	10.67
Relative liver weight	M	2.76	2.88	2.98	3.01
	F	2.87	2.94	3.03	**3.19****
Absolute brain weight (g)	M	2.24	2.24	2.24	2.20
	F	2.00	**2.06***	2.03	1.99
Relative brain weight	M	0.33	0.33	0.33	0.32
	F	0.57	0.61	0.59	0.61
Absolute thyroid weight (mg)	M	40.20	41.63	44.00	**52.71***
	F	29.46	26.35	35.39	30.32
Relative thyroid weight	M	0.006	0.006	0.006	0.008
	F	0.008	0.008	0.010	0.009

*: $p \leq 0.05$; **: $p \leq 0.01$; (Kruskal-Wallis, one sided and Wilcoxon-test, two-sided)

Male animals administered 2500 ppm Boscalid in the food revealed a statistically significant increase in absolute thyroid weight of 31% as compared to the controls.

Females treated with 100 ppm Boscalid in the food showed a statistically significant increase in absolute brain as compared with the controls. However, no macroscopic and microscopic correlates were observed in the course of the necropsy.
Additionally, increase of relative liver weights of 11% were observed in females receiving 2500 ppm of the test substance in the food. No changes of absolute and relative weights were observed in kidneys, adrenal glands, ovaries and testes.

2. Gross lesions

In males, the number of **testes** with cystic degenerations and the number of enlarged thyroid glands or with focus in the thyroid glands were increased.

All other gross lesions occurred either as isolated single incidences or were equally distributed between all groups. Paying particular attention to the target organ liver and the organs of the endocrine system, the single incidences noticed are summarised below.

Table 5.5-8: 24 months feeding rats - macroscopic findings in liver and organs of the endocrine system

Sex	Male				Female			
Dose (ppm)	**0**	**100**	**500**	**2500**	**0**	**100**	**500**	**2500**
Animals examined	**20**	**20**	**20**	**20**	**20**	**20**	**20**	**20**
Liver								
- adhesion	-	-	-	1	-	-	1	-
- cyst	3	5	2	2	6	3	4	2
- focus	20	19	16	16	7	11	12	12
- granular surface	-	-	-	-	1	-	-	-
- mass	2	5	4	2	-	1	-	-
- overgrown by tumour	-	-	-	1	-	-	-	-
- prominent acinar pattern	-	-	-	-	-	-	-	1
Testes								
- calcification	1	1	3	1				
- cystic degeneration	2	3	2	9				
- discoloration	-	-	-	1				
- enlarged	2	1	-	1				
- focus	3	4	6	6				
- mass	3	6	2	7				
- reduced size	3	3	2	1				
Epididymides								
- adhesion	-	-	-	1				
- size reduction	1	-	-	1				
Seminal vesicle								
- discoloration	-	1	-	-				
- reduced size	-	1	3	-				
Prostate								
- discoloration	-	-	1	1				
- oedema	-	1	-	-				
- enlarged	3	1	-	1				
- focus	1	2	1	1				
- induration	1	1	-	-				
- reduced size	-	1	2	-				
Preputial glands								
- abscess	-	1	-	1				
Ovaries								
- cyst					5	10	8	13
- focus					4	2	1	4
- induration					-	-	1	2
- mass					2	2	3	2
Oviduct								
- dilatation					-	1	-	-
Uterus								
- cyst					2	3	-	-
- dilatation					1	1	1	1
- focus					2	-	-	1
- induration					1	3	3	4
- mass					2	2	4	1
- wall thickening					-	1	1	-

Sex	Male				Female			
Dose (ppm)	**0**	**100**	**500**	**2500**	**0**	**100**	**500**	**2500**
Animals examined	**20**	**20**	**20**	**20**	**20**	**20**	**20**	**20**
Mammary gland								
- cyst					-	1	2	1
- mass					2	3	2	3
Adrenal gland								
- enlarged	2	1	-	-	2	1	2	-
- focus	-	2	1	1	-	1	-	-
Adrenal cortex								
- focus	11	9	9	8	16	18	16	19
Thyroid glands								
- discoloration	-	-	-	1	-	-	-	-
- enlarged	-	-	1	1	-	-	1	-
- focus	-	-	1	3	-	1	-	1
- mass	-	-	-	-	-	-	1	-
Pituitary gland								
- cyst	-	1	1	-	-	2	1	-
- enlarged	-	2	-	-	1	1	2	3
- focus	2	2	2	2	4	5	7	8
- mass	2	-	-	1	13	8	6	6

3. Histopathology

Neoplastic findings

In **thyroid glands** slightly increased incidences of follicular cell adenoma as compared to the control animals were recorded in males at the 500 and 2500 ppm dose levels and in females at the 500 ppm dose level [see Table 5.5-9]. The macroscopically observed enlargement of the thyroid gland correlated with follicular cell adenoma observed in one female administered 500 ppm Boscalid in the food and in one male of the 2500 ppm dose group. Histopathologically, these adenomas were composed of well demarcated areas of hyperplastic follicles, sometimes with hypertrophic epithelial cells, and always compression of adjacent parenchyma. Due to the lack of a dose-response relationship, these tumours were considered as not treatment-related, however in the parallel running carcinogenicity study [DocID 2001/1000115] a slight increase of thyroid follicular cell adenomas was seen at the 2500 ppm dose level in animals of both sexes. The increase of the absolute thyroid weight in males of the 2500 ppm dose group can be correlated to isolated, not test-substance related occurrence of some large C-cell adenomas. As many evidences for hepatic enzyme induction are noticed, the higher metabolic activity of hepatocytes would lead to a higher degradation rate of T_3/T_4 hormones and as a consequence to a functional stimulation of the thyroid glands by a feedback mechanism that is evident the slightly increase incidences of follicular cell adenomas in the mid and high dose groups. Thus, this neoplastic findings can be regarded to be not due to an organ specific carcinogenicity of the test substance but to be the consequence of this mechanistic pathway that is assessed to be not relevant to humans.

The number of animals with neoplasms, with more than one primary neoplasm, with metastases, and with benign or malignant neoplasms, as well as the total number of primary and benign or malignant neoplasms were comparable between the control animals and the animals treated with the top-dose [see Table 5.5-9]. As with the exception of liver, kidneys, lungs and thyroid glands as well as organs with macroscopic abnormalities, complete microscopic examinations were only performed on decedents of groups of the 100 and 500 ppm dose levels, and thus the tumour incidences cannot be readily compared with the control and the high dose groups.

For survivors, the number of males with neoplasms and with more than one primary neoplasms was slightly increased at the top-dose. In females, the total number of primary neoplasms was also slightly increased at the top-dose. In general, increase in tumours seen in the top dos animals was considered to reflect the high biologic variation of tumours in aged rodents.

Non-neoplastic findings

In the **liver**, histopathological examinations revealed centrilobular hypertrophy of hepatocytes. Histopathologically, this hypertrophy was characterized by an increase in hepatocellular size and organelles, mainly showing a ground-glass appearance. The centrilobular hypertrophy of hepatocytes has been interpreted to be the consequence of accumulation/proliferation of smooth endoplasmic reticulum due to the compound-mediated enzyme induction. The increase of the relative liver weight in females of the 2500 ppm dose group was correlated to the occurrence of centrilobular hepatocellular hypertrophy which was considered substance-related.

Furthermore, in males treated with the 500 and 2500 ppm Boscalid dose levels, the number of eosinophilic foci of cellular alterations was slightly increased [see Table 5.5-9]. No such finding was made in females at any dose level.

In **thyroid glands**, minimal increased incidences of diffuse follicular cell hypertrophy and focal follicular cell hyperplasia were observed in animals of both sexes treated with the 2500 ppm dose level that corroborate the previously macroscopically observed slight increase in the incidence of thyroid foci of the males in the top dose. Furthermore, the statistically significant increase of the absolute thyroid weight in males of the 2500 ppm group can be also correlated to the occurrence of some follicular cell hyperplasia and some diffuse follicular cell hypertrophies which were considered substance-related. The mode of action as discussed for neoplastic findings is also consistent to apply for the increased incidence of follicular cell hypertrophy and focal, follicular cell hyperplasia [see Table 5.5-9].

The macroscopically increased number of cystic degenerations of **testes** in top-dose males correlated histopathologically mainly with diffuse tubular degenerations or Leydig cell adenomas. Both, the incidence of Leydig cell hyperplasia and Leydig cell adenoma gave no indication of a dose response relationship and these findings were thus considered to be not test substance-related [see Table 5.5-9].

Evaluation on decedents did not reveal any histopathological findings than may be attributed to the earlier death. Mostly, inflammatory lesions or different types of tumours in various organs were noticed.

All other morphology changes detected showed either a single occurrence or no dose-response relationship and were seen to be of incidental or spontaneous nature and ultimately were considered to be not induced by the test substance. Nevertheless, paying particular attention to the organs of the endocrine system, the incidences noticed are additionally summarised in Table 5.5-9.

Table 5.5-9: 24 months feeding rats - microscopic neoplastic and non-neoplastic findings in liver and organs of the endocrine system

Sex	**Male**				**Female**			
Dose (ppm)	**0**	**100**	**500**	**2500**	**0**	**100**	**500**	**2500**
Liver	20	20	20	20	20	20	20	20
- lymphoid infiltation	6	10	7	3	4	6	7	6
- fatty infiltr. focal	2	5	3	1	3	2	-	1
- fatty infiltr. perip.	8	7	4	2	1	5	4	1
- necrosis single cell	-	-	-	1	-	-	-	-
- necrosis, focal	2	1	1	-	1	1	1	-
- necrosis, centrilob.	-	-	-	1	-	-	-	1
- necrosis, lobar	-	-	1	-	-	-	-	-
- congestion	3	1	1	1	2	2	3	-
- cellular alterations	14	20	16	15	8	9	13	10
- clear cell foci	-	3	2	-	-	-	-	-
- basophilic foci	13	18	17	14	7	8	12	9
- eosinophilic foci	3	4	7	9	1	1	2	2
- centril. hypertrophy	-	-	1	6	-	-	-	16
- cyst (s), biliary	1	2	4	1	5	3	1	1
- bile ducts: cyst hyp.	-	-	-	-	1	2	2	1
- bile duct proliferation	9	7	7	7	5	8	3	7
- pigment storage	1	1	-	-	-	1	-	-
- spongiosis/peiosis	7	7	9	8	2	4	2	5
- haematopoiesis	-	1	1	1	1	2	4	1
- ganulocytosis	-	-	-	-	1	-	-	-
- fibrosis capsular	-	-	-	-	-	-	-	1
- adenoma hepatocell.	*4*	*4*	*3*	*3*	*2*	-	-	*2*
- adenocarc. hepatocell.	-	*1*	*2*	-	-	-	-	-
- metastatic sarcoma	-	-	-	-	-	-	*1*	-
Testes	20	14	16	20				
- congestion	-	-	-	1				
- oedema	1	-	-	-				
- calcification focal	2	-	3	4				
- degeneration focal	6	3	5	3				
- degeneration diff.	5	4	3	6				
- Leydig cell hyperplasia	12	7	7	7				
- Leydig cell adenoma	*7*	*9*	*7*	*10*				

Sex	**Male**				**Female**			
Dose (ppm)	**0**	**100**	**500**	**2500**	**0**	**100**	**500**	**2500**
Epididymides	20	4	6	20				
- lymphoid infiltration	2	1	1	1				
- loss of sperm	4	-	-	9				
Seminal vesicle	20	5	8	20				
- inflammation	-	-	1	-				
- atrophy	1	1	3	-				
- dilatation	-	1	-	-				
Prostate	20	9	7	20				
- inflammation	8	5	6	8				
- atrophy	-	-	2	-				
- hyperplasia, focal	6	2	2	3				
- adenoma	*1*	*1*	-	-				
Preputial glands	-	1	-	1				
- inflammation	-	-	-	1				
- cyst squamous	-	1	-	1				
Ovaries					20	14	16	20
- cyst (s)					5	10	12	13
- thrombus					-	-	-	1
- abscess					-	1	-	-
- hyperpl. sex c. focal					1	1	2	3
- hyperpl. sex c. diff.					4	4	2	4
- tumour sex c. benign					*2*	-	*1*	*1*
- tumor gran. c. benign					*2*	*2*	*1*	*2*
- luteoma benign					*1*	-	-	-
Oviduct					20	4	7	20
- dilatation					-	1	-	-
Uterus					20	10	13	20
- dilatation focal					4	4	1	1
- thrombus					1	-	-	-
- cervix, fibrosis					1	4	3	4
- hyperpl. gland. focal					5	2	1	6
- squamous cyst					1	1	-	-
- squamous metaplasia					3	2	1	5
- polyp stromal					*1*	*2*	*1*	*2*
- polyp endometrial					-	-	*1*	-
- sarcoma endom. strom.					*1*	*1*	*2*	*1*
- leiomyosarcoma					-	-	*1*	-
- adenocarcinoma					-	-	*1*	*1*
Mammary gland					20	6	9	20
- cyst (s)					1	1	2	1
- hyperplasia focal					3	1	2	4
- fibroadenoma					*1*	*2*	*1*	*2*
- adenocarcinoma					-	*1*	*1*	*1*

Sex	**Male**				**Female**			
Dose (ppm)	**0**	**100**	**500**	**2500**	**0**	**100**	**500**	**2500**
Adrenal cortex	20	13	15	20	20	18	18	20
- cyst (s)	-	-	-	-	1	-	-	-
- blood filled cyst (s)	4	-	1	2	18	18	17	19
- accessory adr. tissue	8	6	5	7	6	7	5	4
- degeneration focal	-	-	-	-	3	2	3	4
- necrosis	-	-	1	-	2	-	-	-
- haematopoiesis	-	2	2	2	-	1	2	-
- calcification focal	-	-	-	-	1	-	-	-
- fatty change focal	8	6	4	7	-	2	2	-
- hypertrophy focal	5	2	2	2	4	5	2	3
- hyperplasia focal	1	1	2	3	-	4	1	5
- myelolipoma	-	-	-	-	-	*1*	-	-
- adenoma	*1*	*1*	*1*	-	-	-	*1*	-
Adrenal medullas	20	13	15	20	19	18	18	20
- hyperplasia focal	7	4	7	6	8	2	5	5
- tumour benign	*2*	*3*	*3*	*3*	*1*	*2*	*3*	*1*
Thyroid gland	20	20	20	20	20	20	20	20
- cyst (s)	9	8	6	12	11	9	11	11
- mineralisation	-	-	-	1	-	-	-	-
- congestion	-	-	-	1	-	-	-	-
- hypertr. foll. c. diff.	3	1	3	6	-	1	-	4
- hypertr. foll. c. focal	2	3	2	1	6	2	5	2
- hyperplasia. foll. c. diff.	10	9	12	11	8	9	6	12
- hyperplas. foll. c. focal	1	1	2	4	-	-	-	2
- adenoma C-cell	*4*	*2*	*3*	*4*	*3*	*5*	*7*	*4*
- adenoma follic. cell	-	-	*2*	*1*	-	-	*1*	-
Parathyroid gland	20	20	20	18	20	19	20	19
- hyperplasia focal	-	-	1	-	3	1	-	-
- hyperplasia diffuse	1	-	1	-	-	-	-	-
- adenoma	*1*	-	-	-	-	-	-	-
Pituitary gland	20	8	9	20	20	17	17	20
- cyst (s)	6	3	4	2	4	9	5	5
- hyperpl. p. distalis	4	1	3	4	4	2	5	6
- hyperpl. p. intermedia	1	-	-	-	-	1	-	-
- adenoma p.distalis	*2*	*3*	-	*1*	*3*	*7*	*7*	*9*
- adenocarc. p. distalis	-	-	-	-	-	-	*1*	-

III. CONCLUSIONS

The oral administration of Boscalid induced alterations of clinical pathology parameters associated with microsomal enzyme induction and possibly with changes in the nutrition status of animals. Moreover, in females at the 2500 ppm dose level a marginal anaemic process was observed. Target organs identified were the liver and thyroids. Histopathological investigations showed increase in incidence of diffuse thyroid cell hypertrophy in both sexes and increase in thyroid follicular cell hyperplasia in females at the 2500 ppm dose level. In the liver centrilobular hypertrophy was seen at this dose level. The most prominent findings observed in clinical chemistry were associated with hepatic microsomal enzyme induction. Alterations in γ-GT, prothrombin times, total protein, albumin, globulin and cholesterol are caused by the induction of the de novo synthesis of enzymes, clotting factors, various proteins and cholesterol in the liver. Mild anaemic process (decreased haemoglobin, haematocrit, mean corpuscular volume and mean corpuscular haemoglobin) in females at the 2500 ppm and in part in males at the 500 ppm dose level. The administration of 100 ppm was tolerated by male and female rats without any changes that could be attributed to the test substance.
Based on this findings, the no observed adverse effect level (NOAEL) for Wistar rats under the conditions of the study was 100 ppm, that is equivalent to the substance intake of 4.4 mg/kg bw/day and 5.9 mg/kg bw/day for males and females, respectively.

Boscalid - Carcinogenicity study in Wistar rats - Administration in the diet for 24 months ([redacted] et al., 2001)
DocID 2001/1000115

Guidelines: According to OECD 451 (1981), EEC 87/302, EPA/FIFRA (Subdivision F; para. 83-2) and JMAFF
Deviations: None
GLP: Yes
Acceptance: The study has been evaluated and considered acceptable in the EU registration process for the first Annex I inclusion.

Note: The carcinogenicity study in rats was identified as relevant study for setting of the Acceptable Daily Intake (ADI) reference dose. For this reason this study is in detail evaluated here following the OECD format.

Executive summary

Boscalid (94.4% purity, batch N 37) was administered to groups of 50 male and 50 female Wistar rats (source: Boehringer Ingelheim Pharma KG, Germany) at dietary levels of 0, 100, 500, and 2500 ppm for a period of 24 months. These concentrations were equivalent to the substance intake of 0, 4.6, 23 and 116 mg/kg bw/day in males, and 0, 6, 30 and 156 mg/kg bw/day in females. Due to excessive toxicity (severe effects on body weights), the initial 15000 ppm top dose group were discontinued after approximately 17 months of administration. Reduction of body weight and body weight gain was observed in females at the 2500 ppm dose level, whereas food consumption was not affected.
Following a 24-month dietary administration of Boscalid to Wistar rats, adverse effects were observed in animals of dose group ≥ 500 ppm. Treatment-related findings included histopathological changes in liver (centrilobular hypertrophy in both sexes at 2500 ppm and increased incidence of eosinophilic foci of cellular alteration in males ≥ 500 ppm), and increased incidences of thyroid follicular cell adenomas (2500 ppm, both sexes) in combination with increased thyroid weights (2500 ppm, males) and correlating histopathological changes (diffuse follicular cell hypertrophy and focal follicular cell hyperplasia in both sexes).
Based on the results of this 24-month carcinogenicity study, the NOAEL in the rat can be established at 100 ppm (equivalent to 4.6 mg/kg bw/day in males and 6.0 mg/kg bw/day in females).

(DocID 2001/1000115)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:	Boscalid (BAS 510 F; Reg.No. 300 355)
Description:	solid / white
Lot/Batch #:	N37 (Tox-batch III)
Purity:	94.4%
Stability of test compound:	The stability of the test substance was verified by re-analysis via HPLC after the in-life phase of the study. The re-analyzed purity was the same on 27 Oct. 2000, the expiration date was Oct. 2003.
2. Vehicle and/or positive control:	None
3. Test animals:	
Species:	Rat
Strain:	Wistar (Chbb:THOM (SPF))
Sex:	Male and female
Age:	42 / 43 days (males / females at start of administration)
Weight at dosing (mean):	Males: 186.1 (163.5 - 211.5) g Females: 144.7 (128.3 - 159.9) g
Source:	Boehringer Ingelheim Pharma KG, Biberach/Riss, Germany
Acclimation period:	9 / 10 days for males / females
Diet:	Kliba maintenance diet rat/mouse/hamster, meal (Provimi Kliba SA, Kaiseraugst, Switzerland), ad libitum
Water:	Drinking water from bottles, ad libitum
Housing:	Animals were housed individually in type DK III stainless steel wire mesh cages (Becker & Co., Castrop-Rauxel, Germany) with a floor area of about 800 cm². Underneath the cages, waste trays were fixed containing absorbent material (type ¾ dust free embedding, supplied by SSNIFF, Soest, Germany).
Environmental conditions:	
Temperature:	20 - 24°C
Humidity:	30 - 70%
Air changes:	Animals were housed in a fully air-conditioned room
Photo period:	12 hours / 12 hours (Light: form 06.00 a. m. - 06.00 p. m; dark: from 06.00 p. m. - 06.00 a. m.)

B. STUDY DESIGN AND METHODS

1. Dates of experimental work: 26-Jan-1998 - 28-Feb-2001
(In-life dates: 04/19-Feb-1998 (start of administration) to 25-Feb/03-Mar-2000 (necropsy))

2. Animal assignment and treatment:
Boscalid was administered to groups of 50 male and 50 female Wistar rats at dietary concentrations of 0, 100, 500, 2500 ppm daily for 24 months. Due to excessive toxicity at the initial 15000 ppm top dose level (i.e. severe effects on body weight), the treatment was discontinued for both sexes and animals sacrificed after about 17 month test item administration.

3. Test substance preparation and analysis:
At the high concentration, the respective amount of the test substance was directly added to the food in the laboratory mixer. For the remaining test substance concentrations, food of the top dose level was weighed out and mixed with the corresponding amounts of untreated food, depending on the dose group in order to obtain the desired concentrations and mixed for 10 minutes in a laboratory mixer. Usually, the test substance preparations were mixed weekly.
The stability of the test substance in the diet over a period of 32 days at room temperature was verified before the start of the study.
Homogeneity and/or concentration control analyses for the current study and for the parallel carcinogenicity study were performed at the start of the study-period as well as after 3, 6, 9, 12, 15 and 18 month via HPLC/UV. No concentration control analyses were performed toward the end of the study. However, as all analyses up to 18 months were found to be consistent with the nominal test substance concentrations and procedures of test substance preparation were not changed for the remainder of the study it has been considered reasonable to expect that test substance concentrations were correct over the whole study. Homogeneity was analysed by taking 3 samples from the top, middle and bottom of the beaker for the low (100 ppm) and high dose level (15000 ppm).

4. Statistics:
Means and standard deviations of each test group were calculated for several parameters. Further statistical analyses were performed according to following tables:

Statistics of clinical examinations

Parameter	Statistical test
Food consumption, body weight, body weight gain, food efficiency	Parametric one-way analysis using the F-test (ANOVA, two-sided). If the resulting p-value was equal or less than 0.05, a comparison of each group with the control group was performed using DUNNETT's test (two-sided) for the hypothesis of equal means

Statistics of pathology

Parameter	Statistical test
Organ weights	Non-parametric one-way analysis using KRUSKAL-WALLIS test (two-sided). If the resulting p-value was equal or less than 0.05, a pairwise 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:
A check for any moribund or dead animals as well as for clinical signs of toxicity was made twice a day (in the morning and in the late afternoon) on working days and once a day (in the morning) on weekends and public holidays. Once a week, an additional comprehensive clinical examination (including palpation) was carried out.

2. Body weight:
Body weight was determined before the start of the administration period in order to randomize the animals. During the study period, body weight was determined on day 0, once a week during the first 13 weeks of the administration period, thereafter at 4-week intervals, and prior to start of necropsy.
The difference between the body weight on the respective day of weighing and the body weight on day 0 was calculated as body weight gain.

3. Food consumption, food efficiency and compound intake:
Food consumption was determined once a week over a period of 7 days during the first 13 weeks of the administration period, thereafter at 4-week intervals, and prior to start of necropsy. The values were calculated as food consumption in grams per animal and day.
Food efficiency (group means) was calculated based upon individual values for body weight and food consumption:

$$\text{Food efficiency for day x} = \frac{BW_x - BW_Y}{FC_{y\ to\ x}} x\,100$$

BW_x and BW_y = body weight [g] at day x and day y (last weighing date before day x)
$FC_{y\ to\ x}$ = the mean food consumption from day y to x calculated as mean daily food consumption [g] on day x, multiplied by the number of days from day y to day x

The mean daily intake of test substance (group means) was calculated based upon individual values for body weight and food consumption.

$$\text{Substance intake for day x} = \frac{FC_x \times D}{BW_x}$$

BW_x = body weight [g] at day x
FC_x = the mean food consumption [g] for day x
D = dietary dose in ppm

4. Haematology:
Blood samples were taken by decapitation from fasted, anaesthetised animals at the end of the study period to prepared differential blood smears. Leukocyte differential count and white and red blood cell morphologies were determined from the blood smears by light microscopy. The blood smears of the control and highest dose groups were evaluated only.

5. Sacrifice and pathology:

At study termination after 24 month, all animals were sacrificed by decapitation under CO_2-anaesthesia. The exsanguinated animals were necropsied and assessed by gross pathology. The animals which died prematurely or were sacrificed in a moribund state were necropsied as soon as possible after death and assessed by gross pathology.

The following organs were sampled, weighed and examined histopathologically after haematoxylin-eosin staining:

Pathology:											
The following organs were collected (column C), weighed (W) and examined histopathologically (H, ✓: all animals per group and sex, #: all control and high dose animals)											
C	W	H		C	W	H		C	W	H	
✓	✓	#	adrenals	✓			lacrimal gland[%]	✓		#	sternum w. marrow
✓		#	aorta	✓	✓	✓	liver	✓		#	stomach [&]
			bone	✓		✓	lungs	✓	✓	#	testes
✓	✓	#	brain	✓		#	lymph nodes[#]	✓		✓	thymus
✓		#	caecum	✓		#	mammary gland (♀)				tongue
			cervix				nose/nasal cavity	✓	✓	✓	thyroid/parathyroid[α]
✓		#	colon	✓	✓	#	ovaries with oviduct*	✓		#	trachea
✓		#	duodenum	✓		#	pancreas	✓		✓	urinary bladder
✓		#	epididymides				pharynx	✓		#	uterus
✓		#	esophagus	✓		#	pituitary gland	✓		#	vagina
✓		#	eyes	✓		#	prostate				
✓		#	femur’	✓		#	rectum		✓		body (anaesthetised animals)
			gall bladder	✓		#	salivary gland[$]				
✓		✓	gross lesions	✓		#	Sciatic nerve				
			Harderian glands	✓		#	seminal vesicles				
✓		#	heart	✓		#	skeletal muscle				
✓		#	ileum	✓		#	skin				
✓		#	jejunum	✓		#	spinal cord[§]				
✓	✓	✓	kidneys	✓		#	spleen				
’ with knee joint and marrow; [%] extra-orbital; [#] mesenteric and mandibular; * oviduct not weighed; [$] mandibular and sublingual; [§] cervical, thoracic and lumbar cord; [&] fore-stomach and glandular stomach; [α] parathyroid not weighted and assessed histopathologically only in the control and high dose animals											

Gross lesions were examined in all affected animals per dose group and sex.

II. RESULTS AND DISCUSSION

Due to excessive toxicity at the 15000 ppm dose level providing evidence of severe effects on body weight, and which were expected to progress in time, both sexes of this dose group were sacrificed after about 17 month of treatment without further examinations.

A. TEST SUBSTANCE ANALYSES

The stability of the test substance in the diet for a period of 32 days at room temperature was verified analytically with a comparable batch as used for the study. As the diet preparations were stored no longer than about 1 weeks the stability was guaranteed.

The homogeneity of the mixtures was verified at concentrations of 100 and 15000 ppm, as the relative standard deviation was in the range of 1.8 - 3.8 and 1.5 - 1.7%, respectively.
The concentration control analysis revealed values in the range from 89.9% to 104.5% of the nominal concentration for all dose levels. No test substance was determined in control diets.
Since the chronic feeding study in rats and the carcinogenicity study were performed side-by-side with the same dose levels the test substance preparations in the food were done for both studies in the same batches. The results of homogeneity and concentration analysis were the same as for the carcinogenicity study and are summarised in Table 5.5-2.

B. OBSERVATIONS

1. Clinical signs of toxicity

No treatment-related findings were observed. All abnormal clinical signs were equally distributed between control and treated animals and/or occurred in single animals, only.

2. Mortality

The number of decedents and mortality rate until day 728 (i.e. 24 month of treatment) is given in the table below.

Table 5.5-10: 24 months carcinogenicity rat - decedents number and mortality rate

Sex	Parameter	Dose group [ppm]			
		0	100	500	2500
Both	Animals examined	50	50	50	50
Males	Number	9	13	13	16
	Rate (%)	14	24	22	32
Females	Number	14	8	11	10
	Rate (%)	28	16	22	20

The highest mortality occurred in males administered 2500 ppm Boscalid in the food. Many of these deaths occurred towards start of necropsy, their treatment-relation is doubtful and the concomitant chronic study with similar mortality rates (Table 5.5-3) does not support this finding to be test substance related.

C. BODY WEIGHT AND BODY WEIGHT GAIN

Males receiving 500 - 15000 ppm Boscalid in the food showed a statistically significantly increased body weight gain in the first study week. Furthermore, males of the 500 ppm dose group showed increased body weight gain until day 42 of the study. However, this was assessed being incidental and not treatment-related. In females of the 100 ppm dose level statistically significantly lower body weight gain was sporadically observed (day 56, 91) which has been considered to be of incidental nature given the isolated findings and the lack of any indication of a dose response relationship.

In females treated with 15000 ppm in the food significant reduction of the body weight and body weight gain was observed from day 147 onward. As the value was 13.2% below control on day 483 (corresponding body weight gain reduction of 20.9% as compared with the control group), this group and the corresponding males were necropsied prematurely.

Females of the 2500 ppm dose level showed significant body weight gain and body weight reduction from day 287 and 315 onwards, respectively. At study termination, the value was 15.9% below the control (corresponding body weight gain reduction of 24.2% as compared with the control group). This effect was assessed being treatment-related. Details are presented in Table 5.5-11 below.

Table 5.5-11: 24 months carcinogenicity rat - mean body weight and body weight gain

Day	Parameter	Dose level [ppm]									
		Males					Females				
		0	100	500	2500	15000	0	100	500	2500	15000
0	BW [g]	187.9	187.2	186.2	185.5	183.8	144.0	145.9	145.5	145.2	143.1
	SD	± 9.6	± 9.7	± 9.4	± 9.6	± 9.8	± 7.2	± 7.7	± 7.4	± 8.0	± 7.3
	(% of control)	(100)	(99.7)	(99.1)	(98.8)	(97.9)	(100)	(101.3)	(101.0)	(100.8)	(99.4)
28	BW [g]	340.8	342.8	348.4	343.7	334.6	218.4	213.6	214.9	214.7	215.6
	SD	± 24.4	± 20.4	± 16.6	± 18.2	± 19.6	± 16.8	± 16.8	± 15.7	± 18.2	± 15.3
	(% of control)	(100)	(100.6)	(101.5)	(100.9)	(98.2)	(100)	(97.8)	(98.4)	(98.3)	(98.7)
	Δ [g]	152.9	155.6	**161.9***	158.2	150.8	74.4	**67.7***	69.4	69.5	72.5
	SD	± 17.6	± 14.7	± 12.3	± 13.2	± 15.3	± 12.7	± 10.0	± 12.1	± 12.5	± 10.9
	(% of control)	(100)	(101.7)	(105.9)	(103.4)	(98.6)	(100)	(91.0)	(93.3)	(93.4)	(97.4)
56	BW [g]	425.2	427.9	434.1	429.8	421.8	258.1	250.5	252.7	250.0	253.4
	SD	± 35.0	± 26.2	± 25.1	± 28.9	± 28.6	± 20.3	± 18.0	± 19.6	± 20.8	± 18.4
	(% of control)	(100)	(100.6)	(102.1)	(101.1)	(99.2)	(100)	(97.0)	(97.9)	(96.9)	(98.2)
	Δ [g]	237.4	240.7	247.9	244.3	238.0	114.2	**104.5****	107.2	**104.9****	110.3
	SD	± 28.3	±21.8	± 21.1	± 24.1	± 25.3	± 16.0	± 13.7	± 15.8	± 16.0	± 13.5
	(% of control)	(100)	(101.4)	(104.4)	(102.9)	(100.3)	(100)	(91.6)	(93.9)	(91.9)	(96.6)
91	BW [g]	480.1	486.4	493.7	489.4	482.0	284.8	276.9	279.5	276.6	276.0
	SD	± 40.5	± 29.9	± 34.1	± 34.9	± 35.8	± 22.1	± 18.9	± 20.5	± 24.2	± 20.2
	(% of control)	(100)	(101.3)	(102.8)	(101.9)	(100.4)	(100)	(97.2)	(98.1)	(97.1)	(96.9)
	Δ [g]	292.2	299.2	307.5	303.9	298.2	140.8	**130.9***	134.0	**131.4***	132.9
	SD	± 34.5	± 25.8	± 30.6	± 30.2	± 32.5	± 17.7	± 15.2	± 17.0	± 19.3	± 15.4
	(% of control)	(100)	(102.4)	(105.2)	(104.0)	(102.0)	(100)	(93.0)	(95.1)	(93.3)	(94.3)
147	BW [g]	540.5	538.2	549.8	546.9	541.6	304.1	303.5	304.5	298.7	291.4
	SD	± 53.6	± 45.4	± 43.7	± 45.5	± 42.4	± 24.6	± 22.0	± 22.1	± 26.0	± 21.2
	(% of control)	(100)	(99.6)	(101.7)	(101.2)	(100.2)	(100)	(99.8)	(100.1)	(98.2)	(95.8)
	Δ [g]	352.6	351.0	363.7	361.4	357.8	160.1	157.6	159.0	153.6	**148.3****
	SD	± 47.9	± 43.9	± 41.1	± 41.1	± 39.8	± 20.7	± 18.5	± 19.1	± 20.9	± 16.9
	(% of control)	(100)	(99.5)	(103.1)	(102.5)	(101.5)	(100)	(98.5)	(99.3)	(95.9)	(92.7)
315	BW [g]	628.0	637.9	646.4	642.9	624.7	340.2	330.9	329.5	**323.8***	**313.9****
	SD	± 74.6	± 51.1	± 64.7	± 75.2	± 65.2	± 35.0	± 27.0	± 28.1	± 29.7	± 26.6
	(% of control)	(100)	(101.6)	(102.9)	(102.4)	(99.5)	(100)	(97.3)	(96.9)	(95.2)	(92.2)
	Δ [g]	440.2	450.7	460.3	457.4	440.4	196.2	185.0	184.0	**178.6****	**170.8****
	SD	± 69.7	± 49.0	± 63.3	± 72.0	± 62.0	± 32.2	± 23.9	± 26.0	± 25.0	± 23.0
	(% of control)	(100)	(102.4)	(104.6)	(103.9)	(100.1)	(100)	(94.3)	(93.8)	(91.0)	(87.1)
483	BW [g]	681.6	709.0	707.8	711.7	694.2	381.9	362.4	364.9	**347.4****	**331.4****
	SD	± 92.4	± 70.7	± 87.6	± 92.6	± 81.9	± 18.2	± 40.2	± 42.1	± 38.6	± 34.0
	(% of control)	(100)	(104.0)	(103.8)	(104.4)	(81.9)	(100)	(194.9)	(95.5)	(91.0)	(86.8)
	Δ [g]	494.0	521.8	522.0	526.2	510.2	238.0	**216.5***	219.4	**202.3****	**188.3****
	SD	± 88.2	± 69.3	± 87.1	± 89.1	± 78.1	± 46.6	± 37.0	± 41.7	± 34.9	± 30.9
	(% of control)	(100)	(105.6)	(105.7)	(106.5)	(103.3)	(100)	(91.0)	(92.2)	(85.0)	(79.1)
728	BW [g]	699.3	695.9	711.2	700.0		425.8	410.0	397.0	**358.0****	
	SD	± 105.4	± 99.3	± 107.1	± 79.9	-	± 75.7	± 63.9	± 70.0	± 84.1	-
	(% of control)	(100)	(99.5)	(101.7)	(100.1)		(100)	(96.3)	(93.2)	(84.1)	
	Δ [g]	511.4	510.0	525.4	513.7		281.1	263.9	252.6	**213.1****	
	SD	± 101.8	± 100.6	± 105.2	± 78.5	-	± 74.8	± 62.1	± 70.2	± 55.1	-
	(% of control)	(100)	(99.7)	(102.7)	(100.5)		(100)	(93.9)	(89.8)	(75.8)	

BW = body weight; Δ = body weight gain

* = $p \leq 0.05$; ** = $p \leq 0.01$ Anova + Dunnett's test (two-sided)

D. FOOD CONSUMPTION, FOOD EFFICIENCY AND COMPOUND INTAKE

Regarding food consumption and efficiency, several statistically significant increases as well as decreases in animals of both sexes were noticed in the course of the treatment. However, due to the isolated occurrence, these deviations were assessed as being incidental and not related to the treatment.

The approximate, mean daily test substance intake during the administration period is summarised in the Table 5.5-12 below. In order to assure equally spaced intervals for calculation of the mean test substance intake, only the values of days 7, 35, 63, 91 and days 119 to 707 (15000 ppm dose group only up to day 483) were taken into account.

Table 5.5-12: 24 months carcinogenicity rat - substance intake

Substance intake	Dose level [ppm]									
	Males					Females				
	0	**100**	**500**	**2500**	**15000**	**0**	**100**	**500**	**2500**	**15000**
[mg/kg bw/day]	-	4.6	23.0	116.1	768.8	-	6.0	29.7	155.6	1024.4

E. CLINICAL PATHOLOGY

1. Haematology

At the end of the study, no treatment-related changes were observed in the leukocyte differential count as well as in erythrocyte morphology animals of both sexes of the treatment groups.

Various changes in white and red blood cells were observed in peripheral blood of animals of both sexes that were killed in extremis (both, control group and treatment groups). Thus, the observed changes were considered to be spontaneous, incidental and/or age-related. Accordingly, these findings were not assessed being treatment-related.

F. NECROPSY

1. Organ weight

Male animals administered 500 ppm Boscalid in the food revealed a statistically significant increase in absolute **testes** weight of 20.2% and decrease in relative adrenal weight of 14.3% as compared to the controls.

At 2500 ppm males showed a statistically significant elevation in the absolute and relative **thyroid** weight of 17.9% and 16.7%, respectively.

Table 5.5-13: 24 months carcinogenicity rats - summary of absolute and relative (% of terminal body weight) organ weights

Parameter	Sex	Dose level (ppm)			
		0	100	500	2500
Terminal body weight (g)	M F	659.72 401.75	661.06 383.01	682.72 370.26	668.54 **334.01****
Absolute liver weight (g)	M F	18.67 12.33	18.10 12.32	19.12 11.74	19.57 11.64
Relative liver weight	M F	2.84 3.08	2.75 3.23	2.81 3.19	2.96 3.50
Absolute kidney weight (g)	M F	3.96 2.67	4.04 2.73	4.32 **2.54***	4.13 **2.47****
Relative kidney weight	M F	0.61 0.68	0.62 0.73	0.65 0.70	0.61 0.76
Absolute testes weight (g)	M	3.67	3.80	**4.41****	4.13
Relative testes weight	M	0.57	0.59	0.67	0.63
Absolute adrenal weight (mg)	M F	91.34 144.68	99.16 145.45	82.47 140.26	83.82 126.10
Relative adrenal weight	M F	0.014 0.038	0.015 0.038	**0.012*** 0.039	0.013 0.039
Absolute thyroid weight (mg)	M F	38.87 30.58	37.58 29.08	41.70 30.18	**45.82**** 30.60
Relative thyroid weight	M F	0.006 0.008	0.006 0.008	0.006 0.008	**0.007**** 0.009

*: $p \leq 0.05$; **: $p \leq 0.01$; (Kruskal-Wallis, one sided and Wilcoxon-test, two-sided)

Females treated with 500 and 2500 ppm Boscalid in the food showed a statistically significant reduction in absolute **kidney** weight of 4.8% and 7.5%, respectively, as compared with the controls.

2. Gross lesions

In males the number of decedents with enlarged **thyroid gland** was increased with 2 animals in the 500 ppm dose group and one animal in the 2500 ppm dose group.

All other gross lesions occurred either as isolated single incidences or were equally distributed between all groups. Nevertheless, paying particular attention to the target organ liver and the organs of the endocrine system, the single incidences noticed are summarised below.

Table 5.5-14: 24 months carcinogenicity rats - macroscopic findings in liver, kidney and organs of the endocrine system

Sex	**Male**				**Female**			
Dose (ppm)	**0**	**100**	**500**	**2500**	**0**	**100**	**500**	**2500**
Animals examined	**50**	**50**	**50**	**50**	**50**	**50**	**50**	**50**
Liver								
- adhesion	-	1	-	-	-	-	-	2
- cyst	7	6	7	7	12	11	9	16
- deposition	-	-	-	-	1	-	-	-
- discoloration	-	-	1	-	1	-	1	-
- enlarged	-	-	1	-	-	-	-	1
- focus	48	44	41	41	26	30	23	22
- granular surface	-	-	-	-	1	-	-	-
- mass	8	8	10	5	1	3	-	1
- necrosis	1	-	-	-	-	-	-	1
- reduced size	-	-	1	-	-	-	-	-
- overgrown by tumour	-	-	-	-	-	-	-	2
- prominent acinar pattern	1	2	3	2	1	2	1	1
- thrombus	-	-	-	1	-	-	-	-
Kidney								
- concretion	1	-	3	3	-	-	-	-
- cyst	2	3	4	2	-	1	2	2
- discoloration	-	1	1	-	1	-	-	-
- enlarged	-	-	2	-	-	-	-	-
- focus	2	2	-	4	-	-	-	-
- granular surface	13	11	18	13	1	3	2	5
- inflammation	-	-	-	1	-	-	-	-
- mass	-	-	1	1	1	-	-	1
- reduced size	1	-	1	-	-	-	-	-
- overgrown by tumour	-	-	-	-	-	-	-	2
- pelvic dilatation	2	1	3	1	1	-	-	1
- retraction	5	8	6	9	-	1	-	1
Testes								
- calcification	7	8	11	9				
- cystic degeneration	4	4	9	8				
- deposition	-	-	1	-				
- discoloration	-	1	1	-				
- enlarged	2	1	1	1				
- focus	9	12	6	13				
- mass	9	11	15	12				
- reduced size	11	7	6	10				

Sex	Male				Female			
Dose (ppm)	**0**	**100**	**500**	**2500**	**0**	**100**	**500**	**2500**
Animals examined	**50**	**50**	**50**	**50**	**50**	**50**	**50**	**50**
Epididymides								
- deformation	-	-	1	-				
- enlarged	-	1	-	-				
- focus	-	-	-	1				
- size reduction	4	2	4	6				
Seminal vesicle								
- discoloration	-	1	-	1				
- enlarged	-	4	2	-				
- induration	1	-	-	-				
- reduced size	3	2	7	6				
Coagulation gland								
- enlarged	-	-	1	-				
Prostate								
- discoloration	-	1	-	2				
- oedema	3	-	1	-				
- enlarged	1	3	2	1				
- focus	3	-	2	3				
- induration	1	4	4	-				
- mass	2	-	-	1				
- reduced size	-	2	3	3				
Preputial glands								
- abscess	1	2	1	1				
- induration	-	-	-	2				
Ovaries								
- cyst					22	29	21	32
- enlarged					1	-	-	1
- focus					3	7	4	5
- induration					2	-	1	1
- mass					7	4	6	6
- overgrown by tumour					-	-	-	3
Oviduct								
- dilatation					1	-	-	1
Uterus								
- cyst					-	1	3	2
- deformation					1	-	-	-
- dilatation					6	5	7	6
- focus					1	-	1	-
- induration					2	3	2	4
- mass					6	3	2	9
- overgrown by tumour					-	1	-	-
- wall thickening					1	-	1	-
Mammary gland								
- abscess					1	-	-	-
- cyst					2	7	9	6
- mass					9	16	7	7
Adrenal gland								
- cyst	-	-	-	-	1	-	1	-
- enlarged	2	1	1	1	8	6	6	6
- focus	2	1	1	1	2	2	2	1

Sex	Male				Female			
Dose (ppm)	**0**	**100**	**500**	**2500**	**0**	**100**	**500**	**2500**
Animals examined	**50**	**50**	**50**	**50**	**50**	**50**	**50**	**50**
Adrenal cortex								
- cyst	-	-	-	-	-	-	2	-
- focus	16	26	23	21	41	40	41	35
Thyroid glands								
- cyst	1	-	1	-	-	-	-	-
- discoloration	-	-	1	1	-	-	-	-
- enlarged	-	-	2	1	-	-	1	-
- focus	-	-	1	-	1	1	1	-
- mass	2	2	-	3	1	2	1	-
Parathyroid gland								
- enlarged	2	1	1	3	-	-	-	-
Pituitary gland								
- cyst	1	3	3	3	6	3	5	3
- discoloration	-	-	-	-	-	-	-	1
- enlarged	2	1	2	2	12	10	4	9
- focus	5	8	6	6	10	7	8	14
- mass	2	4	3	2	20	24	26	17

3. Histopathology

Neoplastic findings

In **thyroid glands** increased incidences of follicular cell adenoma as compared to the control animals were recorded in males treated at the 500 and 2500 ppm dose levels and in females at the 100 and 2500 ppm dose levels. In males this finding correlated with the macroscopically observed enlargement of the thyroid gland and increased absolute as well as relative organ weight at the 2500 ppm dose level. Histopathologically, the adenomas were composed of well demarcated areas of hyperplastic follicles, with hypertrophic epithelial cells, and compression of adjacent parenchyma. The single occurrence of the follicular cell adenoma in one male of the 500 ppm dose group is regarded to be substance-induced, since two further incidences were observed in males of the same dose group in a parallel running chronic toxicity study [DocID 2001/1000114], increasing the frequency and showing a dose-dependency of that finding. The single incidence at the 100 ppm dose level in females is regarded to be not substance related, since it is in the range of the female historical control data [see Table 5.5-15]. In conclusion the increased incidence at the 2500 ppm dose level in both sexes is considered to be substance-related, albeit the incidence observed in females is within the historical control range, even though at its higher limit.

From the mechanistic perspective the higher metabolic activity of hepatocytes would lead to a higher degradation rate of T_3/T_4 hormones and as a consequence to a functional stimulation of the thyroid glands by a feedback mechanism that is evident to induce the slight increase in incidences of follicular cell adenomas in the mid and high dose groups. Further information including data on specific mechanistic studies to discuss this findings in more detail together with the overall discussion of this findings has been presented in section 5.8 of this supplementary dossier.

Table 5.5-15: 24 months chronic & carcinogenicity rats - incidences of follicular cell adenoma after administration of Boscalid

Parameter		Male				Female			
		0	**100**	**500**	**2500**	**0**	**100**	**500**	**2500**
current carcinogenicity study									
animals examined	N	50	50	50	50	50	50	50	50
incidences	N	0	0	1	4	0	1	0	3
	%	0	0	2	8	0	2	0	6
parallel chronic toxicity study									
animals examined	N	20	20	20	20	20	20	20	20
incidences	N	0	0	2	1	0	0	1	0
	%	0	0	10	5	0	0	5	0
carcinogenicity + chronic toxicity studies									
total animals examined	N	70	70	70	70	70	70	70	70
total incidences	N	0	0	3	5	0	1	1	3
	%	0	0	4.3	7.1	0	1.4	1.4	4.3
Historical data (1981 - 2000)									
animals examined	N	n. s.				1710			
min - max	N	n. s.				0 - 3			
min - max	%	n. s.				0 - 10			

n. s. = not specified

The gravimetrically observed increase of the absolute **testes** weight of males administered 500 ppm Boscalid in the food was considered to be in line with findings of one bilateral and/or some large Leydig cell tumours. The latter findings was devoid of indication of being test substance related with comparable incidences (Table 5.5-16).

The number of females with neoplasms, with more than one primary neoplasm, with metastases, and with benign or malignant neoplasms, as well as the total number of primary and benign or malignant neoplasms were comparable between the control and the top-dose groups. The total number of benign neoplasms was decreased in females at the 2500 ppm dose level. The number of males with neoplasms, with metastases, and with benign or malignant neoplasms, as well as the total number of malignant neoplasms were comparable between the control and the top-dose groups. The total number of males with more than one primary neoplasm and the total number of primary and benign neoplasms were increased in the top-dose males and was considered to be the consequence of the increased incidence of the thyroid gland adenomas.

As with the exception of liver, kidneys, lungs, thymus, urinary bladder and thyroid glands as well as organs with macroscopic abnormalities, complete microscopic examinations were only performed on decedents of groups at the 100 and 500 ppm dose levels, and thus the tumour incidences cannot be readily compared with the control and the high dose groups.

For survivors, the number of females with neoplasms and the number of males with benign neoplasm were slightly decreased at the at the 2500 ppm dose level. For females, it was considered to be a consequence of decreased body weight at study termination. The number of males with more than one primary neoplasms was slightly increased at the top-dose, and was considered to reflect the high biologic variation of tumours in aged rodents.

Non-neoplastic findings

In the **liver**, histopathological examinations revealed centrilobular hypertrophy of hepatocytes with a minimal effect in 2/50 males at the 500 ppm dose level but with increased incidence in 27/50 males and 11/50 females at the 2500 ppm dose level. Histopathologically, this hypertrophy was characterized by an increase in hepatocellular size and organelles, mainly showing a ground-glass appearance, sometimes with presence of eosinophilic droplets. The centrilobular hypertrophy of hepatocytes was considered to be the consequence of accumulation/ proliferation of smooth endoplasmic reticulum due to the enzyme induction stimulated by the test substance.

Furthermore, in males treated at the 500 ppm and 2500 ppm dose levels, eosinophilic foci were slightly increased, but larger ones, showing eosinophilic cytoplasmic inclusions, were present only in males at the 2500 ppm dose level.

In **thyroid glands**, minimal increased incidences of diffuse follicular cell hypertrophy were observed in males at the 500 ppm dose level and in females at the 2500 ppm dose level. Higher incidences were recorded in males at the 2500 ppm dose level that was in line with increased thyroid weights in this sex and dose group. Focal thyroid follicular cell hyperplasia was increased in the animals at the 2500 ppm dose level. Thyroid findings have been linked to liver enzyme as previously described.

In **urinary bladder**, a slightly increased incidence of diffuse papillary transitional cell hyperplasia were noticed in males at 2500 ppm with corresponding inflammation and concretion, and was considered treatment related. Histopathologically, this hyperplasia was characterised by a diffuse thickening of the transitional cell epithelium with papillary growth pattern.

The decrease in absolute **kidney** weights of females treated at the 500 ppm and 2500 ppm dose levels corresponded to the presence and severity of chronic progressive glomerulo-nephropathy which was considered to be not substance related taking in addition into consideration the decrease in body weight of this dose group.

The decrease of relative adrenal weight in males treated at the 500 ppm dose level was supposed to express the biological variation in the presence of focal cortical fatty change and/or cortical hyperplasia that were not substance-related.

Evaluation on decedents did not reveal histopathological findings than may be attributed to the earlier death. Mostly, inflammatory lesions or different types of tumours in various organs were noticed.

All other morphology changes detected were attributed either to be a single occurrence or no dose-response relationship were seen and were thus considered to be of incidental or spontaneous nature. Nevertheless, paying particular attention to the organs of the endocrine system, kidney and the target organ liver, the incidences noticed are additionally summarised below.

Table 5.5-16: 24 months carcinogenicity rats - microscopic neoplastic and non-neoplastic findings in liver, kidney and organs of the endocrine system

Sex	**Male**				**Female**			
Dose (ppm)	**0**	**100**	**500**	**2500**	**0**	**100**	**500**	**2500**
Liver	50	50	50	50	50	50	50	50
- centril. hypertrophy	-	-	2	27	-	-	-	11
- perihepatitis	-	-	-	-	1	-	-	-
- lymphoid infiltation	16	15	14	14	18	23	10	19
- congestion	-	2	-	-	-	-	-	-
- haematopoiesis	2	1	1	1	13	6	1	7
- ganulocytosis	-	-	-	-	2	-	-	-
- thrombus	-	1	2	1	-	1	-	-
- duct. chol.: dilatation	-	-	-	-	1	-	-	-
- fibrosis capsular	1	1	1	-	-	-	-	-
- fatty infiltr. focal	10	7	8	12	8	6	3	3
- fatty infiltr. perip.	9	13	13	8	8	6	5	1
- fatty infiltr. diff.	1	1	-	-	1	-	-	-
- pigment storage	1	-	2	-	6	-	-	1
- spongiosis/peiosis	23	24	23	23	10	12	8	5
- necrosis, focal	1	2	2	3	1	1	1	4
- necrosis, centrilob.	-	1	-	1	1	1	-	1
- haemorrhage	1	-	-	-	-	-	-	1
- remodeling	1	1	1	-	2	-	-	-
- cyst (s), biliary	7	5	8	5	12	10	9	14
- bile ducts: cyst hyp.	1	2	1	1	2	3	6	1
- bile duct proliferation	20	16	13	20	10	18	12	20
- cellular alterations	35	33	39	41	30	39	31	24
- clear cell foci	5	1	5	5	4	5	5	3
- basophilic foci	35	31	37	42	25	39	30	18
- eosinophilic foci	3	4	8	9	-	-	-	-
- NOS[1]	-	1	-	-	7	1	1	7
- *cholangioma*	-	-	-	-	-	-	-	*1*
- *cholangiocarcinoma*	*1*	-	*1*	-	-	-	-	-
- *adenoma hepatocell.*	*11*	*10*	*11*	*12*	*3*	*6*	*3*	*1*
- *adenocarc. hepatocell.*	*1*	*1*	*4*	-	-	-	-	-

[1] NOS = not classifiable hyperplasia

Sex	Male				Female			
Dose (ppm)	**0**	**100**	**500**	**2500**	**0**	**100**	**500**	**2500**
Kidney	50	50	50	50	50	50	50	50
- cyst (s)	2	2	6	3	1	2	2	1
- pelvis: concretion	1	-	4	3	-	-	-	-
- pelvis: dilatation	2	2	3	1	2	-	-	1
- calcificat. pelvis	11	15	16	13	24	26	30	33
- calcificat. cortical	2	4	-	3	19	6	8	16
- pyelitis	3	10	6	7	-	-	1	-
- infarct	1	3	2	1	1	1	-	1
- storage nephrosis	1	3	1	1	-	2	-	3
- nephropathy, chronic	40	40	39	41	33	29	26	27
- pyelonephritis	3	1	2	3	1	-	-	-
- pelvis: hyperplasia	4	6	6	4	9	6	4	6
- hyperplasia: tubular	-	-	-	1	-	-	-	1
- *renal lipoma*	-	-	-	-	*1*	-	-	-
- *adenoma*	-	-	-	*1*	-	-	-	-
- *carcinoma*	-	-	-	-	-	-	-	*1*
Testes	50	41	38	50				
- congestion	-	1	-	-				
- oedema	1	-	-	-				
- thrombus	-	1	-	-				
- calcification focal	5	9	11	10				
- degeneration focal	6	6	8	7				
- degeneration diff.	12	9	13	14				
- arteritis/periarteritis	1	1	1	1				
- mesothelium hyperpl.	-	1	-	-				
- Lyedig cell hyperplasia	19	17	17	18				
- *Leydig cell adenoma*	*18*	*21*	*23*	*23*				
Epididymides	50	15	16	50				
- lymphoid infiltration	4	1	-	1				
- spermatocele	-	-	-	1				
- loss of sperm	14	3	9	17				
- *mesothelioma benign*	-	-	*1*	-				
Seminal vesicle	50	18	19	50				
- inflammation	1	2	1	4				
- atrophy	5	1	7	6				
- dilatation	-	4	2	-				
- hyperplasia focal	2	1	1	1				
Coagulation gland	-	-	1	-				
- dilatation	-	-	1	-				
Prostate	50	22	22	50				
- inflammation	27	15	13	20				
- atrophy	1	-	1	4				
- dilatation glandular	-	2	2	-				
- hyperplasia, focal	16	2	3	18				
- *adenoma*	*3*	*1*	*2*	*3*				

Sex	**Male**				**Female**			
Dose (ppm)	**0**	**100**	**500**	**2500**	**0**	**100**	**500**	**2500**
Preputial glands	1	3	1	1				
- inflammation	1	1	1	-				
- cyst squamous	1	3	1	-				
Urinary bladder	50	50	50	50	50	50	49	49
- haemorrhage	1	-	-	-	-	-	-	-
- concretion	6	5	7	8	-	-	-	-
- arteritis/periarteritis	-	-	-	-	-	1	-	-
- dilatation	5	6	4	8	5	6	7	4
- inflammation	11	16	8	17	1	-	-	-
- hyperpl. tr. focal	1	2	1	1	1	-	1	-
- hyperpl. tr. diff.	3	6	2	3	-	-	-	-
- hyperpl. tr. diff. papill.	7	6	7	13	-	-	-	-
- metapl. squamous	-	1	1	2	-	-	-	-
- leiomyoma	-	-	-	-	-	-	*1*	-
- papilloma squamous	-	*1*	-	*1*	-	-	-	-
- papilloma trans. cell.	*4*	*3*	*3*	*4*	-	-	-	-
Ovaries					50	40	34	50
- cyst (s)					20	28	21	31
- abscess					2	1	1	2
- granuloma					-	1	1	-
- atrophy					4	3	2	-
- hyperpl. sex c. diff.					4	7	8	5
- hyperpl. sex c. focal					4	3	1	2
- tumour sex c. benign					*4*	*2*	*4*	*2*
- tumor gran. c. benign					*2*	*1*	*1*	-
- thecoma benign					*2*	*4*	*1*	*3*
- luteoma benign					*1*	-	-	*1*
- metastatic carcinoma					-	-	-	*1*
Oviduct					49	8	11	50
- dilatation					1	-	-	1
Uterus					50	18	22	50
- dilatation focal					10	5	11	10
- haemorrhage focal					2	-	-	-
- thrombus					-	-	-	1
- atrophy					1	-	-	-
- cervix, fibrosis					2	4	2	5
- squamous cyst					4	2	-	5
- squamous metaplasia					4	5	2	7
- hyperpl. gland. focal					6	4	6	10
- polyp stromal					*5*	*1*	*1*	*4*
- polyp endometrial					*1*	-	-	*2*
- sarcoma endom. strom.					-	*1*	*1*	*2*
- leiomyoma					-	-	-	*2*
- adenocarcinoma					*2*	*1*	*1*	-
-carcinoma squamous					-	*1*	-	-

Sex	Male				Female			
Dose (ppm)	**0**	**100**	**500**	**2500**	**0**	**100**	**500**	**2500**
Mammary gland	-	-	1	-	49	26	24	50
- abscess	-	-	-	-	1	-	-	-
- cyst (s)	-	-	-	-	2	7	8	8
- hyperplasia focal	-	-	-	-	6	1	1	4
- fibroadenoma	-	-	-	-	*7*	*8*	*3*	*3*
- adenoma	-	-	-	-	*1*	*1*	-	-
- adenocarcinoma	-	-	*1*	-	-	*4*	*4*	*4*
- metastatic carcinoma	-	-	-	-	*1*	-	-	-
Adrenal cortex	50	37	35	50	50	46	44	50
- blood filled cyst (s)	3	8	8	4	41	44	38	38
- haemorrhage	1	-	-	-	-	-	-	-
- accessory adr. tissue	14	12	11	8	5	3	5	6
- degeneration focal	-	-	-	-	7	3	4	1
- necrosis	-	-	1	-	-	1	-	-
- granulocytosis	-	-	-	-	-	-	-	1
- haematopoiesis	1	2	2	2	5	-	1	5
- calcification focal	2	2	-	1	1	1	-	3
- fatty change focal	16	12	14	16	3	5	4	1
- hypertrophy focal	5	5	7	3	3	7	6	3
- hyperplasia focal	7	2	5	7	10	10	13	11
- adenoma	-	-	-	*1*	-	*2*	*2*	-
- metastatic carcinoma	-	-	-	-	-	-	-	*1*
Adrenal medullas	50	36	34	50	50	46	43	50
- hyperplasia focal	18	11	11	18	10	15	10	10
- lipoma	-	-	-	-	*1*	-	-	-
- tumour benign	*8*	*6*	*4*	*9*	*5*	*2*	*3*	*4*
- tumour malignant	*1*	*1*	*1*	*1*	-	*2*	*1*	*1*
Thyroid gland	50	50	50	50	50	50	50	50
- congestion	-	-	1	1	1	-	-	-
- cyst (s)	13	14	11	15	8	15	9	15
- arteritis/periarteritis	-	-	-	1	-	-	-	-
- mineralisation	-	-	-	1	-	-	-	-
- inflammation	-	-	-	-	-	-	-	1
- hypertr. foll. c. diff.	2	5	6	22	2	-	-	4
- hypertr. foll. c. focal	2	5	7	3	9	6	6	3
- hyperpl. foll. c. diff.	18	6	6	15	16	16	17	23
- hyperpl. foll. c. focal	1	1	1	9	2	2	1	7
- adenoma C-cell	*5*	*5*	*6*	*3*	*8*	*8*	*7*	*5*
- adenoma follic. cell	-	-	*1*	*4*	-	*1*	-	*3*
- adenocarc. C-cell	-	*2*	-	*1*	-	-	*1*	-
- adenocarc. follic. cell	*1*	-	-	-	-	-	-	-
Parathyroid gland	48	50	49	50	50	47	41	47
- mineralisation	-	-	-	1	-	-	-	-
- hyperplasia focal	4	1	6	6	2	2	2	-
- hyperplasia diffuse	1	-	1	3	-	-	-	-
- adenoma	-	-	-	*1*	-	*1*	-	*1*

Sex	Male				Female			
Dose (ppm)	**0**	**100**	**500**	**2500**	**0**	**100**	**500**	**2500**
Pituitary gland	50	26	23	50	50	44	45	50
- cyst (s)	11	5	-	18	7	10	11	8
- necrosis	-	-	-	1	-	-	-	-
- gliosis pars nervosa	-	-	-	-	-	-	-	1
- hyperpl. p. distalis	16	5	8	11	14	15	9	14
- hyperpl. p. intermedia	1	-	-	-	-	-	-	-
- adenoma p.distalis	*6*	*10*	*8*	*6*	*28*	*27*	*29*	*24*
- adenocarc. p. distalis	-	-	-	-	*3*	*1*	*2*	*2*

III. CONCLUSIONS

The oral administration of Boscalid induced substance-related effects at 500 and 2500 ppm dose levels. The liver and thyroid were identified as target organs. Following a 24-month dietary administration of Boscalid to Wistar rats, adverse effects were observed in animals of dose group ≥ 500 ppm. Treatment-related findings included histopathological changes in liver (centrilobular hypertrophy in both sexes at the 2500 ppm dose level and increased incidence of eosinophilic foci of cellular alteration in males ≥ 500 ppm). Increased incidences of thyroid follicular cell adenomas (2500 ppm, both sexes) in combination with increased thyroid weights (2500 ppm, males) and correlating histopathological changes (diffuse follicular cell hypertrophy and focal follicular cell hyperplasia in both sexes) were observed. From a mechanistic point of view the thyroid changes can be associated with increased metabolism of thyroid hormones (T_3 and T_4) due to increased phase II (conjugation) hepatic activity. The reduced thyroid hormone levels trigger, by means of a feedback mechanism, the release of increased amounts of TSH, in an attempt to restore homeostatic conditions. Due to continued treatment, the metabolic activity of the liver, however, remains elevated resulting in a continuously increased breakdown of thyroid hormone and followed by increased TSH demand. This mechanism is well known to be able to induce a tumorigenic response in the thyroid of the rat.

The administration of 100 ppm Boscalid in the food was tolerated by the male and female rats without any changes that could be attributed to Boscalid-treatment.

Based on this findings, the no observed adverse effect level (NOAEL) for Wistar rats under the conditions of the study was 100 ppm Boscalid in the food, corresponding to the test substance intake of 4.4 mg/kg bw/day and 6.0 mg/kg bw/day for males and females, respectively.

Boscalid - Carcinogenicity study in C57BL mice - Administration in the diet for 18 months ([redacted] et al., 2001)
DocID 2001/1000116

Guidelines: According to OECD 451 (1981), EEC 87/302, EPA/FIFRA (Subdivision F; para. 83-2) and JMAFF
Deviations: None
GLP: Yes
Acceptance: The study has been evaluated and considered acceptable in the EU registration process for the first Annex I inclusion.

Note: The carcinogenicity study in mice was in detail evaluated here following the OECD format in analogy to the chronic and carcinogenicity studies in rats.

Executive summary

Boscalid (94.4% purity, batch N 37) was administered to groups of 50 male and 50 female C57BL mice (source: Centre d'Elevage R. Janvier, France) at dietary levels of 0, 80, 400, 2000 and 8000 ppm Boscalid in the food for a period of 18 months. These concentrations were equivalent to the substance intake of 0, 13, 65, 331 and 1345 mg/kg bw/day in males, and 0, 18, 90, 443 and 1804 mg/kg bw/day in females.
Following an 18-month dietary administration of Boscalid to C57BL mice, adverse effects were observed in animals of the dose group ≥ 400 ppm. Treatment-related findings included reduced body weights (females 8000 ppm, males ≥ 400 ppm). The liver was the target organ with increase in absolute and/or relative weights at dose levels ≥ 400 ppm Boscalid in the food. Histopathological evaluations showed increased incidence of peripheral hypertrophy of hepatocytes at dose levels ≥ 2000 ppm in female mice. Boscalid was not carcinogenic to mice. The administration of 80 ppm was tolerated by the male and female mice without any changes that could be attributed to the-treatment.

Based on the results of this 18-month carcinogenicity study, the NOAEL in the mouse can be established at 80 ppm (equivalent to 13 mg/kg bw/day in males and 18 mg/kg bw/day in females).

(DocID 2001/1000116)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:	Boscalid (BAS 510 F; Reg.No. 300 355)
Description:	Solid / white (powder)
Lot/Batch #:	N37 (Tox-batch III)
Purity:	94.4%
Stability of test compound:	The stability of the test substance was verified by re-analysis via HPLC after the in-life phase of the study. The re-analyzed purity was still 94.4% on 01 Dec. 1999, the expiration date of the batch was Dec. 2001.
2. Vehicle and/or positive control:	None
3. Test animals:	
Species:	Mouse
Strain:	C57BL/6 J Rj
Sex:	Male and female
Age:	About 7 weeks (at start of administration)
Weight at dosing (mean):	Males: 21.2 (19.2 - 22.8) g Females: 18.1 (16.4 - 19.9) g
Source:	Centre d'Elevage R. Janvier, France
Acclimation period:	At least 7 days
Diet:	Kliba maintenance diet rat/mouse/hamster, meal (Provimi Kliba SA, Kaiseraugst, Switzerland), ad libitum
Water:	Drinking water from bottles, ad libitum
Housing:	Animals were housed individually in type MI Makrolon cages with wire mesh tops (Becker & Co., Castrop-Rauxel, Germany) with a floor area of about 200 cm^2. The bedding material (type ¾ dust free embedding) was supplied by SSNIFF, Soest, Germany.
Environmental conditions:	
Temperature:	20 - 24°C
Humidity:	30 - 70%
Air changes:	Animals were housed in a fully air-conditioned room
Photo period:	12 hours / 12 hours (light: form 06.00 a. m. - 06.00 p. m; dark: from 06.00 p. m. - 06.00 a. m.)

B. STUDY DESIGN AND METHODS

1. Dates of experimental work: 3-Feb-1998 - 28-Feb-2001
(In-life dates: 10/25-Feb-1998 (start of administration) to 23-Aug/08-Sep-1999 (necropsy))

2. Animal assignment and treatment:
Boscalid was administered to groups of 50 male and 50 female mice at dietary concentrations of 0, 80, 400, 2000 and 8000 ppm daily for 18 months.

3. Test substance preparation and analysis:
For each preparation the test substance was weighed out and added to the corresponding amounts of food, depending on dose group, and mixed for 10 minutes in a laboratory mixer. Usually, the test substance preparations were mixed in intervals of about 4 weeks.
The stability of the test substance in the diet over a period of 32 days at room temperature was verified before the start of the study.
Concentration control analyses were performed at the start of the study as well as after 3, 6, 9, 12, 15 and 18 month via HPLC/UV. Homogeneity was proven at the start of the study as well as after 3 month by taking 3 samples from the top, middle and bottom of the beaker for the low (80 ppm) and high dose level (8000 ppm).

4. Statistics:
Means and standard deviations of each test group were calculated for several parameters. Further statistical analyses were performed according to following tables:

Statistics of clinical examinations

Parameter	Statistical test
Food consumption, body weight, body weight gain, food efficiency	Parametric one-way analysis using the F-test (ANOVA, two-sided). If the resulting p-value was equal or less than 0.05, a comparison of each group with the control group was performed using DUNNETT's test (two-sided) for the hypothesis of equal means

Statistics of pathology

Parameter	Statistical test
Organ weights	Non-parametric one-way analysis using KRUSKAL-WALLIS test (two-sided). If the resulting p-value was equal or less than 0.05, a pairwise 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:

A check for any moribund or dead animals as well as for clinical signs of toxicity was made twice a day (in the morning and in the late afternoon) on working days and once a day (in the morning) on weekends and public holidays. Once a week, an additional comprehensive clinical examination (including palpation) was carried out.

2. Body weight:

Body weight was determined before the start of the administration period in order to randomize the animals. During the study period, body weight was determined on day 0, once a week during the first 13 weeks of the administration period, thereafter at 4-week intervals, and prior to start of necropsy.
The difference between the body weight on the respective day of weighing and the body weight on day 0 was calculated as body weight gain.

3. Food consumption, food efficiency and compound intake:

Food consumption was determined once a week over a period of 7 days during the first 13 weeks of the administration period, thereafter at 4-week intervals, and prior to start of necropsy. The values were calculated as food consumption in grams per animal and day.
Food efficiency (group means) was calculated based upon individual values for body weight and food consumption:

$$\text{Food efficiency for day x} = \frac{BW_x - BW_Y}{FC_{y\ to\ x}}\, x\, 100$$

BW_x and BW_y = body weight [g] at day x and day y (last weighing date before day x)
$FC_{y\ to\ x}$ = the mean food consumption from day y to x calculated as mean daily food consumption [g] on day x, multiplied by the number of days from day y to day x

The mean daily intake of test substance (group means) was calculated based upon individual values for body weight and food consumption.

$$\text{Substance intake for day x} = \frac{FC_x \times D}{BW_x}$$

BW_x = body weight [g] at day x
FC_x = the mean food consumption [g] for day x
D = dietary dose in ppm

4. Haematology:
Blood samples were taken by tail puncture from non-fasted, non-anaesthetised animals during the administration period, and by decapitation from fasted (about 16 - 18 hours), anaesthetised animals at the end of the study period to prepared differential blood smears. Leukocyte differential count and white and red blood cell morphologies were determined from the blood smears by light microscopy. The blood smears of the control and highest dose groups were evaluated only.

5. Sacrifice and pathology:
At study termination after 18 month, all animals were sacrificed by decapitation under CO_2-anaesthesia. The exsanguinated animals were necropsied and assessed by gross pathology. The animals which died prematurely or were sacrificed in a moribund state were necropsied as soon as possible after death and assessed by gross pathology.
The following organs were sampled, weighed and examined histopathologically after haematoxylin-eosin staining:

Pathology:
The following organs were collected (column C), weighed (W) and examined histopathologically (H, ✓: all animals per group and sex, #: all control and high dose animals)

C	W	H		C	W	H		C	W	H	
✓	✓	#	adrenals	✓			lacrimal gland[%]	✓		#	sternum w. marrow
✓		#	aorta	✓	✓	✓	liver	✓		#	stomach [&]
			bone	✓		✓	lungs	✓	✓	#	testes
✓	✓	#	brain	✓		#	lymph nodes[#]	✓		#	thymus
✓		#	caecum	✓		#	mammary gland (♀)				tongue
			cervix				nose/nasal cavity	✓	✓	#	thyroid/parathyroid[α]
✓		#	colon	✓	✓	#	ovaries with oviduct*	✓		#	trachea
✓		#	duodenum	✓		#	pancreas	✓		#	urinary bladder
✓		#	epididymides				pharynx	✓		#	uterus
✓		#	esophagus	✓		#	pituitary gland	✓		#	vagina
✓		#	eyes	✓		#	prostate				
✓		#	femur’	✓		#	rectum		✓		body (anaesthetised animals)
✓		#	gall bladder	✓		#	salivary gland[$]				
✓		✓	gross lesions	✓		#	Sciatic nerve				
			Harderian glands	✓		#	seminal vesicles				
✓		#	heart	✓		#	skeletal muscle				
✓		#	ileum	✓		#	skin				
✓		#	jejunum	✓		#	spinal cord[§]				
✓	✓	✓	kidneys	✓		#	spleen				

’ with knee joint and marrow; [%] extra-orbital; [#] mesenteric and mandibular; * oviduct not weighed; [$] mandibular and sublingual; [§] cervical, thoracic and lumbar cord; [&] fore-stomach and glandular stomach; [α] parathyroid not weighted

Gross lesions were examined in all affected animals per dose group and sex. Of few organs, the following special stains were performed: Toluidin blue for mast cells and Elastica van Gieson for collagenic fibres.

II. RESULTS AND DISCUSSION

A. TEST SUBSTANCE ANALYSES

The stability of the test substance in the diet for a period of 32 days at room temperature was verified analytically with a comparable batch as used for the study. During the study, the preparations were used usually no longer than 32 days, with some exceptions. On few occasions, preparations were used up to 38 days, however since the stability value after 32 days was 97.9%, the assumption has been considered adequate that a sufficient stability was still given for periods slightly exceeding the confirmed period of test substance stability.

The results of homogeneity and concentration analysis are summarised below:

Table 5.5-17: Analysis of diet preparations for homogeneity and test-item content

Dose level [ppm]	Sampling	Analysis	Concentration [ppm] Mean ± SD[§]	Relative standard deviation [%]	Mean % of nominal concentration[§]
80 ppm	09.02.98	09.02.98	73.1 ± 0.3[#]	0.3[#]	91.4
	28.04.98	28.04.98	87.6 ± 2.7[#]	3.1[#]	109.5
	24.08.87	24.08.87	77.2		96.5
	16.11.98	18.11.98	87.4		109.3
	08.02.99	08.02.99	88.0		110.0
	03.05.99	06.05.99	79.9		99.9
	26.07.99	30.07.99	78.8		98.5
average			**81.7 ± 6.0**		**102.1 ± 7.3**
400 ppm	09.02.98	09.02.98	394		98.5
	28.04.98	28.04.98	370		92.4
	24.08.87	24.08.87	410		102.6
	16.11.98	18.11.98	397		99.2
	08.02.99	08.02.99	373		93.2
	03.05.99	06.05.99	383		95.8
	26.07.99	30.07.99	389		97.3
average			**388 ± 14**		**97.0 ± 3.6**
2000 ppm	09.02.98	09.02.98	1983		99.2
	28.04.98	28.04.98	1874		93.7
	24.08.87	24.08.87	1844		92.2
	16.11.98	18.11.98	2011		100.5
	08.02.99	08.02.99	1853		92.6
	03.05.99	06.05.99	1911		95.6
	26.07.99	30.07.99	1932		96.6
average			**1915 ± 64**		**95.8 ± 3.4**
8000 ppm	09.02.98	09.02.98	8088 ± 144[#]	1.8[#]	101.1
	28.04.98	28.04.98	7633 ± 50[#]	0.6[#]	95.4
	24.08.87	24.08.87	7381		92.3
	16.11.98	18.11.98	7861		98.3
	08.02.99	08.02.99	7382		92.3
	03.05.99	06.05.99	7834		97.9
	26.07.99	30.07.99	7525		94.1
average			**7672 ± 267**		**95.6 ± 3.5**

[#] based on mean values of the three individual samples (homogeneity samples)
[§] Values may not calculate exactly due to rounding of figures

The homogeneity of the mixtures was verified at concentrations of 80 and 8000 ppm in the diet, as the relative standard deviation was in the range of 0.3 - 3.1% and 0.6 - 1.8%, respectively.
The concentration control analysis revealed values in the range of 91.4% to 110.0% of the nominal concentration for all dose levels. No test substance was determined in control diets.

B. OBSERVATIONS

1. Clinical signs of toxicity

No treatment-related findings were observed. All findings were equally distributed between control and treated animals and/or occurred in single animals, only. These findings were therefore assessed as spontaneous in nature and/or considered to reflect the range of biological variation.

2. Mortality

No treatment-related mortalities occurred during the study period.
Most animals survived until the scheduled study termination, the number of decedents and the not treatment-related mortality rate are summarised below:

Table 5.5-18: 18 months carcinogenicity mice - decedents number and mortality rate

Sex	Parameter	Dose group [ppm]				
		0	80	400	2000	8000
Both	Animals examined	50	50	50	50	50
Males	Number	3	5	4	8	3
	Rate (%)	6	10	8	16	6
Females	Number	3	3	2	1	1
	Rate (%)	6	6	4	2	2

C. BODY WEIGHT AND BODY WEIGHT GAIN

In males treated at the dose level of 400, 2000 and 8000 ppm Boscalid in the diet, body weight and body weight gain were significantly decreased with increasing incidence and severity at higher dose levels. At the top-dose level, the impairment of the body weight development was observed throughout the study period. At study termination, the body weight (the body weight gain) of males at the 400. 2000 and 8000 ppm dose level was 9.2 (23.8)%, 6.8 (18.1)% and 5.4 (13.6)% below the control animals, respectively. These effects were assessed being treatment-related.

At the low dose this effect was observed on some days, mainly at study initiation with some additional findings sporadically distributed over the study period. However, as these deviations were of minor magnitude (maximum up to 4.7% below the control), and no statistically significance was obtained at study termination, these deviations were assessed being incidental in nature and not treatment-related.

In females treated at the 8000 ppm dose level, significant reduction of the body weight and body weight gain was observed from day 231 onwards. As the value was 7.4% below control (corresponding body weight gain reduction of 20.5% as compared with the control group) at study termination, this effect was assessed being treatment-related.

Females treated with lower dose levels of 80, 400 and 2000 ppm overall had comparable body weight and body weight gain with sporadic statistically significant differences to control animals. The differences consisted of lower as well as higher body weight and body weight gain. Because of the isolated occurrence of these findings and a lack of dose-response relationship, all these deviations were assessed being incidental in nature and not treatment-related.

Table 5.5-19: 18 months carcinogenicity mice- mean body weight and body weight gain

Day	Parameter	Dose level [ppm]									
		Males					Females				
		0	80	400	2000	8000	0	80	400	2000	8000
0	BW [g]	21.2	21.3	21.2	21.2	21.2	18.1	18.0	18.0	18.2	18.0
	SD	± 0.8	± 0.8	± 0.8	± 0.7	± 0.6	± 0.8	± 0.7	± 0.7	± 0.7	± 0.8
	(% of control)	(100)	(100.4)	(100.0)	(100.2)	(100.0)	(100)	(99.6)	(99.7)	(100.5)	(99.8)
14	BW [g]	22.8	**22.2****	22.5	**22.0****	**22.1****	19.2	18.8	**18.7***	**18.7***	18.9
	SD	± 0.9	± 1.2	± 1.1	± 1.3	± 1.1	± 0.9	± 1.1	± 1.0	± 1.0	± 1.0
	(% of control)	(100)	(97.0)	(98.4)	(96.3)	(96.8)	(100)	(98.2)	(97.2)	(97.3)	(98.2)
	Δ [g]	1.7	**0.9****	1.3	**0.8****	**1.0****	1.1	0.8	**0.6****	**0.5****	0.8
	SD	± 0.7	± 1.1	± 1.0	± 1.1	± 1.0	± 0.6	± 0.9	± 0.7	± 0.9	± 0.8
	(% of control)	(100)	(53.9)	(77.3)	(46.7)	(56.7)	(100)	(74.6)	(57.7)	(45.6)	(72.4)
84	BW [g]	28.6	28.0	**27.2****	**27.3****	**26.9****	22.3	22.0	**21.6****	22.2	22.1
	SD	± 2.0	± 1.8	± 2.5	± 1.7	± 1.4	± 1.1	± 1.0	± 1.1	± 1.0	± 1.1
	(% of control)	(100)	(98.1)	(95.2)	(95.7)	(94.3)	(100)	(98.5)	(96.8)	(99.4)	(98.9)
	Δ [g]	7.4	6.8	**6.0****	**6.1****	**5.8****	4.2	4.0	**3.6****	4.0	4.0
	SD	± 1.8	± 1.6	± 2.3	± 1.4	± 1.2	± 0.8	± 0.8	± 0.9	± 0.9	± 0.9
	(% of control)	(100)	(91.6)	(81.2)	(82.6)	(77.9)	(100)	(93.7)	(84.4)	(94.9)	(95.1)
147	BW [g]	30.7	29.9	29.7	**29.3****	**28.6****	23.3	**22.3****	**22.3****	22.9	22.7
	SD	± 2.6	± 2.3	± 2.6	± 2.5	± 1.8	± 1.6	± 1.5	± 1.5	± 1.5	± 1.5
	(% of control)	(100)	(97.4)	(96.6)	(95.3)	(93.1)	(100)	(95.8)	(95.4)	(98.4)	(97.4)
	Δ [g]	9.5	8.6	**8.5***	**8.0****	**7.4****	5.2	**4.3****	**4.2****	4.8	4.7
	SD	± 2.5	± 2.2	± 2.2	± 2.1	± 1.6	± 1.2	± 1.4	± 1.3	± 1.3	± 1.5
	(% of control)	(100)	(90.6)	(89.0)	(84.4)	(77.7)	(100)	(82.5)	(80.7)	(91.1)	(89.0)
259	BW [g]	32.5	31.7	31.6	**31.0***	**30.5****	24.9	**24.1***	**23.9****	24.7	**24.0***
	SD	± 3.2	± 3.1	± 3.0	± 3.1	± 2.6	± 1.7	± 1.4	± 1.4	± 2.0	± 1.4
	(% of control)	(100)	(97.5)	(97.2)	(95.3)	(93.9)	(100)	(96.7)	(96.0)	(99.1)	(96.5)
	Δ [g]	11.3	10.4	10.4	9.7*	9.3**	6.8	**6.1***	**5.9****	6.5	**6.0****
	SD	± 3.1	± 2.9	± 2.8	± 2.8	± 2.4	± 1.5	± 1.2	± 1.2	± 1.7	± 1.3
	(% of control)	(100)	(92.0)	(91.8)	(86.0)	(82.4)	(100)	(88.8)	(86.1)	(95.5)	(87.6)
427	BW [g]	33.2	31.6	**30.8****	**30.3****	**29.7****	26.5	**24.8****	**24.8****	25.9	**24.5****
	SD	± 3.9	± 3.3	± 3.4	± 3.6	± 2.6	± 2.6	± 2.3	± 2.2	± 3.0	± 1.9
	(% of control)	(100)	(95.0)	(92.6)	(91.2)	(89.4)	(100)	(93.6)	(93.6)	(97.8)	(92.4)
	Δ [g]	12.1	**10.3***	**9.6****	**9.1****	**8.5****	8.4	**6.8****	**6.8****	7.7	**6.4****
	SD	± 3.7	± 3.1	± 3.1	± 3.2	± 2.6	± 2.2	± 2.2	± 2.0	± 2.6	± 1.9
	(% of control)	(100)	(85.3)	(79.8)	(75.3)	(70.8)	(100)	(80.7)	(80.6)	(92.0)	(76.4)
546	BW [g]	34.4	33.8	**32.6***	**32.1****	**31.3****	28.0	27.6	27.1	28.0	**25.9****
	SD	± 4.1	± 3.8	± 3.9	± 3.3	± 2.5	± 3.1	± 2.6	± 3.0	± 3.6	± 2.8
	(% of control)	(100)	(98.1)	(94.6)	(93.2)	(90.8)	(100)	(98.6)	(96.9)	(99.9)	(92.6)
	Δ [g]	13.3	12.5	**11.5***	**10.9***	**10.1****	9.9	9.6	9.1	9.8	**7.9****
	SD	± 3.9	± 3.5	± 3.7	± 2.9	± 2.3	± 2.9	± 2.5	± 2.7	± 3.3	± 2.7
	(% of control)	(100)	(94.0)	(86.4)	(81.9)	(76.2)	(100)	(97.1)	(92.0)	(99.2)	(79.5)

BW = body weight; Δ = body weight gain

* = $p \leq 0.05$; ** = $p \leq 0.01$

D. FOOD CONSUMPTION, FOOD EFFICIENCY AND COMPOUND INTAKE

Regarding food consumption and efficiency, several statistically significant increases as well as decreases in animals of both sexes were noticed in the course of the treatment. However, due to the isolated occurrence or lack of dose-response relationship, these deviations were assessed as being incidental and not related to the treatment.

The approximate, mean daily test substance intake during the administration period is summarised in the Table 5.5-20 below. In order to assure equally spaced intervals for calculation of the mean test substance intake, only the values of days 7, 35, 63, 91 and days 119 to 539 were taken into account. During the study some spilling of food by the mice occurred in all groups which adds to the approximate nature of these calculations of test substance intake per day.

Table 5.5-20: 18 months carcinogenicity mice- substance intake

Substance intake	Dose level [ppm]									
	Males					Females				
	0	80	400	2000	8000	0	80	400	2000	8000
[mg/kg bw/day]	-	13	65	331	1345	-	18	90	443	1804

E. CLINICAL PATHOLOGY

1. Haematology

One year after administration start and at the end of the study, no treatment-related changes were observed in the leukocyte differential count as well as in erythrocyte morphology animals of both sexes administered Boscalid at the 8000 ppm dose level.

For animals which were killed in extremis changes in white and red blood cells were observed in peripheral blood of animals of both sexes in the dose groups as well as in control group animals. Thus, the observed changes were considered to be spontaneous, incidental and/or age-related. Accordingly, these findings were not assessed being treatment-related.

F. NECROPSY

1. Organ weight

Male animals administered the dose levels of 400 - 8000 ppm showed a statistically significant and dose-dependent increase in relative **liver** weight of 5.3 - 28.2%. Furthermore, top-dose males showed statistically significant increase in absolute liver weight of 16.3% as compared to the control males. Females treated with 2000 and 8000 ppm Boscalid in the food showed a statistically significant and dose-dependent increase in absolute and relative liver weights. This liver effect was assessed being treatment-related.

At the 2000 and 8000 ppm dose levels , male mice showed statistically significant reduction of absolute **kidney** weights of 7.3% and 12.4% as compared with the controls, respectively. Relative kidney weights were not affected. As no correlates were observed by macroscopy and microscopy examinations, this effect was considered to be incidental in nature and not treatment related. At the 400 and 8000 ppm dose levels, female mice showed statistically significant reduction of absolute kidney weights of about 4.5% as compared with the controls. As no correlates were observed by macro- and microscopy, this effect was considered to be incidental in nature and not treatment related. Relative kidney weights were not affected in females.

Males at all dose levels showed a statistically significant increase in relative t**estes** weights. This effect was, however, did not show a clear dose-response relationship and there were no differences in absolute testes weights. This finding has thus been associated with the slightly lower body weights and this conclusion has been confirmed by the absence of correlates in the macroscopic and microscopic examination of the organ. This effect was considered to be incidental in nature and not treatment related.

Relative **brain** weight was statistically significantly increased in males from the dose level of 400 ppm onwards and in females at the 8000 ppm dose level.

However, the absolute brain weight in all treatment groups were comparable with the control and no corroborating findings were observed in further macroscopic and histopathological evaluation. This effect was assessed being not treatment-related.

All males showed a statistically significant increase of absolute and relative **adrenal** weights. However, this has been associated with the fact that both, the absolute and relative adrenal weights in control animals were the lowest in the set of historical control animals (Table below) since all absolute as well as relative adrenal weight values were within the range of historical control data and the effect was devoid of a dose response. This effect was assessed to be incidental in nature and not treatment-related.

Table 5.5-21: 18 months carcinogenicity mice - summary of absolute (mg) and relative (% of terminal body weight) organ weights

Parameter	Sex	Dose level (ppm)				
		0	80	400	2000	8000
Terminal body weight (g)	M	31.71	31.05	**29.78***	**29.11****	**28.64****
	F	25.09	24.50	24.22	25.05	**23.45****
Absolute liver weight (mg)	M	1260.04	1235.09	1245.70	1329.14	**1465.47****
	F	1166.65	1251.41	1150.35	**1261.00****	**1289.25****
Relative liver weight	M	3.99	4.00	**4.20****	**4.57****	**5.12****
	F	4.68	5.11	4.77	**5.06****	**5.52****
Absolute kidney weight (mg)	M	463.85	457.31	446.33	**430.05****	**406.17****
	F	404.22	398.94	**386.40****	402.88	**386.16****
Relative kidney weight	M	1.48	1.49	1.51	1.49	1.42
	F	1.63	1.64	1.61	1.63	1.66
Absolute testes weight (mg)	M	209.68	220.378	213.47	220.33	215.36
Relative testes weight	M	0.67	**0.72***	**0.72***	**0.76****	**0.76****
Absolute brain weight (mg)	M	480.30	483.80	483.83	482.45	483.32
	F	488.28	490.20	488.54	490.85	483.67
Relative brain weight	M	1.54	1.59	**1.65****	**1.67****	**1.70****
	F	1.97	2.02	2.04	1.99	**2.08****
Absolute adrenal weight (mg)	M	3.53	**4.02****	**4.13****	**4.05****	**4.15****
	F	8.67	8.41	8.58	8.75	9.25
Historical control	M	**5.02 (3.53 - 6.50)**				
	F	**9.68 (8.67 - 11.64)**				
Relative adrenal weight	M	0.011	**0.013****	**0.014****	**0.014****	**0.015****
	F	0.035	0.035	0.036	0.035	**0.04****
Historical control[#]	M	**0.017 (0.011 - 0.024)**				
	F	**0.037 (0.030 - 0.044)**				

*: $p \leq 0.05$; **: $p \leq 0.01$; (Kruskal-Wallis, one sided and Wilcoxon-test, two-sided);

[#] in brackets the range of the historical control data is given

2. Gross lesions

Macroscopic evaluations revealed no substance-related findings in treated animals of both sexes.

All other gross lesions, including the macroscopically diagnosed “masses”, occurred either as isolated single incidences or were equally distributed between all groups. Nevertheless, paying particular attention to the target organ liver and the organs of the endocrine system as well as lung, the incidences noticed are summarised below.

Table 5.5-22: 18 months carcinogenicity mice - macroscopic findings in liver, lung and organs of the endocrine system

Sex	**Male**					**Female**				
Dose (ppm)	**0**	**80**	**400**	**2000**	**8000**	**0**	**100**	**400**	**2000**	**8000**
Animals examined	**50**	**50**	**50**	**50**	**50**	**50**	**50**	**50**	**50**	**50**
Liver										
- discoloration	-	1	-	1	-	-	1	-	-	-
- enlarged	-	-	-	-	1	2	1	1	1	-
- focus	8	3	6	4	1	3	5	3	11	4
- granular surface	-	-	1	-	-	1	-	-	2	2
- mass	-	2	1	-	-	2	2	3	-	1
- reduced size	-	-	-	-	-	-	-	-	1	-
- prominent acinar pattern	-	-	1	-	-	-	-	-	-	-
- retraction	1	-	-	-	-	-	-	-	-	-
- torsion	1	-	-	-	2	-	-	1	2	1
Lung										
- (broncho)pneumonia	-	-	-	-	-	1	-	-	-	-
- atelectasis	-	-	-	-	-	-	1	-	-	-
- discoloration	-	1	-	-	-	-	-	1	1	-
- focus	4	1	-	-	1	3	1	1	-	3
- mass	-	4	-	-	1	-	1	1	2	-
Kidneys	50	50	50	50	50	50	50	50	50	50
- cyst	2	3	2	1	1	-	-	-	1	-
- granular surface	8	4	5	-	3	1	-	-	1	1
- hydronephrosis	-	-	1	-	-	-	-	-	-	-
- retraction	9	7	8	5	7	1	4	4	12	7
- deformation	-	-	-	-	-	-	-	-	-	1
- discoloration	-	-	-	-	-	1	-	-	-	2
- focus	-	-	-	-	-	-	1	-	-	-
- mass	-	-	-	-	-	-	-	-	-	-
- pelvic dilation	-	-	-	-	-	-	-	-	-	1
Testes										
- calcification	-	-	-	1	-					
- enlarged	1	-	-	1	-					
- focus	-	-	-	1	-					
- reduced size	1	-	-	1	-					

Sex	Male					Female				
Dose (ppm)	**0**	**80**	**400**	**2000**	**8000**	**0**	**100**	**400**	**2000**	**8000**
Animals examined	**50**	**50**	**50**	**50**	**50**	**50**	**50**	**50**	**50**	**50**
Epididymides										
- abscess	-	1	-	-	-					
- focus	-	-	-	-	1					
Seminal vesicle										
- discoloration	1	1	1	2	-					
- enlarged	40	39	40	36	39					
- focus	-	-	-	1	1					
- induration	-	1	2	-	-					
- mass	-	-	1	-	-					
- necrosis	-	-	-	-	1					
- reduced size	-	-	1	2	1					
- rupture	-	-	-	1	-					
Coagulation gland										
- necrosis	-	-	-	-	1					
Prostate										
- discoloration	1	-	-	-	-					
Ovaries										
- cyst						2	5	5	6	2
- discoloration						-	-	3	-	1
- enlarged						-	-	-	1	-
- focus						17	14	8	6	8
Oviduct										
- cyst						1	1	-	-	-
Uterus										
- cyst						13	12	16	17	14
- dilatation						-	2	2	1	1
- focus						-	-	1	3	4
- mass						1	-	1	1	-
Vagina										
- mass						-	-	1	-	-
Mammary gland										
- mass						-	1	-	-	-
Pituitary gland										
- cyst	1	-	-	-	-	-	-	-	1	1
- enlarged	-	-	-	-	-	2	1	1	2	-
- focus	2	-	1	-	-	14	9	10	17	13
- mass	-	-	-	-	-	3	4	3	2	3

3. Histopathology

Neoplastic findings

In the **lung**, adenomas were observed in males at the incidence of 6/3/0/2/0 for males and 1/0/0/0/1 for females at dose levels of 0, 80, 400, 2000, 8000 ppm. Carcinoma were observed in males at the incidence of 2/4/0/0/0 for males and 0/0/0/1/0 for females at dose levels of 0, 80, 400, 2000, 8000 ppm. [see Table 5.5-23]. Since the incidences were comparable to those of the controls or were isolated, the neoplastic lung findings were assessed to be not treatment-related.

All other neoplastic findings occurred also either singly or were biologically equally distributed over the control and treatment groups.

The number of males with neoplasms, with one primary neoplasm, with benign or malignant neoplasms, as well as the total number of primary, benign, and malignant neoplasms were slightly decreased in the top-dose group, as compared with the control group. The number of males with two and more primary neoplasms and with systemic neoplasms, as well as the total number of systemic neoplasms were comparable between the control and the top-dose groups. The decreased numbers of neoplasms in males was considered to be incidental in nature.

The number of females with neoplasms, with one and with two and more primary neoplasm, with benign, malignant, and systemic neoplasms, as well as the total number of primary, benign, malignant, and systemic neoplasms were comparable between the control and the top-dose groups.

Non-neoplastic findings

In the **liver**, histopathological examinations revealed a minimal or slight peripheral hypertrophy of hepatocytes in females of the 2000 ppm dose level and in animals of both sexes of the 8000 ppm dose level. Furthermore, a slight enlargement of hepatocytes in the periportal (peripheral) zones of the liver was observed. The cytoplasm of these liver cells stained eosinophilic and appeared delicate granular.

Additionally, a shift from diffuse to centrilobular fatty infiltration of hepatocytes with correlating peripheral hypertrophy (more pronounced at the 2000 ppm dose level) was observed in females at dose levels from 800 ppm onwards.

Furthermore, the incidences of minimal or slight oval cell proliferation was increased in top-dose females, however without dose-response relationship. Oval cell proliferation was graded minimal or slight in most affected animals with few or small areas only.

These findings were in line with liver weight effects in females of the in 2000 ppm dose level and the top-dose animals of both sexes and were assessed as being treatment-related.

All other findings in the liver were of single or low incidences or did not reveal biologically relevant differences as compared with the control animals. Therefore these lesions were considered being incidental or spontaneously in nature and not treatment-related.

In **adrenal glands**, decreased incidences of focal atrophy in the adrenal cortex in the top-dose males were noticed, and was considered to be a toxic effect. Females had no finding of this type. The observed increase of the adrenal weights in males (absolute and relative weight) and top-dose females (relative weight) were not corroborated by any macroscopic and microscopic finding and thus was assessed to be not treatment-related.

The observed changes in **kidney and brain** weight as well as **testes** weight in males were not corroborated by any macroscopic and microscopic finding and thus were assessed to be not treatment-related.

The occurrence of focal or **multifocal lymphoid cell infiltrations** in liver, gall bladder, epdidymides and prostate were assessed being incidental in nature, as the incidence of this finding varied between control and top-dose animals and was inconsistent, since the number of affected animals was partly higher in controls, partly in the top-dose mice.

Evaluation on decedents did not reveal any histopathological findings that may be attributed to the earlier death. Mostly, erosions or ulcers in the glandular stomach, inflammatory lesions, haemorrhages, and different types of tumours in various organs were noticed.

All other morphology changes detected did show either a single occurrence or no dose-response relationship and were seen to be of incidental or spontaneous nature and were not induced by the test substance. Nevertheless, paying particular attention to the target organ liver and the organs of the endocrine system as well as the lung, the incidences noticed are additionally summarised in table below.

Table 5.5-23: 18 months carcinogenicity mice - microscopic neoplastic and non-neoplastic findings in liver, gall bladder, lung and organs of the endocrine system

Sex	Male					Female				
Dose (ppm)	**0**	**80**	**400**	**2000**	**8000**	**0**	**80**	**400**	**2000**	**8000**
Liver	50	50	50	50	50	50	50	50	50	50
- lymphoid infiltation	43	35	36	34	34	37	39	38	43	45
- inclusions	-	-	-	-	-	-	1	-	-	-
- fatty infiltr. focal	9	1	6	4	2	11	3	3	6	2
- fatty infiltr. centr.	38	39	39	41	37	10	11	25	25	37
- fatty infiltr. diff.	10	7	7	3	10	35	35	22	23	10
- amyloidosis	-	-	1	-	-	-	-	-	-	-
- necrosis, focal	4	1	1	-	1	1	1	1	4	1
- pigment storage	1	2	3	2	6	11	2	5	10	11
- cyst (s), biliary	-	-	-	-	-	-	1	-	-	-
- oval cell proliferation	4	2	2	-	6	7	3	2	6	15
- bile duct proliferation	-	-	-	-	1	-	-	-	-	-
- remodeling	-	-	-	-	-	-	-	-	-	1
- hypertrophy, periph.	-	-	-	-	29	-	-	-	10	45
- hypertrophy, central	1	-	-	-	1	-	-	-	-	-
- cellular alterations	2	2	1	2	4	-	2	1	2	1
- basophilic foci	2	1	-	2	3	-	1	-	2	-
- eosinophilic foci	1	1	1	-	1	-	1	1	-	1
- focus, not specified	-	-	-	-	1	-	-	-	-	-
- adenoma hepatocell.	-	*1*	-	-	-	*1*	-	-	-	*2*
- hemangioma	-	-	-	-	-	-	-	*1*	-	-
- carcinoma hepatocell.	-	-	*1*	-	-	-	-	-	-	-
Gall bladder	50	7	4	7	50	50	5	2	20	49
- lymphoid infiltration	10	-	1	-	18	7	-	-	-	8
- intramural cyst		-	-	-	-		1	-	-	-
- hypertrophy, focal	-	-	-	-	-		-	-	-	1
Lung	50	50	50	50	50	50	50	50	50	50
- lymphoid infiltr.	7	6	6	1	4	11	14	10	11	11
- calcification, focal	-	-	-	-	1	-	-	-	-	-
- eosinophilic crystal	1	1	-	-	1	1	3	2	3	1
- pneumonia	-	1	-	-	2	-	-	-	-	-
- alv. histiocytosis	2	-	-	1	2	2	1	2	3	2
- hyperplasia, bronch.	1	-	-	2	-	-	-	2	-	1
- adenoma, bnonch.-alv.	*6*	*3*	-	*2*	-	*1*	-	-	-	*1*
- carcinoma, bronch.-alv.	*2*	*4*	-	-	-	-	-	-	*1*	-

Sex	**Male**					**Female**				
Dose (ppm)	**0**	**80**	**400**	**2000**	**8000**	**0**	**80**	**400**	**2000**	**8000**
Kidneys	50	50	50	50	50	50	50	50	50	50
- cyst	3	4	2	1	2	-	1	-	-	-
- lymphoid infiltration	44	39	44	36	41	41	40	39	39	39
- hydronephrosis	-	-	1	-	-	-	-	-	-	-
- arteritis	-	-	1	1	-	-	-	-	-	-
- osseus metaplasia	-	1	-	-	-	2	-	1	2	1
- calcification focal	2	1	1	-	-	2	-	-	1	2
- necrosis, papilla	-	-	1	-	-	-	-	-	-	-
- amyloidosis	2	1	5	1	1	3	2	4	4	4
- pelvic dilation	-	-	-	1	2	-	-	-	-	1
- nephropathy, chronic	44	42	36	39	48	31	39	41	42	31
- double pelvis	-	-	-	-	-	-	1	1	-	1
- hemorrahage, focal	-	-	-	-	-	-	1	-	-	-
- storage nephrosis	-	-	-	-	-	-	2	2	-	-
- hemangiosarcoma	-	-	-	-	-	-	-	-	1	-
Testes	50	5	4	12	50					
- calcification, tubul.	1	-	-	-	-					
- tub. dilation, diff.	-	-	-	1	-					
- giant cells	-	-	-	-	1					
- degeneration focal	25	1	2	4	26					
- spermatocele	-	-	-	1	-					
- hyperplas. Leydig, focal	1	-	-	-	-					
- hyperplas. Leydig diff.	5	-	-	1	-					
Epididymides	50	6	4	8	50					
- lymphoid infiltration	20	-	1	1	10					
- loss of sperm	1	1	-	-	2					
- spermatocele	-	1	-	-	2					
Seminal vesicle	50	43	43	40	50					
- dilatation	27	31	33	28	26					
- atrophy	-	-	1	3	1					
- rupture	-	-	-	-	2					
- haemorrhage, focal	-	-	-	-	1					
- amyloidosis	-	-	3	1	-					
- inflammation	5	1	-	-	-					
- hyperplasia, focal	1	-	-	-	-					
Coagulation gland					1					
- haemorrhage	-	-	-	-	1					
Prostate	50	5	4	8	50					
- lymphoid infiltration	12	-	-	1	6					
- haemorrhage	2	-	-	-	-					
Ovaries						50	22	18	13	50
- cyst (s)						16	15	7	9	13
- bursa ovarica cystic						-	1	1	-	-
- atrophy						14	4	4	4	16
- angiectasis						-	-	-	-	1
- epithelial hyperpl.						2	-	-	-	1
- hyperplasia, cystic						-	-	-	-	1
- *cystadenoma*						-	-	*1*	-	-
- *hemangioma*						-	-	*1*	-	-

Sex	Male					Female				
Dose (ppm)	**0**	**80**	**400**	**2000**	**8000**	**0**	**80**	**400**	**2000**	**8000**
Oviduct						50	20	18	14	50
- cyst (s)						4	1	-	1	-
- papilloma						-	-	-	-	*1*
Uterus						50	17	22	22	50
- inflammation						1	-	-	-	1
- dilatation						-	3	2	2	-
- endometrial hyperplas.						42	14	18	19	47
- hyperplasia squam.						1	-	-	-	-
- fat storing macroph.						-	-	-	1	1
- polyp stromal						*1*	-	-	-	-
- polyp glandular						*1*	-	-	-	-
- leiomyoma						-	-	-	-	*1*
Vagina						50	8	12	8	50
- hyperplasia, squam.						2	-	-	-	-
- adenoma, sebaceous						-	-	*1*	-	-
Mammary gland						50	4	1	2	50
- leiomyosarcoma						-	*1*	-	-	-
Adrenal cortex	50	5	4	8	50	50	4	2	2	50
- accessory adr. tissue	1	-	-	2	1	2	-	1	-	2
- lipogenic pigment	38	1	2	1	41	48	2	-	2	50
- atrophy, focal	33	-	2	-	4	-	-	-	-	-
- hypertrophy focal	10	1	-	2	7	1	-	-	-	1
- hyperplasia subcap. foc.	3	-	1	-	4	5	-	-	-	-
- hyperplasia subcap. diff.	-	-	-	-	-	1	-	-	-	-
- hyperplasia, focal	4	-	-	-	-	-	-	-	-	-
- adenoma	*1*	-	-	-	-	-	-	-	-	-
Adrenal medulla	50	5	4	8	50	50	4	2	2	50
- hyperplasia focal	-	1	-	-	1	-	-	-	-	-
Thyroid gland	50	5	4	8	50	50	4	2	2	50
- lymphoid infiltr.	1	-	-	-	2	-	-	-	-	4
- arteritis	1	-	-	-	-	-	-	-	1	-
- ectopic thymic tissue	-	-	-	-	-	1	2	-	-	-
- cyst (s)	-	-	-	-	1	1	-	-	-	-
- hyperpl. foll. focal	-	-	-	-	-	-	-	-	-	2
Parathyroid gland	49	5	4	8	49	46	4	1	2	48
- cyst (s)	2	-	-	-	1	-	-	-	-	1
- amyloidosis	-	-	-	-	-	-	-	-	-	1
Pituitary gland	49	4	5	7	49	50	18	15	23	49
- cyst (s), p. distalis	17	1	2	3	13	5	1	-	1	3
- hyperpl. p. distalis	2	-	-	-	1	15	6	3	6	19
- adenoma p.distalis	*1*	-	-	-	1	*16*	*8*	*12*	*17*	*15*
- adenoma. p. interm.	-	-	-	-	-	*1*	-	-	-	-

III. CONCLUSIONS

The oral administration of Boscalid to C57BL mice at dose levels of 80, 400, 2000 and 8000 ppm in the food induced substance-related effects from 400 ppm onwards (i.e. impairment of the body weight and body weight gain). The liver was the target organ with increase in absolute and/or relative weights at dose levels $\geq$ 400 ppm. Histopathological evaluations showed increased incidence of periportal hypertrophy of hepatocytes at dose levels $\geq$ 2000 ppm in female mice.

The administration of 80 ppm was tolerated by the male and female mice without any changes that could be attributed to the-treatment.

Boscalid was not carcinogenic to mice. Based on this findings, the no observed adverse effect level (NOAEL) for C57BL mice under the conditions of the study was 80 ppm which is equivalent to the substance intake of 13 mg/kg bw/day and 18 mg/kg bw/day for males and females, respectively.

CA 5.6 Reproductive Toxicity

All studies in M-CA 5.6 have already been peer-reviewed by the Rapporteur Member State Germany and national competent authorities following submission of the original dossier for Annex I inclusion (2000):

An adequate set of reproductive toxicity and prenatal developmental toxicity studies has been evaluated and has been considered acceptable. The endpoints were fixed in the European Commission Review Report for the active substance Boscalid (SANCO/3919/2007 –Rev 5, 21 January 2008).
For the reviewer's convenience, these studies are summarised below following the Monograph (2002), and the tabulated summary is provided in Table 5.6-1. The results of the studies have been summarised in more detail as compared to the Monograph to assist the classification and labelling of Boscalid and evaluation due in chapter M-CA 5.8.3. Where the evaluation of the applicants comes to deviating conclusions as previously drawn in the Monograph this has been duly marked in this evaluation.

Table 5.6-1: Summary of reproductive toxicity and prenatal toxicity studies performed with Boscalid

Study	NOAEL mg/kg bw/day	LOAEL mg/kg bw/day	Main adverse effect	Reference BASF DocID
2-generation study in Wistar rats 0, 100, 1000 & 10000 ppm	Parental toxicity 11* (100 ppm)	113* (1000 ppm)	≥1000 ppm: Increased incidence in hepatocellular hypertrophy 10000 ppm: Increase in liver weight and hepatocellular degeneration; reduced body weight gain and feed intake	[redacted] et al 2001/1000117
	Fertility 1165* (10000 ppm)	-	No adverse effects observed	
	Offspring toxicity **113**** (1000 ppm)	1165** (10000 ppm)	10000 ppm: Reduced body weight gain 10000 ppm: Increase in male F2 pup mortality during days 0-4 p.p.	
Prenatal developmental toxicity in Wistar rats 0, 100, 300 & 1000 mg/kg bw	Maternal toxicity 1000	-	No adverse effects observed	[redacted] and [redacted] 2000/1015001
	Developmental toxicity **1000****	-	No adverse effects observed **Increased incidence of incomplete ossification of the thoracic centrum not test substance mediated****	
Prenatal developmental toxicity in Himalayan rabbits 0, 100, 300 & 1000 mg/kg bw	Maternal toxicity 100	300	300 mg/kg bw/day: 1 doe with abortion & reduced / discoloured faeces 1000 mg/kg bw/day: 3 does with abortion; reduced feed intake, body weight & body weight gain	[redacted] and [redacted] 2000/1013425
	Developmental toxicity 300	1000	1000 mg/kg bw/day: Increased incidence of incomplete ossification of the thoracic centrum	

* Values [mg/kg bw/day] adjusted according to actual feed intake data, refer to Addendum 2 (24 May 2006) to the Draft Assessment Report (= Monograph of 8 November 2002)
**** Conclusion of the applicant deviating from previous conclusions at EU level**

2-generation reproduction toxicity study in Wistar rats

Boscalid had no adverse effects on the reproductive performance or fertility of the F0 and F1 parental animals in dose groups up to the top dose level of 10000 ppm.

NOTE: In the initial evaluation as laid down in the Monograph (2002), the conversion of the feed's test substance concentration into test substance intake (in terms of mg/kg bw/day) was done based on the JMPR default factor of 15. Following further consultation at EU level this approach was changed by finally applying the measured mean feed consumption data of the study which concluded that 10000 ppm is corresponding to (at least) 1165 mg/kg bw/day (see Addendum 2 to the DAR of May 26, 2006). The corresponding conversions of the lower dose levels were 113 mg/kg bw/day for 1000 ppm and 11 mg/kg bw/day for 100 ppm test substance concentration in the feed. The NOAEL for fertility was concluded to be 1165 mg/kg bw/day (10000 ppm dose group) based on mating and fertility indices for F0 and F1 parental females and sperm parameters of F0 and F1 parental males.

Signs of general toxicity/systemic effects occurred in both parental generations at 1000 and 10000 ppm. At the dose level of 10000 ppm reduced body weight and body weight gain was observed during parts of the study. Liver weight was statistically increased from 1000 ppm onward with corresponding dose-related histopathological changes in both parental generations with increasing incidence and severity of centrilobular hypertrophy and hepatocyte degeneration. The NOAEL proposed for parental toxicity is 100 ppm corresponding to 11 mg/kg bw/day.

Substance related signs of toxicity in the offspring were seen at 1000 ppm and 10000 ppm.
At the dose level of 10000 ppm a slightly increased perinatal mortality was seen in F2 litters (viability index: 86%) and body weight (gain) was reduced in both F1 and F2 litters. Mean body weight (gain) was also reduced in male F2 pups of the 1000 ppm dose group. However, more detailed evaluation by the applicant within this renewal process suggests that this effect is linked to the extraordinary high number of pups and concomitant low pup weights in a single dam. Additional covariance analysis with inclusion of the litter size as co-variable provides sufficient evidence this finding to be not test substance related. Organ weight changes in pups at the top dose level were considered to be secondary to the reduced body weight. Thymus weight (absolute and relative) and spleen weight (absolute) were reduced in F2 pups at lower dose levels with no clear dose response and without similar findings in F1 pups. In the absence of a histopathological correlate in the spleen of the parental generation the reduction of spleen weight is considered to be related to reduced body weight rather than a specific test substance related effect. Further information providing evidence of the findings in thyroids and thymus to be not a boscalid mediated effect of specific immunotoxicity has been presented in M-CA 5.8.2. All other findings in absolute and/or relative F2 pup organ weight were assessed as incidental and without toxicological significance.

The NOAEL for offspring was previously set at 11 mg/kg bw/day (100 ppm) as a conservative approach based on reduced body weight and body weight gain of F2 male pups at the 1000 ppm dose level. The previous conclusion is not any more supported and the new evaluation supports the NOAEL at 1000 ppm (113 mg/kg bw/day) instead.

Developmental toxicity study in rats

In the developmental toxicity study in rats the NOAEL for maternal toxicity was 1000 mg/kg bw/day. The NOAEL for developmental toxicity was 1000 mg/kg bw/day according to the revised evaluation of the applicant. Incomplete ossification of the thoracic centrum was observed at the highest dose tested (1000 mg/kg bw/day) in the absence of overt maternal toxicity, however, with this effect being in the range of the revised historical control data. The effect was also devoid of a dose response relationship. More detailed discussion on the relevance of developmental findings notably the incomplete ossification of the thoracic centrum is presented with regard to the applicability of the CLP criteria below. There was no evidence of malformations up to the highest dose tested.

Developmental toxicity study in rabbits

In the rabbit developmental toxicity study, incomplete ossification of the thoracic centrum was also observed at significantly increased litter incidence and for affected foetuses per litter at the highest dose level (1000 mg/kg bw/day). At this dose level, there was overt maternal toxicity (clinical signs, reduced body weight and body weight gain, increased incidence of abortion). At 300 mg/kg bw there was still one finding of abortion and reduced/discoloured faeces. Thus the NOAEL for maternal toxicity was set at 100 mg/kg bw/day and for developmental toxicity was set at 300 mg/kg bw/day, respectively. More detailed discussion on the relevance of developmental findings, notably the incomplete ossification of the thoracic centrum is presented with regard to the applicability of the CLP criteria below. There was no evidence of malformations up to the highest dose tested.

NOTE: In the review process of Boscalid the effects in both prenatal developmental toxicity studies were in more detail discussed in Addendum 2 of May 26, 2006, section 2.3.4. The question raised was as to whether an acute reference dose should be set for Boscalid on the basis that a reduction in the ossification of vertebral structures (thoracic centra and to a lesser extent sacral arches) was seen at the dose of 1000 mg/kg bw/day in both species. This finding has finally been considered to be of minor toxicological significance as such variations, although often indicative of embryo-foetal toxicity, represent only a few hours in relative development of foetuses at the time of sacrifice. Moreover, in the absence of malformations of the underlying cartilaginous structures no true adversity was associated with this type of change. This conclusion has also been supported by the new evaluation of findings in the rat.

Based on the available data on the reproductive toxicity, classification and labelling of Boscalid for this endpoint is not required according to the criteria laid down in Regulation (EU) No. 1272/2008 (CLP, see discussion on CLP criteria below). It has also been concluded in the review process for the first Annex I inclusion that Boscalid does not warrant classification and labelling according to reproductive health effects (as laid down in the European Commission Review Report for the active substance Boscalid (SANCO/3919/2007 –Rev 5, 21 January 2008)). The limited newly available screening data are not considered to affect these conclusions drawn for Boscalid.

Reproductive toxicity (Regulation (EU) N° 283/2013, Annex Part A, point 5.6)
In the light of all available information, the NOAEL values that have been derived from the studies and the conclusions for the relevant endpoints in regard to the current re-registration process are as summarized below:

Reproduction toxicity

Reproduction target / critical effect	**Parental toxicity**: Reduced body weight and body weight gain in parental animals identified as statistically significant in F1 males. Increase in liver weight with histo-pathological alterations. **Fertility**: No adverse effect observed **Offspring toxicity**: Pup body weight gain reduced in the presence of parental toxicity.	No classification See discussion on CLP criteria below
Relevant parental NOAEL	11 mg/kg bw/day	
Relevant reproductive NOAEL	1165 mg/kg bw/day	
Relevant offspring NOAEL	**113 mg/kg bw/day***	

*** New conclusion of the applicant deviating from previous conclusions at EU level**

Developmental toxicity

Developmental target / critical effect	**Rat**: <u>Maternal toxicity:</u> No overt adverse effects <u>Developmental toxicity:</u> No adverse effects observed. Increased incidence of delayed ossification of thoracic centrum at 1000 mg/kg bw/day not test substance mediated. **Rabbit**: <u>Maternal toxicity:</u> Reduced body weight and body weight gain, increased incidence of abortions. <u>Developmental toxicity:</u> Increased incidence of delayed ossification of thoracic centrum at 1000 mg/kg bw/day.	No classification See discussion on CLP criteria below
Relevant maternal NOAEL	**Rat**: 1000 mg/kg bw/day **Rabbit**: 100 mg/kg bw/day	
Relevant developmental NOAEL	**Rat**: **1000 mg/kg bw/day*** **Rabbit**: 300 mg/kg bw/day	

*** New conclusion of the applicant deviating from previous conclusions at EU level**

Comparison with CLP criteria

For the purpose of classification for reproductive toxicity according to the criteria of the CLP (Regulation 1272/2008/EC), substances are allocated to one of two categories. Within each category, effects on sexual function and fertility, and on development, are considered separately. In addition, effects on lactation are allocated to a separate hazard category.

Category 1: Known or presumed human reproductive toxicant
Substances are classified in Category 1 for reproductive toxicity when they are known to have produced an adverse effect on sexual function and fertility, or on development in humans or when there is evidence from animal studies, possibly supplemented with other information, to provide a strong presumption that the substance has the capacity to interfere with reproduction in humans. The classification of a substance is further distinguished on the basis of whether the evidence for classification is primarily from human data (Category 1A) or from animal data (Category 1B).

Category 1A: Known human reproductive toxicant
The classification of a substance in Category 1A is largely based on evidence from humans.

Category 1B: Presumed human reproductive toxicant
The classification of a substance in Category 1B is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects. However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in Category 2 may be more appropriate.

Category 2: Suspected human reproductive toxicant
Substances are classified in Category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development, and where the evidence is not sufficiently convincing to place the substance in Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification. Such effects shall have been observed in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects.

Evaluation of Boscalid with regard to CLP criteria

Category 1A: Known human reproductive toxicant

Conclusion: Boscalid is not a known human reproductive toxicant. Therefore, Category 1A does not apply to this active substance.

Category 1B: Presumed human reproductive toxicant
The classification of a substance in Category 1B is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects

In case of information on reproductive and/or developmental toxicity with inadequate evidence to justify Category 1B, alternatively Category 2 may be applicable as precautionary step.

Category 2: Suspected human reproductive toxicant
Substances are classified in Category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development, and where the evidence is not sufficiently convincing to place the substance in Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification. Such effects shall have been observed in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects.

Boscalid was tested in a 2-generation study in rats and developmental toxicity studies in rats and rabbits.

In the **2-generation study** the dose levels chosen were up to 10000 ppm in the feed administered to parental generations and the offspring (corresponding to 1165 mg/kg bw/day).
Signs of general toxicity/systemic effects occurred in both parental generations at 1000 and 10000 ppm. At the dose level of 10000 ppm reduced body weight and body weight gain was observed during parts of the study. Liver weight was statistically increased from 1000 ppm onward with corresponding dose-related histopathological changes in both parental generations with increasing incidence and severity of centrilobular hypertrophy and hepatocyte degeneration. Dose levels of 1000 ppm and 10000 ppm have thus been identified to elicit adverse effects in parental generations with the liver to be the primary target organ.

No adverse effects on reproductive organs have been observed that could be associated with the test substance administration in any sex and generation at any dose level. No adverse effects on male reproduction parameters such as mating index and fertility index were observed up to the top dose level. No changes of sperm parameters of statistical significance and/or biological relevance were identified which are considered to justify for classification of Boscalid as a reproductive toxicant. Female reproduction parameters such as mating index, fertility index, gestation index and life birth-index were not adversely affected by the treatment at any dose level. There were few isolated findings such as marginal reduction of gestation time, reduced number of implantation sites per dam and concomitant reduced number of pups delivered at lower dose levels. All these findings occurred in single generations only and did not evidence a dose-response relationship. At the top dose level of 10000 ppm marginal findings of reduced implantation sites per dam, post implantation loss per litter and reduced number of pups per dam were observed in individual generations with lacking evidence in the other generation. These findings would require interpretation in the light of maternal toxicity seen at the dose level of 10000 ppm (notably 1165 mg/kg bw/day) and have been considered as secondary effects. Survival was marginally but statistically significantly reduced at the 10000 ppm dose level in F2 offspring. This finding was still within the historical range of control animals in the test institute. Reduced body weight and body weight gain of the offspring at the 10000 ppm dose level (notably F2 offspring) as well as maternal findings (notably increased absolute and relative liver weight with corresponding histopathological alterations) are indicative of this dose level having elicited adverse effects for which the reduced viability index in F2 offspring at the top dose cannot be ruled out.

Sex ratios of male and female pups were not different when investigated at the day of birth and day 21 post-partum. Sexual maturation was not different over treatment groups as compared to control animals for both males and females on the basis of findings on preputial separation and vaginal opening.

Based on the findings in this 2-generation study in rats there is adequate evidence that Boscalid does not adversely affect the sexual function, fertility or development of the offspring. Marginal effects as discussed above need to be considered secondary to concomitant toxic effects seen in the parental generation and/or the offspring.

The same conclusion can be drawn from the developmental neurotoxicity study in Wistar rats (see MCA Section 5.7.1) performed with the same dose levels. Female reproductive performance was not affected. The fertility index was 80, 86, 86 and 94% for the control, 100, 1000 and 10000 ppm dose groups respectively. The duration of gestation was comparable with 21.8 days for control animals and gestation of 21.8, 21.6 and 21.7 days for the 100, 1000 and 10000 ppm dose groups. Gestation index was 100 % over all groups. The number of offspring, live and stillborn and viability did not show any treatment-related difference between control and treated animals. The life-birth index was thus comparable over all groups (98 - 100%). Offspring viability and postnatal mortality was not affected by the treatment. The sex distribution and sex ratio of live offspring on the day of birth and day 21 p.p. was unaffected. The mean body weight and body weight gain of the male and female offspring was consistently reduced in this study, a finding similar to that of the 2-generation study.
No adverse effects could be observed on the onset of sexual maturation as shown by vaginal opening and preputial separation.

Boscalid was tested in addition in prenatal toxicity studies in rats and rabbits. Boscalid did not cause malformations in the prenatal developmental toxicity study in Wistar rats and in Himalayan rabbits.

Prenatal toxicity study in rats: In foetuses of rats administered the limit dose of 1000 mg/kg bw/day during day 6-19 of gestation increased incidence of incomplete ossification of the thoracic centrum was observed as the only statistically significant finding. No other developmental effects to which biological relevance could be attributed were observed. The evaluation of the data within the first Annex I inclusion process came to the conclusion that the increased incidence of incomplete ossification of the thoracic centrum has been test substance mediated with the study findings being out of the historical control range. The latter conclusion was mainly based on the old terminology of historical control data applied at the time of data evaluation. New evaluation with consideration of the harmonized terminology allow the conclusion that the incidences of incomplete ossification of thoracic centrum (= “thoracic vertebral body/bodies incompletely ossified" according to the “old terminology”) vary between 1.4 – 9.7% if expressed on a foetus per litter basis as opposed to 1.8% -3.5% of the initial evaluation and are thus within historical control data. Therefore they do not support a test substance mediated effect if the overall findings including the evaluation of the overall rate of skeletal variations and the biological variability for rat skeletal variations in general are taken into consideration. The effect notably is devoid of a clear dose response with regard to the foetal and litter incidences as well as the percentage of affected foetuses per litter because the intermediate dose level of 300 mg/kg bw/day would be expected to exert some increase in these incidences, too. However, the incidences (foetal, litter and percentage of affected foetuses per litter) of the intermediate dose are factually lower than the control values. As supportive finding, the total rates of skeletal variations were similar between the controls and the treatment groups and did not show a dose-response relationship.

Prenatal toxicity study in rabbits: The administration of Boscalid during day 7-28 of gestation lead to distinct maternal toxicity as elicited by abortions occurring in 1 female at 300 mg/kg bw/day and in 3 females of the 1000 mg/kg bw/day dose group. One female at 1000 mg/kg bw/day delivered prematurely and was killed. Food consumption was markedly reduced at 1000 mg/kg bw/day (-26%) throughout the treatment period. Mean terminal body weight was statistically significantly reduced at this dose level and the body weight gain was markedly reduced (-81%). Boscalid did not cause malformations in the prenatal developmental toxicity study in rabbits. The only prominent finding was increased incidence of “incomplete ossification of the thoracic centrum” observed in foetuses of dams administered the limit dose of 1000 mg/kg bw/day. No other developmental effects of biological relevance were observed. The percentage of affected foetuses and the litter incidence was statistically identified to be different to controls. This finding has been reviewed in the light of the changes in terminology as previously explained for the rat. New evaluation with consideration of the harmonized terminology allow the conclusion that the incidences of “incomplete ossification of thoracic centrum” vary between 0.0 – 17.3% if expressed on a foetus per litter basis. Thus, the percentage of affected foetuses per litter (8.3%) is considered to be within the historical control data. This is considered indicative of the increased incidence of “incomplete ossification of thoracic centrum” to be not a direct biological effect of developmental toxicity mediated by the test substance administration.

The conclusion that the increased incidence of this variation is not a test substance specific effect is mainly supported by these aspects: Concomitant severe maternal toxicity suggesting these findings to be secondary to systemic maternal toxicity, and the findings being within the historical control range.

Summary evaluation: The 2-generation study in rats gave no indication of adverse effects on the sexual function and development. Therefore, Category 1B is not considered to apply nor are the findings considered to qualify for Category 2. In the prenatal toxicity studies performed in rats and rabbits no malformations were observed. The only prominent finding was incomplete ossification of the thoracic centrum. This variation occurred at statistically increased levels in both species at the limit dose of 1000 mg/kg bw/day. In both species this finding was within the historical control data of the test institute and can thus be considered to be within the normal range of biological variation of the test species. Besides, in the rat study this finding was devoid of a dose response with the next lower dose level of 300 mg/kg bw/day having an even lower incidence of this finding as the untreated control group in the study. Moreover, it needs to be taken into consideration that the overall incidence of variations gave no difference between control animals and the treatment groups including the limit dose of 1000 mg/kg bw/day. No corroborative findings at other parts of the vertebral column were evident which would support delayed ossification to be a relevant finding and thus the potential adversity of this finding. The adversity of incomplete ossification of the thoracic column has finally been discussed at EU level within the first Annex I inclusion process and considered to be of minor toxicological significance as such variations, although often indicative of embryo-foetal toxicity, represent only a few hours in relative development of foetuses at the time of sacrifice. Moreover, in the absence of malformations of the underlying cartilaginous structures no true adversity was associated with this type of change (see Addendum No 2 of May 26, 2006).

In the rabbit prenatal toxicity study the increased incidence of incomplete ossification of the thoracic centrum at 1000 mg/kg bw/day was in the presence of severe maternal toxicity and was also within the historical control data. In the lower dose group there was no evidence of this finding to be different to the control. In the overall conclusion, this finding in rats and rabbits has not been considered to qualify for Category 1B nor for Category 2.

Conclusion: Category 1B or Category 2 is not considered to apply to Boscalid

Conclusion on classification and labelling

As overall conclusion the active substance Boscalid does not qualify for classification with regard to reproductive toxicity according to Regulation 1272/2008/EC.

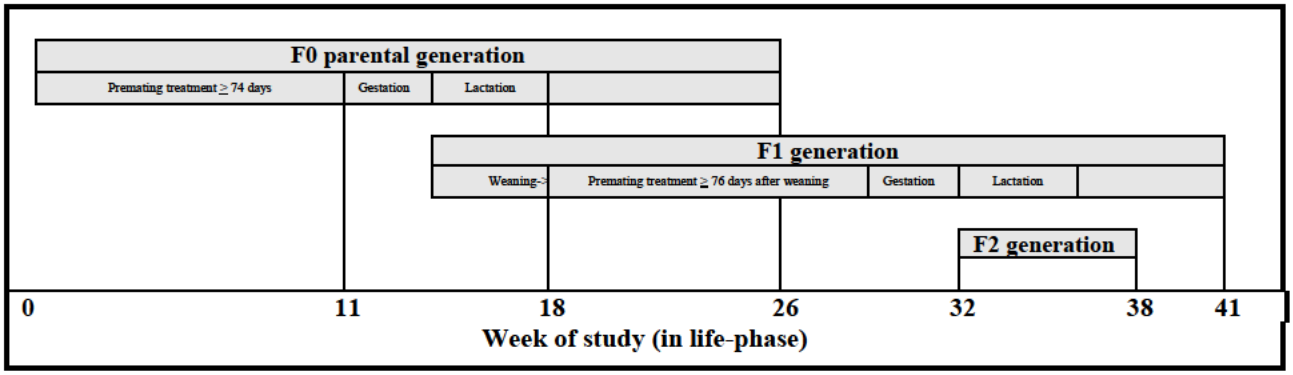
CA 5.6.1 Generational studies

BAS 510 F – Two-generation reproduction toxicity study in Wistar rats – Continuous dietary administration ([redacted] et al, 2001)
DocID 2001/1000117

Guidelines: According to OECD 416 (draft document, June 2000), EEC 87/302, EPA OPPTS 870.3800 and JMAFF
Deviations: None
GLP: Yes
Acceptance: The study has been evaluated and considered acceptable in the EU registration process for the first Annex I inclusion.

Note: This study report has been part of the documentation for the first Annex I inclusion process but a more detailed summary is presented here to assist in discussion on classification and labelling and evaluation due in M-CA 5.8.3 (ED related properties)

Groups of 25 male and female Wistar rats (Chbb:THOM (SPF), source: Boehringer Ingelheim Pharma KG, Germany) were administered Boscalid (batch N37; purity 94.4 %) at concentrations of 0, 100, 1000 and 10000 ppm via feed. Test substance concentrations were kept constant over the administration period. The experimental design of the study is outlined below.



Feed with the test substance was offered throughout the pre-mating, mating, gestation and lactation phases of the study, and the offspring were exposed to the test diets throughout the lactation period. The premating period for F0 was ≥ 74 days and for F1 was ≥ 76 days.
For mating cohabitation of one female with one male was maintained for up to 3 weeks to produce F1 and F2 litters. At day 4 post-partum (p.p.) individual litters with more than 8 pups were standardized by pup culling to maintain 4 males and 4 females or the nearest possible sex distribution the individual litter allowed to achieve. F1 and F2 offspring not selected at standardisation on day 4 p.p. or at weaning for the F1 parental generation were sacrificed; tissues and organs grossly and microscopically examined and organ weights recorded. Termination of the in-life phase was with sacrifice of the F1 parental generation and the F2 offspring. The parental animal and the pup state of health was checked each day, and parental animals were examined for their mating and reproductive performances.

Food consumption of the F0 and F1 parents was determined regularly during premating (once weekly over a period of 7 days each), and weekly during gestation (days 0, 7, 14, 20) and lactation periods (days 1, 4, 7, 14). In general, body weights of F0 and F1 parents were determined once weekly. However, during gestation and lactation F0/F1 females were weighed on days 0, 7, 14 and 20 of gestation, and on days 1, 4, 7, 14 and 21 after birth. Oestrus cycle data were evaluated for F0 and F1 generation females over a three week period prior to mating until evidence of mating was given. Moreover, the oestrus stage of each female was determined on the day of scheduled sacrifice. Sperm parameters (motility, sperm head count, morphology) were assessed in all F0 and F1 generation males at scheduled sacrifice or shortly thereafter. The F1 and F2 pups were sexed and weighed on the day after birth and on days 4, 7, 14 and 21 p.p. Their viability was recorded. All pups were examined macroscopically at necropsy (including weight determinations of brain, spleen and thymus in one pup/sex/litter). Sexual maturation (day of preputial separation/vaginal opening) of all pups selected to become F1 parental generation animals was investigated.
All F0 and F1 parental animals were assessed by gross pathology (including weight determinations of several organs) and subjected to an extensive histopathological examination, special attention being paid to the organs of the reproductive system. A quantitative assessment of primordial follicles, growing follicle and antral follicles in the ovaries was performed for all control and high dose F0 and F1 parental females.

Findings
The stability of the test substance over the study period was shown by reanalysis. The stability and homogeneity of the test item in the diet were analytically verified and the correctness of the test substance concentrations chosen was analytically demonstrated.

Parental mortality, clinical symptoms and feed consumption

No mortality and no clinical symptoms considered treatment related were observed in both parental generations in any of the treatment groups. Feed consumption was not affected by the test substance in any sex and any dose group. Details on the test substance intake based on actual feed consumption data are shown in the table below.

Table 5.6.1-1: 2-generation rat-summary of test substance intake

	Test substance intake (mg/kg bw/day)					
Feed concentration	**100 ppm**		**1000 ppm**		**10000 ppm**	
Generation	**F0**	**F1**	**F0**	**F1**	**F0**	**F1**
Males	10.1	12.3	101.2	123.9	1034.5	1295.4
Males mean	**11**		**113**		**1165**	
Females pre-mating	10.7	12.5	106.8	124.7	1062.0	1299.6
Females gestation	8.7	9.2	88.7	94.2	907.4	952.9
Females lactation*	14.8	14.8	149.4	155.2	1456.7	1456.1
Females mean	**12**		**120**		**1189**	
*For days 1-14 post-partum						

Parental body weight and body weight gain

Body weight and body weight gain of parental males and females was affected in the high dose. In F1 males, statistically significant decrease of body weight was observed during the major part of the 18 week treatment period with terminal reduced body weight and body weight gain by about 9%. Detailed information is presented in Table 5.6.1-2 below. No such effects were observed in F0 males.

Table 5.6.1-2: F1 males: body weight and body weight gain (g)

	0 ppm	**100 ppm**	**1000 ppm**	**10000 ppm**
Week 0	72.2 ± 10.48	75.6 ± 8.8	71.8 ± 8.34	67.4 ± 8.71
Week 2	1.76 ± 17.55	178.5 ± 15.63	175.8 ± 15.2	**164.7*** ± 12.27
Week 4	278.0 ± 24.2	277.2 ± 20.06	273.1 ± 20.31	**254.6**** ± 16.29
Week 8	391.7 ± 37.27	386.8 ± 32.29	388.1 ± 33.75	**381.1*** ± 24.73
Week 10 (end of mating)	428.6 ± 45.63	421.2 ± 33.15	422.6 ± 38.63	**401.1*** ± 25.58
Week 18 (end of in-life phase)	519.5 ± 64.11	502.8 ± 46.47	499.5 ± 51.96	**473.0*** ± 36.31
Weight gain (overall)	447.4 ± 64.13	427.2 ± 42.19	427.7 ± 49.20	**405.6*** ± 34 98
Significantly different from control: *p ≤ 0.05, **p ≤ 0.01 (Dunnett-test (two sided))				

In F0 females of the high dose group body weight gain was statistically significantly reduced during week 6 and 7 of pre-mating and day 7-14 of gestation as isolated findings. No such findings were recorded in F1 females although there was statistically reduced feed consumption between day 4 and 14 of lactation.

Parental organ weights and histopathological findings

Organs with changes in weight were liver, kidneys, spleen and brain. Findings in these organs are summarized in the Table 5.6.1-3 and Table 5.6.1-4 below.

Table 5.6.1-3: F0 and F1 males: organ weights of liver, kidneys and spleen

	Selected organ weights							
	F0 males				**F1 males**			
Dose level (ppm)	**0**	**100**	**1000**	**10000**	**0**	**100**	**1000**	**10000**
Body weight (g)	459	463	452	455	503	484	483	**456****
Liver abs (g)	16.64 ± 2.70	16.24 ± 2.68	16.59 ± 3.05	17.13 ± 2.43	17.77 ± 3.93	18.34 ± 4.07	17.73 ± 3.10	18.09 ± 1.87
Liver rel (%)	3.61 ± 0.45	3.50 ± 0.40	3.66 ± 0.50	3.76 ± 0.28	3.53 ± 0.55	3.79 ± 0.74	3.68 ± 0.58	**3.97**** ± 0.25
Kidneys abs (g)	2.99 ± 0.29	2.94 ± 0.23	2.85 ± 0.20	2.87 ± 0.22	3.06 ± 0.39	3.01 ± 0.22	2.96 ± 0.35	2.74 ± 0.24
Kidneys rel (%)	0.65 ± 0.04	0.64 ± 0.04	0.63 ± 0.05	0.63 ± 0.03	0.61 ± 0.04	0.62 ± 0.04	0.61 ± 0.04	0.60 ± 0.04
Spleen abs (g)	0.872 ± 0.14	0.856 ± 0.09	**0.774*** ± 0.1	**0.714**** ± 0.11	0.901 ± 0.12	0.866 ± 0.17	**0.757*** ± 0.09	**0.708**** ± 0.14
Spleen rel (%)	0.19 ± 0.03	0.19 ± 0.02	**0.17**** ± 0.02	**0.16**** ± 0.02	0.18 ± 0.02	0.18 ± 0.02	**0.16**** ± 0.02	**0.16**** ± 0.03
Brain abs (g)	2.095 ± 0.072	2.087 ± 0.084	2.072 ± 0.075	2.073 ± 0.078	2.123 ± 0.094	2.131 ± 0.099	2.114 ± 0.074	**2.063*** ± 0.073
Brain rel (%)	0.459 ± 0.039	0.453 ± 0.032	0.460 ± 0.033	0.459 ± 0.042	0.428 ± 0.046	0.443 ± 0.033	0.442 ± 0.046	0.455 ± 0.031
Significantly different from control: *p ≤ 0.05, **p ≤ 0.01; Kruskall-Wallis + Wilcoxon-test (two sided)								

Table 5.6.1-4: F0 and F1 females: organ weights of liver, kidneys and spleen

	Selected organ weights							
	F0 females				**F1 females**			
Dose level (ppm)	**0**	**100**	**1000**	**10000**	**0**	**100**	**1000**	**10000**
Body weight (g)	273	267	267	264	283	277	275	282
Liver abs (g)	9.86 ± 1.49	9.75 ± 1.15	10.08 ± 1.06	**11.46**** ± 1.26	10.41 ± 1.09	10.48 ± 1.23	10.51 ± 1.11	**12.61**** ± 1.62
Liver rel (%)	3.60 ± 0.43	3.65 ± 0.34	3.79 ± 0.37	**4.35**** ± 0.41	3.68 ± 0.37	3.77 ± 0.47	3.83 ± 0.36	**4.47**** ± 0.51
Kidneys abs (g)	2.08 ± 0.17	1.98* ± 0.17	2.01 ± 0.20	**1.93**** ± 0.17	1.99 ± 0.16	1.93 ± 0.18	1.94 ± 0.20	**1.86**** ± 0.29
Kidneys rel (%)	0.76 ± 0.05	0.74 ± 0.06	0.76 ± 0.07	0.73 ± 0.04	0.71 ± 0.05	0.69 ± 0.06	0.71 ± 0.06	**0.66**** ± 0.09
Spleen abs (g)	0.61 ± 0.10	0.59 ± 0.08	0.56 ± 0.06	0.55 ± 0.06	0.63 ± 0.07	0.61 ± 0.08	**0.58*** ± 0.07	**0.57**** ± 0.13
Spleen rel (%)	0.22 + 0.03	0.22 + 0.03	0.21 + 0.03	0.21 + 0.04	0.22 ± 0.03	0.22 ± 0.03	0.21 ± 0.03	**0.20**** ± 0.04
Brain abs (g)	1.932 ± 0.102	1.936 ± 0.062	1.974 ± 0.068	1.900 ± 0.068	1.948 ± 0.074	1.934 ± 0.057	**2.006**** ± 0.064	1.963 ± 0.072
Brain rel (%)	0.709 ± 0.037	0.728 ± 0.049	0.743 ± 0.043	0.724 ± 0.050	0.691 ± 0.049	0.697 ± 0.042	**0.733**** ± 0.051	0.698 ± 0.042
Significantly different from control: *p ≤ 0.05, **p ≤ 0.01 (Kruskall-Wallis + Wilcoxon-test (two sided))								

Organ weight changes were dose dependent and gained statistical significance in the liver and spleen in either sex or generation.

Corresponding dose-related histopathological changes were observed in the liver of both parental generations with increasing incidence and severity of centrilobular hypertrophy and hepatocyte degeneration in the dose groups of 1000 ppm and 10000 ppm. Detailed information on the incidence of effects in the liver is provided in Table 5.6.1-5 below. Spleen weights were statistically significantly decreased at dose levels of 1000 ppm and 10000 ppm. Histopathological evaluation gave no morphologic correlate to the decrease of weight, all functional units of the red and white pulp were comparable between treatment groups and control groups. The absolute weight of kidneys was decreased in females of both generations at the 10000 ppm dose level and in the F0 generation at 100 ppm dose level. This finding has been considered to be of incidental nature as there is no dose response relationship, no corresponding findings were in the F0 generation or in male animals at all, relative kidney weights were unchanged at 100 ppm and 1000 ppm dose levels and no histological correlate could be found.
Other effects were found to be of spontaneous nature without dose relationship. Absolute brain weight of F1 males at the 10000 ppm dose level was decreased correlating with the statistically significantly decreased body weight as relative brain weights were not different to control animals.
Both, absolute and relative brain weight of F1 females at the 1000 ppm dose level were increased. In the absence of a dose relationship and no such indication of changes in the F0 generation this finding has been considered as incidental.

Table 5.6.1-5: F0 and F1 parental generation: incidence of histopathological findings in the liver

	F0 parental generation at dose level (ppm)				**F1 parental generation at dose level (ppm)**			
	0	**100**	**1000**	**10000**	**0**	**100**	**1000**	**10000**
Incidence of centrilobular hypertrophy*								
Males	0 / 25	0 / 25	9 / 25	25 / 25	1 / 25	0 / 25	10 / 25	25 / 25
Females	0 / 25	0 / 25	6 / 25	25 / 25	0 / 25	0 / 25	8 / 25	25 / 25
Hypertrophy grading 1/2/3								
Males	-	-	8 / 1 / 0	2 / 23 / 0	1/ 0 / 0	-	10 / 0 / 0	7 / 15 / 3
Females	-	-	6 / 0 / 0	8 / 16 / 1	-	-	4 / 2 / 2	9 / 15 / 1
Incidence of hepatocyte degeneration*								
Males	0 / 25	1 / 25	0 / 25	3 / 25	0 / 25	0 / 25	0 / 25	8 / 25
Females	0 / 25	0 / 25	0 / 25	1 / 25	0 / 25	0 / 25	0 / 25	0 / 25
Hepatocyte degeneration grading 1/2/3/4								
Males	-	0/0/1/0	-	0/3/0/0	-	-	-	0/5/2/1
Females	-	-	-	0/1/0/0	-	-	-	-
*Incidence of animals affected / animals investigated								

No treatment related effects on reproductive organs were observed in either generation or sex. Detailed information on organ weights is provided in the tables below. Absolute and relative mean ovary weights were recorded in F1 females at the 100 ppm dose (Table 5.6.1-7). However, there was no dose response and the histopathological evaluation of the high dose group gave no indication of this finding to have been caused by the test substance.

Table 5.6.1-6: F0 and F1 males: weights of reproductive organs

	Reproductive organ weights							
	F0 males				**F1 males**			
Dose level (ppm)	**0**	**100**	**1000**	**10000**	**0**	**100**	**1000**	**10000**
Body weight (g)	459	463	452	455	503	484	483	**456****
Testes abs (g)	3.734 ± 0.28	3.874 ± 0.32	3.562 ± 0.64	3.818 ± 0.31	3.816 ± 0.34	3.916 ± 0.29	3.872 ± 0.51	3.814 ± 0.42
Testes rel (%)	0.817 ± 0.08	0.841 ± 0.08	0.791 ± 0.15	0.847 ± 0.11	0.769 ± 0.10	0.814 ± 0.07	0.805 ± 0.09	0.839 ± 0.09
Epididymides abs (g)	1.296 ± 0.10	1.296 ± 0.16	1.255 ± 0.15	1.292 ± 0.09	1.352 ± 0.09	1.344 ± 0.08	1.326 ± 0.09	1.295 ± 0.12
Epididymides rel (%)	0.284 ± 0.03	0.282 ± 0.04	0.278 ± 0.04	0.287 ± 0.03	0.272 ± 0.03	0.279 ± 0.02	0.277 ± 0.03	0.285 ± 0.03
Cauda epididymides abs (g)	0.545 + 0.07	0.554 + 0.05	0.522 + 0.08	0.552 + 0.06	0.547 + 0.05	0.540 + 0.05	0.533 + 0.04	0.524 + 0.08
Cauda epididymides rel (%)	0.119 ± 0.02	0.120 ± 0.01	0.116 ± 0.02	0.123 ± 0.02	0.110 ± 0.02	0.112 ± 0.01	0.111 ± 0.01	0.115 ± 0.02
Semical vesicle abs (g)	1.404 ± 0.16	1.387 ± 0.16	1.370 ± 0.15	1.359 ± 0.17	1.372 ± 0.18	1.321 ± 0.19	1.265 ± 0.25	1.317 ± 0.25
Semical vesicle rel (%)	0.307 ± 0.04	0.301 ± 0.04	0.305 ± 0.04	0.301 ± 0.04	0.278 ± 0.05	0.276 ± 0.05	0.265 ± 0.06	0.290 ± 0.05
Prostrate abs (g)	1.432 ± 0.22	1.413 ± 0.14	1.314 ± 0.17	1.374 ± 0.20	1.318 ± 0.22	1.212 ± 0.16	1.230 ± 0.24	1.224 ± 0.30
Prostrate rel (%)	0.313 ± 0.05	0.308 ± 0.04	0.292 ± 0.04	0.304 ± 0.05	0.264 ± 0.04	0.252 ± 0.04	0.255 ± 0.04	0.268 ± 0.06
Significantly different from control: *p ≤ 0.05, **p ≤ 0.01 (Kruskall-Wallis + Wilcoxon-test (two sided))								

Table 5.6.1-7: F0 and F1 females: weights of reproductive organs

	Selected organ weights							
	F0 females				**F1 females**			
Dose level (ppm)	**0**	**100**	**1000**	**10000**	**0**	**100**	**1000**	**10000**
Body weight (g)	273	267	267	264	283	277	275	282
Ovaries abs (mg)	141.60 ± 20.09	134.52 ± 17.31	142.40 ± 22.09	143.24 ± 16.26	151.24 ± 19.12	**135.12**** ± 14.93	140.64 ± 22.01	137.76 ± 23.51
Ovaries rel (%)	0.052 ± 0.005	0.050 ± 0.006	0.054 ± 0.009	0.055 ± 0.007	0.053 ± 0.006	**0.049**** ± 0.005	0.051 ± 0.007	0.049 ± 0.010
Uterus abs (g)	0.625 ± 0.15	0.752 ± 0.30	0.760 ± 0.32	0.661 ± 0.20	0.725 ± 0.18	0.705 ± 0.21	0.683 ± 0.19	0.707 ± 0.22
Uterus rel (%)	0.231 ± 0.062	0.282 ± 0.112	0.284 ± 0.114	0.252 ± 0.080	0.256 ± 0.061	0.254 ± 0.079	0.251 ± 0.076	0.252 ± 0.081
Significantly different from control: *p ≤ 0.05, **p ≤ 0.01 (Kruskall-Wallis + Wilcoxon-test (two sided))								

Effects on fertility

Oestrous cycle

Oestrous cycle length evaluated daily in all F0 and F1 female parental rats for a minimum of 3 weeks before mating gave no differences between control and dose groups. The mean cycle length was 5.2, 5.2, 5.2 and 5.3 days for F0 females and 5.6, 5.2, 5.4 and 5.5 days for F1 females at the dose levels of 0, 100, 1000 and 10000 ppm.

Male reproductive performance and sperm analysis

Male reproductive performance was unaffected by the treatment. The mating index for F0 parental males was 100% in all groups. For F1 parental males the mating index was 100% except the top dose where 92% was recorded based on two females without sperm positive findings. In one of these females concomitant histopathological finding was fibroplasia of the cervix uteri and dilation of the uterus horns which ultimately may have contributed to the failure of mating. Overall, the low incidence of mating failure has been considered to be of incidental nature.
The fertility index for F0 parental males varied between 88% and 100% without a clear relation to the dose levels chosen. The fertility index for F1 parental males varied between 88% and 100%. Two males of the mid dose and three males of the high dose did not prove fertility. However, from the high dose group two F1 parental females also had slightly irregular oestrous cycles towards the determination period with one of them showing fibroplasia of the cervix uteri and dilation of the uterus horns in addition. F1 parental males did not show histopathological correlates that could be associated with the lack of fertility. Overall, findings were within the range of historical control data and have thus been considered to be of incidental nature.
Sperm analysis gave no treatment related adverse effects in the F0 and F1 parental generation for sperm counts, normal and abnormal sperm and sperm motility. The percentage of motile sperms in F1 parental males was slightly but significantly reduced in the high dose group as compared to control. (83% versus 89%). This finding, however, was within historical control data with a range of 65% to 99% for the period investigated. In the light of the genital organs being without treatment-related effects (for both, organ weights and histology) these findings are considered to be not treatment related.
Findings are summarized in the Table 5.6.1-8 below.

Table 5.6.1-8: F0 and F1 males: reproduction parameters and sperm analysis

	Parental generation	Dose level (ppm)				Historical control range used in the study report
		0	**100**	**1000**	**10000**	
Mating index	F0	100%	100%	100%	100%	96-100%
	F1	100%	100%	100%	92%§	
Fertility index	F0	100%	100%	88%	100%	88-100%
	F1	100%	100%	92%	88%#	
Sperm count Testis (10^6/g)	F0	100			98	
	F1	87			85	
Sperm count C. epididymidis ($x10^6$/g)	F0	671			662	
	F1	705			679	
Normal sperm	F0	95.6%			96.0%	
	F1	97.3%	95.7%	95.8%	94.7	
Abnormal sperm	F0	4.4%			4.0%	
	F1	2.7%	4.3%	4.2%	5.3%	1.5–4.6%
Abnormal sperm F1 >4% (N / 25)		N = 5	N = 7	N = 7	N = 10	
Sperm motility	F0	89%	86%	83%	89%	65-99%
	F1	89%	88%	87%	83%*	
§ Two parental females affected: female Nos. 388 and 396 with slightly irregular oestrous cycle and fibroplasia of the cervix uteri and dilation of the uterus horns in female No 388						
# Three parental females affected: female Nos. 388 and 396 with slightly irregular oestrous cycle and fibroplasia of the cervix uteri and dilation of the uterus horns in female No 388, female No. 399 without finding						
Significantly different from control: *$p \leq 0.05$ (Wilcoxon-test with Bonferoni-Holm adjustment)						

Female reproductive performance

Mating index was 100% in all groups of parental females except of the high dose F1 females with a slight reduction to 92% due to mating failure of two females. In this dose group F1 females Nos. 388 and 396 evidenced to have slightly irregular oestrous cycle, and fibroplasia of the cervix uteri and dilation of the uterus horns was in addition found in F1 female No 388.
Mating times until sperm was detected (day 0 p.c.) were 2.8 days for F0 control and 2.3 days for F1 control females. The range of mating times for the respective dose groups was between 2.1 days and 2.9 days, thus reflecting the range of biological variation inherent in this train of rats.
Fertility indices were 100% in both control groups and varied between 100% and 88% for F0 females and between 100% and 92% for F1 females. With regard to F0 females the lower fertility index of 88% observed in the mid dose were due to failure of pregnancy in three animals. No corroborative histological finding was made in these animals and due to lack of a dose response relationship this finding has not been considered substance induced. For F1 females slightly reduced fertility indices were observed in the mid (92%) and high dose group (96%). Two sperm positive F1 females of the mid dose and one female of the high dose group did not become pregnant. All these females were devoid of a histopathological correlate that may have explained the failure of pregnancy. Similar to the F0 generation the additional lack of a dose response thus suggests these findings to be of incidental nature rather than test substance related.

Duration of gestation varied between 21.7 to 22.0 days over both generations and was very similar including the duration of 21.7 days in the F1 mid dose identified as statistically significantly decreased (*p<0.05), however, without a dose-response relationship if compared with the other dose groups. The numbers of **implantation sites** were comparable over both generations and dose groups. For the **mean of implantation sites** (implantation sites/dam) a slight, however statistically identified (*p<0.05) decrease was observed in F0 females of the low and high dose group. However, these findings have been considered to lack of a clear dose response relationship and was not evident at all in the F1 females. As this parameter has an inherent wide variability (range of 11.4- 18.8, HOOD, R.D. Handbook of Developmental Toxicology, CRC Press, Boca Raton New York, London, Tokyo) the reduced mean number of implantations has been considered to be of spontaneous nature and without biological relevance.
Total post-implantation loss and **mean post-implantation loss** gave no statistically identified differences and were thus considered as being not affected by the treatment. As corresponding consequence for F0 females (i.e. reduced mean implantation sites and no effects on post-implantation loss) the number of **pups delivered/dam** was reduced gaining statistical significance for the same dose groups of F0 females as for mean of implantation sites (i.e. pups delivered/dam: 15.8, 13.2*, 14.3. 13.0**) as a correlated finding. No such finding was made in the F1 females.
Post-implantation loss per litter was slightly increased at the top dose group gaining statistical significance (*p<0.05) for the F1 generation.
The **gestation index** was not affected in any dose group and generation. For F0 females the control group gave a value of 96% as compared to a range of 96 to 100% for the dose groups. In F1 females the gestation index was overall 100%. The **number of dams with stillborn pups** in the dose groups did not exceed the level of those in F0 and F1 control groups (i.e. F0: N = 8 and F1: N = 7) and were in general lower, notably in the top dose groups (i.e. F0: N = 3 and F1: N = 6).
Dams with pups all stillborn did not occur in F0 and F1 controls and in any of the dose groups.
The **number of pups live-born** and the **number of pups stillborn** gave no apparent differences to control F0 and F1 females. The **life-birth index** was 96% and 97% for F0 and F1 control females and in the range of 97% to 99% for the dose groups.

Table 5.6.1-9: F0 and F1 females: reproduction parameters

	Parental generation	Dose level (ppm)				Historical control range used in the study report
		0	**100**	**1000**	**10000**	
Females pregnant (n)	F0	25	25	22	25	
	F1	25	25	23	22	
Mating index	F0	100%	100%	100%	100%	
	F1	100%	100%	100%	92%	
Fertility index	F0	100%	100%	88%	100%	
	F1	100%	100%	92%	96%	
Mating until sperm detection (day 0 p.c.)	F0	2.8	2.2	2.4	2.5	
	F1	2.3	2.1	2.7	2.9	
Duration of gestation (days)	F0	21.8	21.8	21.9	21.8	
	F1	22.0	22.0	**21.7***	21.8	
Implantation sites - total (n)	F0	418	389	358	364	
	F1	385	377	393	335	
Implantation sites/dam (n)	F0	17.4	**15.6***	16.3	**15.2***	
	F1	15.4	15.1	17.1	15.2	
Post-implantation loss total (n)	F0	40	60	46	54	
	F1	29	41	36	50	
Post-implantation loss/dam (n)	F0	1.6	2.4	2.1	2.2	
	F1	1.2	1.6	1.6	2.3	
Post-implantation loss/litter	F0	12.8%	14.7%	13.7%	18.6%	
	F1	7.0%	10.8%	9.1%	15.4%*	
Dams with live-born pups (n)	F0	24	25	22	24	
	F1	25	25	23	22	
Gestation index	F0	96%	100%	100%	96%	
	F1	100%	100%	100%	100%	
Dams with stillborn pups (n)	F0	8	2	7	3	
	F1	7	3	7	6	
Dams with pups all stillborn (n)	F0	0	0	0	0	
	F1	0	0	0	0	
Pups delivered total (n)	F0	379	329	312	313	
	F1	356	336	357	285	
Pups delivered/dam (n)	F0	15.8	**13.2***	14.2	**13.0****	11.1 to 16.4 pups/litter
	F1	14.2	13.4	15.5	13.0	
Pups live-born total (n)	F0	364	327	299	309	
	F1	344	332	348	276	
Pups stillborn total (n)	F0	15	2	13	4	
	F1	12	4	9	9	
Life-birth index	F0	96%	99%	96%	99%	
	F1	97%	99%	97%	97%	

* $p \leq 0.05$; ** $p \leq 0.01$ (Dunnet-test two sided or Fisher's exact test one sided)

Findings in offspring

Pup clinical findings: Both, F1 and F2 offspring did not show clinical findings up to weaning that could be attributed to the test substance administration.

Pup survival: Perinatal survival of pups was not affected in the F1 offspring with viability indices of 95%, 93%, 92% and 94% at the dose levels of 0, 100, 1000 and 10000 ppm. For F2 offspring the viability index was 93%, 91%, 97% and 86%** at the dose levels of 0, 100, 1000 and 10000 ppm. The reduced viability index of the low dose level essentially has been attributed to excessive pup mortality in one litter (F1 female No 311 with in-proper nursing of pups having almost empty stomach). This slightly reduced viability index has thus been considered to be of incidental nature. For the high dose F2 offspring, the viability index was significantly reduced (i.e. 86%**), albeit being still within the historical control range of 83% - 99%. No corresponding finding was made in the respective F1 group. Subsequent mortality following culling of offspring until weaning (days 4 p.p. until 21) did not show differences of biological relevance as evidenced by lactation indices of 99% to 100% for both the F1 and F2 control animals and the dose groups. However, reduced body weight and body weight gain of the offspring at the 10000 ppm dose level (notably F2 offspring, Table 5.6.1-11) as well as maternal findings (notably increased absolute and relative liver weight, Table 5.6.1-4 and corresponding histopathological findings, Table 5.6.1-5) are indicative of this dose level having elicited adverse effects for which the reduced viability index in F2 offspring at the top dose cannot be ruled out.

Sex ratio: The sex ratio of F1 and F2 pups at day 0 and day 21 did not reveal differences between control and treated groups.

Table 5.6.1-10: F1 and F2 offspring: survival parameters and sex ratio

	Offspring	Dose level (ppm)				Historical control range used in the study report
		0	**100**	**1000**	**10000**	
Number of litters (n)	F1	24	25	22	24	
	F2	25	25	23	22	
Dams with liveborn pups (n)	F1	24	25	22	24	
	F2	25	25	23	22	
Dams with stillborn pups (n)	F1	8	2	7	3	
	F2	7	3	7	6	
Pups liveborn total (n)	F1	364	327	299	309	
	F2	344	332	348	276	
Pups cannibalized total (n)	F1	2	**14****	3	4	
	F2	7	2	0	6	
Pups dead# day 1-4 total (n)	F1	17	22	21	17	
	F2	21	30	7	38	
Pups surviving day 1-4 total (n)	F1	347	305	276	291	
	F2	321	301	339	**238****	
Viability index day 4 (pre-culling)	F1	95%	93%	92%	94%	
	F2	93%	91%	97%	**86%****	83% - 99%
Pups culled on day 4 total (n)	F1	156	114	111	112	
	F2	122	110	155	69	
Pups on day 4 total post-culling (n)	F1	191	191	165	179	
	F2	199	181	184	169	
Pups on day 21 (n)	F1	190	191	164	178	
	F2	199	181	184	168	
Lactation index	F1	99%	100%	99%	99%	
	F2	100%	100%	100%	99%	
Sex ratio day 0 (% live males)	F1	48.9%	51.1%	50.2%	47.2%	
	F2	47.7%	53.3%	50.9%	47.5%	
Sex ratio day 21 (% live males)	F1	49.5%	51.8%	50.0%	47.2%	
	F2	49.2%	53.0%	48.9%	50.6%	

* $p \leq 0.05$; ** $p \leq 0.01$ (Dunnet-test two sided or Fisher's exact test one sided)
# Pups dead = $\sum$ pups died, sacrificed moribund and cannibalized

Pup body weight: In both, F1 and F2 offspring of the high dose group mean body weight was significantly reduced starting with day 21 for F1 offspring and with day 7 for F2 offspring. The body weight gain from day 4 until 21 was also identified as statistically significant in both sexes of F1 and F2 pups.
Detailed information on the body weight and body weight gain is presented in Table 5.6.1-11 below.

Table 5.6.1-11: F1 and F2 offspring: body weight and body weight gain

	Offspring	Dose level (ppm)			
		0	100	1000	10000
Pup weight males (g)	F1				
Day 1		6.2	6.4	6.3	6.4
Day 4 pre-culling		8.7	9.0	8.8	8.8
Day 4 post-culling		8.7	9.2	8.9	8.8
Day 7		14.2	14.7	14.5	13.7
Day 14		30.5	30.8	31.0	29.0
Day 21		50.3	50.6	51.8	46.7*
Body weight gain day 4 to 21		41.7	41.6	43.0	37.9*
Day 1	F2	6.5	6.4	6.2	6.3
Day 4 pre-culling		9.5	9.3	8.6*	9.1
Day 4 post-culling		9.6	9.3	8.7	9.0
Day 7		15.4	14.7	13.9*	13.9*
Day 14		32.7	31.6	30.9	29.1**
Day 21		54.6	52.4	50.8*	47.0**
Body weight gain day 4 to 21		45.2	43.1	42.2*	37.9**
Pup weight females (g)	F1				
Day 1		5.9	6.0	6.0	6.1
Day 4 pre-culling		8.3	8.5	8.7	8.6
Day 4 post-culling		8.4	8.6	8.8	8.6
Day 7		13.7	13.9	14.1	13.4
Day 14		30.0	29.7	30.5	28.4
Day 21		48.7	48.0	49.3	45.2*
Body weight gain day 4 to 21		40.4	39.4	40.5	36.6**
Day 1	F2	6.2	6.2	5.9	6.0
Day 4 pre-culling		9.0	9.0	8.3	8.7
Day 4 post-culling		9.1	9.0	8.5	8.7
Day 7		14.7	14.7	13.6	13.5
Day 14		31.5	31.1	30.4	28.4**
Day 21		51.6	50.8	49.3	45.5**
Body weight gain day 4 to 21		42.5	41.6	41.0	36.8**
* $p \leq 0.05$; ** $p \leq 0.01$ (Dunnet-test two sided)					

In the mid dose group isolated findings of reduced body weight was observed in F2 males gaining statistical significance (*p<0.05) on day 4 pre-culling, day 7 and 21. Body weight reduction was about 9.5% at day 4 pre-culling, 9.7% at day 7 and 7.7% and day 21 for F2 males relative to control. Detailed review of the F2 litters revealed one dam (No. 357) to have delivered pups with remarkably low birth weight (mean: 5.3 g at lactation day 1) combined with a high number of 19 pups. To further consider the influence of the litter size on the pup weight a covariance analysis was performed. A comparison of each dose group with the control group was performed using the DUNNETT test (two-sided) for the hypothesis of equal means. In this analysis the litter size was included as co-variable. The results are shown in Table 5.6.1-12. Statistical analyses were performed using the SAS procedure PROC Mixed. With consideration of the litter size these results suggest that the reduction of F2 male pup weight may be incidental and is not a true test substance related finding.

Table 5.6.1-12: F2 offspring males: co-variance analysis of body weight

	Offspring	**Dose level (ppm)**			
		0	**100**	**1000**	**10000**
Males Pup weight (g)					
Day 1	F2	6.5	6.4	6.2	6.3
Day 4 pre-culling		9.5	9.3	8.6	**9.1***
Day 4 post-culling		9.6	9.3	8.7	**9.0***
Day 7		15.4	14.7	13.9	**13.9****
Day 14		32.7	31.6	30.9	**29.1****
Day 21		54.6	52.4	50.8	**47.0****
* p ≤ 0.05; ** p ≤ 0.01 (Dunnett test with Covariable litter size two sided)					

No effects on body weights and body weight development were observed in low dose F1 and F2 pups as well as mid dose female pups and F1 male pups.

Organ weights and gross necropsy findings in offspring
Concomitant to the decreased terminal F1 and F2 pup body weight and body weight change, absolute and/or relative organ weight of statistical significance were observed in brain, thymus and spleen. Detailed information is presented in the Table 5.6.1-13 below.

Table 5.6.1-13: F1 and F2 offspring: absolute and relative organ weights

		F1 pups				F2 pups			
Dose level (ppm)		**0**	**100**	**1000**	**10000**	**0**	**100**	**1000**	**10000**
Organ	Sex								
Brain, abs (g)	M	1.459	1.464	1.461	1.430	1.489	1.461	1.469	1.462
	F	1.409	1.423	1.433	1.410	1.435	1.426	1.430	1.413
Brain, rel (%)	M	2.899	2.888	2.784	**3.048***	2.710	2.837	**2.970****	**3.123****
	F	2.848	2.924	2.956	**3.095***	2.838	2.772	2.942	**3.107****
Thymus, abs (g)	M#	0.175	0.175	0.179	0.164	0.200	**0.166****	**0.176***	**0.162****
	F	0.180	0.174	0.193	**0.162***	0.193	0.181	0.195	0.175
Thymus, rel (%)	M	0.343	0.342	0.343	0.348	0.363	**0.316****	0.353	0.345
	F	0.362	0.353	0.394	0.355	0.382	**0.349***	0.398	0.380
Spleen, abs (g)	M	0.222	0.213	0.222	0.201	0.260	0.242	**0.215****	**0.189****
	F	0.232	0.211	0.220	0.199	0.238	0.237	0.218	**0.196***
Spleen, rel (%)	M	0.431	0.409	0.424	0.423	0.467	0.459	0.429	**0.397****
	F	0.461	0.426	0.448	0.436	0.468	0.456	0.443	0.422

M = male, F = female
# NB: In the draft assessment report (November 08, 2002) thymus weights of males and females combined were erroneously reported for thymus weights of males. Correct values reported in this evaluation
* $p \leq 0.05$, ** $p \leq 0.01$ (Kruskal-Wallis and Wilcoxon-test (two-sided))

Organ weight changes in pups at the top dose level were considered to be secondary to the reduced body weight. Thymus weight (absolute and relative) and spleen weight (absolute) were reduced in F2 male pups at lower dose levels with no clear dose response and without similar findings in F1 pups. In the absence of a histopathological correlate in the spleen of the parental generation the reduction of spleen and thymus weight is considered to be related to the reduced body weight of F2 males rather than a specific test substance related effect. Further information providing evidence of the findings in thyroids and thymus to be not a Boscalid mediated effect of specific immunotoxicity has been presented in M-CA 5.8.2 (see Doc ID 2003/1025755). The detailed evaluation of come to the conclusion that Boscalid did not reveal any signs of immunotoxicity when administered via the diet to male Wistar rats over a period of 4 weeks. The NOAEL for the immunotoxicity as well as for systemic toxicity was determined to be 10000 ppm (736.2 mg/kg bw/day; highest dose tested).
All other findings in absolute and/or relative F2 pup organ weight were assessed as incidental and without toxicological significance.

Pup gross necropsy findings: No treatment-related findings at pup necropsy were made.
Pup gross necropsy examination was performed in stillborn pups, pups that died inter-currently, in culled and surplus pups. In F1 and F2 offspring a few macroscopic findings were at necropsy such as incisors sloped, hernia diaphragmatica, empty stomach, focal necrosis of liver, dilated renal pelvis or kinky tail. For none of these findings any indication of being test substance related was identified. A more detailed evaluation of necropsy findings is presented in the Table 5.6.1-14 below.

Table 5.6.1-14: F1 and F2 offspring: incidence of cross necropsy findings

	F1 pups				F2 pups			
Dose level (ppm)	**0**	**100**	**1000**	**10000**	**0**	**100**	**1000**	**10000**
Litters evaluated	24	24	22	23	25	25	23	22
Pups evaluated	327	265	258	257	349	334	357	279
- Live	312	263	245	253	337	330	348	270
- Stillborn	15	2	13	4	12	4	9	9
Post mortem autolysis	6 (3)	2 (2)	6 (5)	9 (5)	4 (4)	3 (3)	5(3)	18 (7)
Partly cannibalized	1	0	0	0	0	0	0	0
Incisors sloped	2 (2)	2 (2)	0	0	2 (2)	1	1	0
Hernia diaphragmatica	1	1	0	0	0	0	1	0
Stomach empty	0	0	0	0	0	2 (1)	0	0
Focal necrosis of liver	1	0	1	0	0	0	0	0
Dilated renal pelvis	2 (2)	3 (3)	0	3 (3)	0	0	0	1
Kinky tail	0	1	0	0	0	0	0	0
Total of pups affected	13 (9)	9 (6)	7 (6)	13 (7)	6 (6)	6 (5)	7 (5)	19 (7)
Affected pups per litter	4.1	3.5	2.8	5.0	1.6	1.5	1.8	6.2
( ) Numbers in brackets refer to litter incidence								

Sexual maturation

Male and female **F_1** pups selected to become **F_1** parental animals were examined for sexual maturation. No treatment-related effects on sexual maturation were observed. For female pups of the top dose group there was an isolated finding of retarded maturation (*$p<0.05$) on day 31 with 16 out of 25 females having completed vaginal opening relative to 24 out of 24 female pups in the control group. However, inspection on the following day gave no statistical difference any more suggesting this finding to be incidental rather than test substance related. No further differences to the control group could be observed in any of the treatment groups until finalization of inspections on day 37.

For male pups no difference in the time needed for preputial separation could be seen in any of the treatment groups. Detailed information is presented in the Table 5.6.1-15 below.

Table 5.6.1-15: F1 offspring: sexual maturation

Sex/parameter investigated	**Females/vaginal opening**				**Males/preputial separation**			
Dose level (ppm)	**0**	**100**	**1000**	**10000**	**0**	**100**	**1000**	**10000**
Pups investigated	25	25	25	25	25	25	25	25
Time until criterion (days)	30.6	31.0	30.7	31.6	44.5	44.1	44.0	44.5

Conclusions

Under the conditions chosen in this 2-generation reproduction toxicity study in Wistar rats the NOAEL for systemic toxicity in F0 and F1 parental rats has been based on organ weight changes in the liver with corresponding dose-related histopathological changes in both parental generations with increasing incidence and severity of centrilobular hypertrophy and hepatocyte degeneration in the dose groups of 1000 ppm and 10000 ppm. The NOAEL proposed thus is at the dose level of 100 ppm corresponding to 11 mg/kg bw/day.

The NOAEL for fertility is 1165 mg/kg bw/day (10000 ppm dose group) based on mating and fertility indices for F0 and F1 parental females and sperm parameters of F0 and F1 parental males.

The NOAEL for offspring was previously set at 11 mg/kg bw/day (100 ppm) as a conservative approach based on reduced body weight and body weight gain of F2 male pups at the 1000 ppm dose level. This previous conclusion is not any more supported and the new evaluation supports the NOAEL at 1000 ppm (113 mg/kg bw/day) instead.

CA 5.6.2 Developmental toxicity studies

BAS 510 F – Prenatal developmental toxicity study in Wistar rats – Oral administration – gavage- ([redacted]., 2000)
DocID 2000/1015001

Guidelines: According to OECD 414 (draft document, March 1998), EEC 87/302, EPA OPPTS 870.3700 and JMAFF
Deviations: None
GLP: Yes
Acceptance: The study has been evaluated and considered acceptable in the EU registration process for the first Annex I inclusion.

Note: This study report has been part of the documentation for the first Annex I inclusion process but a more detailed summary is presented here to assist in discussion on classification and labelling and evaluation due in M-CA 5.8.3 (ED related properties)

Groups of 25 mated female Wistar rats (Chbb:THOM (SPF), source: Boehringer Ingelheim Pharma KG, Germany, 10-11 weeks of age) were administered by oral gavage Boscalid (batch N37; purity 94.4 %), suspended in 0.5 % Tylose CB 30.000 in doubly distilled water, at doses of 0, 100, 300 and 1000 mg/kg bw/day, from days 6 to 19 (inclusive) of gestation. Dosing volume was 10 mL/kg bw in each group. Dams were investigated for mortality, signs of toxicity, body weight (change), food consumption and gross examination at necropsy. Terminal investigations on day 20 of gestation included a detailed examination of the reproductive tract during necropsy for numbers of corpora lutea in ovaries, implantations, number and distribution of foetuses, resorptions and extent of pre and post-implantation losses. Foetuses were examined for external, visceral (soft tissue) and skeletal abnormalities. Following the unscheduled deaths of 3 animals/dose group (in 100 and 1000 mg/kg bw/day dose groups) due to gavage error, animals were replaced to complement the treatments groups.

Findings
The stability of the test substance was proven by reanalysis. The stability and homogeneity of the test substance preparation was analytically verified and the correctness of the concentrations chosen was demonstrated.

Findings in dams
Maternal toxicity: There were no test substance-related deaths or overt clinical signs of toxicity in dams. Three dams each in the 100 and 1000 mg/kg bw/day dose groups were found dead or were sacrificed following apparent gavage error on gestation days 7-9. Feed consumption, mean body weight and body weight gain did not show any test substance-related differences between dose groups (Table 5.6.2-1).

Table 5.6.2-1: Prenatal toxicity in rats: Feed consumption and body weight in rats during days 6 to 19 of gestation

Dose level [mg/kg bw/day]	0	100	300	1000
Feed consumption [g/animal/day]				
Day 0 to 6	21.5	21.5	22.0	21.6
Day 6 to 19	26.2	25.2	26.2	25.8
Day 0 to 20	24.8	24.2	25.2	24.7
Body weight [g]				
Day 0	215.8	214.6	215.4	214.1
Day 6	244.1	243.7	247.5	244.8
Day 19	350.7	336.6	354.5	347.7
Day 20	365.9	350.1	371.8	363.3
Body weight gain [g]				
Day 0 to 6	28.3	29.2	32.2	30.7
Day 6 to 19	106.6	92.9	107.0	102.9
Day 0 to 20	150.1	135.5	156.4	149.1

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

Mean gravid uterus weights did not show any treatment-related differences (Table 5.6.2-2).

Table 5.6.2-2: Prenatal toxicity in rats: Mean gravid uterus weights and net body weight change

Dose level [mg/kg bw/day]	0	100	300	1000
Gravid uterus (g)	83.8	70.1	84.8	78.8
Carcass (g)	282.1	279.9.	287.0	284.4
Net weight change from day 6 (g)	38.1	36.2	39.5	39.6

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

Developmental toxicity: Pregnancy rates were 88 %, 82 %, 88 % and 86 % for the control, 100, 300 and 1000 mg/kg bw/day dose groups respectively. There were no differences of biological relevance in the mean number of corpora lutea and implantation sites or regarding the pre- and post-implantation losses and viable foetuses.
Sex distribution, mean placental weights, mean foetal body weights were comparable with controls. Details are presented in Table 5.6.2-3 below.

Table 5.6.2-3: Prenatal toxicity in rats: Caesarean section data

Dose level [mg/kg bw/day]		**0**	**100**	**300**	**1000**
Pregnancy status					
- mated	[n]	25	28	25	28
- pregnant	[n]	22	23	22	24
Conception rate	[%]	88	82	88	86
- aborted	[n]	0	0	0	0
- premature birth	[n]	0	0	0	0
- dams with viable foetuses	[n]	22	18	22	21
- dams with all resorptions	[n]	0	2	0	0
- mortality	[n]	0	3	0	3
- pregnant at terminal sacrifice	[n]	22	20	22	21
Cesarean section data[a]					
- Corpora lutea	[n]	16.8	15.4	17.0	15.9
total number	[n]	369	308	375	333
- Implantation sites	[n]	16.1	13.9	16.0	14.6
total number	[n]	254	278	352	306
- Pre-implantation loss	[%]	4.1	9.6	6.5	8.6
- Post-implantation loss	[%]	9.7	17.8	5.3	6.2
- Resorptions	[n]	1.6	1.6	0.9	0.9
total number	[n]	35	32	19	19
- Early resorptions		1.5	1.4	0.9	0.9
total number	[n]	34	27	19	17
- Late resorptions		0.0	0.3	0.0	0.1
total number	[n]	1	5	0	2
- Dead foetuses	[n]	0	0	0	0
- Live foetuses	[n]	14.5	13.7	15.1	13.7
total number	[n]	319	246	333	287
- Total live female foetuses	[n]	6.5	6.6	7.5	6.9
total number	[n]	142	118	165	144
Mean	[%]	39.8	44.3	47.8	54.4
- Total live male foetuses	[n]	8.0	7.1	7.6	6.8
total number	[n]	177	128	168	143
Mean	[%]	50.5	47.0	46.9	48.4
- Percent live females		44.5	48.0	49.5	50.2
- Percent live males		55.2	52.0	50.5	49.8
Placental weights	[g]	0.45	0.45	0.44	0.44
- male foetuses	[g]	0.45	0.45	0.45	0.44
- female foetuses	[g]	0.44	0.45	0.44	0.44
Mean foetal weight	[g]	3.9	3.8	3.7	3.9
- males	[g]	4.0	3.9	3.8	4.0
- females	[g]	3.8	3.7	3.6	3.8

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

Findings in foetuses

There was no treatment-related increase in the incidences of external and soft tissue abnormalities. External malformations were seen in two females of the low dose group with anophthalmia. No further findings were made in the other dose groups and the control group. This finding has been considered to be isolated, at low incidence and within the historical control data. Some increase in the incidence of variations was observed: Dilatation of renal pelvis, ureter, and cerebral ventricles. None of these gained statistical significance in any of the dose groups when compared to control incidences. The incidences failed to show a dose response and were within historical control ranges. The same has been considered to apply for the total of foetal soft tissue variations. Details of findings are presented in Table 5.6.2-4.

Table 5.6.2-4: Prenatal toxicity in rats: Incidence of soft tissue (visceral) malformations and variations

Dose level [mg/kg bw/day]		0	100	300	1000	Historical control* Mean (%)	Historical control* Range (%)
Litters evaluated	[n]	22	18	22	21		
Foetuses evaluated	[n]	155	118	159	139		
Live	[n]	155	118	159	139		
Dead	[n]	0	0	0	0		
Total visceral malformations							
Foetal incidence	[n, (%)]	2 (1.3)	1 (0.8)	0 (0.0)	0 (0.0)		
Litter incidence	[n, (%)]	2 (9.1)	1 (5.6)	0 (0.0)	0 (0.0)		
Affected foetuses/litter	mean% ± SD	2.2 (7.6)	0.9 (3.9)	0 (0.0)	0 (0.0)		
Total visceral variations							
Foetal incidence	[n, (%)]	18 (12)	21 (18)	22 (14)	28 (20)	I (13.4)	I(5.4-20.5)
Litter incidence	[n, (%)]	12 (55)	11 (61)	12 (55)	15 (71)	I (55.3)	I(27.3-80.0)
Affected foetuses/litter	mean% ± SD	12.6 ± 14.08	17.6 ± 19.37	13.0 ± 14.55	21.1 ± 18.7	I (13.5)	I(5.0-21.0)
Individual visceral variations							
Dilated renal pelvis							
Foetal incidence	[n, (%)]	18 (12)	20 (17)	22 (14)	27 (19)	I (13.4) II (13.6)	I(5.4-20.5) II (7.6-21.6)
Litter incidence	[n, (%)]	12 (55)	10 (56)	12 (55)	15 (71)	I (55.3) II (50.4)	I(27.3-80.0) II (30.4-68.0)
Affected foetuses/litter	mean% ± SD	12.6 ± 14.08	16.7 ± 19.81	13.0 ± 14.55	20.5 ± 18.77	I (13.5) II (14.4)	I(5.0-21.0) II (7.5-21.9)
Dilated ureter/hydroureter							
Foetal incidence	[n, (%)]	2 (1.3)	1 (0.8)	6 (3.8)	3 (2.2)	I (0.6) II (1.4)	I (0-1.2) II (0.5-3.6)
Litter incidence	[n, (%)]	2 (9.1)	1 (5.6)	5 (23)	3 (14)	I (4.3) II (7.0)	I (0-8.0) II (3.2-14.3)
Affected foetuses/litter	mean% ± SD	1.4 ± 4.57	0.8 ± 3.37	3.4 ± 6.97	2.7 ± 7.15	I (0.7) II (1.6)	I (0-1.2) II (0.5-5.9)
Dilated cerebral ventricle							
Foetal incidence	[n, (%)]	0 (0.0)	1 (0.8)	0 (0.0)	1 (0.7)		
Litter incidence	[n, (%)]	0 (0.0)	1 (5.6)	0 (0.0)	1 (4.8)		
Affected foetuses/litter	mean% ± SD	0 ± 0.0	0.9 ± 3.9	0 ± 0.0	0.6 ± 2.7		

* I = Recent historical control dada (2 studies 1997-1998) with updated classification of foetal findings as malformations and variations
II = Old historical control data (10 studies, 1996-1997) with classification of foetal findings as malformations, variations and retardations

No increase in skeletal malformations was observed. Skeletal malformations were limited to mal-positioned and bipartite ossification of sternebra with the incidence of foetuses (litters) affected of 2(2), 1(1), 1(1) and 0(0) in the dose groups 0, 100, 300 and 1000 mg/kg bw/day.
There was increased incidence of incomplete ossification of the thoracic centrum with the incidence of foetuses (%) affected of 5 (3.0%), 4 (3.1%), 3 (1.7%) and 14 (9.5%) in the dose groups 0, 100, 300 and 1000 mg/kg bw/day. Litter incidences were 14%, 17%, 9% and 48% and the percentage of affected foetuses per litter was 3.0%, 3.4%, 1.6% and 9.2% in the dose groups 0, 100, 300 and 1000 mg/kg bw/day. No other parts of the vertebral column were affected. For the high dose group, both the foetal incidence (9.5%) and litter incidence (48%) were beyond the historical control range as evaluated at the time of dossier preparation. The total of foetal skeletal variations with incidences of 87.8%, 86.8%, 91.8% and 89.4 % in the dose groups 0, 100, 300 and 1000 mg/kg bw/day was unaffected. Details of findings are presented in Table 5.6.2-5.

NOTE:
Several important modifications of the respective test guidelines for prenatal developmental toxicity studies have been made between 1998 and 2001 (e.g. prolongation of the administration period, double-staining of the foetal skeletons). Moreover, terminology of developmental abnormalities has been internationally harmonised within these years (WISE, D. et al., 1997; CHAHOUD; I. et al., 1999). Thus, parts of the terminology used in the applicant's test institute had to be revised accordingly for studies being performed from about the year 2000 onwards. Due to these changes some of the reports performed in the transition phase between "old" and "new" test guideline requirements and "old" and "new" terminology (which also applies to this prenatal toxicity study) contained individual and summary tables with "new terminology", but historical control data was mainly based on the "old terminology" (see also footnotes on page 40 of the study report). This may have led to some problems concerning readability and interpretation of the results.
Moreover, further consideration should be given to the point that findings, although appearing at statistically significantly increased incidence levels in treatment groups may be lacking of toxicological relevance for several reasons (e.g. no relation to dosing; isolated finding without any corroborative, associated effects; finding within actual or historical control ranges) and thus are not considered as substance-induced. The incidence of the finding "incomplete ossification of thoracic centrum (unchanged cartilage)" is statistically significantly increased at the top dose (1000 mg/kg body weight/day) and the respective values are shown to be beyond the historical control data (according to the "old terminology") as shown on Table 5.6.2-5.

However, the increased occurrence of "incomplete ossification of thoracic centrum" is not considered to have any biological relevance for the following reasons:

Firstly, the total rates of skeletal variations (Table 5.6.2-5) were similar between the controls and the treatment groups and did not show a dose-response relationship (mean percentage of affected foetuses/litter: 87.8%, 86.8%, 91.8% and 89.4 % in the dose groups 0, 100, 300 and 1000 mg/kg bw/day). Moreover, other skeletal variations occurred at increased rates in the concurrent control at rates being also outside the historical control ranges. The variation "cervical rib (cartilage not present)", for example, occurred at the following mean percentages of affected foetuses/litter 5.6%, 3.5%, 2.2% and 1.0 % in the dose groups 0, 100, 300 and 1000 mg/kg bw/day; historical control data range on a level as shown for the top dose (Table 5.6.2-5 and Tab. IIIB-029 of the study report: 1.1 – 1.3% on the basis of mean foetuses per litter).

Secondly, a full set of historical control data on all relevant foetal findings for the studies mentioned on page I-43 of the study report and following the considerations as laid down under the "NOTES" of above is attached in Appendix 1 of this chapter M-CA 5.6.

According to the data shown in attachment 1 the historical control incidences of "incomplete ossification of thoracic centrum" vary between 1.4 – 9.7% if expressed on a foetus per litter basis as opposed to 1.8% -3.5% of the initial evaluation (Table 5.6.2-5). Thus, the statistically identified increase of 9.2% of affected foetuses per litter would need to be seen in the light of this revised evaluation of historical control data based on the same terminology for the findings in the study and in the historical control data. In conclusion the increase is within historical control data and does not support a test substance mediated effect if the overall findings including the evaluation of the overall rate of skeletal variations, the biological variability for rat skeletal variations in general are taken into consideration.

Table 5.6.2-5: Prenatal toxicity in rats: Incidence of skeletal malformations and variations

Dose level [mg/kg bw/day]		**0**	**100**	**300**	**1000**	**Historical control[#]**	
						Mean (%)	**Range (%)**
Litters evaluated	[n]	22	18	22	21		
Foetuses evaluated	[n]	164	128	174	148		
Live	[n]	164	128	174	148		
Dead	[n]	0	0	0	0		
Total skeletal malformations (mal-positioned and bipartite sternebra, unchanged cartilage only)							
Foetal incidence	[n, (%)]	2 (1.2)	1 (0.8)	1 (0.6)	0 (0.0)		
Litter incidence	[n, (%)]	2 (9.1)	1 (5.6)	1 (4.5)	0 (0.0)		
Affected foetuses/litter	mean% ± SD	1.3 (4.2)	0.8 (3.3)	0.6 (3.05)	0 (0.0)		
Total skeletal variations							
Foetal incidence	[n, (%)]	145 (88)	111 (87)	160 (92)	132 (89)	272/341 (79.8)	78.1-81.6
Litter incidence	[n, (%)]	22 (100)	18 (100)	22 (100)	21 (100)	47/47 (100)	100
Affected foetuses/litter	mean% ± SD	87.8 ± 12.1	86.8 ± 17.78	91.8 ± 12.79	89.4 ± 13.49	79.9	78.0-82.0
Individual skeletal variations							
Cervical rib (*cartilage not present*)							
Foetal incidence	[n, (%)]	9 (5.5)	5 (3.9)	4 (2.3)	1 (0.7)	1.2	0.6-1.6
Litter incidence	[n, (%)]	6 (27)	4 (22)	4 (18)	1 (4.8)	6.4	4.5-8.0
Affected foetuses/litter	mean% ± SD	5.6 ± 11.2	3.5 ± 7.31	2.2 ± 4.87	1.0 ± 4.36	1.2	1.1-1.3
Incomplete ossification of the thoracic centrum (*unchanged cartilage*)							
Foetal incidence	[n, (%)]	5 (3.0)	4 (3.1)	3 (1.7)	14 (9.5)	9/341 (2.6)	1.9-3.3
Litter incidence	[n, (%)]	3 (14)	3 (17)	2 (9.1)	10* (48)	9/47 (19.1)	13.6-24.0
Affected foetuses/litter	mean% ± SD	3.0 ± 8.16	3.4 ± 8.28	1.6 ± 5.65	**9.2*** ± 11.95	2.7	1.8-3.5 **1.4-9.7[§]**
Incomplete ossification of the thoracic centrum (*cartilage not stained due to technical error*)							
Foetal incidence	[n, (%)]	0 (0.0)	0 (0.0)	0 (0.0)	3 (2.0)		
Litter incidence	[n, (%)]	0 (0.0)	0 (0.0)	0 (0.0)	1 (4.8)		
Affected foetuses/litter	mean% ± SD	0 ± 0.0	0 ± 0.0	0 ± 0.0	1.8 ± 8.18		

* $p < 0.05$, ** $p < 0.01$Fisher's exact test one sided, Wilcoxon test one-sided

[#] = Recent historical control dada (2 studies 1997-1998) with updated classification of foetal findings as malformations and variations

[§] = new evaluation of historical control data

Discussion of findings

The administration of Boscalid during day 6-19 of gestation did not lead to maternal toxicity in Wistar rats. There were no test substance-related deaths or overt clinical signs of toxicity, nor did feed consumption, mean body weight and body weight gain show test substance-related differences. However, other information provides evidence that the administration of Boscalid at similar dose levels results in liver weight increase after exposure periods similar to the administration during day 6-19 of gestation: In a 14-day feeding study (see M-CA 5.8.2/1, Doc ID 1999/10522) Boscalid was administered to Wistar rats at the dose level of 15000 ppm in the food (corresponding to about 1500 mg/kg bw/day in females). Similar to the prenatal toxicity study no effects on mortality, overt clinical signs of toxicity and body weight were observed. Liver weight, however, was increased by 23% in female rats, with concomitant increase of CYP P450 content and proliferation/accumulation of the smooth endoplasmatic reticulum in zone 3 hepatocytes, as well as glycogen depletion. It is thus reasonable to assume, albeit there was absence of overt clinical toxicity in the prenatal toxicity study that a comparable level of liver toxicity was occurring when 1000 mg/kg bw/day were administered to dams.

Boscalid did not cause malformations in the prenatal developmental toxicity study in Wistar rats.

In foetuses of dams administered the limit dose of 1000 mg/kg bw/day during day 6-19 of gestation increased incidence of incomplete ossification of the thoracic centrum was observed as the only prominent finding. No other developmental effects to which biological relevance could be attributed were observed. The evaluation of the data within the first Annex I inclusion process came to the conclusion that the increased incidence of incomplete ossification of the thoracic centrum has been test substance mediated with the study findings being out of the historical control range. The latter conclusion was mainly based on the terminology of historical control data applied at this time of evaluation. The new evaluation with consideration of the harmonized terminology as discussed in detail under “NOTES” (see above) allows the conclusion that the incidences of incomplete ossification of thoracic centrum (= “thoracic vertebral body/bodies incompletely ossified" according to the “old terminology”) vary between 1.4 – 9.7% if expressed on a foetus per litter basis as opposed to 1.8% -3.5% of the initial evaluation. Thus, the statistically identified increase of 9.2% of affected foetuses per litter would need to be seen in the light of this revised evaluation of historical control data based on the same terminology for the findings in the study. In conclusion the increase is within historical control data and does not support a test substance mediated effect. This is underlined if the overall findings including the evaluation of the overall rate of skeletal variations, the biological variability for rat skeletal variations in general are taken into consideration. The effect notably is devoid of a clear dose response with regard to the foetal and litter incidences as well as the percentage of affected foetuses per litter because the intermediate dose level of 300 mg/kg bw/day would be expected to exert some increase in these incidences, too. However, the incidences (foetal, litter and percentage of affected foetuses per litter) of the intermediate dose were factually lower than the control values. As supportive finding, the total rates of skeletal variations were similar between the controls and the treatment groups and did not show a dose-response relationship either. It is furthermore reasonable to expect that liver toxicity was present in dams at the dose level of 1000 mg/kg bw/day. No developmental effects were observed at the dose level of 300 mg/kg bw/day.

Conclusions
Boscalid did not cause malformations in the prenatal developmental toxicity study in rats.

For maternal toxicity the NOAEL has been set at 1000 mg/kg bw/day based on the absence of overt toxicity.

The proposed NOAEL for developmental toxicity in rats is 1000 mg/kg bw/day which is mainly based firstly on the revised evaluation of the historical control data showing the incidence of the finding “incomplete ossification of the thoracic centrum” to be within the range of biological variation of this strain of rats in the test institute and secondly on the absence of a dose response relationship if the lower dose levels are taken into consideration.

The previous NOAEL for developmental toxicity of 300 mg/kg bw/day is **not** supported anymore.

BAS 510 F – Prenatal developmental toxicity study in Himalayan rabbits – Oral administration – gavage- ([redacted] 2000)
DocID 2000/1013425

Guidelines: According to OECD 414 (draft document, March 1998), EEC 87/302, EPA OPPTS 870.3700 and JMAFF
Deviations: None
GLP: Yes
Acceptance: The study has been evaluated and considered acceptable in the EU registration process for the first Annex I inclusion.

Note: This study report has been part of the documentation for the first Annex I inclusion process but a more detailed summary is presented here to assist in discussion on classification and labelling and evaluation due in M-CA 5.8.3 (ED related properties)

Groups of 25 female Himalayan rabbits (Chbb:HM; source: Boehringer Ingelheim Pharma KG, Germany, 34-36 weeks of age) were administered Boscalid by gavage (batch N37; purity 94.4 % purity), suspended in 0.5 % Tylose CB 30.000 in doubly distilled water. Doses administered were 0, 100, 300 and 1000 mg/kg bw/day, from days 7 to 28 (inclusive) of gestation (post-insemination). Food consumption and health status were recorded on a daily basis throughout the study period and body weights at 2-3 day intervals. On day 29 post-insemination, all surviving females were killed and assessed by gross pathology (including weight determination of the unopened uterus and the placentae). For each dam, corpora lutea were counted and the number and distribution of implantation sites (differentiated as resorptions, live and dead foetuses) were determined. The foetuses were removed from the uterus, sexed, weighed and further investigated for any external, soft tissue and skeletal findings.

Findings
The stability of the test substance was proven by reanalysis. The stability and homogeneity of the test substance preparation was analytically verified and the correctness of the concentrations chosen was demonstrated.

Findings in dams

Maternal toxicity: One control and one female at 300 mg/kg bw/day were found dead. The deaths were considered to be due to misgavage based on the necropsy findings. One female at 100 mg/kg bw/day was found to be not pregnant and was excluded from further evaluation. Abortions occurred in 1 female at 300 mg/kg bw/day and in 3 females of the 1000 mg/kg bw/day dose group. One female at 1000 mg/kg bw/day delivered prematurely and was killed. Reduced defaecation was observed in 1 female at 300 mg/kg bw/day a few days prior to abortion and discoloured faeces were observed in 1 female at 1000 mg/kg bw/day before abortion.

Food consumption was markedly reduced at 1000 mg/kg bw/day (-26%) throughout the treatment period if compared with controls. Mean terminal body weight was statistically significantly reduced (-4.7%) at 1000 mg/kg bw/day compared with controls and body weight gain for the period of day 7 –28 was markedly reduced (-81%) compared with controls. Mean terminal body weights, carcass weights and body weight gain was normal and comparable to controls at dose levels of ≤ 300 mg/kg bw/day. Details are presented in Table 5.6.2-6. Mean gravid uterine weights were not indicative of adverse effects over all dose groups if compared with control animals. The low dose females had a statistically significantly decreased net body weight change (Table 5.6.2-7). However, this has been caused by the extraordinary high gravid uterus weight rather than to be a true adverse finding in the low dose group. Details of findings are summarized in Table 5.6.2-6 and Table 5.6.2-7 below.

Table 5.6.2-6: Prenatal toxicity in rabbits: Feed consumption and body weight in rats during days 7 to 28 of gestation

Dose level [mg/kg bw/day]	0	100	300	1000
Food consumption [g/animal/day]				
Day 0 to 7	95.0	98.7	93.2	92.3
Day 7 to 28	76.1	69.0	68.3	56.2 (-26%)
Day 0 to 29	80.5	76.2	74.6	65.2
Body weight [g]				
Day 0	2733	2698	2723	2700
Day 7	2744	2752	2755	2742
Day 28	2873	2878	2880	2734* (-4.6%)
Day 29	2889	2891	2895	2752* (-4.7%)
Body weight gain [g]				
Day 0 to7	11.0	53.9*	31.6	41.5
Day 7 to 28	146.8	125.5	115.0	27.8** (-81%)
Day 0 to 29	174.7	193.0	170.4	96.1

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

Table 5.6.2-7: Prenatal toxicity in rabbits: Mean gravid uterus weights and net body weight change

Dose level [mg/kg bw/day]	0	100	300	1000
Gravid uterus (g)	288.1	359.7*	320.5	298.4
Carcass (g)	2601.1	2531.6	2574.4	2453.8*
Net weight change from day 7 (g)	-125.3	-220.6**	-190.3	-233.3**

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

Pregnancy rates were 100 %, 96 %, 100 % and 100 % for the control, 100, 300 and 1000 mg/kg bw/day dose groups respectively. There were no differences of biological relevance in the mean number of corpora lutea and implantation sites or regarding the pre- and post-implantation losses and viable foetuses.
Sex distribution, mean placental weights, mean foetal body weights were comparable with controls (Table 5.6.2-8).

Table 5.6.2-8: Prenatal toxicity in rabbits: Caesarean section data

Dose level [mg/kg bw/day]		**0**	**100**	**300**	**1000**
Pregnancy status					
- mated	[n]	25	25	25	25
- pregnant	[n]	25	24	25	25
conception rate	[%]	100	96	100	100
- aborted	[n]	0	0	1	3
- premature birth	[n]	0	0	0	1
- dams with viable foetuses	[n]	23	24	22	21
- dams with all resorptions	[n]	1	0	1	0
- mortality	[n]	1	0	2	3
- pregnant at terminal sacrifice	[n]	24	24	23	21
Cesarean section data[a]					
- Corpora lutea	[n]	8.7	9.2	8.6	9.1
total number	[n]	209	220	197	192
- Implantation sites	[n]	6.8	8.3*	7.7	7.3
total number	[n]	164	199	177	153
- Pre-implantation loss	[%]	22.5	9.5*	9.9*	20.2
- Post-implantation loss	[%]	19.9	7.7	16.8	14.2
- Resorptions	[n]	1.2	0.7	1.4	0.8
total number	[n]	29	16	32	17
- Early resorptions		0.9	0.6	1.1	0.5
total number	[n]	22	14	25	11
- Late resorptions		0.3	0.1	0.3	0.3
total number	[n]	7	2	7	6
- Dead foetuses	[n]	0	0	0	0
- Live foetuses	[n]	5.9	7.6*	6.6	6.5
total number	[n]	135	183	145	136
- Total live female foetuses	[n]	3.1	3.6	3.6	3.7
total number	[n]	72	87	79	77
Mean	[%]	44.6	43.1	46.0	46.7
- Total live male foetuses	[n]	2.7	4.0**	3.0	2.8
total number	[n]	63	96	66	59
Mean	[%]	39.0	49.1	41.0	39.1
- Percent live females		53.3	47.5	54.5	56.6
- Percent live males		46.7	52.5	45.5	43.4
Placental weights	[g]	4.2	4.1	4.3	4.0
- male foetuses	[g]	4.2	4.1	4.3	4.1
- female foetuses	[g]	4.2	4.0	4.2	3.8
Mean foetal weight	[g]	36.5	34.9	37.3	34.9
- males	[g]	36.2	34.8	37.2	34.9
- females	[g]	36.4	35.0	36.6	34.4

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

In conclusion in this developmental toxicity study in Himalayan rabbits the oral administration of Boscalid from day 7 to 28 post insemination elicited overt maternal toxicity at the dose level of 1000 mg/kg bw/day based on increased incidence of abortions, reduced feed consumption. Means of terminal body weight, body weight gain, carcass weight and corrected body weight gain were significantly reduced if compared to control. Possibly, the dose of 300 mg/kg bw/day caused abortion in one dam. No adverse effects were observed in dams of the 100 mg/kg bw/day dose level.

Findings in foetuses

There were no gross abnormalities found at necropsy regarding external and soft tissue examination. External malformations occurred in low incidence and without dose relationship comprising spina bifida (1 only at 100 mg/kg bw/day) and one foetus with meningoencepahalae and microcephaly at 300 mg/kg bw/day. In one foetus thread-like tail at 300 mg/kg bw/day was observed. There were no incidents for the control and the 1000 mg/kg bw/day groups. The skeletal malformations observed did not show any dose relationship and the occurrences were within the normal control range. The only developmental effect observed at some increased incidence (without statistical significance) was paw hyperflexion occurring in comparable number with foetal incidence of 3.7%, 2.2%, 2.8% and 1.5% for the dose groups of 0, 100, 300 and 1000 mg/kg bw/day, and litter incidence was 17%, 13%, 18% and 9.5%. Paw hyperflexion is a commonly observed, reversible external variation. No findings attributable to test substance related malformations/variations were made. Soft tissue examination gave no test substance related findings. A variety of visceral (soft tissue) malformations were observed in all groups including the control group. The highest number of malformation was either observed in the control group or isolated findings occurred in dose groups without dose relationship. As no statistically significant differences were observed and the malformations occurred either singly, without dose response-relationship none of these visceral malformations were considered to be treatment-related. For soft tissue variations mal-positioned carotid branch was observed in the control and all dose groups. Mal-positioned carotid branch is a common finding in the strain of rabbits used in this study. This finding has been lacking of a dose response with historical control data for foetal incidence and litter incidence to be within historical control data (Table 5.6.2-9) and has thus been considered to be not induced by the test substance.

There was increased incidence of incomplete ossification of the thoracic centrum. Foetal incidence was 0.7%, 2.2%, 4.1% and 12% for the dose groups of 0, 100, 300 and 1000 mg/kg bw/day, and litter incidence was 4.3%, 17%, 14% and 33%. Affected foetuses per litter were 0.7%, 1.8%, 2.9% and 8.3%. Both litter incidence and affected foetuses per litter were identified as statistically significant for the dose group of 1000 mg/kg bw/day with dose related increase. No associated finding was noted in the other parts of the vertebral column. All other skeletal variations observed gave no indication of being related to the test substance and/or were within the historical range of control data investigated. For the total of skeletal findings incidences were comparable to the concurrent control with the foetal incidence of 61%, 69%, 59% and 62% for the dose groups of 0, 100, 300 and 1000 mg/kg bw/day, and litter incidence was 91%, 100%, 95% and 90%. Affected foetuses per litter were 59.7%, 68.2%, 59.8% and 56.5%

NOTE:
The occurrence of the finding “incomplete ossification of thoracic centrum” (Table 5.6.2-9) is statistically significantly increased at the top dose (1,000 mg/kg body weight/day) and the respective value has been discussed to be beyond the range of historical control data in course of the first evaluation for the Annex I inclusion of Boscalid.
However, due to the changes in terminology of foetal findings (as explained above and in the footnote on page 044 of the study report), it should be taken into account that the finding “incomplete ossification of thoracic centrum” (classified as a variation according to CHAHOUD, I. et al., 1999) was designated as “thoracic vertebral body/bodies incompletely ossified" according to the "old terminology" and had been classified as a “retardation”. Attachment 2 lists incidences of “skeletal retardations” for several prenatal developmental toxicity studies in Himalayan rabbits performed at BASF’s test institute in the past using the “old terminology”. It becomes obvious, that the finding “thoracic vertebral body/bodies incompletely ossified" (which is equivalent to the finding “incomplete ossification of thoracic centrum”) occurs at a distinctly higher rate (range: 0.0 – 17.3% affected foetuses/litter) in this historical control data set than in the present study at the top dose group (8.3% affected foetuses/litter).
Moreover, the increased occurrence of “incomplete ossification of thoracic centrum” is not considered to have biological relevance as the total rates of skeletal variations in the rabbit study in question were similar between the controls and the substance-treated groups and did not show a dose-response relationship (mean percentage of affected foetuses/litter: 59.7%, 68.2%, 59.8% and 56.5%) and thus were actually lowest at the top dose. According to the data shown in appendix 2 the incidences of “incomplete ossification of thoracic centrum” (= “thoracic vertebral body/bodies incompletely ossified" according to the “old terminology”) vary between 0.0 – 17.3% if expressed on a foetus per litter basis. Thus, the value of 8.3% as calculated for the rabbit prenatal developmental toxicity study is fully covered by historical control data and does not suggest a substance-mediated effect if the overall rate of skeletal variations and the biological variability for rabbit skeletal variations in general and for “incomplete ossification of thoracic centrum” (= “thoracic vertebral body/bodies incompletely ossified" according to the “old terminology”) in particular are taken into consideration.

Table 5.6.2-9: Prenatal toxicity in rabbits: Incidence of soft tissue (visceral) malformations and variations

Dose level [mg/kg bw/day]		0	100	300	1000	Historical control[#] Mean (%)	Range (%)
Litters evaluated	[n]	23	24	22	21		
Foetuses evaluated	[n]	135	183	145	136		
Live	[n]	135	183	145	136		
Dead	[n]	0	0	0	0		
Total visceral malformations							
Foetal incidence	[n, (%)]	5 (3.7%)	5 (2.7%)	3 (2.1%)	0 (0.0%)		
Litter incidence	[n, (%)]	4 (17%)	5 (21%)	2 (9.1%)	0 (0.0%)		
Affected foetuses/litter	mean% ± SD	3.3 (7.8%)	3.5 (8.1%)	1.8 (6.0%)	0 (0.0%)		
Total visceral variations							
Foetal incidence	[n, (%)]	7 (5.2%)	15 (8.2%)	9 (6.2%)	12 (8.8%)		
Litter incidence	[n, (%)]	6 (26%)	9 (38%)	4 (18%)	9 (43%)		
Affected foetuses/litter	mean% ± SD	5.3 ± 9.8	7.3 ± 11.3	6.1 ± 16.2	7.5 ± 10.9		
Individual visceral variations							
Mal-positioned carotid branch							
Foetal incidence	[n, (%)]	7 (5.2%)	15 (8.2%)	9 (6.2%)	12 (8.8%)	2	0.0-11.4
Litter incidence	[n, (%)]	6 (26%)	9 (38%)	4 (18%)	9 (43%)	9.9	0.0-58.3
Affected foetuses/litter	mean% ± SD	5.3 ± 9.8	7.3 ± 11.3	6.1 ± 16.2	7.5 ± 10.9	1.9	0.0-11.5

* p < 0.05, ** p < 0.01Fisher's exact test one sided, Wilcoxon test one-sided
[#] = Recent historical control dada (8 studies 1995-1998)

Table 5.6.2-10: Prenatal toxicity in rabbits: Incidence of skeletal malformations and variations

Dose level [mg/kg bw/day]]		0	100	300	1000	Historical control[#] Mean (%)	Range (%)
Litters evaluated	[n]	23	24	22	21		
Foetuses evaluated	[n]	135	183	145	136		
Live	[n]	135	183	145	136		
Dead	[n]	0	0	0	0		
Total skeletal malformations							
Foetal incidence	[n, (%)]	2 (1.5%)	5 (2.7%)	5 (3.4%)	2 (1.5%)		
Litter incidence	[n, (%)]	2 (8.7%)	3 (13%)	4 (18%)	2 (9.5%)		
Affected foetuses/litter	mean% ± SD	1.2 (4.1%)	2.3 (6.3%)	2.6 (5.9%)	1.9 (6.2%)		
Total skeletal variations							
Foetal incidence	[n, (%)]	82 (61%)	127 (69%)	86(59%)	84 (62%)		
Litter incidence	[n, (%)]	21 (91%)	24 (100%)	21 (95%)	19 (90%)		
Affected foetuses/litter	mean% ± SD	59.7 ± 31.7	68.2 ± 20.0	59.8 ± 26.5	56.5 ± 26.7		
Individual skeletal variations							
Incomplete ossification of the thoracic centrum							
Foetal incidence	[n, (%)]	1 (0.7%)	4 (2.2%)	4 (4.1%)	16 (12%)	0.1	0.0-0.6
Litter incidence	[n, (%)]	1 (4.3%)	4 (17%)	3 (14%)	7 (33%)*	0.7	0.0-4.2
Affected foetuses/litter	mean% ± SD	0.7 ± 3.5	1.8 ± 4.2	2.9 ± 9.0	**8.3**** ± 17.2	0.1	0.0-0.5 **0.0-17.3**[§]

* p < 0.05, ** p < 0.01Fisher's exact test one sided, Wilcoxon test one-sided
[#] = Recent historical control dada (8 studies 1995-1998) according to evaluation as made for the first Annex I inclusion
[§] = new evaluation of historical control data

Discussion of findings
The administration of Boscalid during day 7-28 of gestation lead to distinct maternal toxicity as elicited by abortions occurring in 1 female at 300 mg/kg bw/day and in 3 females of the 1000 mg/kg bw/day dose group. One female at 1000 mg/kg bw/day delivered prematurely and was killed. Food consumption was markedly reduced at 1000 mg/kg bw/day (-26%) throughout the treatment period. Mean terminal body weight was statistically significantly reduced (-4.7%) at this dose level and the body weight gain was markedly reduced (-81%). Boscalid did not cause malformations in the prenatal developmental toxicity study in rabbits. The only prominent finding was increased incidence of “incomplete ossification of the thoracic centrum” observed in foetuses of dams administered the limit dose of 1000 mg/kg bw/day. No other developmental effects of biological relevance were observed. The percentage of affected foetuses and the litter incidence was statistically identified to be different to controls. This finding has been reviewed in the light of the changes in terminology. The new evaluation with consideration of the harmonized terminology as discussed in detail under “NOTES” (see above) allow the conclusion that the incidences of “incomplete ossification of thoracic centrum” (= “thoracic vertebral body/bodies incompletely ossified" according to the “old terminology”) vary between 0.0 – 17.3% if expressed on a foetus per litter basis. Thus, the percentage of affected foetuses per litter (8.3%) is considered to be within the historical control data. This is considered indicative of the increased incidence of “incomplete ossification of thoracic centrum” to be not a direct biological effect of developmental toxicity mediated by the test substance administration. The conclusion that the increased incidence of this variation is not a test substance specific effect is mainly supported by concomitant severe maternal toxicity suggesting these findings to be secondary to systemic maternal toxicity. This conclusion is further supported if the overall rate of skeletal variations and the biological variability for rabbit skeletal variations in general and for “incomplete ossification of thoracic centrum” (= “thoracic vertebral body/bodies incompletely ossified" according to the “old terminology”) in particular are taken into consideration.

Conclusions
Boscalid did not cause malformations in the prenatal developmental toxicity study in rabbits.

For maternal toxicity the NOAEL has been set at 100 mg/kg bw/day based on incidences of abortions increasing at dose levels of $\geq$ 300 mg/kg bw/day as well as adverse effects on food consumption and body weight effects at 1000 mg/kg bw/day.

No developmental effects were observed at the dose level of 300 mg/kg bw/day. The NOAEL for developmental toxicity in rabbits has been set at 300 mg/kg bw/day. The increased incidence of the finding “incomplete ossification of the thoracic centrum”, however, is considered to be secondary to severe maternal toxicity and thus not a direct test substance mediated effect and is also within the historical control data of this specific variation.

Literature data

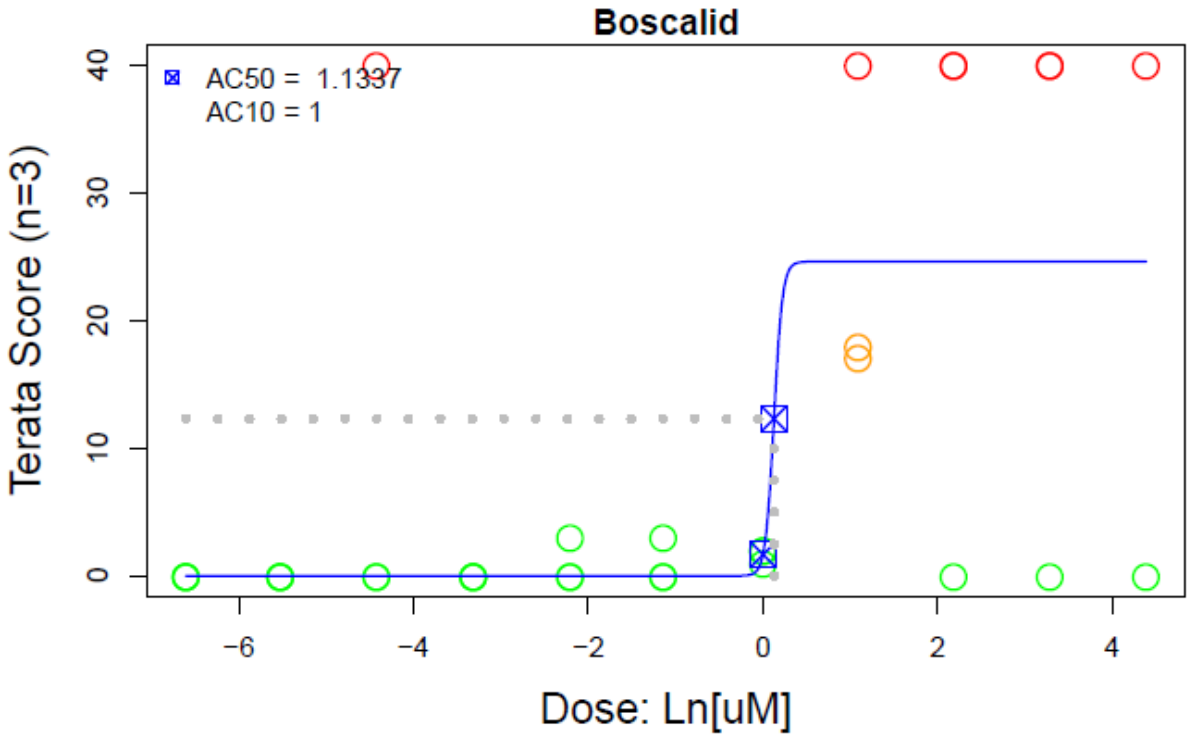
Report: CA 5.6.2/1
Padilla S. et al., 2011a
Zebrafish developmental screening of the ToxCast Phase I chemical library
2012/1368722
Guidelines: none
GLP: no

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 screening for developmental toxicity. All exposures were by immersion from 6–8 h post fertilisation (hpf) to 5 days post fertilisation (dpf); nominal concentration range of 1 nM–80 µM. 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 40 was calculated for Boscalid based on the single concentration study, indicating a positive response. An AC_{50} of 1.1337 µM was derived for Boscalid in the dose-response experiment. A steep increase of the Terata Score was observed for Boscalid around the AC_{50} of 1.1337 µM indicating that malformations may only occur within a very narrow concentration range. The occurrence of larvae with malformations is considered to be of rather equivocal relevance, as no dose-response relationship was observed and a high variation of individual scores occurred at high doses (larvae within the normal range and non-viable larvae).

Figure 5.6.2-1 Boscalid Tox Cast evaluation – results of zebrafish (Danio rerio) developmental toxicity screening



Classification of the study: supplemental information

Appendix 1

Summary of historical control data on foetal variations in Wistar rats from BASF studies (Projects 96132, 96/168, 96188, 97142, 98070 and 99011 - Evaluation period of March 04, 1997 to December 16, 1999)

16-JAN-03

TABLE : 97140_JAP

HISTORICAL CONTROL DATA FOR TOTAL MALFORMATIONS AND TOTAL VARIATIONS

SPECIES: RAT
STRAIN: WISTAR
SUPPLIER: Thomae/BOEHRING.

	FETUSES				LITTERS				AFFECTED FETUSES/LITTER		
	POOLED		BY STUDY		POOLED		BY STUDY		POOLED	BY STUDY	
	N	%	LO%	HI%	N	%	LO%	HI%	MEAN%	LO%	HI%
NUMBER EVALUATED	1693				122						
TOTAL FETAL MALFORMATIONS	17	1.00	0.00	1.96	14	11.48	0.00	20.83	1.03	0.00	2.07
TOTAL FETAL VARIATIONS	860	50.80	44.68	57.99	122	100.00	100.00	100.0	50.89	44.90	57.42

Appendix 1 continued

16-JAN-03

TABLE :

SPECIES RAT
STRAIN WISTAR
SUPPLIER Thomae/BOEHRING.

HISTORICAL CONTROL DATA
FETAL SKELETAL VARIATIONS

	FETUSES N	FETUSES %	%RANGE (per study)		LITTERS N	LITTERS %	%RANGE (per study)		AFFECTED FETUSES/LITTER MEAN %	RANGE (per study)	
NUMBER EVALUATED	876				122						
Live	876										
Dead	0										
V EXTRA STERNEBRAL OSSIFICATION SITE	2	0.2	0.0	0.7	2	1.6	0.0	5.0	0.3	0.0	1.0
V SHORT RIB (13TH)	89	10.2	6.7	12.2	51	41.8	33.3	50.0	9.8	5.8	12.4
V CERVICAL RIB	23	2.6	0.6	5.5	14	11.5	4.5	25.0	2.8	0.7	6.7
V SUPERNUMERARY RIB (14TH)	10	1.1	0.0	2.5	9	7.4	0.0	13.6	1.1	0.0	2.2
V ABSENT RIB (13TH)	1	0.1	0.0	0.6	1	0.8	0.0	4.5	0.1	0.0	0.6
V INCOMPLETE OSSIFICATION OF METACARPAL	1	0.1	0.0	0.7	1	0.8	0.0	5.0	0.1	0.0	0.6
V INCOMPLETE OSSIFICATION OF METATARSAL	1	0.1	0.0	0.7	1	0.8	0.0	5.0	0.1	0.0	0.6
TOTAL FETAL SKELETAL VARIATIONS	730	83.3	70.9	95.9	122	100.0	100.0	100.0	83.7	71.8	96.0

OBSERVATION CODE: V=Variation

Appendix 1 continued

16-JAN-03

TABLE :

SPECIES RAT HISTORICAL CONTROL DATA
STRAIN WISTAR FETAL SKELETAL VARIATIONS
SUPPLIER Thomae/BOEHRING.

	FETUSES N	FETUSES %	%RANGE (per study)		LITTERS N	LITTERS %	%RANGE (per study)		AFFECTED FETUSES/LITTER MEAN	RANGE (per study) %		
NUMBER EVALUATED	876				122							
Live	876											
Dead	0											
V INCOMPLETE OSSIFICATION OF HYOID	1	0.1	0.0	0.7	1	0.8	0.0	5.0	0.1	0.0	0.6	
V UNOSSIFIED HYOID	4	0.5	0.0	2.0	2	1.6	0.0	5.0	0.5	0.0	2.1	
V INCOMPLETE OSSIFICATION OF INTERPARIETAL	24	2.7	0.0	6.1	18	14.8	0.0	35.0	2.7	0.0	6.2	
V BIPARTITE OSSIFICATION OF SUPRAOCCIPITAL	6	0.7	0.0	1.4	6	4.9	0.0	10.0	0.6	0.0	1.4	
V SUPRAOCCIPITAL HOLE(S)	330	37.7	20.2	62.2	107	87.7	76.0	100.0	38.0	20.4	61.9	
V EXTRA OSSIFICATION SITE (BETW. PARIETAL AND INTERPARIETAL)	3	0.3	0.0	1.4	3	2.5	0.0	10.0	0.4	0.0	1.7	
V INCOMPLETE OSSIFICATION OF PARIETAL	3	0.3	0.0	0.7	3	2.5	0.0	5.0	0.3	0.0	0.7	
V INCOMPLETE OSSIFICATION OF SUPRAOCCIPITAL	1	0.1	0.0	0.7	1	0.8	0.0	4.8	0.1	0.0	0.7	
V INCOMPLETE OSSIFICATION OF SKULL	6	0.7	0.0	4.1	4	3.3	0.0	15.0	0.7	0.0	4.3	
V BASIOCCIPITAL HOLE(S)	1	0.1	0.0	0.7	1	0.8	0.0	5.0	0.1	0.0	0.7	
V INTERPARIETAL FISSURE	27	3.1	0.0	12.9	18	14.8	0.0	54.2	3.3	0.0	13.5	
V INCOMPLETE OSSIFICATION OF FRONTAL	1	0.1	0.0	0.6	1	0.8	0.0	4.2	0.1	0.0	0.6	
V BASISPHENOID FISSURE	6	0.7	0.0	8.1	4	3.3	0.0	40.0	0.7	0.0	8.2	
V INTERPARIETAL HOLE(S)	3	0.3	0.0	1.8	2	1.6	0.0	8.3	0.3	0.0	1.6	
V INCOMPLETE OSSIFICATION OF CERVICAL ARCH	1	0.1	0.0	0.6	1	0.8	0.0	4.2	0.1	0.0	0.5	

Appendix 1 continued

16-JAN-03

TABLE :

SPECIES RAT — HISTORICAL CONTROL DATA
STRAIN WISTAR — FETAL EXTERNAL VARIATIONS
SUPPLIER Thomae/BOEHRING.

	FETUSES N	%RANGE (per study) %	%	%	LITTERS N	%RANGE (per study) %	%	%	AFFECTED FETUSES/LITTER MEAN	RANGE (per study) %	%	%
NUMBER EVALUATED	1693				122							
Live	1693											
Dead	0											
TOTAL FETAL EXTERNAL VARIATIONS	0	0.0	0.0	0.0	0	0.0	0.0	0.0	0.0	0.0	0.0	

SPECIES RAT — HISTORICAL CONTROL DATA
STRAIN WISTAR — FETAL SOFT TISSUE VARIATIONS
SUPPLIER Thomae/BOEHRING.

	FETUSES N	%RANGE (per study) %	%	%	LITTERS N	%RANGE (per study) %	%	%	AFFECTED FETUSES/LITTER MEAN	RANGE (per study) %	%	%
NUMBER EVALUATED	817				122							
Live	817											
Dead	0											
Y DILATED CEREBRAL VENTRICLE	1	0.1	0.0	0.7	1	0.8	0.0	4.8	0.1	0.0	0.7	
Y DILATED RENAL PELVIS	130	15.9	5.4	20.5	75	61.5	27.3	80.0	15.5	5.0	21.0	
Y DILATED URETER	10	1.2	0.0	4.2	10	8.2	0.0	30.0	1.2	0.0	4.2	
TOTAL FETAL SOFT TISSUE VARIATIONS	130	15.9	5.4	20.5	75	61.5	27.3	80.0	15.5	5.0	21.0	

OBSERVATION CODE: Y=Variation

Appendix 1 continued

16-JAN-03

TABLE :

SPECIES RAT
STRAIN WISTAR
SUPPLIER Thomae/BOEHRING.

HISTORICAL CONTROL DATA
FETAL SKELETAL VARIATIONS

	FETUSES N	%	%RANGE (per study)		LITTERS N	%	%RANGE (per study)		AFFECTED FETUSES/LITTER MEAN	RANGE (per study)	
			%	%			%	%	%	%	%
NUMBER EVALUATED	876				122						
Live	876										
Dead	0										
V INCOMPLETE OSSIFICATION OF THORACIC CENTRUM	35	4.0	1.3	9.8	26	21.3	9.5	40.0	4.1	1.4	9.7
V DUMBBELL OSSIFICATION OF THORACIC CENTRUM	155	17.7	9.5	24.5	73	59.8	40.0	70.8	18.0	9.6	25.1
V HEMICENTRIC THORACIC CENTRUM	1	0.1	0.0	0.5	1	0.8	0.0	4.0	0.1	0.0	0.5
V BIPARTITE OSSIFICATION OF THORACIC CENTRUM	9	1.0	0.0	4.1	9	7.4	0.0	30.0	1.1	0.0	4.3
V UNOSSIFIED THORACIC CENTRUM	1	0.1	0.0	0.6	1	0.8	0.0	4.2	0.1	0.0	0.5
V INCOMPLETE OSSIFICATION OF LUMBAR ARCH	1	0.1	0.0	0.5	1	0.8	0.0	4.0	0.1	0.0	0.6
V SUPERNUMERARY LUMBAR VERTEBRA	1	0.1	0.0	0.6	1	0.8	0.0	4.5	0.1	0.0	0.8
V INCOMPLETE OSSIFICATION OF SACRAL ARCH	49	5.6	0.0	21.6	20	16.4	0.0	50.0	5.4	0.0	21.6
V UNOSSIFIED SACRAL ARCH	1	0.1	0.0	0.6	1	0.8	0.0	4.2	0.1	0.0	0.5
V INCOMPLETE OSSIF. OF SACRAL ARCH	9	1.0	0.0	5.5	6	4.9	0.0	25.0	0.9	0.0	4.8
V UNOSSIFIED STERNEBRA	86	9.8	4.4	15.5	41	33.6	16.0	45.0	9.8	4.2	15.0
V INCOMPLETE OSSIFICATION OF STERNEBRA	221	25.2	14.2	58.1	92	75.4	64.0	100.0	25.1	14.3	58.4
V HEMICENTRIC STERNEBRA	102	11.6	7.4	16.7	57	46.7	29.2	60.0	11.7	7.0	17.7
V MISSHAPEN STERNEBRA	407	46.5	18.9	55.4	116	95.1	80.0	100.0	46.5	19.2	54.8
V BIPARTITE OSSIFICATION OF STERNEBRA	14	1.6	0.5	2.7	12	9.8	4.0	19.0	1.6	0.6	3.0

Appendix 2

Summary of historical control data on foetal variations in Himalayan rabbits from BASF studies (Projects 91107, 92064, 92080, 92082, 92086, 92100, 93051, 93055, 93064, 94040, 95097, 96076, 96135- Evaluation period of January 01, 1993 to December 12, 1997))

16-JAN-03

TABLE :

SPECIES RABBIT
STRAIN HIMALAYAN RABBIT
SUPPLIER ALL

HISTORICAL CONTROL DATA
FETAL SKELETAL RETARDATIONS

DATES: 01-JAN-93 - 12-DEC-97

	FETUSES N	FETUSES %	%RANGE (per study)		LITTERS N	LITTERS %	%RANGE (per study)		AFFECTED FETUSES/LITTER MEAN	AFFECTED FETUSES/LITTER %	RANGE (per study)	
			%	%			%	%		%	%	%
NUMBER EVALUATED	1353				204							
Live	1351											
Dead	2											
R SKULL INCOMPLETELY OSSIFIED	8	0.6	0.0	2.9	8	3.9	0.0	20.0	0.5	0.0	2.7	
R HYOID BONE INCOMPLETELY OSSIFIED	143	10.6	0.0	46.3	63	30.9	0.0	94.1	10.8	0.0	48.8	
R INTERPARIETAL AND/OR PARIETAL BONES INCOMPLETELY OSSIFIED	12	0.9	0.0	4.3	9	4.4	0.0	23.5	0.9	0.0	5.9	
R ENLARGED FRONTAL FONTANELLE	2	0.1	0.0	1.0	2	1.0	0.0	6.7	0.1	0.0	0.7	
R HYOID BONE NOT OSSIFIED	1	0.07	0.0	0.8	1	0.5	0.0	5.6	0.1	0.0	0.8	
R CERVICAL VERTEBRAL BODY/BODIES INCOMPLETELY OSSIFIED	61	4.5	0.0	14.0	40	19.6	0.0	58.8	4.3	0.0	12.4	
R CERVICAL VERTEBRAL BODY/BODIES DUMBBELL-SHAPED	1	0.07	0.0	0.8	1	0.5	0.0	5.6	0.1	0.0	0.8	
R THORACIC VERTEBRAL BODY/BODIES INCOMPLETELY OSSIFIED	28	2.1	0.0	18.3	19	9.3	0.0	73.3	1.8	0.0	17.3	
R THORACIC VERTEBRAL BODY/BODIES DUMBBELL-SHAPED (SYMMETR.)	2	0.1	0.0	1.0	2	1.0	0.0	7.1	0.1	0.0	1.0	
R THORACIC VERTEBRAL BODY/BODIES DUMBBELL-SHAPED	1	0.07	0.0	0.9	1	0.5	0.0	5.9	0.1	0.0	1.2	
R LUMBAR VERTEBRAL ARCH(ES) INCOMPLETELY OSSIFIED	27	2.0	0.0	12.1	21	10.3	0.0	53.3	1.7	0.0	11.1	
R SACRAL VERTEBRAL ARCH(ES) INCOMPLETELY OSSIFIED	9	0.7	0.0	3.5	8	3.9	0.0	17.6	0.6	0.0	2.8	
R SACRAL VERTEBRAL ARCH(ES) NOT OSSIFIED	1	0.07	0.0	0.9	1	0.5	0.0	5.9	0.0	0.0	0.6	

OBSERVATION CODE: R=Retardation

Attachment 2 / Page 1 of 2

Appendix 2 continued

16-JAN-03

TABLE :

SPECIES RABBIT
STRAIN HIMALAYAN RABBIT
SUPPLIER ALL

HISTORICAL CONTROL DATA
FETAL SKELETAL RETARDATIONS

DATES: 01-JAN-93 - 12-DEC-97

	FETUSES N	FETUSES %	%RANGE (per study)		LITTERS N	LITTERS %	%RANGE (per study)		AFFECTED FETUSES/LITTER MEAN	AFFECTED FETUSES/LITTER %RANGE (per study)	
NUMBER EVALUATED	1353				204						
Live	1351										
Dead	2										
R SCAPULA(E) INCOMPLETELY OSSIFIED	2	0.1	0.0	0.9	2	1.0	0.0	5.9	0.2	0.0	1.2
R STERNEBRA(E) NOT OSSIFIED	283	20.9	12.4	32.0	130	63.7	35.7	80.0	21.1	12.2	30.4
R STERNEBRA(E) INCOMPLETELY OSSIFIED OR REDUCED IN SIZE	360	26.6	12.6	34.4	158	77.5	46.2	93.3	26.0	13.2	35.6
R RIB(S) INCOMPLETELY OSSIFIED	2	0.1	0.0	0.9	2	1.0	0.0	5.9	0.2	0.0	1.2
R METACARPAL BONES NOT OSSIFIED	1	0.07	0.0	1.0	1	0.5	0.0	7.1	0.1	0.0	1.0
R TALUS INCOMPLETELY OSSIFIED	30	2.2	0.0	9.6	20	9.8	0.0	33.3	1.9	0.0	8.1
R OS PUBIS INCOMPLETELY OSSIFIED	3	0.2	0.0	1.7	3	1.5	0.0	11.8	0.2	0.0	1.3
TOTAL FETAL SKELETAL RETARDATIONS	770	56.9	31.1	83.5	195	95.6	85.7	100.0	56.6	31.9	84.3

OBSERVATION CODE: R-Retardation

Attachment 2 / Page 2 of 2

CA 5.7 Neurotoxicity Studies

The core studies in M-CA 5.7 have already been peer-reviewed by the Rapporteur Member State Germany and national competent authorities following submission of the original Dossier for Annex I inclusion (2000). For the sake of completeness and accuracy one amendment to the study report on acute oral neurotoxicity has been included in this evaluation which does not alter the overall conclusions of this study.

An adequate set of neurotoxicity studies (acute, sub-chronic and developmental neurotoxicity) has been evaluated and has been considered acceptable. The endpoints were fixed in the Monograph (08 November 2002) and in the European Commission Review Report for the active substance Boscalid (SANCO/3919/2007 –Rev 5, 21 January 2008) no further information on neurotoxicity was required.
For the reviewer's convenience, these studies are summarised below as extracted from the Monograph (2002), and the tabulated summary is provided in Table 5.7-1.

Table 5.7-1: Summary of neurotoxicity studies performed with Boscalid

Study	Target	NOAEL mg/kg bw/day	LOAEL mg/kg bw/day	Effects	Reference BASF Doc #
Acute oral neurotoxicity Wistar rat 0, 500, 1000 & 2000 mg/kg bw/d	Neurotoxicity	2000	-	No specific neurotoxic effects	[redacted] et al 2000/1018638 & [redacted] Amendment 2003/1001523
	General toxicity	1000	2000	2000 mg/kg bw/d: Piloerection in 2/10 females on day 0	
90-day oral neurotoxicity Wistar rat 0, 150, 1500 & 15000 ppm	Neurotoxicity	1050 / 1273 males / females (15000 ppm)	-	No effects observed	[redacted] et al 2001/1000113
Developmental neurotoxicity Wistar rats 0, 100,1000 & 10000 ppm	Maternal toxicity	1442 (10000 ppm)	-	No effects observed	[redacted] et al 2001/1000118
	Offspring General toxicity	14 (100 ppm)	147 (1000 ppm)	1000 ppm: Reduced body weight and body weight gain at day 1-4-p.p. 10000 ppm: Reduced body weight and body weight gain until weaning Reduced absolute brain weight and brain length (male pups, day 11 p.p. only)	
	Offspring Neurotoxicity	1442 (10000 ppm)	-	No effects observed	

Three oral neurotoxicity studies were performed with Boscalid.

In the acute neurotoxicity study the test substance was administered to Wistar rats by gavage up to the dose level of 2000 mg/kg bw/day. No signs indicative of specific neurotoxic effects were observed. Findings were restricted to piloerection observed in 2 out of 20 rats which has been considered to reflect an unspecific clinical symptom as the consequence of the high dose administered. No signs of toxicity were observed in animals at dose levels of 1000 mg/kg bw and 500 mg/kg bw/day.
The NOAEL for acute neurotoxicity study in rats was 2000 mg/kg bw/day based on the absence of evidence of neurotoxicity in both sexes. The NOAEL for general toxicity was 1000 mg/kg bw/day based on transient piloerection in 2 out of 20 rats at 2000 mg/kg bw/day.
In the 90-day feeding neurotoxicity study in Wistar rats there were no test substance related adverse effects and there were no signs of neurotoxicity at any dose level. The NOAEL has been considered to be 15000 ppm corresponding to 1050 mg/kg bw/day in males and 1273 mg/kg bw/day in females.
In the developmental neurotoxicity study performed in Wistar rats, slight reduction in offspring body weight and body weight gain was observed at the dose levels of 10000 ppm and 1000 ppm. These effects were in line with similar effects observed in the 2-generation study in rats, where at these dose levels parental toxicity was noted in form of hepatotoxicity. No signs of developmental toxicity was observed at any dose level. In this study the following no observed adverse effect levels were found:
The NOAEL (developmental neurotoxicity) was found to be 10000 ppm (1442 mg/kg bw/day)
The NOAEL (reproductive toxicity) was found to be 100 ppm (14 mg/kg bw/day)
The NOAEL (maternal toxicity) was found to be 10000 ppm (1442 mg/kg bw/day)

CA 5.7.1 Neurotoxicity studies in rodents

BAS 510 F –Acute oral neurotoxicity study in Wistar rats (et al, 2000)
Doc ID 2000/1018638

Guidelines: According to OECD 424 (July 1997), EEC 92/32, EPA OPPTS 870.6200
Deviations: None
GLP: Yes
Acceptance: The study has been evaluated and considered acceptable in the EU registration process for the first Annex I inclusion.

Note: This study report has been part of the documentation for the first Annex I inclusion process and a short summary is therefore presented here

Groups of 10 male and 10 female fasted Wistar rats (Chbb:THOM (SPF), source: Boehringer Ingelheim Pharma KG, Germany, with all animals at 49 days of age at start of the treatment) were administered Boscalid (batch: N 46, purity 96.3%) suspended in 0.5% aqueous solution of carboxymethylcellulose (CMC) as a single oral dose by gavage at concentrations of 0, 500, 1000 or 2000 mg/kg bw. The mean body weight was 243 g (220 – 268 g) for males and 164 g (133 – 188 g) for females. The administration volume was 20 mL/kg bw. The animals were observed up to 2 weeks after dosing. The general state of health of the rats was examined daily. Body weight was determined on day -7 (prior to dosing), day 0 (test substance administration), day 7 and day 14. Functional observational batteries (FOB) and motor activity measurements were carried out in all animals on day -7, on day 0 (within few hours after dosing), as well as on days 7 and 14. Functional observational battery of tests (FOBs) consisted of passive observations in the home cage followed by removal from the home cage and open field observations in a standard arena. Thereafter sensorimotor tests and reflex tests were performed. Measurement of motor activity was performed in the dark with 4 infrared beams per cage over a period of 60 minutes. Five animals per sex and dose were anaesthetised and killed by perfusion fixation and subjected to neuropathological examinations. Visible organs were assessed by gross necropsy and sections from the brain, spinal cord and peripheral nervous system were prepared and examined by light microscopy. The remaining animals were sacrificed under CO_2-anaesthesia without any further examinations.

Findings
The stability of the test substance in CMC, homogeneity and concentration control analysis (97 – 102%) confirmed the doses chosen. There was no mortality during the study period. There were no treatment-related clinical signs of toxicity or effects on body weight development. Piloerection was observed in two female rats on day 0 at the dose level of 2000 mg/kg bw, however, in the absence of further indicators of neurotoxicity it was considered to be an unspecific sign of toxicity and not a specific test substance related effect.

Quantitative observations including faeces, rearing, grip strength measurement and landing foot splay test gave no treatment-related findings. The only exception was a statistically significant decrease in grip strength observed in high dose males on day 7. No such response was observed on day 0 and 14 in male or in female animals. It was therefore not regarded as test substance related effect. Motor activity measurements did not reveal any treatment-related differences. Light microscopic investigations of the peripheral and central nervous system did not reveal any neuropathological changes in the organ samples examined.

No signs of neurotoxicity were observed. The NOAEL for acute neurotoxicity study in rats was 2000 mg/kg bw based on the absence of evidence of neurotoxicity in both sexes. The NOAEL for general toxicity was 1000 mg/kg bw based on transient piloerection in 2 out of 20 rats at 2000 mg/kg bw.

New Information

Report:	CA 5.7.1/1 [redacted] 2003a Amendment No. 1 to the report: BAS 510 F - Acute oral neurotoxicity study in Wistar rats 2003/1001523
Guidelines:	EEC 92/32, EPA 870.6200
GLP:	yes (certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

This amendment was issued to correct typing errors. In the summary (page I-12) and the discussion of results (page I-41) of the study report the dose groups laid down by typing error were 100, 300 and 2000 mg/kg bw instead of the correct doses of 500, 1000 and 2000 mg/kg bw.

These typing errors were corrected by amendment.

BAS 510 F –Subchronic oral neurotoxicity study in Wistar rats ([redacted] et al, 2001)
Doc ID 2001/1000113

Guidelines:	According to OECD 424 (July 1997), EEC 92/32, EPA OPPTS 870.6200
Deviations:	None that compromised the validity of the study
GLP:	Yes
Acceptance:	The study has been evaluated and considered acceptable in the EU registration process for the first Annex I inclusion.

Note: This study report has been part of the documentation for the first Annex I inclusion process and a short summary is therefore presented here

Boscalid (batch: N 46, purity 96.3%) was administered to groups of 10 male and 10 female Wistar rats (Chbb:THOM (SPF), source: Boehringer Ingelheim Pharma KG, Germany,) at dietary concentrations of 0, 150, 1500 or 15000 ppm for 3 months. At the start of treatment all rats were 49 days old and mean body weight was 248 g (232 – 271 g) for males and 177 g (159 – 194 g) for females. Food and water consumption were determined once a week. Body weight was determined once a week and on the days when functional observational batteries were performed. A check of the general state of health was made at least daily. Furthermore, the animals were thoroughly examined and palpated once a week. Functional observational batteries (FOB) and motor activity measurements were carried out in all animals on day –7 (prior to the start of the administration) as well as on days 22, 50 and 85. FOBs consisted of passive observations in the home cage followed by removal from the home cage and open field observations in a standard arena. Thereafter sensorimotor tests and reflex tests were performed. Measurement of motor activity was performed in the dark with 4 infrared beams per cage over a period of 60 minutes. Five animals per sex and dose were anaesthetised and killed by perfusion fixation and subjected to neuropathological examinations.

Visible organs were assessed by gross necropsy and sections from the brain, spinal cord and peripheral nervous system were prepared and examined by light microscopy. The remaining animals were sacrificed under CO_2-anaesthesia without any further examinations.

Findings
The stability and homogeneity of the test substance was proven by analysis. The correctness of test substance concentrations was confirmed by analysis. Mean test substance intake corresponded to 10.5, 103.1 and 1050 mg/kg bw/day in males and 12.7, 124.5 and 1272.5 mg/kg bw/day in females for the dietary concentrations of 150, 1500 and 15000 ppm.
There were no deaths or treatment-related signs of toxicity. There were no significant intergroup differences in food consumption and body weight. FOBs and motor activity tests did not provide any treatment-related findings. There were no gross lesions found at termination. No statistically significant differences were observed in organ weight measurement of the brain. The only histopathological finding was a single incidence (grade 1) of axonal degeneration in the lumbar ganglia of one male and one female at 15000 ppm. This finding has been considered to be spontaneous in nature and not test substance related.

The NOAEL in the 3-month neurotoxicity study in rats was 15000 ppm corresponding to 1050 mg/kg bw/day in males and 1272.5 mg/kg bw/day in females based on the absence of neurotoxicity up to the highest dose tested.

BAS 510 F –Developmental neurotoxicity study in Wistar rats (et al, 2001)
Doc ID 2001/1000118

Guidelines:	According to OECD draft guideline 426 (October 1999), EPA OPPTS 870.6200
Deviations:	None that compromised the validity of the study
GLP:	Yes
Acceptance:	The study has been evaluated and considered acceptable in the EU registration process for the first Annex I inclusion.

Note: This study report has been part of the documentation for the first Annex I inclusion process and a short summary is therefore presented here

Report: CA 5.7.1/2
Hasting C.E., 2002a
BAS 510 F Developmental neurotoxicity study: Response to EPA request for additional data
2002/5004332
Guidelines: none
GLP: none (evaluation paper)

Note: Additional information which has not been reviewed for the first Annex I inclusion process of Boscalid

Boscalid (batch: N 46, purity 96.3%) was administered to groups of 35 mated female Wistar rats (Chbb:THOM (SPF), source: Boehringer Ingelheim Pharma KG, Germany,) at dietary concentrations of 0, 100, 1000 or 10000 ppm from day 6 post coitum (p.c.) until day 21 post-partum (p.p.). The dams were allowed to litter and rear the total offspring until day 4 p.p. (until standardisation of offspring) and until day 21 p.p. for the standardised offspring. After the offspring were weaned (day 21 p.p.), the dams were sacrificed without any further examinations.

The state of health of the dams and offspring was checked each day. Nesting, littering and lactation behaviour of dams was checked each day. Feed consumption of the dams was determined regularly during gestation (days 0, 6, 13 and 20) and lactation (days 1, 7 and 14). Body weights of the dams were determined regularly during gestation (days 0, 6, 13 and 20) and lactation (days 1, 7, 14 and 21). A detailed clinical examination outside the cage (open field observations) was performed in 10 dams/group on days 7 and 14 p.c. and on days 7 and 14 p.p. Details of parameters evaluated in open field examinations is shown in the Table 5.7.1-1 below.

Table 5.7.1-1: Rat developmental neurotoxicity: parameters assessed in open field examinations of dams and selected offspring

• Behaviour during handling • Posture • Activity/arousal level • Abnormal movements • Palpebral closure • Urine • Fur • Salivation • Tremors	• Gait abnormalities • Exophthalmos • Pupil size • Skin • Respiration • Convulsions • Lacrimation • Faeces (appearance/consistency) • Other abnormalities

Female fertility index, gestation index, and live birth index were calculated.

The offspring was examined (number of offspring per litter, sex, live or stillborn and gross changes) as soon as possible on the day of birth. They were weighed on the day after birth and on day 4, 11, 17 and 21 p.p. and after weaning in weekly intervals. Viability was recorded. Sexual maturation (day of preputial separation/vaginal opening) of all selected offspring and the respective body weights were determined.

After parturition only those litters were used for further examination which consisted of at least 8 offspring and whose date of littering (day o p.p.) was within three consecutive days. The offspring's state of health including viability and mortality was checked each day. Body weight was recorded on the day of birth (day 0), on day 4, 7, 14, 21 p.p. and after weaning once a week. Sex ratio was determined on the day 0 and day 21 p.p. Following standardisation on day 4 p.p. selected pups from each standardised litter were allocated to five subsets (I-V). The subsets consisted of 10 pups per sex and dose group as shown in the Table 5.7.1-2 below.

Table 5.7.1-2: Rat developmental neurotoxicity: scope of investigations (subsets)

Subsets	Number of pups selected	Test procedure
I	10/sex/group	Day 11 p.p.: Perfusion fixation, brain weights and neuropathology of the brain
II	10/sex/group	Open field observation (OFO) and motor activity (MA)
III	10/sex/group	Auditory startle test and day 60 (+2) p.p.: Perfusion fixation, brain weights and neuropathology
IV	10/sex/group	Day 21 (+2) p.p.: Learning and memory test
V	10/sex/group	Day 60 (+2) p.p.: Learning and memory test

The sexual maturation of subsets II, III and V were investigated daily beginning with day 27 p.p. for females (vaginal opening) and day 40 p.p. for males (preputial separation). Body weight was in addition recorded on the day when sexual maturation was stated to have occurred.

Detailed clinical examinations were performed outside the cage (open field observations) including measurement of motor activity, auditory startle, learning and memory (water maze test) in selected offspring. Neuropathological examinations and measurements were performed in offspring selected for perfusion fixation on day 11 p.p. (subset I) and 60 p.p. (subset III):

Brain weight (incl. olfactory bulb) and morphometric measurements of length and width of the whole brain.

The organs and tissues removed and examined by light microscopy are shown in Table 5.7.1-3.

Table 5.7.1-3: Rat developmental neurotoxicity: histopathological examinations of tissues

Tissue sections in pups fixed by perfusion on day 11(subset I) and 60 (subset III)	
Brain (cross sections of olfactory bulb, frontal lobe, parietal lobe with diencephalon, midbrain with occipital and temporal lobe, pons, cerebellum, medulla oblongata)* Eyes with retina and optical nerve Olfactory epithelium (nose cavity level III)	Spinal cord (cervical, thoracial, lumbar sections) Pituitary gland Gasserian ganglia with nerve Gastrocnemius muscle All gross lesions
Additional tissue sections examined in pups fixed by perfusion on day 60 (subset III)	
Dorsal root ganglion Dorsal fibre root Ventral fibre root	Proximal sciatic nerve Proximal tibial nerve (at knee) Distal tibial nerve (at lower leg)
*Tissues from control and high dose group only	

All offspring not required for any examinations, all offspring not maintained after standardization (culling) and all offspring of subsets II, IV and V (on completion of the test) were sacrificed without further examination.

Findings
The stability, concentrations and homogeneity of the test substance in the diet were analytically confirmed.

Findings in dams
There were no mortality or treatment-related signs of toxicity in any dam of any dose group. Alopecia was found in few animals as isolated findings. Feed consumption and body weight gain of dams were not affected by the treatment. This is considered to also include the transient, statistically significantly increased feed consumption in the mid dose during day 6 to 20 of gestation and in the high dose during day 13 to 20 of gestation as well as the transient, statistically significantly decreased feed consumption in the low and high dose group during day 7 to 14 of lactation. Detailed data are provided in the table below.

Table 5.7.1-4: Rat developmental neurotoxicity: summary of feed consumption

	Feed substance intake (grams/animal/day)			
	0 ppm	**100 ppm**	**1000 ppm**	**10000 ppm**
Dams: Gestation				
Day 0 to 6	17.3	17.6	17.5	17.6
Day 6 to 13	19.1	19.2	**21.1****	19.0
Day 13 to 20	19.7	20.0	**23.4****	**23.4****
Day 6 to 20	19.4	19.6	22.3	21.2
Dams: Lactation				
Day 1 to 7	34.1	33.0	34.9	33.6
Day 7 to 14	50.8	**47.9***	48.3	**47.8***
Day 1 to 14	42.4	40.4	41.6	40.7
* p<0.05, ** p<0.01 Dunnett test (two-sided)				

The test substance intake of dams resulting from the feed consumption is presented in the Table 5.7.1-5 below.

Table 5.7.1-5: Rat developmental neurotoxicity: summary of test substance intake

	Test substance intake (mg/kg bw/day)		
Dams	**100 ppm**	**1000 ppm**	**10000 ppm**
Gestation period	9.6	108.6	1031.6
Lactation period	18.3	186.0	1853.1
Mean	14	147	1442

Body weight and body weight gain of dams was unaffected by the treatment. This has been considered to include the transient statistically significantly increased body weight gain during gestation days 13 to 20 and during lactation day 7 to 14 in the high dose group as well as the marginally retarded body weight gain in the mid dose group during lactation days 1 to 21.

Detailed clinical examinations and cage side open field observations did not show any treatment-related differences between groups during gestation and lactation. All findings were equally distributed between treatment and control groups or were considered to have occurred incidentally in individual animals only.

Female reproductive performance

Fertility index was 80, 86, 86 and 94% for the control, 100, 1000 and 10000 ppm dose groups respectively. The duration of gestation was comparable with 21.8 days for control animals and gestation of 21.8, 21.6 and 21.7 days for the 100, 1000 and 10000 ppm dose groups. Gestation index was 100 % over all groups. The number of offspring, live and stillborn and viability did not show any treatment-related difference between control and treated animals. The life-birth index was thus comparable over all groups (98 - 100%). Details of results are presented in Table 5.7.1-6 below.

Table 5.7.1-6: Rat developmental neurotoxicity: reproduction parameters

	Dose level (ppm)			
	0	**100**	**1000**	**10000**
Females mated (n)	35	35	35	35
Females pregnant (n)	28	30	30	33
Fertility index	80%	86%	86%	94%
Duration of gestation (days)	21.8	21.8	21.6	21.7
Dams with live-born pups (n)	28	30	30	33
Gestation index	100%	100%	100%	100%
Dams with stillborn pups (n)	1	5	0	2
Dams with pups all stillborn (n)	0	0	0	0
Pups delivered total (n)	264	310	288	339
Pups delivered/dam (n)	9.4	10.3	9.6	10.3
Pups live-born total (n)	263	304	288	337
Pups stillborn total (n)	1	6	0	2
Life-birth index	100%	98%	100%	99%

Findings in offspring

Offspring viability and postnatal mortality was not affected by the treatment. The sex distribution and sex ratio of live offspring on the day of birth and day 21 p.p. was unaffected. All differences observed were regarded to be of spontaneous nature. Detailed evaluation of the data is presented in the Table 5.7.1-7 below.

Table 5.7.1-7: Rat developmental neurotoxicity: survival parameters and sex ratio

	Dose level (ppm)			
	0	**100**	**1000**	**10000**
Total number of litters (n)	28	30	30	33
Litters with live-born pups (n)	28	30	30	33
Litters with stillborn pups (n)	1	5	0	2
Litters with pups all stillborn (n)	0	0	0	0
Pups delivered total (n)	264	310	288	339
Pups live-born total (n)	263	304	288	337
Pups stillborn total (n)	1	6	0	2
Pups died (n)	3	1	0	1
Pups sacrificed moribund (n)	0	1	0	1
Pups cannibalized (n)	1	1	0	2
Pups accidental death (n)	0	0	0	0
Pup mortality on day 0 (n)	0	0	0	0
Pup mortality on day 1 to 4 (n)	4	0	0	2
Pup mortality on day 5 to 7 (n)	0	0	0	0
Pup mortality on day 8 to 14 (n)	0	2	0	1
Pup mortality on day 15 to 21 (n)	0	0	0	1
Live pups per litter, day 0 (n)	9.4	10.1	9.6	10.2
Live pups per litter, day 4 pre-culling (n)	7.6	9.1	8.7	10.2
Live pups per litter, day 4 post culling (n)	7.6	7.2	7.1	7.9
Live pups per litter, day 11 (n)	7.6	6.9	6.4	7.5
Live pups per litter, day 17 (n)	6.9	6.2	5.7	6.9
Live pups per litter, day 21 (n)	6.9	6.2	5.7	6.8
Sex ratio, day 0 (% live males)	46.0	46.7	49.0	54.0
Sex ratio, day 21 (% live males)	46.9	48.9	50.0	52.2

Clinical observations in the offspring gave no effects that could be considered as test substance related. Incidental findings such as lesion of the hind-limb, alopecia and poor general state associated with piloerection were made over all groups.

Pup body weight

Mean body weights in the high dose group of males were statistically significantly reduced by 14% at day 4 p.p. and lasted up to weaning on day 21 p.p. (-7.4%). The respective body weight gain in males of the high dose group was statistically significantly reduced by 32% at day 4 p.p. For the period p.p. 4 to p.p. 21 body weight was still reduced by 5.4%, however, without being identified as statistically significant.

Mean body weights in the high dose group of females were statistically significantly reduced by 16% at day 4 p.p. and lasted up to weaning on day 21 p.p. (-6.8%). The respective body weight gain in females of the high dose group was statistically significantly reduced by 32% at day 4 p.p. For the period p.p. 4 to p.p. 21 body weight was still reduced by 4.4%, however, without being identified as statistically significant.

In the mid dose group there was transient statistically significant reduction in body weight (males: -8.2%, females: -9.4%) and body weight gain (-21% in both, males and females) at day 4 p.p. only. Until day 17 compensatory growth was observed with both males and females having exceeded body weights of the control groups. No adverse effect on body weight and body weight gain could be observed in the low dose. Detailed information is presented in Table 5.7.1-8 below.

Table 5.7.1-8: Rat developmental neurotoxicity: pup body weight and body weight gain – lactation phase

		Dose level (ppm)			
		0	**100**	**1000**	**10000**
Pup weight (gain) at day (g)	**Sex**				
Day 1	Males	6.4	6.2 *(-3.1%)*	6.3 *(-1.6%)*	6.1 *(-4.7%)*
Day 4 pre-culling		9.8	9.3 *(-5.1%)*	**9.0*** *(-8.2%)*	**8.4**** *(-14%)*
Day 4 post-culling		9.8	9.3 *(-5.1%)*	**9.0*** *(-8.2%)*	**8.4**** *(-14%)*
Day 11		21.5	22.6 *(+5.1%)*	21.1 *(-1.9%)*	**19.6**** *(-8.8%)*
Day 17		34.4	**37.0**** *(+7.6%)*	35.6 *(+3.5%)*	**32.3*** *(-6.1%)*
Day 21		47.1	49.3 *(+4.6%)*	48.0 *(+1.9%)*	**43.6**** *(-7.4%)*
Body weight gain day 1 to 4		3.4	3.1 *(-8.8%)*	**2.7**** *(-21%)*	**2.3**** *(-32%)*
Body weight gain day 4 to 11		11.8	**13.3**** *(+12.7%)*	12.1 *(+2.5%)*	11.2 *(-5.1%)*
Body weight gain day 11 to 17		12.9	**14.4**** *(+11.6%)*	**14.6**** *(+13.2%)*	12.7 *(-1.6%)*
Body weight gain day 17 to 21		12.7	12.3 *(-3.1%)*	12.3 *(-3.1%)*	**11.3**** *(-11%)*
Body weight gain day 4 to 21		37.3	**40.0*** *(+7.2%)*	39.0 *(+4.6%)*	35.6 *(-5.4%)*
Day 1	Females	6.2	5.9 *(-4.8%)*	6.0 *(-3.2%)*	**5.8*** *(-6.5%)*
Day 4 pre-culling		9.6	9.1 *(-5.2%)*	**8.7**** *(-9.4%)*	**8.1**** *(-16%)*
Day 4 post-culling		9.6	9.1 *(-5.2%)*	**8.7**** *(-9.4%)*	**8.1**** *(-16%)*
Day 11		21.2	22.1 *(-4.2%)*	20.5 *(-3.3%)*	**19.1**** *(-9.9%)*
Day 17		33.7	**36.0**** *(+6.8%)*	34.4 *(+2.1%)*	**31.5**** *(-6.5%)*
Day 21		45.6	47.6 *(+4.4%)*	46.1 *(+1.1%)*	**42.5**** *(-6.8%)*
Body weight gain day 1 to 4		3.4	3.1 *(-8.8%)*	**2.7**** *(-21%)*	**2.3**** *(-32%)*
Body weight gain day 4 to 11		11.6	**13.1**** *(+12.1%)*	11.9 *(+2.6%)*	11.0 *(-5.2%)*
Body weight gain day 11 to 17		12.5	**13.9**** *(+11.2%)*	**13.8**** *(+10.4%)*	12.4 *(-0.8%)*
Body weight gain day 17 to 21		11.9	11.6 *(-2.5%)*	11.7 *(-1.7%)*	**11.0*** *(-7.6%)*
Body weight gain day 4 to 21		36.0	**38.6**** *(+7.2%)*	37.5 *(+4.2%)*	34.4 *(-4.4%)*
* $p \leq 0.05$; ** $p \leq 0.01$ (Dunnet-test two sided)					

No adverse effects on body weight and body weight gain were observed in those treatment groups maintained after weaning (subsets II to V). Detailed information on the data is presented in the Table 5.7.1-9 below.

Table 5.7.1-9: Rat developmental neurotoxicity: pup body weight and body weight gain- after weaning-

			Dose level (ppm)			
			0	**100**	**1000**	**10000**
Subset	**Pup weight (gain) at week (g)**	**Sex**				
II	Week 0	Males	49.7	**55.4***	53.7	50.3
	Week 1		87.2	94.6	92.0	88.7
	Week 5		254.3	258.7	253.7	254.0
	Body weight gain week 0 to 5		204.7	203.3	200.0	204.5
	Week 0	Females	49.4	54.4	49.3	47.0
	Week 1		81.8	87.9	82.6	78.2
	Week 5		164.3	173.7	163.5	167.4
	Body weight gain week 0 to 5		114.9	119.3	114.2	120.5
III	Week 0	Males	52.6	54.7	52.7	50.3
	Week 1		90.5	93.4	90.4	85.2
	Week 5		257.8	255.8	259.4	253.5
	Body weight gain week 0 to 5		205.1	199.2	206.7	203.2
	Week 0	Females	48.5	52.6	52.4	49.5
	Week 1		81.2	84.4	85.3	81.4
	Week 5		167.4	165.4	170.7	165.7
	Body weight gain week 0 to 5		118.9	112.8	118.3	116.2
IV	Week 0	Males	51.4	52.4	50.9	49.9
	Week 1		90.0	87.5	88.1	84.7
	Body weight gain week 0 to 1		38.6	35.1	37.2	34.8
	Week 0	Females	49.1	53.6	51.7	48.5
	Week 1		81.5	87.8	84.0	77.8
	Body weight gain week 0 to 1		32.4	34.2	32.4	29.3
V	Week 0	Males	49.9	**56.9***	53.5	51.0
	Week 1		88.5	**98.3***	93.1	89.9
	Week 6		277.2	295.0	283.4	283.7
	Body weight gain week 0 to 6		227.3	238.1	229.9	232.7
	Week 0	Females	51.2	53.5	53.2	48.7
	Week 1		86.7	85.3	87.5	80.0
	Week 6		182.6	178.6	180.2	179.5
	Body weight gain week 0 to 6		131.4	125.1	127.0	130.8
* $p \leq 0.05$; ** $p \leq 0.01$ (Dunnet-test two sided)						

Sexual maturation
No adverse effects could be observed on the onset of sexual maturity. Detailed information on the mean time until vaginal opening and preputial separation is presented in the Table 5.7.1-10 below.

Table 5.7.1-10: Offspring: sexual maturation

Sex/parameter investigated	Females/vaginal opening				Males/preputial separation			
Dose level (ppm)	**0**	**100**	**1000**	**10000**	**0**	**100**	**1000**	**10000**
Pups investigated	30	30	30	30	30	30	30	30
Time until criterion (days)	32.5	32.2	32.8	32.8	43.4	43.0	44.2	43.3

Detailed clinical examinations outside the cage – open field observations
No substance related findings were observed during the detailed clinical observations outside the cage in males and females. For motor activity no statistically significant or biologically relevant deviations were observed. For auditory startle response isolated statistically significant deviations were observed on day 24 p.p. in males and females of the low and mid dose. However, further analysis of auditory startle findings was made in the supporting evaluation document (MCA 5.7.1/2). The exclusion of one male of the low dose group at PND 24 (due to its moribund state) does not further support statistical identification the mean startle amplitude to be affected. Mean startle amplitudes fell within historical control data of the last three years.
No such effect was observed on day 60 p.p. Based on the isolated occurrence and the lack of a dose-response relationship this finding has been considered as being incidental.
The learning and memory test (water maze test) gave no indication of the learning and memory capacity of males and females being impaired by the test substance which includes a slight albeit statistically significantly decreased re-learning result of females of the mid dose group of subset IV. No such findings were observed in either sex in subset V.

Neuropathological examination of different brain regions and the spinal cord overall gave no substance related differences in morphology when investigated on day 11 p.p. and day 60 p.p. The only findings were some axonal degeneration of peripheral nerves with similar incidence and severity in control and treatment groups. Overall these findings have been considered to be of spontaneous nature and not related to treatment.

Morphometric measurements of the brain did not reveal differences of treatment groups as compared to control for neocortex, caudate nucleus, caudate putamen, corpus callosum and cerebellum. There were a few statistically significant differences in brain weight and morphology when investigated in pups sacrificed on day 11 and day 60.
In offspring sacrificed on day 11 p.p. there was statistically significantly decreased body weight for both males and females in the high dose group. Absolute brain weight was statistically significantly reduced in both sexes of this dose group, but relative brain weights were unaffected. In females of the low and mid dose relative brain weights were statistically significantly decreased with whereas terminal body weights were exceeding that of control animals. The linear measurements of the overall maximum brain length gave a statistically significant decrease in the males of the high dose concomitant to decreased body weight. For females of the high dose group the length of the right hippocampus was slightly but statistically significantly reduced.

Additional evaluation of the hippocampus length in the low and mid dose groups gave no significant differences to control groups. No consistent dose related change in the size of hippocampi could be observed. No significant differences could be observed in males. Therefore the marginally statistically significant effect in decrease of size in the high dose females on one side only of the hippocampus was considered to most likely have resulted by chance. This is further supported by the lack of neuropathological findings.
Overall, the decreased body weights and concomitant decreased absolute brain weights were considered to be treatment related effect in both sexes at day 11 p.p. The findings within this subgroup have been considered to be in line with the impaired body weight gain observed during lactation (Table 5.7.1-8). The reduced length of brain in the high dose males has been considered associated with the reduced body weight. The slightly reduced length of the right hippocampus observed in the high dose females has been considered to be incidental, given the absence of this finding in the left hippocampus of females and in males at all.
In offspring sacrificed on day 60 p.p. neither terminal body weight nor brain weight parameters were affected by the treatment. The only finding was a slightly but significantly reduced brain width in females of the high dose group. No other changes in linear measurements of major brain areas including the hippocampus were observed.

Table 5.7.1-11: Rat developmental neurotoxicity: neuropathological findings in pups sacrificed on day 11 p.p.

Sex/parameter investigated	**Males**				**Females**			
Dose level (ppm)	**0**	**100**	**1000**	**10000**	**0**	**100**	**1000**	**10000**
Pups investigated	10	10	10	10	10	10	10	10
Terminal body weight (g)[#]	21.77	22.71	21.46	**19.81***	20.92	**22.69***	21.20	**19.03***
Brain weight, absolute (g)[#]	1.299	1.287	1.242	**1.215***	1.267	1.231	1.205	**1.179****
Brain weight, relative (%)[#]	5.997	5.712	5.812	6.15	6.07	**5.443****	**5.692***	6.235
Brain length (cm)[&]	1.711	1.706	1.676	**1.658***	1.684	1.683	1.666	1.655
Brain width (cm)[&]	1.398	1.411	1.387	1.380	1.401	1.361	1.371	1.375
Hippocampus right, length (µm)[§]	1201	1238[!]	1277[!]	1167	1207	1274[!]	1214[!]	**1111***
Hippocampus left, length (µm)[§]	1180	1243[!]	1267[!]	1155	1174	1291[!]	1234[!]	1101

* $p \leq 0.05$; ** $p \leq 0.01$
[#] Kruskal-Wallis-H & Wilcoxon test (two sided)
[&] Wilcoxon test (two sided) with Bonferroni-Holm adjustment
[§] Wilcoxon test (one sided)
[!] Not included in the original report, however, part of additional evaluation document DocID 2002/5004332

Table 5.7.1-12: Rat developmental neurotoxicity: neuropathological findings in pups sacrificed on day 60 p.p.

Sex/parameter investigated	Males				Females			
Dose level (ppm)	**0**	**100**	**1000**	**10000**	**0**	**100**	**1000**	**10000**
Pups investigated	10	10	10	10	10	10	10	10
Terminal body weight (g)[#]	264.99	260.42	264.72	258.24	172.09	170.18	173.10	168.21
Brain weight, absolute (g)[#]	2.020	2.043	2.058	1.976	1.907	1.899	1.928	1.866
Brain weight, relative (%)[#]	0.768	0.788	0.781	0.770	1.111	1.121	1.116	1.113
Brain length (cm)[&]	2.100	2.094	2.092	2.077	2.045	2.049	2.062	2.028
Brain width (cm)[&]	1.541	1.561	1.569	1.542	1.522	1.506	1.524	**1.502***
Hippocampus right, length (µm)[§]	1830	-	-	1871	1803	-	-	1788
Hippocampus left, length (µm)[§]	1833	-	-	1833	1810	-	-	1808

* $p \leq 0.05$; ** $p \leq 0.01$
[#] Kruskal-Wallis-H & Wilcoxon test (two sided)
[&] Wilcoxon test (two sided) with Bonferroni-Holm adjustment
[§] Wilcoxon test (one sided)

Conclusions
Overall, general toxicity occurred in the pups at the high dose level of 10000 ppm as evidenced by reduced pup weight and pup weight gain during the pre-weaning period. Associated decreases in absolute brain weight brain length and possibly right hippocampus size were observed. There were slight signs of impaired physical development as evidenced by transient reduced body weight and retarded body weight gain at the dose level 1000 ppm. Effects were marginal and at day 4 p.p. only, followed by compensatory growth of males and females until day 17.
Under the conditions chosen in this study Boscalid had no adverse effects on the embryonic, foetal and postnatal development of the nervous system in Wistar rats following the administration in the feed at test substance concentrations of up to 10000 ppm.

The **NOAEL** (**developmental neurotoxicity)** was found to be 10000 ppm (1442 mg/kg bw/day)
The **NOAEL** (**reproductive toxicity)** was found to be 100 ppm (14 mg/kg bw/day)
There were no signs of general maternal toxicity up to the high dose level.
The **NOAEL** (**maternal toxicity)** was found to be 10000 ppm (1442 mg/kg bw/day)

CA 5.7.2 Delayed polyneuropathy studies

No neurotoxic effects were observed in any of the studies evaluated. Hence, no need for delayed neurotoxicity testing was identified for Boscalid.

CA 5.8 Other Toxicological Studies

Some of the studies in M-CA 5.8 have already been peer-reviewed by the Rapporteur Member State Germany and national competent authorities following submission of the original dossier for Annex I inclusion (2000). For the convenience of the reviewer, these are summarized below as extracted from the Monograph (2002) including addenda together with the new studies conducted since then. Where new information has been added this has been duly marked. This specifically applies to new studies performed with metabolites of Boscalid to further assist in consumer risk assessment and new information in regard to thyroid effects of the active substance.

Chapter M-CA 5.8.1 is essentially dealing with the following items

- Detailed review of metabolites occurring in animal matrices (including the rat) and plant matrices to further identify the need for more detailed consumer assessment.
- The potential occurrence of metabolites in groundwater and implications for further testing following the guidance document on the assessment of relevance of metabolites in groundwater (SANCO/221/2000 –rev. 10 of 25 February 2003) has been considered.
- Some information on 2-parachlorobenzoic acid has been included for the sake of completeness and consistency with the previous Annex I inclusion process. 2-parachlorobenzoic acid (i.e. metabolite M510F64) is considered to potentially occur in surface water and sediment and risk assessments for the aquatic life have duly been performed in the respective sections. However, following the current regulations there was no need to perform a human risk assessment for this metabolite.

Chapter M-CA 5.8.2 is essentially dealing with the following items

- Supplementary studies to investigate the mechanism of Boscalid related to the thyroid effects observed in various repeat dose studies of sections CA 5.3 through CA 5.7.
- Additional information on immunotoxicity which has already been included in the documentation of Boscalid for the first Annex I inclusion process at a later stage (not reflected in the Monograph of November 08, 2002).

CA 5.8.1 Toxicity studies of metabolites

Review of relevance of metabolites occurring in animal matrices (including the rat) and plant matrices

For the review on metabolites occurring in the various matrices of animal and plant origin the information from the following studies have been used as shown in Table 5.8.1-1. More detailed information on the rat metabolism has been presented in M-CA 5.1 of this dossier for the rat metabolism which was newly added to the data base since the first Annex I inclusion process. All other relevant information can be found in the Monograph of Boscalid (November 2002) and the Addendum of May 2006.

Table 5.8.1-1: List of studies on metabolism in various matrices of animal and plant origin

Study type	Study (crop/matrix)	BASF DocID (plus relevant report amendments)	Comment
Rat metabolism	Tissues and excreta including bile	2000/1017220	Evaluated in previous review
Rat metabolism	Tissues and excreta including bile	2003/1012629	New information included in CA 5.1
Crop metabolism	Grape (foliar)	2000/1014860	Evaluated in previous review
Crop metabolism	Lettuce (foliar)	1999/11240	Evaluated in previous review
Crop metabolism	Beans (foliar)	2000/1014861	Evaluated in previous review
Processing	Hydrolysis	1998/10878	Evaluated in previous review
Processing	Peas	2000/1014885	Evaluated in previous review
Succeeding crop (confined)	Lettuce, radish, wheat	2000/1014862	Evaluated in previous review
Livestock metabolism	Goat	2000/1012353 2000/1017221	Evaluated in previous review
Livestock metabolism	Hen	2000/5154	Evaluated in previous review

In Table 5.8.1-2 shown below a detailed review of metabolites occurring in animal matrices (including the rat) and plant matrices is presented. The occurrence of individual metabolites qualitatively and quantitatively in terms of the percentage of total radioactivity applied (%TRR) has been demonstrated in the light of the presence of these metabolites in the rat metabolism studies to assist in the identification of their relevance with regard to the consumer risk evaluation. The range of findings in TRR proportions is shown for the metabolites in rats to account for the fact that the dosing comprised a low and high dose, and single dosing plus multiple dosing was done in line with the respective study guidelines. A number of rat metabolites were found with no further quantification given in the study reports due to their occurrence in negligible amounts.

In these cases a qualitative rather than quantitative characterization of their occurrence as “trace amounts” or “minor amounts” is given in the overview below. For the further consumer safety evaluation none of these metabolites has been considered to be of relevance.

For Boscalid the major principle of metabolism in animals consists of two key transformation steps:

- Hydroxylation of the aromatic ring system
- Glutathione conjugation of the pyridine and the diphenyl ring system

These steps are common in the majority of metabolism studies with the differences observed mainly relating to the quantity of metabolites. In plants Boscalid substantially remains unchanged thus forming the major part of the residue definition. Where differences to this pattern are evident the toxicological relevance is in more detail evaluated below. The effect of processing on the nature of plant residues was investigated in a hydrolysis study. Boscalid was shown to be stable under all conditions tested and no formation of toxicologically relevant metabolites other than those of the unprocessed plant items occurred. The same conclusion applies for residues in rotational crops. More detailed information on this is provided in M-CA 6.5 (Effects on processing) and M-CA 6.6 (Residues in rotational crops).

Table 5.8.1-2: Review of metabolites occurring in animal matrices (including the rat) and plant matrices

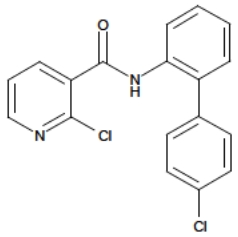
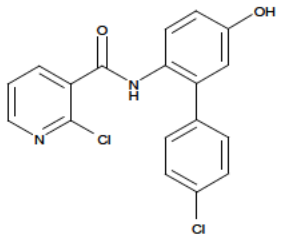
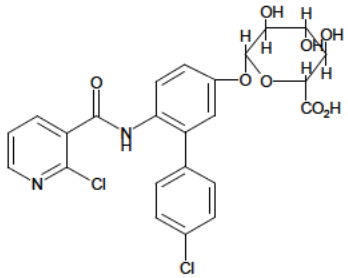
Metabolite		Occurrence (% of TRR)			
Substance Code	**Chemical structure**	**Rat**	**Plant**	**Poultry**	**Ruminants**
Parent Boscalid		Feces: 29.4 – 85.2% Urine: 0.0 -1.03% Plasma: < 0.01% Kidney: 0.01 – 0.03% Liver: 0.01 – 0.03%	Grapes: ≥92.2% Lettuce: 99.3% Beans: 97% in green beans 72% dry seeds >80% dry pods Confined rotational crop: Lettuce: 94.1% Radish roots: 92.8% Wheat grain: 35.4%	Egg: 35.5% Fat: 93.3% Liver[1]: Not detected Muscle: 0.0025 mg/kg (<0.01 mg/kg, not further characterized) Excreta: 4.1%	Muscle: 20.4% Fat: 34.6% Kidney: 2.5% Liver: 5.0% (5.7[1]) Milk: 3.2% (7.9%[1])
M510F01 Reg. No. 398794		Feces: 2.5 – 24.5% Urine: 0.5 – 15.8% Bile: 0.28 – 1.71% Plasma: < 0.01% Kidney: ≤ 0.01% Liver: 0.03 – 0.13%	Not detected	Egg: 26.9% Liver: 5.6% Excreta: 75.5%	Muscle: 20.6% Fat: 26.3% Kidney: 8.6% Liver: 2.9% (6.4%[1]) Milk: 14.9% (19.0%[1]) Urine: 70.8% Feces
M510F02		Urine: 0.08 – 14.33% Bile: 4.78 – 19.27% Plasma: ≤ 0.01% Kidney: 0.01 – 0.03% Liver: 0.2 – 0.38%	Not detected	Egg: 17.3% Excreta: 0.8%	Muscle: 11.9% Kidney: 50.3% Milk: 6.4% (not detected[1]) Urine: 2.4% together with isomer F

Table 5.8.1-2: Review of metabolites occurring in animal matrices (including the rat) and plant matrices

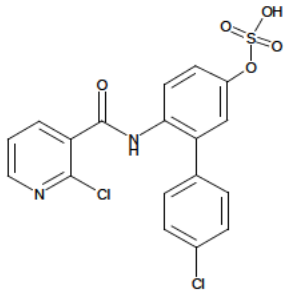
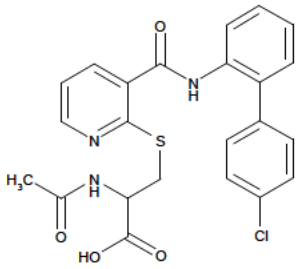
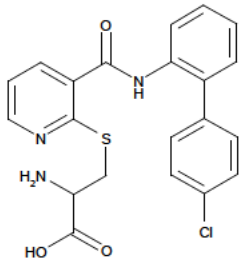
Metabolite		Occurrence (% of TRR)			
Substance Code	**Chemical structure**	**Rat**	**Plant**	**Poultry**	**Ruminants**
M510F03		Urine: 0.0 – 1.63% Bile: 0.21 – 1.48% Kidney: < 0.01%	Not detected	Not detected	Not detected
M510F04		Urine: 0.01 – 0.22%	Not detected	Not detected	Not detected
M510F05		Feces: 0.0 – 4.87% Urine: 0.03 – 0.59% Bile: 3.59 – 14.24% Kidney: < 0.01 – 0.06% Liver: 0.01 – 0.04%	Not detected	Not detected	Urine: 2.0% together with M510F16 and M510F59/ 6.3% together with M510F16 and M510F59 (no clear separation of peaks possible)

Table 5.8.1-2: Review of metabolites occurring in animal matrices (including the rat) and plant matrices

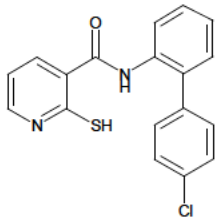
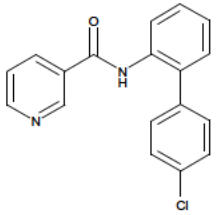
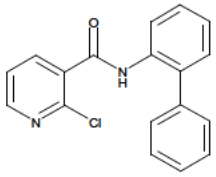
Metabolite		Occurrence (% of TRR)			
Substance Code	**Chemical structure**	**Rat**	**Plant**	**Poultry**	**Ruminants**
M510F06		Feces: 1.04 -12.24% Plasma: < 0.01% Kidney: < 0.01% Liver: 0.03 – 0.05%	Not detected	Not detected	Not detected
M510F08		Urine: Trace amounts only	Not detected	Not detected	Not detected
M510F09		Feces: Trace amounts only	Not detected	Not detected	Not detected

Table 5.8.1-2: Review of metabolites occurring in animal matrices (including the rat) and plant matrices

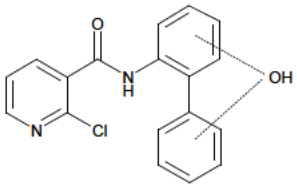
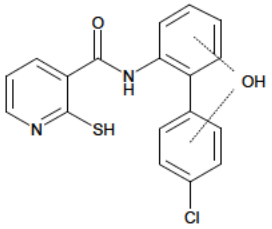
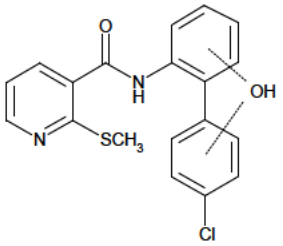
Metabolite		Occurrence (% of TRR)			
Substance Code	**Chemical structure**	**Rat**	**Plant**	**Poultry**	**Ruminants**
M510F10		Urine: Trace amounts only	Not detected	Not detected	Not detected
M510F11		Feces:0.14 - 2.31%	Not detected	Not detected	Not detected
M510F12		Urine: 0.04 – 0.34%	Not detected	Not detected	Not detected

Table 5.8.1-2: Review of metabolites occurring in animal matrices (including the rat) and plant matrices

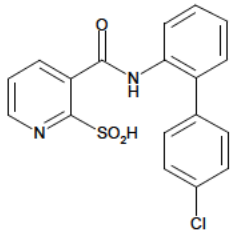
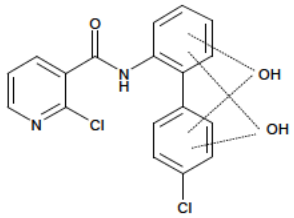
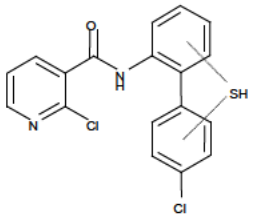
Metabolite		Occurrence (% of TRR)			
Substance Code	**Chemical structure**	**Rat**	**Plant**	**Poultry**	**Ruminants**
M510F13		Urine: Trace amounts only	Not detected	Not detected	Not detected
M510F14		Urine: Trace amounts only	Not detected	Not detected	Not detected
M510F15		Urine: Trace amounts only	Not detected	Not detected	Not detected

Table 5.8.1-2: Review of metabolites occurring in animal matrices (including the rat) and plant matrices

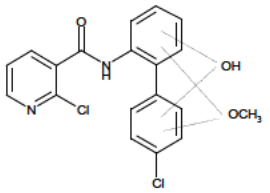
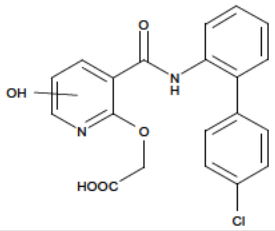
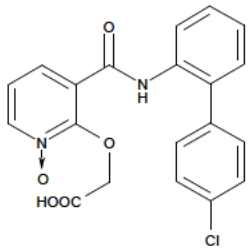
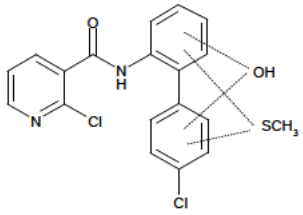
Metabolite		Occurrence (% of TRR)			
Substance Code	**Chemical structure**	**Rat**	**Plant**	**Poultry**	**Ruminants**
M510F16		Urine: Trace amounts only	Not detected	Not detected	Urine: 2.0% together with M510F05 and M510F59/ 6.3% together with M510F05 and M510F59 (no clear separation of peaks possible)
M510F18		Urine: Minor amounts only	Not detected	Not detected	Not detected
M510F19		Urine: Minor amounts only	Not detected	Not detected	Not detected
M510F20		Feces: 0.51 – 8.32% Urine: 0.0 – 0.57%	Not detected	Not detected	Urine: 0.7%

Table 5.8.1-2: Review of metabolites occurring in animal matrices (including the rat) and plant matrices

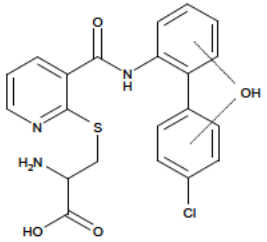
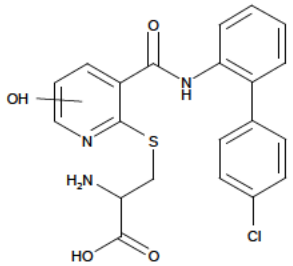
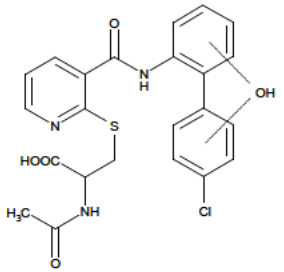
Metabolite		Occurrence (% of TRR)			
Substance Code	**Chemical structure**	**Rat**	**Plant**	**Poultry**	**Ruminants**
M510F22		Urine: Trace amounts only Bile: 0.0 – 0.1% (combined with M510F23)	Not detected	Not detected	Urine: 2.2%
M510F23		Bile: 0.0 – 0.1% (combined with M510F22)	Not detected	Not detected	Not detected
M510F28		Urine: Trace amounts only	Not detected	Not detected	Not detected

Table 5.8.1-2: Review of metabolites occurring in animal matrices (including the rat) and plant matrices

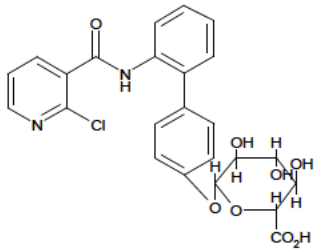
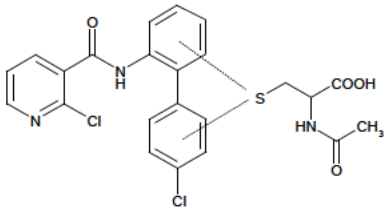
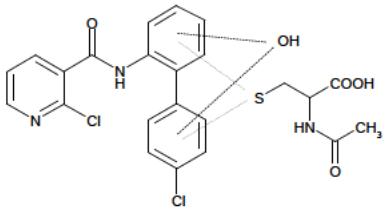
Metabolite		Occurrence (% of TRR)			
Substance Code	**Chemical structure**	**Rat**	**Plant**	**Poultry**	**Ruminants**
M510F29		Urine: Minor amounts only	Not detected	Not detected	Not detected
M510F32		Urine: Trace amounts only	Not detected	Not detected	Not detected
M510F33		Urine: Trace amounts only	Not detected	Not detected	Not detected

Table 5.8.1-2: Review of metabolites occurring in animal matrices (including the rat) and plant matrices

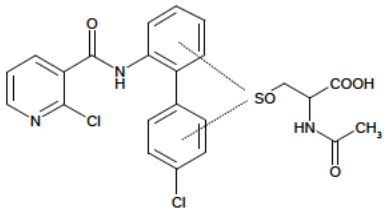
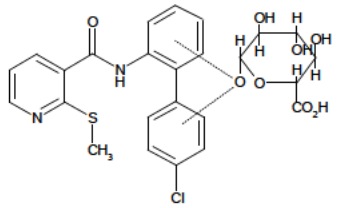
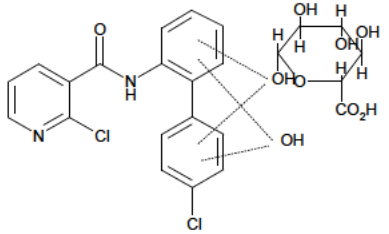
Metabolite		Occurrence (% of TRR)			
Substance Code	**Chemical structure**	**Rat**	**Plant**	**Poultry**	**Ruminants**
M510F34		Urine: Trace amounts only	Not detected	Not detected	Not detected
M510F39		Urine: Minor amounts only	Not detected	Not detected	Not detected
M510F40		Urine: Trace amounts only	Not detected	Not detected	Not detected

Table 5.8.1-2: Review of metabolites occurring in animal matrices (including the rat) and plant matrices

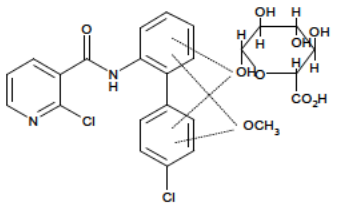
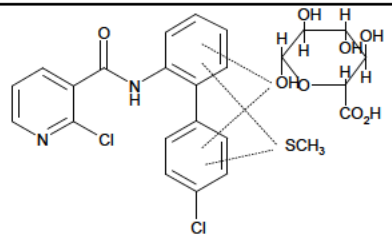
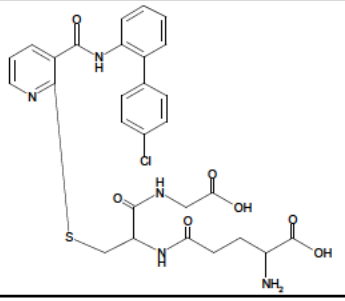
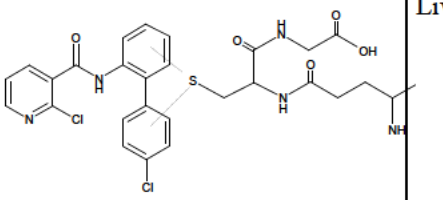
Metabolite		Occurrence (% of TRR)			
Substance Code	**Chemical structure**	**Rat**	**Plant**	**Poultry**	**Ruminants**
M510F41		Urine: Trace amounts only	Not detected	Not detected	Urine: 0.7% together with isomer and M510F59
M510F42		Feces: 0.0 – 0.2% Urine: 0.03 – 1.45% Kidney: < 0.01% Liver: ≤ 0.03%	Not detected	Not detected	Not detected
M510F43		Liver: 0.14 – 0.26%	Not detected	Not detected	Not detected
M510F45		Liver: 0.05 – 0.1%	Not detected	Not detected	Not detected

Table 5.8.1-2: Review of metabolites occurring in animal matrices (including the rat) and plant matrices

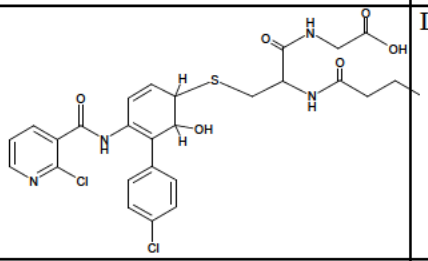
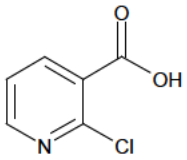
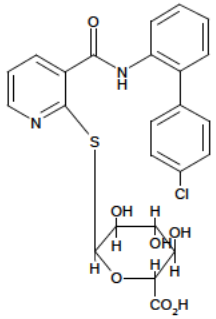
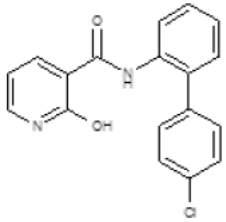
Metabolite		Occurrence (% of TRR)			
Substance Code	**Chemical structure**	**Rat**	**Plant**	**Poultry**	**Ruminants**
M510F46 or isomer		Liver: 0.03 – 0.24%	Not detected	Not detected	Not detected
M510F47 Reg. No. 107371 CAS 2942-59-8		Urine: 0.0 – 0.1% Liver: <0.01%	Green bean: 2.8% Bean pods (fresh): 2.15% Bean seeds (fresh): 9.97% Bean pods (dry): 1.1% Bean seeds (dry): 1.7%	Not detected	Not detected
M510F48		Feces: 0.38 – 6.50% Urine: 0.02 – 2.28% Plasma: < 0.01% Kidney: < 0.01% - 0.02%	Not detected	Not detected	Not detected
M510F49 Reg. No 391572		Not detected	Not detected	Liver[1]: 12.7%	Milk[1]: 7.7% Liver[1]: 11.4%

Table 5.8.1-2: Review of metabolites occurring in animal matrices (including the rat) and plant matrices

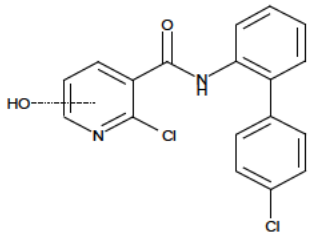
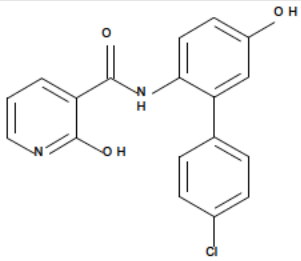
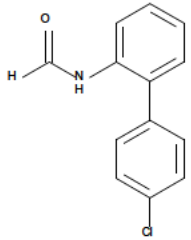
Metabolite		Occurrence (% of TRR)			
Substance Code	**Chemical structure**	**Rat**	**Plant**	**Poultry**	**Ruminants**
M510F50		Bile: Trace amounts only	Not detected	Not detected	Urine:0.9%
M510F51 Reg. No. 4035208		Not detected	Not detected	Liver[1]: 21.7%	Milk[1]: 12.2% Liver[1]: 6.6%
M510F52 Reg. No. 4035211		Not detected	Not detected	Liver[1]: 42.1% (marker for bound residues)	Liver[1]: 35.4% (marker for bound residues)

Table 5.8.1-2: Review of metabolites occurring in animal matrices (including the rat) and plant matrices

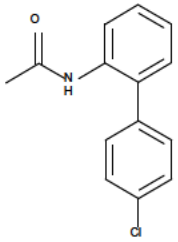
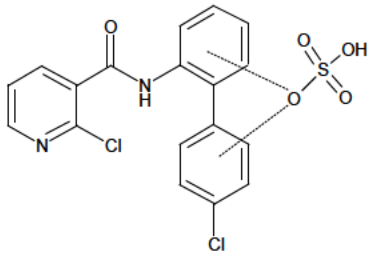
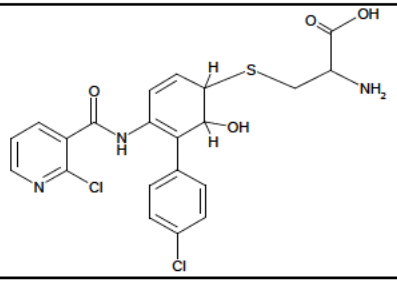
Metabolite		Occurrence (% of TRR)			
Substance Code	**Chemical structure**	**Rat**	**Plant**	**Poultry**	**Ruminants**
M510F53 Reg. No. 4035210		Not detected	Not detected	Not detected	Milk[1]: 11.2% Liver[1]: 43.6% (marker for bound residues)
M510F54		Not detected	Not detected	Egg: 1.9% Excreta: 2.1%	Urine: 2.2%
M510F57 or isomer		Bile: 0.41 – 1.32%	Not detected	Not detected	Not detected

Table 5.8.1-2: Review of metabolites occurring in animal matrices (including the rat) and plant matrices

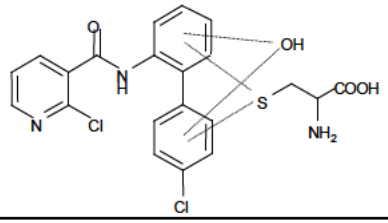
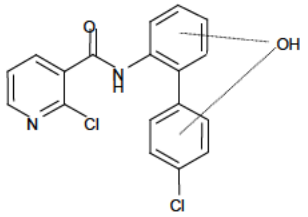
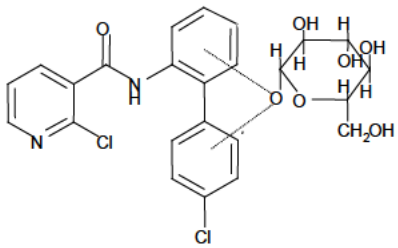
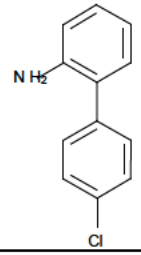
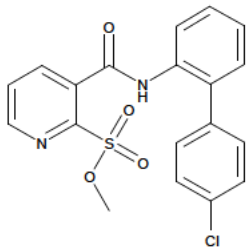
Metabolite		Occurrence (% of TRR)			
Substance Code	**Chemical structure**	**Rat**	**Plant**	**Poultry**	**Ruminants**
M510F58		Bile: 0.09 – 0.27%	Not detected	Not detected	Not detected
M510F59		Not detected	Not detected	Not detected	Urine: 0.7% together with M510F41 and its isomer/2.0% together with M510F05 and M510F16/6.3% with M510F05 and M510F16/5.0% activity (no clear separation of peaks possible)
M510F61		Not detected	Confined rotational crop: Radish roots: 10.9%	Not detected	Not detected
M510F62 Reg. No. 363487 CAS 1204-44-0		Not detected	Amount <0.01 mg/kg: Bean plant: 0.02% Green bean: 0.6% Bean seeds (fresh): 0.7% **Impurity findings without relevance to consumers**	Not detected	Not detected

Table 5.8.1-2: Review of metabolites occurring in animal matrices (including the rat) and plant matrices

Metabolite		Occurrence (% of TRR)			
Substance Code	**Chemical structure**	**Rat**	**Plant**	**Poultry**	**Ruminants**
M510F63		Feces: 0.0 – 4.01%	Not detected	Not detected	Not detected

* TRR total applied radioactivity in metabolism studies

1 Measured after microwave treatment and not bioavailable

Toxicological relevance assessment of metabolites in plant and animal matrices, and metabolites potentially occurring in ground water

General provisions

The toxicological relevance assessment of the existing metabolites has been conducted, taking into consideration the presence and amount of the metabolites in matrices of animal and plant origin as well as metabolites potentially occurring in ground water above the EU established threshold level of 0.1 µg/L. Relevant toxicological information available within BASF SE was in addition taken into account. With regard to the evaluation of chemical similarity the general proposals given e.g. by the EFSA Scientific Opinion on Evaluation of the Toxicological Relevance of Pesticide Metabolites for Dietary Risk Assessment [EFSA Journal 2012;10(07):2799] were followed. The following general molecular modifications were considered to probably not cause higher toxicity of the metabolites as compared to the parent compound:

- Simple demethylation of the ring or side chain
- Simple hydroxylation of the ring system without any cleavage of the ring
- Hydroxylation of another ring position
- Conjugation of molecules with amino acids

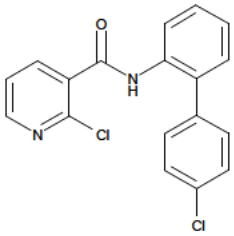
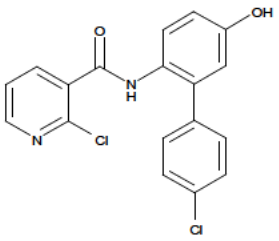
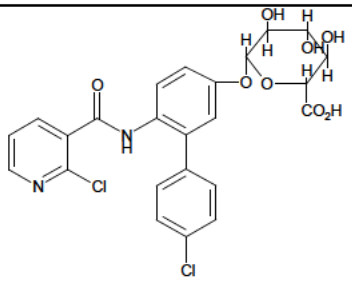
Comparison was made to the parent compound as well as to the same metabolites occurring in the rat metabolism or other similar rat metabolites the extent of which suggest adequate co-testing within the toxicological data base as generated for Boscalid technical material. Consideration has also been given to metabolites contained in Boscalid technical material as impurities of the toxicology batches N 26, N 37 or N 46.

Note: In the detailed assessment on toxicological relevance of metabolites presented below the evaluations No. I to No. VIII do not necessarily follow the same sequence as the metabolites listing of Overview 5.8.1-2. However, all metabolites occurring in plant and animal matrices as well as all metabolites identified to potentially be present in groundwater have been addressed. The following metabolites have been evaluated in more detail below:

- I: Metabolites M510F01 and M510F02 as group
- II: Metabolite M510F47
- III: Metabolite M510F61
- IV: Metabolite M510F54
- V: Metabolite M510F49
- VI Metabolite M510F62
- VII Other metabolites occurring in urine and/or faeces of goats
- VIII Metabolites M510F52 and M510F53 found in liver and milk

Detailed assessment on toxicological relevance of metabolites

I Metabolites M510F01 and M510F02 as phase I and phase II transformation steps of the parent compound Boscalid

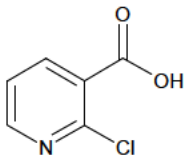
Boscalid	M510F01	M510F02
		
	Occurrence in animal matrices: Yes Occurrence in plant matrices: No	Occurrence in animal matrices: Yes Occurrence in plant matrices: No

The metabolite M510F01 has been found in milk, muscle, fat, kidney and liver in the goat metabolism study as well as the feeding study, and also in eggs and liver in the hen metabolism study. M510F02 was found in milk, muscle and kidney of goats and in eggs of hens. In the **hen**, both metabolites were found in eggs at levels greater than 10% of TRR and metabolite M510F01 was also found in liver at a level of about 6% of TRR. Main route of excretion is as metabolite M510F01 (up to 75% of TRR). In the **goat** both metabolites can occur at levels greater than 10% of TRR in muscle and fat. Metabolite M510F01 can also be present in milk at levels greater than 10% of TRR whereas metabolite M510F02 was present in this matrix at up to about 6% of TRR. The main route of excretion is via urine with substantial amounts of TRR found for both metabolites in kidneys and subsequently in urine. Detailed information on the extent of occurrence of both metabolites is presented in Overview 5.8.1-2. In conclusion metabolites M510F01 and M510F02 are considered relevant for consumer risk assessment.

In the **rat** both metabolites M510F01 and M510F02 are formed at levels greater than 10% of TRR followed by excretion via urine. Detailed information on the extent of occurrence of both metabolites is presented in Overview 5.8.1-2. Both metabolites are therefore considered to have been adequately co-tested in the toxicology data base of Boscalid. The metabolism of Boscalid via hydroxylation to M510F01 followed by glucuronidation to M510F02 is considered as a common pathway of metabolism and supposedly detoxification. This is also supported by the 'EFSA Scientific Opinion on Evaluation of the Toxicological Relevance of Pesticide Metabolites for Dietary Risk Assessment' suggesting the molecular alteration by such as simple hydroxylation of the ring system without any cleavage of the ring system (including subsequent phase II steps of metabolism) to probably not causing higher toxicity of the metabolites as compared to the parent compound.

In the overall conclusion the toxicological relevance of metabolites M510F01 and M510F02 has been adequately addressed. No toxicological concern has been identified for metabolites M510F01 and M510F02 based on the extensive co-testing of these metabolites within the toxicology studies performed with Boscalid, as well as based on the nature of their molecular structure if the principles of the respective EFSA guidance are followed.

II Metabolite M510F47

M510F47	Occurrence	TRR (mg/kg)	TRR (%)
	Green bean	0.003	2.80
	Seeds	0.007	9.97
	Pods	0.002	2.15
	Dry seeds	0.002	1.72
	Dry pods	0.015	1.11
Occurrence in animal matrices: No Occurrence in plant matrices: Yes Potential occurrence in groundwater: No, below the EU established threshold level of 0.1 µg/L			

M510F47 was only found in the **bean** metabolism study in the following matrices: Green beans, seeds and pods, as well as in dry pods and dry seeds. Metabolite M510F47 was found at levels of up to 10% of TRR. However, in terms of absolute residue levels, the maximum of 0.007 mg/kg is considered to be low. Metabolite M510F47 was also identified to eventually occur as a groundwater metabolite (see Doc N 4, Section 3 'Relevance of metabolites in groundwater'). For metabolite M510F47 the maximum PEC in groundwater was calculated to be 0.057 µg/L, i.e. the EU established threshold of 0.1 µg/L for further toxicological testing is not exceeded. Nevertheless, for precautionary reasons the toxicology data requirements according to SANCO/221/2000 –rev. 10 of 25 February 2003 have been fulfilled for substances of groundwater occurrence within the limits of 0.1 µg/L but not exceeding 0.75 µg/L and the required set of genotoxicity studies is presented below together with additional toxicology information on metabolite M510F47. In conclusion metabolite M510F47 is considered relevant for consumer risk assessment.

Metabolite M510F47 was found to a limited extent in liver and urine not exceeding 0.1% of TRR in the rat metabolism study (see Overview 5.8.1-2) which does not account for adequate co-testing of this metabolite in the toxicology data base of the parent compound Boscalid. As M510F47 is not part of the residue definition in plants, it is treated as a Cramer Class III metabolite. The TTC threshold value for Cramer Class III is 0.0015 mg/kg bw/day. The evaluation following the TTC approach based on the Cramer Class III is considered adequate given the absence of neurotoxic alerts such as for organophosphates, carbamates or pyrethroids. There is also no alert for mutagenic properties of M510F47. On the CLP inventory of the ECHA webpage several self-classifications are listed [see http://echa.europa.eu/de/information-on-chemicals/cl-inventory-database/-/cl-inventory/view-notification-summary/125453] which are ranging from "not classified" to the proposal that 2-chloronicotinic acid is skin and eye irritating (Skin Irritation 2, H315 and Eye Irritation 2, H319) as well as potentially causing respiratory irritation (STOT RE 3, H335). In addition information on acute oral toxicity in Wistar rats (BASF DocID 1998/10872) and a set of mutagenicity studies [Ames test (BASF DocID 1998/10812), CHO-HPRT test (BASF DocID 2015/1093631), and in vitro micronucleus assay in V79 cells (BASF DocID 2015/1022707)] has been included in this dossier and is presented in more detail below. Metabolite M510F47 has been shown to have low acute toxicity in rats when applied by the oral route and did not show genotoxic properties. All this information supports the application of the Cramer Class III approach for M510F47 to be appropriate.

In the overall conclusion the toxicological relevance of metabolite M510F47 has been adequately addressed. No toxicological concern has been identified. The TTC approach based on Cramer Class III has been considered acceptable given the absence of neurotoxic alerts and the metabolite M510F47 to be devoid of genotoxic properties.

The information on acute oral toxicity and genotoxicity (Ames test, CHO-HPRT test, micronucleus assay in vitro) is presented below:

2-Chlornicotinsaeure - Acute oral toxicity in rats ([redacted] 1998) 1998/10872

Guidelines: EEC 96/54, OECD 423
GLP: yes
(certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Note: This study report has been part of the documentation for the first Annex I inclusion process and a short summary is therefore presented here

Groups of 3 male and 3 female Wistar rats (Chbb:Thom (SPF); source: Dr. K. Thomae GmbH, Biberach, Germany) were administered Reg. No. 107371 (2-chloronicotinic acid, batch: 01174-232, purity: 99.8%) once at concentration of 2000 mg/kg bw as a suspension in aqua bidest. (dose volume of 10 mL/kg bw) by gavage and were observed for a period of 14 days. Mortality was observed twice daily on working days and daily on weekends and public holidays. Clinical signs of toxicity were recorded at least daily. Body weight was determined just before application (day 0), weekly thereafter and at the end of the study period (before fasting period). Gross necropsy was performed at the end of the study period (day 14).
The stability of the test item over the study period as well as the aqueous test item preparations for a time period of 96 hours and the homogeneous distribution were confirmed analytically by HPLC.
No mortalities occurred and no signs of toxicity were observed during the study period. Body weight and body weight gain were unaffected by the test item. At necropsy, no pathological changes were observed that could be attributed to administration of the test material.

Conclusion
The oral LD_{50} of 2-chloronicotinic acid (Reg. No. 107371) is greater than 2000 mg/kg bw in male and female Wistar rats.

Salmonella typhimurium/Escherichia coli reverse mutation assay (standard plate test and preincubation test) with 2-Chlornicotinsaeure (Engelhardt G.,Hoffmann H.D., 1998) 1998/10812

Guidelines: OECD 471, EEC 92/69 B 13, EEC 92/69 B 14
GLP: yes
(certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Note: This study report has been part of the documentation for the first Annex I inclusion process and a short summary is therefore presented here

S. typhimurium strains TA 98, TA 100, TA 1535 and TA 1537, and a strain of *E. coli* WP2 uvrA were exposed with Reg. No. 107371 (2-chloronicotinic acid, batch: 01174-232, purity: 99.8%) using DMSO as a solvent in the presence and absence of metabolic activation (liver S-9 mix of Aroclor 1254-induced Sprague Dawley rats) for 48 - 72 hours at 37°C. Vehicle and appropriate positive controls were included in each experiment with and without metabolic activation. The test item was tested via standard plate test (SPT) and the pre-incubation test (PIT) in concentrations ranged from 20 to 5000 µg/plate and from 125 to 2000 µg/plate, respectively.
The stability of the test item over the study period as well as the test item preparations in DMSO for a time period of 4 hours were confirmed analytically. The homogeneity of the test item was guaranteed on account of the high purity.
Precipitation of the test item was observed at 5000 µg/plate. Bacteriotoxicity of the test item (reduced his^- or trp^- background growth, decrease in the number of his^+ or trp^+ revertants, reduction in the titer) was observed at concentrations ≥ 1000 and ≥ 2500 µg/plate in PIT and SPT, respectively. An increase in the number of his^+ and trp^+ revertants was not observed in SPT and PIT either without S-9 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.
According to the results of the study, the test substance Reg. No. 107371 is not mutagenic in the Ames reverse mutation assay under the experimental conditions chosen.

Report:	CA 5.8.1/1 Schulz M.,Landsiedel R., 2015 a Reg.No. 107371 (metabolite of BAS 510 F, Boscalid) - In vitro gene mutation test in CHO cells (HPRT locus assay) 2015/1093631
Guidelines:	OECD 476, (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.17 No. L 142, EPA 870.5300
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Note: This study report has **not** been part of the documentation for the first Annex I inclusion process and a full evaluation is therefore presented here to assist in the discussion on relevant metabolites in food items

EXECUTIVE SUMMARY

Reg. No. 107371 (Metabolite of BAS 510 F, Batch: L80-188, Purity: 100%) was tested in vitro for its ability to induce forward mutations in mammalian cells by assessing the mutation of the HPRT locus in Chinese Hamster CHO cells. Two independent experiments were conducted in the presence and absence of metabolic activation. Based on the results of a preliminary cytotoxicity assay concentrations of up to 1600 µg/mL were used in the main experiment. The treatment intervals for both experiments in the presence and absence of metabolic activation were 4 hours. EMS and DMBA served as positive controls in the experiments without and with metabolic activation, respectively. After the incubation period treatment media were replaced by culture medium in both experiments and the cells were incubated for one week for expression of mutant cells. This was followed by incubation of cells in selection medium containing 6-thioguanine for about 1 week.

The test substance did not exhibit any pronounced toxicity up to the highest recommended concentration (1600 µg/mL) in the presence and absence of metabolic activation. Precipitation of the test substance was not observed in at any concentration tested. Cell morphology was not influenced by treatment. The test substance did not cause any relevant increase in the mutant frequencies either without S9 mix or after the addition of a metabolizing system in two experiments performed independently.

Based on the results of the study it is concluded that under the conditions of this test Reg. No. 107371 (Metabolite of BAS 510 F) does not induce forward mutations in mammalian cells in vitro.

(BASF DocID 2015/1093631)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material Reg. No. 107371 (Metabolite of BAS 510 F)
Description: Solid, colorless to white
Lot/Batch #: L80-188
Purity: 100%
Stability of test compound: The stability of the test substance under storage conditions was guaranteed by the sponsor. The homogeneity of the test substance was guaranteed on account of the high purity and ensured by mixing prior to preparation of test substance solutions.
Solvent used: Dimethylsulfoxide (DMSO), 1% final concentration in culture medium

2. Control Materials:
Negative control: A negative control was not employed in this study
Vehicle control: 1% (v/v) DMSO in culture medium
Positive control -S9: Ethylmethanesulfonate (EMS) 400 µg/mL
Positive control +S9: 7,12-Dimethylbenz[a]anthracene (DMBA) 1.25 µg/mL

3. Activation: S9 was produced from the livers of induced male Wistar rats. The rats received 80 mg/kg bw phenobarbital and β-naphthoflavone per gavage on 3 consecutive days. The S9-mix was prepared freshly prior to each experiment. For this purpose, a sufficient amount of S9-fraction is thawed at room temperature and 1 volume of S9-fraction is mixed with 9 volumes of S9-supplement (cofactors). This preparation, the so-called S9-mix, was kept on ice until used.
The S9-mix was prepared immediately before use and had the following composition:

Component	Concentration
Phosphate buffer (pH 7.4)	15 mM
Glucose 6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM

4. Test organism: Chinese hamster ovary (CHO) cells were used. CHO cells have a high proliferation rate (doubling time about 12-16 h), high plating efficiency (about 90%) and karyotype with a modal number of 20 chromosomes. Stocks of the CHO cell line were maintained at -196°C in liquid nitrogen. Each batch used for mutagenicity testing was checked for mycoplasma contamination. The week prior to treatment, spontaneous HPRT-deficient mutants were eliminated from the stock cultures by growing the cells for 3 to 4 days in pretreatment medium (see below).

5. Culture media:

Culture medium: Ham's F12 medium with L-glutamine and hypoxanthine supplemented with 10% (v/v) fetal calf serum (FCS).

Pretreatment medium: ("HAT" medium): FCS-supplemented Ham's F12 medium with L-glutamine and hypoxanthine containing per mL 13.6 µg hypoxanthine, 0.18 µg aminopterin and 3.88 µg thymidine.

Selection medium: ("TG" medium): L-Glutamine- and FCS-supplemented, hypoxanthine-free Ham's F12 medium with 6-thioguanine at a final concentration of 10 µg/mL

All media were supplemented with
- 1% (v/v) penicillin/streptomycin (10000 IU / 10000 µg/mL)
- 1% (v/v) amphotericine B (250 µg/mL)

6. Locus examined: hypoxanthine-guanine-phosphoribosyl transferase (H(G)PRT)

7. Test concentrations:

a) Preliminary toxicity assay: Nine concentrations ranging from 6.3 to 1600 µg/mL (~10 mM)

b) Mutation assay:

1st experiment

(4-h exposure): 200, 400, 800, 1600 µg/mL with/without metabolic activation

2nd experiment

(4-h exposure): 250, 500, 1000, 1600 µg/mL with/without metabolic activation

3rd experiment

(4-h exposure): 250, 500, 1000, 1600 µg/mL with metabolic activation

B. TEST PERFORMANCE:

1. Dates of experimental work: 24-Nov-2014 to 09-Apr-2015

2. Preliminary cytotoxicity assay:

Cytotoxicity was assessed by determination of the cloning efficiency. About 200 cells were incubated in 25-cm² flasks with various test substance concentrations in serum-free Ham's F12 medium for about 4 hours (with and without metabolic activation) after an attachment period of 24 hours. At the end of the exposure period, the cells were washed with Hanks' balanced salt solution (HBSS), covered with Ham's F12 and incubated for a further 6 to 8 days. After this incubation period, colonies were fixed, stained and counted. In addition to the cloning efficiency the following parameters were measured: pH, osmolarity and the determination of precipitates (solubility).

3. Mutation Assay:

Pretreatment of Cells:	Cells with a passage number ≥ 2 after thawing from the frozen cells stock were seeded into 75 cm^2-flasks and incubated for 3-4 days with "HAT" medium during the week prior to treatment to eliminate spontaneous HPRT-deficient mutants. Afterwards, a passage into culture medium followed and the cells were incubated for further 3-4 days.
Cell treatment:	For each test group, about $1x10^6$ cells per flask (175 cm²) were seeded into flasks containing about 20 mL Ham's F12 medium supplemented with 10% FCS and incubated for about 20 – 24 hours with 5% CO_2 at 37°C and ≥ 90% humidity for cell attachment. 2x2 flasks (A/B) were used for each test group. After the cell attachment period the medium was replaced by treatment medium. The test article, dissolved in 200 µL DMSO, was added to the culture medium. Without S9 mix 20 mL Ham's F12 medium without FCS and 200 µL test substance preparation were used. With S9 mix 16 mL Ham's F12 medium without FCS, 200 µL test substance preparation and 4 mL S9 mix were used. Concurrent negative and positive controls were tested in parallel. The cells were exposed for 4 hours both with and without S9-mix at 5% CO_2, 37°C and ≥ 90% humidity.

Expression:	After the exposure period, the treatment medium was replaced by 20 mL Ham's F12 medium with 10% FCS after having been rinsed several times with Hanks' balanced salt solution (HBSS). Subsequently, the flasks were incubated for another 3 days and then subcultured (1st passage). After an entire expression period of 6-8 days, the cells were transferred into selection medium ("TG" medium) at the 2nd passage.
Selection:	For the mutant selection, six 75-cm^2 flasks each were seeded with $3x10^5$ cells from each treatment group in selection medium (TG medium) and incubated for about 6 to 7 days. At the end of the selection period, colonies were fixed with methanol, stained with Giemsa and counted.
Determination of Cytotoxicity:	Cloning efficiency 1 (survival): The survival (cloning efficiency 1; CE_1) was determined in parallel to the mutagenicity test. Approximately 200 cells per dose group were seeded into duplicate 25 cm^2 flasks using 5 mL Ham`s F12 medium with 10% FCS. After about 24 hour attachment period the cells were incubated with vehicle, test substance or the positive control for 4 hours as described above. Following exposure, cells were rinsed several times with HBSS. Finally, cells were cultured in 5 mL Ham's F12 medium incl. 10% (v/v) FCS. Cloning efficiency 2 (viability): The viability (cloning efficiency 2; CE_2) was determined after the expression period. About 200 cells were separated during the transfer into selection medium and seeded in two flasks (25 cm^2) containing 5 mL Ham's F12 medium incl. 10% (v/v) FCS each. After seeding of the cells, the flasks were incubated for 6 – 8 days to form colonies. These colonies were fixed, stained and counted.

Calculations:

Mutant frequency: Uncorrected mutant frequency:

$$MF_{uncorr} = \frac{\text{total number of mutant colonies}}{\text{number of seeded cells}} \times 10^6$$

Corrected mutant frequency:

$$MF_{corr} = \frac{MF_{uncorr}}{CE_{2\,absolute}} \times 100$$

Cloning efficiency (CE,%) absolute:

$$CE_{absolute} = \frac{total\ number\ of\ colonies\ in\ the\ test\ group}{total\ number\ of\ seeded\ cells\ in\ the\ test\ group} x100$$

relative, in comparison to control:

$$CE_{relative} = \frac{CE_{absolute}\ of\ the\ test\ group}{CE_{absolute}\ of\ the\ vehicle\ control} x100$$

4. Check for further parameters:

pH was measured at least for the top concentrations and vehicle control with and without S9 mix. Osmolarity was measured at least for the top concentrations and for vehicle control with and without S9 mix. Possible test substance precipitation was checked immediately after treatment of the test cultures. The test cultures of all test groups were examined microscopically at the end of the exposure period with regard to cell morphology, which allows conclusions to be drawn about the attachment of the cells.

5. Statistics:

An appropriate statistical trend test (MS EXCEL function RGP) was performed to assess a dose-related increase of mutant frequencies. The number of mutant colonies obtained for the test substance treated groups was compared with that of the respective vehicle control groups. A trend is judged as statistically significant whenever the one-sided p-value (probability value) is below 0.05 and the slope is greater than 0. However, both, biological and statistical significance will be considered together.

6. Evaluation criteria:

The test chemical is considered positive in this assay if the following criteria are met:

- Increases of the corrected mutation frequencies ($MF_{corr.}$) both above the concurrent vehicle control values and the historical negative control range.
- Evidence of reproducibility of any increase in mutant frequencies.
- A statistically significant increase in mutant frequencies and the evidence of a dose-response relationship.

Isolated increases of mutant frequencies above the historical negative control range (i.e. 15 mutants per 10^6 clonable cells) or isolated statistically significant increases without a dose-response relationship may indicate a biological effect but are not regarded as sufficient evidence of mutagenicity.

A test substance is generally considered negative in this test system if:

- The corrected mutation frequency ($MF_{corr.}$) in all dose groups is within the historical control range and is not significantly above the concurrent negative control.

II. RESULTS AND DISCUSSION

A. MUTANT FREQUENCY

Two independent experiments were conducted in the presence and absence of metabolic activation. Based on the results of a preliminary cytotoxicity assay concentrations of 200 µg/mL to 1600 µg/mL were used in the main experiment. No increase in the number of mutant colonies was observed either with or without S9 mix. The mutant frequencies at any dose were close to the range of that of the concurrent negative control values and within the range of the historical control data. Unfortunately, the 2nd experiment in the presence of metabolic activation failed the acceptance criteria of this study as the positive control group treated with DMBA did not lead to the expected increase in mutant frequencies. It has to be speculated that a technical error occurred when preparing the positive control preparation. Besides, no relevant increase in the number of mutant colonies was obtained after treatment with the test substance. However, this experimental part was repeated, designated 3rd Experiment, and the results are not further discussed in this study report evaluation.
Treatment with the positive controls EMS and DMBA resulted in a marked increase in the number of mutant colonies as well as of mutant frequencies in all experiments (except the 2nd experiment, see above), thus demonstrating the sensitivity of the test.

B. CYTOTOXICITY

Based on the preliminary toxicity test, the concentration of 1600 µg/mL was chosen as the top dose.
Cytotoxic effects, as indicated by clearly reduced cloning efficiencies of about or below 20% of the respective negative control values were not observed in all experiments in the absence and presence of S9 mix.

C. CELL MORPHOLOGY

Cell attachment was not influenced at any dose evaluated. The cell morphology was “Fibroblast-like cells” at any test substance concentration.

D. TREATMENT CONDITIONS

The pH value of the test substance preparation was adjusted by adding small amounts of NaOH. The pH values in culture medium were not influenced by test substance treatment. In the 2nd Experiment in the absence and presence of S9 mix, osmolarity was slightly increased at 1600 µg/mL compared to the respective vehicle control group (566 mOsm versus 450 mOsm or 500 mOsm versus 381 mOsm, respectively). However, this observation did not lead to any adverse biological effect. Besides, this observation on osmolarity was not confirmed in any further experimental part of this study.
In this study, in the absence and the presence of S9 mix, no precipitation in culture medium was observed up to the highest required test substance concentration.

Table 5.8.1-3: Gene mutation in mammalian cells - 1st experiment

Test group	Number of mutant colonies (A/B)[a]	Mutant frequency (per 10^6 cells)		CE_1 (survival) (4h after treatment; approx. 200 cells/flask seeded		CE_2 (viability) (at the end of the expression period; approx. 200 cells/flask seeded)	
				Cloning efficiency (%)		Cloning efficiency (%)	
		Non corrected	Corrected[b]	absolute	relative	absolute	relative
Without metabolic activation; 4-hour exposure period							
Vehicle (DMSO)	8/12	5.56	5.43	79.6	100	103.1	100
Test item							
200 µg/mL	2/5	1.94	2.05	82.9	104	97.5	94.5
400 µg/mL	0/3	0.83	0.82	81.1	102	103.5	100.4
800 µg/mL	2/4	1.11	1.03	83.8	105	104.9	101.7
1600 µg/mL	6/1	1.94	1.96	76.0	95	103.8	100.6
Positive control EMS							
400.0 µg/mL	145/158	84.17	89.76	70.1	88	93.8	90.9
With metabolic activation; 4-hour exposure period							
Vehicle (DMSO)	1/2	0.83	0.85	79.5	100	98.6	100
Test item							
200 µg/mL	18/4	6.11	6.06	68.8	87	98.4	99.7
400 µg/mL	1/1	0.56	0.56	65.4	82	99.1	100.5
800 µg/mL	6/3	2.50	2.64	69.5	87	94.0	95.3
1600 µg/mL	7/0	1.94	2.11	64.6	81	88.9	90.1
Positive control DMBA							
1.25 µg/mL	138/196	92.78	108.91	60.9	77	86.0	87.2

[a] number of colonies 7 days after seeding 3 x 10^5 cells/flask into selection medium

[b] correction on the basis of absolute cloning efficiency 2 (viability) at the end of the expression period

Table 5.8.1-4: Gene mutation in mammalian cells - 2nd and 3rd experiment

Test group	Number of mutant colonies (A/B)[a]	Mutant frequency (per 10^6 cells)		CE_1 (survival) (4h after treatment; approx. 200 cells/flask seeded		CE_2 (viability) (at the end of the expression period; approx. 200 cells/flask seeded)	
				Cloning efficiency (%)		Cloning efficiency (%)	
		Non corrected	Corrected[b]	absolute	relative	absolute	Relative
Without metabolic activation; 4-hour exposure period							
Vehicle (DMSO)	9/1	2.78	2.76	89.0	100	92.5	100
Test item							
250 µg/mL	9/14	6.39	6.71	94.6	106.3	95.3	103.0
500 µg/mL	4/4	2.22	2.33	91.8	103.1	95.6	103.4
1000 µg/mL	3/4	1.94	2.05	88.1	99.0	94.9	102.6
1600 µg/mL	11/2	3.61	3.80	84.3	94.7	94.9	102.6
Positive control EMS							
400 µg/mL	387/255	178.33	228.90	77.3	86.8	78.5	84.9
With metabolic activation; 4-hour exposure period[#]							
Vehicle (DMSO)	1/1	0.56	0.63	77.9	100	87.6	100
Test item							
250 µg/mL	5/18	6.39	7.40	84.3	108.2	86.8	99.0
500 µg/mL	1/5	1.67	1.93	85.5	119.8	88.8	101.3
1000 µg/mL	4/2	1.67	1.99	83.5	107.2	83.3	95.0
1600 µg/mL	3/5	2.22	2.59	81.6	104.8	84.8	96.7
Positive control DMBA							
1.25 µg/mL	338/327	184.72	238.73	80.5	103.4	77.5	88.4

[a] number of colonies 7 days after seeding 3 x 10^5 cells/flask into selection medium
[b] correction on the basis of absolute cloning efficiency 2 (viability) at the end of the expression period
[#] Third experiment due to experimental failure in complying with acceptance criteria

III. CONCLUSION

Based on the results of the study it is concluded that under the conditions of this test Reg.No. 107371 (Metabolite of BAS 510 F) does not induce forward mutations in the HPRT locus in CHO cells under the experimental conditions chosen.

Report:	CA 5.8.1/2 Schulz M.,Landsiedel R., 2015 b Reg.No. 107371 (metabolite of BAS 510 F, Boscalid) - In vitro micronucleus assay in V79 cells (cytokinesis block method) 2015/1022707
Guidelines:	OECD 487 (2010), Commission Regulation EU No. 640/2012 of 06 July 2012 - B.49: In vitro Mammalian Cell Micronucleus Test
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Note: This study report has **not** been part of the documentation for the first Annex I inclusion process and a full evaluation is therefore presented here to assist in the discussion on relevant metabolites in food items

EXECUTIVE SUMMARY

Reg.No. 107371 (Batch: L80-188; Purity: 100%) was tested in vitro for its potential to induce micronuclei in V79 Chinese Hamster lung fibroblasts (clastogenic or aneugenic activity). Two independent experiments were carried out with and without the addition of liver S9 mix from induced rats (exogenous metabolic activation). The vehicle DMSO served as negative control, EMS served as positive control in the absence of metabolic activation and cyclophosphamide as positive control in the presence of metabolic activation. Cells were incubated with the test substance at concentrations in the range of 49.4 to 1580 µg/mL. Two independent experiments were performed where the cells were incubated for four (with and without S9 mix) or 24 hours (without S9 mix) and were harvested at 24 h or 44 h. Following exposure to the test or control substances, the cell cultures were incubated with Cytochalasin B, subsequently fixed and DNA and cytoplasm stained. Cytotoxicity parameters and number of micronucleated cells were determined in at least 1000 binucleated cells/culture, i.e. 2000 cells for each test group.

Cytotoxicity was not indicated by a dose-dependent decrease of proliferative activity of treated cells; no effect on cell count or proliferation index (CBPI) was observed up to the highest applied test substance concentration in all experimental parts of this study. Effects on cell attachment/morphology and precipitation in culture medium were not observed in both experiments.

The test substance did not lead to a biologically relevant increase in the number of micronucleated cells either without S9 mix or after the addition of a metabolizing system in two experiments performed independently of each other. One statistically significant finding at an intermediate concentration and in presence of metabolic activation has not been affecting this conclusion as considered as biologically irrelevant. The increase in the frequencies of micronuclei induced by the positive control substances EMS and CPP clearly demonstrated the sensitivity of the test system and of the metabolic activity of the S9 mix employed.

In conclusion, Reg.No. 107371 is considered not to have a chromosome-damaging (clastogenic) effect nor to induce numerical chromosomal aberrations (aneugenic activity) under in vitro conditions in V79 cells in the absence and the presence of metabolic activation.

(DocID 2015/1022707)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material Reg.No. 107371 (Metabolite of BAS 510 F, Boscalid)

Description: Solid, colorless to white

Lot/Batch #: L80-188

Purity: 100%

Stability of test compound: The test substance was stable over the study period under the storage conditions (expiry date: June 2016). The homogeneity of the test substance was ensured by mixing prior to preparation of test substance formulations. The stability of the test substance at room temperature in the vehicle DMSO over a period of 4 hours was verified.

Vehicle used: Dimethylsulfoxide (DMSO)

2. Control Materials:

Negative: No negative control was employed in this study.

Vehicle/solvent control: DMSO

Positive control: Without metabolic activation:

Ethylmethanesulfonate (EMS, 400 and 500 µg/mL; dissolved in minimum essential medium with Earle's salt (MEM) without fetal calf serum (FCS))

With metabolic activation:

Cyclophosphamide (CPP, 0.5 and 1.0 µg/mL; dissolved in MEM without FCS)

3. Activation: S9 was produced from the livers of rats pretreated with β-naphthoflavone/phenobarbital. The S9-mix was prepared freshly prior to each experiment. For this purpose, a sufficient amount of S9-fraction was thawed at room temperature and 1 volume of S9-fraction was mixed with 9 volumes of S9-supplement (cofactors). This preparation, the so called S9-mix, was kept on ice until used.

The concentrations of the co-factors in the S9-mix were as follows:

Component	***Concentration***
Phosphate buffer (pH 7.4)	15 mM
Glucose 6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM

4. Test organism: V79 Chinese hamster lung fibroblasts

5. Culture medium / conditions:

Culture media: Minimum essential medium with Earle's salts (MEM) containing a L-glutamine source supplemented with 10% (v/v) fetal calf serum (FCS), 1% (v/v) penicillin / streptomycin (10 000 IU / 10 000 µg/mL) and 1% (v/v) amphotericin B (250 µg/mL). During exposure to the test substance in the presence of S9 mix, MEM was used without FCS supplementation.

Cell culture: Deep-frozen cell stocks were thawed at 37°C in a water bath, and volumes of 0.5 mL were transferred into 25 cm² plastic flasks containing about 5 mL MEM supplemented with FCS. Cells were grown with 5% (v/v) CO_2 at 37°C and ≥ 90% humidity and subcultured twice weekly. Cell monolayers were suspended in culture medium after detachment with 0.25% (w/v) trypsin solution.

Cell cycle and harvest time: The cell cycle of the untreated V79 cells lasts for about 12-14 hours under the selected culture conditions. Thus, a harvest time of 24 hours is about 2 times the normal cell cycle length. V79 cells are an asynchronous cell population, i.e. at the time of test substance treatment there are different cell stages. Since the effect on these cell stages may vary for different test substances, more than one harvest time after treatment may be appropriate. Furthermore, substance-induced mitotic delay may considerably delay the first post-treatment mitosis. Therefore, delayed harvest times (e.g. 44 hours) and prolonged exposure periods (e.g. 24 hours treatment) were considered.

6. Test concentrations:

Micronucleus assay

- Experiment I: 49.4, 98.8, 197.5, 395, 790, 1580 µg/mL
with / without metabolic activation, 4-hour exposure

- Experiment II: 98.8, 197.5, 395, 790, 1580 µg/mL
with metabolic activation, 4-hour exposure

98.8, 197.5, 395, 790, 1580 µg/mL
without metabolic activation, 24-hour exposure

B. TEST PERFORMANCE

1. Dates of experimental work: 03-Nov-2014 to 16-Apr-2015

2. Dose selection

Following the requirements of the current guidelines a test substance should be tested up to a maximum concentration of 5 mg/mL, 5 µL/mL or 10 mM, whichever is the lowest.

In case of toxicity, the top concentration should produce 55 ± 5% cytotoxicity (based on determinations of the relative increase in cell count [RICC] and/or proliferation index [CBPI] and/or replicative index [RI]) compared to the respective vehicle control. For relatively insoluble test substances at least one concentration should be scored showing no precipitation in culture medium at the end of exposure period.

Preliminary cytotoxicity assay

A cytotoxicity pretest was performed following the method described for the main experiment. 1580 µg/mL of the substance was used as top concentration. The cells were prepared at a harvest time of 24 hours (about 2 cell cycles) after 4 and 24 hours exposure time without S9 mix and after 4 hours exposure time with S9 mix. As indication of test substance toxicity the relative increase in cell count (RICC) and cell attachment (morphology) were determined for dose selection. Additionally, the pH-value, osmolarity and solubility were determined.

In the pretest the pH value was not influenced by the addition of the test substance preparation to the culture medium at the concentrations tested. However, a slight pH shift was observed at the highest required concentration prior to testing. Therefore, the pH of the stock solution was adjusted to a physiological value prior to application using small amounts of NaOH. No test substance precipitation in the vehicle DMSO was observed in the stock solution (Test group: 1580 µg/mL) or during exposure.

After 4 and 24 hours treatment in the absence of S9 mix no cytotoxicity indicated by reduced cell numbers of about or below 40 - 50% was observed. In contrary, in the presence of S9 mix, a slightly reduced cell count was observed after treatment with 1580 µg/mL.

3. Micronucleus test

Seeding and treatment of the cultures

A single cell suspension with the required cell count (3-5 x10^5 cells per culture, depending on the schedule) was prepared in MEM incl. 10% (v/v) FCS. 5 mL cell suspension was transferred into 25 cm² cell culture flasks using a dispenser. After attachment (20-24 h) the medium was removed and replaced by the treatment medium.

The cultures were treated according to the following scheme:

	Without S9 mix		With S9 mix	
	Experiment I	Experiment II	Experiment I	Experiment II
Exposure time	4 h	24 h	4 h	4 h
Recovery time	20 h	-	20 h	40 h
Harvest time	24 h	24 h	24 h	44 h

At the end of the exposure period, the treatment medium was removed and the cultures were rinsed twice with 5 mL HBSS (Hanks Balanced Salt Solution). Subsequently, 5 mL MEM (incl. 10% [v/v] FCS) supplemented with Cytochalasin B (CytB, final concentration: 3 µg/mL; stock: 0.6 mg/mL in DMSO) was added and incubated at 37°C, 5% (v/v) CO_2 and ≥ 90% relative humidity for the respective recovery time.

In the case of 24-hour continuous exposure, CytB was added to the treatment medium at start of treatment, and cell preparation was started directly at the end of exposure. In cells exposed for four hours without S9 mix and harvested for cell preparation after 44 hours, CytB was supplemented 24 hours before preparation of the cultures.

Cell harvest, preparation of slides and staining

Just before preparation the culture medium was completely removed. Single cell suspensions were prepared from each test group by trypsination. Then, the cell numbers per flask of each single cell suspension were determined using a cell counter. Subsequently, $5x10^4$ cells per slide were centrifuged at 1400 rpm for 7 minutes onto labelled slides using a Cytospin centrifuge. After drying, the slides were fixed in 90% (v/v) methanol for 10 minutes.

Before scoring, the slides were stained with a mixture of 4',6-diamidino-2-phenylindole dihydrochloride (DAPI; stock: 5 mg/mL) and propidium iodide (PI, stock: 5 mg/mL) in Fluoroshield™ at a concentration of 0.25 µg/mL each. By the use of the combination of both fluorescence dyes it is possible to differentiate between DNA (DAPI; excitation: 350 nm, emission: 460 nm) and cytoplasm (PI; excitation: 488 nm, emission: 590 nm).

4. Cytotoxicity evaluation

Cell count

Before preparing the cytospin slides the cell count was determined from trypsinized cultures. The relative cell count of 45% indicates 55% cytotoxicity/cytostasis.

Cytokinesis-block proliferation index (CBPI)

To describe a cytotoxic effect the CBPI ("Cytokinesis-block proliferation index") was determined in at least 1000 cells per culture and cytotoxicity is expressed as % cytostasis. A CBPI of 1 (i.e. all cells are mononucleated) is equivalent to 100 % cytostasis.

$$CBPI = \frac{(MONC \times 1) + (BINC \times 2) + (MUNC \times 3)}{n}$$

CBPI	Cytokinesis-block proliferation index
n	Total number of cells
MONC	Mono-nucleated cells
BINC	Bi-nucleated cells
MUNC	Multi-nucleated cells

$$\text{Cytostasis (\%)} = 100 - 100 \times \frac{(CBPI_T - 1)}{CBPI_C - 1}$$

T	Test substance
C	Solvent control

Cell morphology
At the end of the treatment period, the test cultures of all test groups were examined microscopically with regard to cell morphology, which is a further indication for cytotoxicity.

5. Micronucleus evaluation

The dose selection for scoring was based on slide/cell quality, number of analyzable cells, and nuclear fragmentation. Evaluation of the slides was performed by fluorescence microscopy. At least 1000 binucleated cells per culture (i.e. 2000 binucleated cells per test group) were evaluated for cytogenetic damage on coded slides. The frequency of micronucleated cells was reported as % micronucleated cells. The analysis of the micronuclei was carried out based on the following criteria:

- The diameter of the micronucleus is less than 1/3 of the main nucleus
- The micronucleus and main nucleus retain the same color
- The micronucleus is not linked to the main nucleus and is located within the cytoplasm of the cell
- Only cells clearly surrounded by a membrane were scored

6. Acceptability criteria

The in vitro micronucleus assay is considered acceptable if the following criteria are met:

- The quality of the slides must allow the evaluation of a sufficient number of analyzable cells.
- The rate of micronuclei in the solvent controls falls within the range of the test laboratory's recent negative control data.
- The rate of micronuclei in the positive controls both with and without S9 mix induced a distinct increase in the number of micronucleated cells.

7. Assessment criteria

A test substance is considered "negative" in the in-vitro micronucleus test if the following criteria are met:

- the number of micronucleated cells in the test groups is not distinctly increased above the concurrent vehicle control
- the number of micronucleated cells in the test groups is within the test laboratory's recent negative control data range

A test substance is considered "positive" in the in-vitro micronucleus test if the following criteria are met:

- a significant, dose-related and reproducible increase in the number of cells containing micronuclei was observed
- The number of micronucleated cells exceeded both the value of the concurrent vehicle control and the range of the laboratory's recent negative control data

8. Statistics:

The statistical evaluation of the data was carried out using an appropriate statistical analysis. The proportion of cells containing micronuclei was calculated for each test group. A comparison of the micronucleus rates of each test group with the concurrent vehicle control group was carried out for the hypothesis of equal proportions (i.e. one-sided Fisher's exact test, MUVIKE software, BASF SE).

If the results of this test were statistically significant compared with the respective vehicle control, labels (* $p \leq 0.05$, ** $p \leq 0.01$) have been be printed in the tables.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS AND TREATMENT CONDITIONS

All formulations were prepared freshly before treatment. The stability of the test substance at room temperature in the vehicle DMSO over a period of 4 hours was verified analytically.

Osmolarity and pH values were not influenced by test substance treatment. However, the pH of the stock solutions was adjusted to a physiological value using small amounts of NaOH. No precipitation of the test substance in culture medium was observed.

B. CYTOTOXICITY

In both main experiments in the absence and the presence of S9 mix no growth inhibition indicated by reduced cell counts of about or below 50% of control was observed up to the highest required test substance concentrations. No reduced proliferative activity was observed either after 4 hours exposure interval in the absence and presence of S9 mix or after 24 hours continuous test substance treatment in the test groups scored for cytogenetic damage.

Cell attachment / cell morphology was not adversely influenced (grade > 2) at any concentration tested for the occurrence of micronuclei.

C. MICRONUCLEUS ASSAY

In this study, no biologically relevant increase in the number of micronucleated cells was observed either without S9 mix or after the addition of a metabolizing system. In both experiments in the absence and presence of metabolic activation after 4 and 24 hours treatment with the test substance, the obtained percentages of micronucleated cells (0.1 - 0.7%) were close to the concurrent vehicle control values (0.1 - 1.1% micronucleated cells) and were clearly within the historical negative control data range (0.1 - 1.8% micronucleated cells).

In the 2nd Experiment in the presence of S9 mix a single statistically significant value (0.6% micronucleated cells) compared to the respective vehicle control was obtained at an intermediate concentration of 790 µg/mL. However, this statistical finding has been considered to be without biological relevance as

- the statistical significance is based on the low rate of micronucleated cells in the concurrent vehicle control group (0.1% micronucleated cells in this experiment)
- was without indication of being dose-related
- did not reproduce in the other independent experiments
- was in the range of the laboratory's recent negative control data

The positive control substances EMS (without S9 mix; 400 µg/mL) and CPP (with S9 mix; 0.5 µg/mL) induced statistically significant increased micronucleus frequencies in both independently performed experiments. In this study, in the absence and presence of metabolic activation the frequency of micronucleated cells (2.7 – 5.4% micronucleated cells) was clearly above the range of our historical negative control data range (0.1 - 1.8% micronucleated cells) and within our historical positive control data range (2.3 – 26.6% micronucleated cells).

Table 5.8.1-5: Results of in-vitro micronucleus test in V79 cells – without S9-mix

Without metabolic activation (S9 mix): 4-hour exposure period, harvest at 24 hours					
Experiment I			Cytotoxicity		Genotoxicity
Test item	Conc. [µg/mL]	Precipi–tation	Cell count [%]	CBPI [%]	Micronucleated cells (%)
Vehicle (DMSO)		n.d.	100.0	0.0	1.1
Test substance	49.4	-	95.3	n.d.	n.d.
	98.8	-	90.3	n.d.	n.d.
	197.5	-	93.4	n.d.	n.d.
	395.0	-	81.8	-0.8	0.7
	790.0	-	86.5	-0.3	0.5
	1580.0	-	80.0	0.2	0.2
Positive control (EMS)	400.0	n.d.	91.1	-0.4	**3.1****
Without metabolic activation (S9 mix): 24-hour exposure period, harvest at 24 hours					
Experiment II			Cytotoxicity		Genotoxicity
Test item	Conc. [µg/mL]	Precipi–tation	Cell count [%]	CBPI [%]	Micronucleated cells (%)
Vehicle (DMSO)		n.d.	100.0	0.0	0.3
Test substance	98.8	-	106.0	n.d.	n.d.
	197.5	-	105.8	n.d.	n.d.
	395.0	-	96.6	-8.1	0.4
	790.0	-	88.8	-4.7	0.4
	1580.0	-	99.7	4.2	0.1
Positive control (EMS)	400.0	n.d.	184.6	1.4	**2.7****

* $p \leq 0.05$, ** $p \leq 0.01$ Fisher's exact test (one sided) with Bonferroni-Holm adjustment
n.d. = not determined

Table 5.8.1-6: Results of in-vitro micronucleus test in V79 cells – with S9-mix

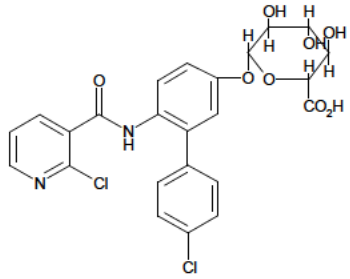
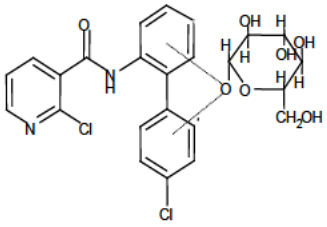
With metabolic activation (S9 mix): 4-hour exposure period, harvest at 24 hours					
Experiment I			Cytotoxicity		Genotoxicity
Test item	Conc. [µg/mL]	Precipi–tation	Cell count [%]	CBPI [%]	Micronucleated cells (%)
Vehicle (DMSO)		n.d.	100.0	0.0	0.7
Test substance	49.4	-	119.5	n.d	n.d.
	98.8	-	107.9	n.d.	n.d.
	197.5	-	111.2	n.d.	n.d.
	395.0	-	127.9	-0.8	0.7
	790.0	-	119.6	-1.4	0.4
	1580.0	-	113.5	-4.6	0.4
Positive control (CPP)	0.5	n.d.	110.0	22.7	**5.4****
With metabolic activation (S9 mix): 4-hour exposure period, harvest at 44 hours					
Experiment II			Cytotoxicity		Genotoxicity
Test item	Conc. [µg/mL]	Precipi–tation	Cell count [%]	CBPI [%]	Micronucleated cells (%)
Vehicle (DMSO)		n.d.	100.0	0.0	0.1
Test substance	98.8	-	81.3	n.d.	n.d.
	197.5	-	85.1	n.d.	n.d.
	395.0	-	87.0	-2.1	0.4
	790.0	-	76.7	-2.1	**0.6***
	1580.0	-	77.8	-0.3	0.2
Positive control. (CPP)	0.5	n.d.	91.9	-8.3	**3.5****
Historical control (4 h exposure/24 h harvest)					
Mean ± SD			-	-	0.8 ± 0.3
Range			-	-	0.1 – 1.8

* $p \leq 0.05$, ** $p \leq 0.01$ Fisher's exact test (one sided) with Bonferroni-Holm adjustment
n.d. = not determined

III. CONCLUSION

In conclusion, it can be stated that under the experimental conditions chosen, the test substance did not induce micronuclei as determined by the in vitro micronucleus test in V79 cells. Therefore, Reg. No. 107371 (Metabolite of BAS 510 F, Boscalid) is considered to be non-mutagenic in this in vitro micronucleus test in the presence and absence of metabolic activation.

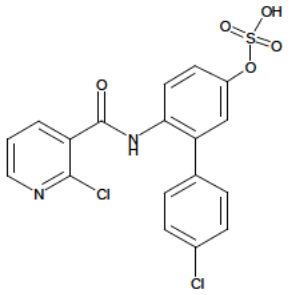
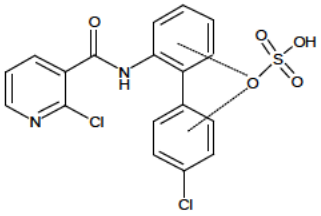
III Metabolite M510F61

M510F02	M510F61	Occurrence	TRR (mg/kg)	TRR (%)
		Radish root	0.006	10.9
		Wheat forage	0.102	-
		Wheat straw	0.423	-
	Occurrence in animal matrices: No Occurrence in plant matrices: Yes, radish root and wheat matrices			

M510F61 was found in the confined rotational crop study (BASF DocID 2000/1014862) in radish tops and roots, as well as in wheat forage and straw. Radish tops are no food item but radish roots do. Wheat forage and wheat straw can be part of the livestock's feed with potential transfer of residues to edible parts of animal origin. Therefore, metabolite M510F61 is considered a relevant metabolite. M510F61 is not part of the residue definition in plants and a separate consumer exposure assessment has thus been performed in section M-CA 6.9.
The metabolite M510F61 was not found in the rat metabolism study. However, this glucuronide of the parent compound Boscalid has close structural similarity with the major rat metabolite M510F02. For comparison, both structures are presented above. Both glucuronides are considered to be the consequence of phase II metabolism of the respective hydroxylate of the parent compound which is M510F01 in case of M510F02. In the rat metabolism the analogous hydroxylate of M510F61 was also detected (M510F10, see Overview 5.8.1-2). Metabolite M510F10 was found in urine of rats in trace amounts only and thus suggests that M510F61 could be formed as well, but the preferential pathway of metabolism appears to be via M510F01 to M510F02 instead (see document M-CA 5.6). The toxicology data base of Boscalid technical material includes extensive co-testing of M510F02. In the absence of neurotoxic and genotoxic effects of Boscalid (including its metabolites) and the close structural similarity of metabolite M510F61 to the main metabolite M510F02 the TTC approach on the basis of Cramer Class III is considered adequate for human risk assessment. Adequate information on the absence of neurotoxicity has been provided in M-CA 5.7 of this supplemental dossier. There is also no alert for mutagenic properties of Boscalid (see M-CA 5.4 of this supplemental dossier) which is considered to also apply for the metabolites originating from the predominant degradation pathway via M510F01 to M510F02 given the negative results in genotoxicity testing in the presence of metabolic activation systems applied in the assays.

In the overall conclusion the toxicological relevance of metabolite M510F61 has been adequately addressed. No toxicological concern has been identified. The TTC approach based on Cramer Class III has been considered adequate given the results of studies with co-testing of the similar metabolite M510F02.

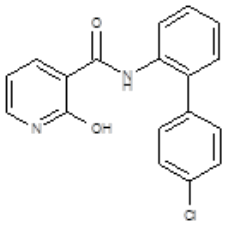
IV Metabolite M510F54

M510F03	M510F54	Occurrence	TRR (%)
		Egg	1.9%
	Occurrence in animal matrices: Yes Occurrence in plant matrices: No		

In food items the metabolite M510F54 has been found only in eggs of the hen metabolism study. Besides the occurrence in eggs this metabolite was found in the faeces of hen (2.1% of TRR) and in urine of the goat (2.2% of TRR). The metabolite is not included in the residue definition. As M510F54 is not part of the residue definition, it is treated as a Cramer Class III.
The metabolite M510F54 was not found in the rat metabolism study. However, this sulphonate derivative of the parent compound Boscalid has close structural similarity with the rat metabolite M510F03 which was detected in the urine up to 1.6% of TRR and in the bile up to 1.5% of TRR. The simple difference is in the position of the sulphonate moiety at the biphenyl structure of the molecule which could not clearly be elucidated in case of M510F54. The structure of M510F03 instead is the sulphonate moiety at the ortho-substitution position of the aminobiphenyl ring and is presented above for the purpose of comparison. Both sulphonate derivatives are considered to be the consequence of phase II metabolism of the respective hydroxylate of the parent compound which is M510F01 in case of M510F03. In the rat metabolism the analogous hydroxylate of M510F54 was also detected (M510F10, see Overview 5.8.1-2). Metabolite M510F10 was found in urine of rats in trace amounts only and thus suggests that M510F54 could be formed as well, but the preferential pathway of metabolism appears to be via glucuronidation of M51F01 to M510F02 instead. For 50 mg/kg bw applied in the rat metabolism study the M510F03 proportion of 1.6% of the TRR applied corresponds to about 0.8 mg/kg bw. The predicted consumer exposure is 0.00007 mg/kg bw/day (i.e. 4.5% of ADI for UK infants, see DocID 2015/1245132 in M-CA 6.9) for M510F54 and thus distinctly lower as M510F03 present in the rat.
In the absence of neurotoxic and genotoxic effects of Boscalid technical material (including its metabolites) and the structural similarity of metabolite M510F54 the TTC approach on the basis of Cramer Class III is considered adequate for human risk assessment. The comparison of exposure values from M510F54 and M510F03 suggests that the similar metabolite has been co-tested in the toxicology data base of Boscalid technical material at considerably higher amounts and is thus considered to provide additional confirmation that the TTC approach chosen includes an adequate level of conservatism.

In the overall conclusion the toxicological relevance of metabolite M510F54 has been adequately addressed. No toxicological concern has been identified. The TTC approach based on Cramer Class III has been considered adequate given structural similarity of metabolite M510F54 to the rat metabolite M510F03.

V Metabolite M510F49

M510F49	Occurrence	Concentration (µg/L)
	Groundwater	0.3
Occurrence in animal matrices: No (seen as artefact originating from bound residues) Occurrence in plant matrices: No Potential occurrence in groundwater: Yes, exceeding the EU threshold level of 0.1 µg/L		

Metabolite M510F49 (Reg. No. 391572) was identified to potentially be present in the groundwater (see Doc N 4, Section 3 'Relevance of metabolites in groundwater'). For metabolite M510F49 the maximum PEC in groundwater was calculated to be 0.3 µg/L, which means that the EU established threshold of 0.1 µg/L is exceeded and further testing is required according to the data requirements of SANCO/221/2000 –rev. 10 of 25 February 2003 for substances of groundwater occurrence within the limits of 0.1 µg/L but not exceeding 0.75 µg/L. In conclusion metabolite M510F49 is considered relevant for consumer risk assessment. The metabolite was not detected in the rat metabolism study.

For potential groundwater metabolites occurring at predicted levels of 0.1 µg/L but not exceeding 0.75 µg/L a set of genotoxicity studies comprising of bacterial cell and mammalian cell systems and a chromosome aberration study is required. Study reports of these tests are presented below (DocIDs 2000/1024059, 2014/1190533 & 2001/1031863), complemented by study reports on acute oral toxicity in rats (DocID 2001/1021303) and a report on the 90-day feeding study in rats (DocID 2015/1204964). The latter study was performed for the safeguard of additional crops and uses of Boscalid following the Annex I renewal for which the prediction of groundwater concentrations is anticipated to result in higher concentrations than for the representative uses of this supplemental dossier. In this case a reference value specific to M510F49 may be required and provisions for this case have already been made within this AIR3 approval process.

In conclusion the metabolite has been shown to be devoid of genotoxicity and is of low acute oral toxicity. In the 90-day feeding study the administration of metabolite M510F49 (i.e. Reg.No. 391572) via the diet to male and female Wistar rats for 3 months caused no test substance-related adverse signs of systemic toxicity at a concentration of 15000 ppm in male and female Wistar rats. Therefore, under the conditions of the study the no observed adverse effect level (NOAEL) was 15000 ppm in male (968 mg/kg bw/d) and in female (1082 mg/kg bw/d) Wistar rats. For the derivation of the ADI based on the NOAEL originating from a subchronic feeding study the additional assessment factor to be taken into consideration is 2 on top of the standard assessment factor of 100 usually applied to derive the ADI from animal studies. This approach follows the scientific opinion on the "Guidance on selected default values to be used by the EFSA Scientific Committee, Scientific Panels and Units in the absence of actual measured data" (EFSA Journal 2012;10(3):2579).

Thus, the proposed ADI for the risk evaluation of consumers in regard to metabolite M510F49 (in groundwater) would calculate as:

$$ADI = \frac{NOAEL(90-day-rat)}{100x2}$$

$$ADI = \frac{968mg/kgbw/day}{100x2}$$

The proposed ADI for metabolite M510F49 is 4.8 mg/kg bw/day consequently.

In the overall conclusion the toxicological relevance of metabolite M510F49 has been adequately addressed. No toxicological concern has been identified. No genotoxic properties have been noted for this metabolite and thus the occurrence in the groundwater within the limits of limits of 0.1 µg/L but not exceeding 0.75 µg/L is acceptable. No further evaluation of the toxicological data package specific to metabolite M510F49 is required at the present time. However, for the potential extension of uses post AIR3 of Boscalid, provisions have been made for the case of exceeding the groundwater concentration above the limit of 0.75 µg/L. For this case the present 90-day feeding study allows to suggest the ADI specific to M510F49 to be 4.8 mg/kg bw/day.

The above mentioned study reports are in detail shown below:

Report: CA 5.8.1/3
[redacted] 2001 a
Reg.No. 391572 - Acute oral toxicity study in Wistar rats
2001/1021303
Guidelines: OECD 423, EEC 96/54 B 1, EPA 870.1100
GLP: yes
(certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Note: This study report has **not** been part of the documentation for the first Annex I inclusion process and a full evaluation is therefore presented here to assist in the discussion on relevant metabolites in groundwater

EXECUTIVE SUMMARY

A single dose of 2000 mg/kg bw of Reg.No. 391572 (batch: 01742-59; purity: 99.7%) suspended in olive oil was given to three fasted male and female animals by gavage. Animals were observed for 14 days. No mortality occurred in the administration groups. Accordingly, the oral LD_{50} was found to be greater than 2000 mg/kg bw:

Rat, oral: LD_{50} > 2000 mg/kg bw

No clinical signs and findings were observed. The mean body weights of the administration groups increased throughout the study period. No macroscopic pathologic abnormalities were noted in the animals examined at the end of the observation period.
Under the conditions of this study the median lethal dose of Reg.No. 391572 after oral administration was found to be greater than 2000 mg/kg bw in rats.

DocID (2001/1021303)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: Reg.No. 391572

Description:	Solid; crystalline/white
Lot/Batch #:	01742-59
Purity/content:	99.7%
Stability of test compound:	The stability of the test substance in the vehicle was determined indirectly by the concentration control analysis.

2. Vehicle: Olive oil

3. Test animals:

Species:	Rat
Strain:	Wistar / Crl:WI (GLX/BRL/HAN)IGS BR
Sex:	male/female
Age:	approximately 8 -12 weeks (males) and 14-18 weeks (females)
Weight at dosing (mean):	205 - 218 g (males), 187 - 203 g (females)
Source:	Charles River Laboratories, Deutschland, Sandhofer Weg 7, 97633 Sulzfeld, Germany
Acclimation period:	At least 1 week
Diet:	Kliba-Labordiät, Provimi Kliba SA, Kaiseraugst, Basel, Switzerland, ad libitum
Water:	Tap water, ad libitum
Housing:	Single housing in stainless steel wire mesh cages, type DK-III (Becker & Co., Castrop-Rauxel, FRG)
Environmental conditions:	
Temperature:	20 - 24 °C
Humidity:	30 - 70 %
Air changes:	Central air-conditioning
Photo period:	Alternating 12-hour light and dark cycles, artificial light from 6 am to 6 pm

B. STUDY DESIGN AND METHODS

1. Dates of work: 05-Oct-2000 - 24-Oct-2000

2. Animal assignment and treatment:

A single dose of 2000 mg/kg bw of the test material suspension in olive oil was given to three fasted male and female animals by gavage. Clinical signs and symptoms were recorded several times on the day of administration and afterwards at least once each workday for the individual animals up to 14 days post-administration. A check for any dead or moribund animal was made twice each workday and once on Saturdays, Sundays and public holidays. Individual body weights were determined shortly before administration (Day 0), weekly thereafter and at the end of the study. The animals were sacrificed by CO_2-inhalation and subjected to necropsy including gross pathological examination on the last day of the observation period or as soon as possible after death in case of animals that died before. The animals were fasted at least 16 h before killing with CO_2.

II. RESULTS AND DISCUSSION

A. MORTALITY

No mortality occurred in the administration groups.

B. CLINICAL OBSERVATIONS

No clinical signs and findings were observed.

C. BODY WEIGHT

The mean body weights of the administration groups increased throughout the study period. The mean weights were 212 g, 272 g, and 297 g at d0, d7, and d13 for the male animals. For the females body weights of 200 g, g, and 226 g at d0, d7, and d13, respectively.

D. NECROPSY

No abnormalities were observed at gross necropsy.

III. CONCLUSION

Under the conditions of this study, the oral LD_{50} in rats for Reg.No. 391572 was determined to be greater than 2000 mg/kg bw.

Report: CA 5.8.1/4
Engelhardt G.,Hoffmann H.D., 2000 a
Salmonella typhimurium / Escherichia coli - Reverse mutation assay (standard plate test and preincubation test) with Reg.No. 391572
2000/1024059
Guidelines: OECD 471 (1997), EEC 2000/32 B.13/B.14
GLP: yes
(certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Note: This study report has **not** been part of the documentation for the first Annex I inclusion process and a full evaluation is therefore presented here to assist in the discussion on relevant metabolites in groundwater

EXECUTIVE SUMMARY

S. typhimurium strains TA98, TA100, TA1535 and TA1537 and E. coli strain WP2 uvrA were exposed to Reg.No. 391572 (batch: 01742-59, purity: 99.7%) using DMSO as a solvent in the presence and absence of metabolic activation in two independent experiments. Triplicate plates were used per dose and per test condition. Vehicle and positive controls were included in each experiment.

In the Ames standard plate test (SPT) Reg.No. 391572 was tested in concentrations of 20 to 5000 µg/plate with and without S9 mix (Aroclor-induced rat liver S9 mix). In the preincubation assay the test item was tested in concentrations of 4 to 5000 µg/plate also with and without metabolic activation.

Precipitation of the test substance was found from about 500 µg/plate onward. A slight decrease in the number of revertants was occasionally observed depending on the strain and test conditions from about 2000 – 2500 µg/plate onward. An increase in the number of his^+ or trp^+ revertants was not observed both in the standard plate test and in the pre-incubation test either without S-9 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.

According to the results of the study, Reg.No. 391572 is not mutagenic in the Ames standard plate or preincubation test under the experimental conditions of the study.

(BASF DocID 2000/1024059)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material Reg.No. 391572

Description: Solid (crystals), white

Lot/Batch #: 01742-59 (= ZHP LJ-No.: 30155-47)

Purity: 99.7%

Stability of test compound: The stability of the test substance in DMSO and water over a period of 4 h or 96 h has been verified analytically.

Solvent used: DMSO

2. Control Materials:

Negative control: In parallel to each experiment, negative control experiments were carried out in order to check for possible contaminants (sterility control) and to determine the spontaneous mutation rate (vehicle control).

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	Concentration
TA 100	N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)	DMSO	5 µg/plate
TA 1535	N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)	DMSO	5 µg/plate
TA 1537	9-Aminoacridine (AAC)	DMSO	100 µg/plate
TA 98	4-nitro-o-phenylendiamine (NOPD)	DMSO	10 µg/plate
E. coli WP2 uvrA	4-nitroquinoline-N-oxide (4-NQO)	DMSO	5 µg/plate

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

Strain	Mutagen	Solvent	Concentration
TA 100	2-aminoanthracene	DMSO	2.5 µg/plate
TA 1535	2-aminoanthracene	DMSO	2.5 µg/plate
TA 1537	2-aminoanthracene	DMSO	2.5 µg/plate
TA 98	2-aminoanthracene	DMSO	2.5 µg/plate
E. coli WP2 uvrA	2-aminoanthracene	DMSO	60 µg/plate

To demonstrate the efficacy of the rat liver S9 mix in this assay, the S9 batch was characterized with benzo(a)pyrene as recommended in the current guideline.

3. Activation:

S9 was produced from the livers of induced male Sprague-Dawley rats. The rats received a single intraperitoneal injection of 500 mg/kg bw Aroclor 1254. Five days after administration the animals were sacrificed and the livers are prepared. Aliquots of the S9-mix are deep frozen and stored at -70°C to -80°C. The S9-mix was prepared freshly prior to each experiment. For this purpose, a sufficient amount of S9-fraction is thawed at room temperature and 1 volume of S9-fraction is mixed with 9 volumes of S9-supplement (cofactors). This preparation, the so-called S9-mix, was kept on ice until used.

The rat liver S9-mix was prepared immediately before use and had the following composition:

Component	Concentration
Phosphate buffer (pH 7.4)	15 mM
Glucose 6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM
S9	10%

4. Test organisms:

S. typhimurium strains: TA98, TA100, TA1535, TA1537
Salmonella typhimurium:
The Salmonella strains are checked for the following characteristics at regular intervals: deep rough character (rfa); UV sensitivity (uvrB); ampicillin resistance (R factor plasmid).

E. coli:

The E. coli strain is checked for UV sensitivity.

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

5. Test concentrations:

Plate incorporation assay: In the first experiment triplicate plates were prepared for each concentration (neg. control; 20, 100, 500, 2500 and 5000 µg/plate and positive controls at the concentrations indicated above) and conditions (i.e. with and without rat liver S9 mix) for all tester strains indicated above.

Preincubation assay: In the second experiment the test article / vehicle / positive control substance, S9 mix were incubated at 37°C for the duration of about 20 minutes. Triplicate plates were prepared for each concentration (neg. control; 4, 20, 100, 500 and 2000 µg/plate and positive controls at the concentrations indicated above) for all tester strains indicated above.

B. TEST PERFORMANCE:

1. Dates of experimental work: 29-September-2000 (start of assays), finalization date: 28-Nov-2000

2. Plate incorporation assay:

To test tubes containing 2-mL portions of warm soft agar (containing 0.5 mM histidine + 0.5 mM biotin for Salmonella strains or 0.5 mM tryptophan for E. coli), 0.1 mL test solution or vehicle, 0.1 mL fresh bacterial culture, 0.5 mL S9-mix (in tests with metabolic activation) or 0.5 mL phosphate buffer (in tests without metabolic activation) were added in the given order. After mixing, the samples are poured onto Vogel-Bonner agar plates (minimal glucose agar plates for Salmonella strains) or minimal agar plates (E. coli). After incubation at 37°C for 48 - 72 hours in the dark, the bacterial colonies (his^+ revertants) are counted.

3. Preincubation assay:

100 µL of test solution or vehicle, 0.1 mL bacterial suspension and 0.5 mL S9 mix were incubated at 37°C for about 20 minutes. Subsequently, 2 mL of soft agar was added and, after mixing, the samples are poured onto the agar plates.

After incubation in the dark for 48 – 72 hours at 37°C revertant colonies were counted.

4. Titer determination:

The titer was additionally determined in experimental parts with S9 mix using the vehicle control and the two highest doses. The procedure followed in both experiments (plate incorporation and pre-incubation) was the same as described above for the individual experiments with the exception that the soft agar used contained maximal amino acid solution (5 mM histidine + 0.5 mM biotin or 0.5 mM tryptophan). After the incubation in the dark for 48 – 72 hours at 37°C the number of bacterial colonies was determined.

5. Statistics:

No special statistical tests were performed.

6. Evaluation criteria:

The test chemical is considered positive in this assay if the following criteria are met:

- A dose-related and reproducible increase in the number of revertant colonies, i.e. about doubling of the spontaneous mutation rate in at least one tester strain either without S9 mix or after adding a metabolizing system.

The test chemical is considered nonmutagenic in this assay if the following criteria are met:

- The number of revertants for all tester strains were within the historical negative control range under all experimental conditions in two experiments carried out independently of each other.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

The stability of the test substance at room temperature in the vehicle DMSO and in water was found to be stable for 4 h and 96 h, respectively.

B. TOXICITY

A preliminary cytotoxicity assay was not performed in this laboratory. Cytotoxicity was determined in the course of the mutation assay.

A slight decrease in the number of revertants was occasionally observed depending on the strain and test conditions from about 2000 – 2500 µg/plate onward.

C. MUTATION ASSAYS

Neither in the plate incorporation nor in the preincubation experiment with and without metabolic activation caused a biologically relevant increase in number of revertants observed in any strain tested [see Table 5.8.1-7]. The positive controls yielded revertant numbers in the range expected for the respective strains and thus demonstrated the sensitivity of the test system.

Test item precipitation was observed from about 500 µg/plate onward.

Table 5.8.1-7: Bacterial gene mutation assay with Reg. No. 391572 - Mean number of revertants

Experiment 1: Standard plate test										
Strain	TA 98		TA 100		TA 1535		TA 1537		E. coli	
Metabol. activation	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9
Neg. control (DMSO)	33±3	47±3	111±9	109±2	18±1	17±1	10±2	11±2	31±1	41±2
Test item										
20 µg/plate	32±4	46±2	106±5	105±4	15±2	16±3	13±4	10±2	35±4	36±2
100 µg/plate	30±7	37±2	120±14	99±7	15±1	16±2	10±2	8±3	33±3	31±3
500 µg/plate	28±7	41±3	95±4	99±2	15±5	17±3	6±1	7±2	31±5	39±10
2500 µg/plate	21±2	27±2	93±8	96±6	10±3	13±3	6±2	5±0	26±4	37±9
5000 µg/plate	18±2	23±4	96±3	90±2	11±6	11±1	4±2	5±1	28±1	32±3
Pos. control§	513±13	860±107	571±31	675±55	164±67	838±28	88±9	522±88	546±19	234±17
Experiment 2: Preincubation assay										
Strain	TA 98		TA 100		TA 1535		TA 1537		E. coli	
Metabol. activation	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9
Neg. control (DMSO)	35±1	32±1	105±4	104±3	18±1	18±1	9±2	10±2	27±3	36±4
Test item										
4 µg/plate	34±6	33±5	108±4	106±5	18±2	17±3	10±2	9±3	23±2	36±1
20 µg/plate	32±7	26±5	105±1	105±6	14±3	16±1	9±1	9±1	21±3	36±7
100 µg/plate	24±5	23±1	107±6	102±2	13±3	14±2	11±1	8±2	20±5	36±3
500 µg/plate	25±3	26±1	101±2	96±3	14±2	13±2	6±2	7±2	21±3	35±3
2500 µg/plate	23±1	17±2	88±6	91±3	11±3	11±2	6±0	5±2	21±4	33±3
Pos. control§	541±34	793±75	530±20	836±26	150±20	701±79	97±5	365±48	229±14	579±36

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

III. CONCLUSION

According to the results of the present study, the test substance Reg.No. 391572 is not mutagenic in the Ames standard plate test and in the preincubation test under the experimental conditions chosen here.

Report: CA 5.8.1/5
[redacted] 2001 a
Cytogenetic study in vivo with Reg.No. 391572 in the mouse micronucleus test after two intraperitoneal administrations
2001/1031863
Guidelines: OECD 474 (1997), EEC 2000/32 B.12
GLP: yes
(certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Note: This study report has **not** been part of the documentation for the first Annex I inclusion process and a full evaluation is therefore presented here to assist in the discussion on relevant metabolites in groundwater

EXECUTIVE SUMMARY

Reg.No. 391572 (Batch: 01196-217; Purity: 98.9%) was tested for chromosomal damage (clastogenicity/aneugenicity) in NMRI mice using the micronucleus test method. For this purpose, the test substance, suspended in 0.5% CMC, was administered twice (within 24 h) intraperitoneally to groups of 5 male mice at dose levels of 500, 1000, and 2000 mg/kg body weight in a volume of 20 mL/kg body weight. The vehicle served as negative and cyclophosphamide/vincristine as positive controls. The animals were sacrificed 24 hours after the second administration, the bone marrow of the two femora was prepared from each animal. After staining of the preparations, 2000 polychromatic erythrocytes were evaluated per animal and investigated for micronuclei. The normocytes occurring per 2000 polychromatic erythrocytes were also recorded.

The administration of Reg.No. 391572 did not lead to any biologically relevant increase in the number of polychromatic erythrocytes containing micronuclei. The rate of micronuclei was mostly close to the concurrent negative control and was within the range of the historical control data. A slight inhibition of erythropoiesis was occasionally detected in individual animals of the 1000 and 2000 mg/kg bw dose group. Clinical signs observed at 1000 and 2000 mg/kg bw included poor general state and squatting posture, which were fully reversible within 48 h after dose administration. No signs of systemic toxicity were observed in any of the animals treated with the positive control substances or the vehicle. The positive control chemicals cyclophosphamide and vincristine led to the expected increase in the rate of polychromatic erythrocytes containing micronuclei, thus demonstrating the sensitivity of the test system. Thus, under the experimental conditions of this study, Reg.No. 391572 did not induce cytogenetic damage (clastogenic or aneugenic) in bone marrow cells of NMRI mice in vivo.

(BASF DocID 2001/1031863)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material Reg.No. 391572

Description:	Solid (crystals), white
Lot/Batch #:	01196-217
Purity:	98.9%
Stability of test compound:	The of the test substance and test substance preparations was verified analytically.
Vehicle used:	0.5% CMC formulation

2. Control Materials:

Negative control:	A negative control was not employed in this study
Solvent control:	0.5% CMC formulation
Positive control:	Cyclophosphamide (CPP) 20 mg/kg bw (dissolved in deionized water); Vincristine sulfate (VCR) 0.15 mg/kg bw (dissolved in deionized water)

3. Test animals:

Species:	Mouse
Strain:	NMRI
Sex:	Male for the main study; male and female for the range finding study
Age:	5-8 weeks
Mean body weight at dosing:	27.0 g
Source:	Charles River Laboratories, Research Models and Services Germany GmbH, Sulzfeld, Germany
Number of animals per dose:	5 males per control and treatment group
Acclimation period:	At least 5 days
Diet:	Standardized pelleted feed (Kliba Haltungsdiät, Provimi Kliba SA, Kaiseraugst, Switzerland), ad libitum
Water:	Tap water, ad libitum
Housing:	Single housing in Makrolon cages, type MI

4. Environmental conditions:

Temperature:	20-24 °C
Humidity:	30-70%
Air changes:	not indicated (central air-conditioning)
Photo period:	12-hour light-dark cycle (06:00 - 18:00, 18:00 - 06:00)

5. Test compound doses:

Range finding test:	2000 mg/kg bw
Micronucleus assay:	500, 1000 and 2000 mg/kg bw
	The test substance was administered twice by intraperitoneal injection using an application volume of 10 mL/kg bw.

B. TEST PERFORMANCE:

1. Dates of experimental work: 22-Jan-2001 to 25-Jan-2001

2. Preliminary range finding test

In a pretest for the determination of the intraperitoneal toxicity, male and female animals were treated twice by intraperitoneal injection with a test substance dose of 2000 mg/kg bw (highest dose recommended in the respective OECD guideline). No mortality occurred. Clinical signs observed included poor general state and squatting posture, showing no distinct symptomatic differences between male and female animals. Thus, only male animals were used for the cytogenetic investigations.

3. Micronucleus test

Treatment and sampling:

Groups of 5 male mice were treated twice within a 24 h time period with either the vehicle or 500, 1000, or 2000 mg test substance per kg bodyweight by intraperitoneal injection. The application volume was 10 mL/kg bw and the volume to be applied was calculated based on actual weight on the day of administration. The positive control substance CPP and VCR were administered once and the mice sacrificed after 24 h. The animals were observed for evident clinical signs of toxicity throughout the study.

24 hours after the second administration the mice were sacrificed and the two femora were prepared free of all soft tissue. After cutting off the epiphyses the bone marrow was flushed out in a centrifugation tube with fetal calf serum (FCS) and subsequently centrifuged at 300 x g for 5 minutes. The supernatant was discharged and the pellet resuspended in FCS.

Slide preparation

One drop of the suspension was applied on a clean microscopic slide and smears were prepared. After air drying the smears were stained with May-Grünwald solution or Wrights solution, rinsed, and finally stained with Giemsa solution. Cover slips were mounted with Corbit-Balsam. The slides were coded prior to microscopic evaluation.

Slide evaluation

In general, 2000 polychromatic erythrocytes (PCEs) from each male animal of every test group were evaluated and investigated for micronuclei (MN). The normochromatic erythrocytes (NCEs) that occurred were also scored.

The increase in the number of micronuclei in polychromatic erythrocytes of treated animals as compared to the vehicle control group provides an index of a chromosome-breaking (clastogenic) effect or of a spindle activity (aneugenic) of the substance tested.

The ratio of polychromatic to normochromatic erythrocytes was calculated. An alteration of this ratio indicates a toxic effect on erythropoiesis and thus, that the test substance actually reached the target organ.

4. Statistics

The statistical evaluation of the data was carried out using the program system MUKERN (BASF SE). The number of micronuclei in polychromatic erythrocytes was analyzed. A comparison of the dose group with the vehicle control was carried out using the Wilcoxon test for the hypothesis of equal medians. Here, the relative frequencies of cells with micronuclei of each animal were used. If the results of this test were significant, labels (* for $p \leq 0.05$, ** for $p \leq 0.01$) were printed with the group means in the tables. This test was performed one-sided.

5. Evaluation criteria

The test substance is considered “positive” in this assay if the following criteria are met:

- A statistically significant and dose-related increase in the number of PCEs containing micronuclei.
- The number of PCEs containing micronuclei has to exceed both the concurrent vehicle control value and the range of the historical vehicle control data.

A test substance is generally considered “negative” in this test system if:

- The number of cells containing micronuclei in the dose groups is not statistically significant increased above the concurrent vehicle control value and is within the range of the historical vehicle control data.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

Stability, homogeneity and concentration control analyses

The stability of the test substance in the vehicle was verified analytically. The homogeneity of the test substance in the vehicle was guaranteed by constant stirring during the removal and administration of the test substance preparation and indirectly by analytical determination of 3 individual samples of each concentration. The mean concentrations were determined as 46.1, 98.5, and 183.7 mg/mL at nominal concentrations of 50, 100 and 200 mg/mL, respectively. This corresponds to a recovery rate in the range of 91.9-98.5% and is thus, within the expected range (90-110%).

B. PRELIMINARY RANGE FINDING TEST

In the pretest the recommended highest dose of 2000 mg/kg body weight was survived by all animals. Clinical signs observed included poor general state and squatting posture, showing no distinct symptomatic differences between male and female animals.

C. MICRONUCLEUS ASSAY

Clinical examinations

The two intraperitoneal administrations of the vehicle in a volume of 10 mL/kg body weight was tolerated by all animals without any signs or symptoms. The administration of the test substance led to evident signs of toxicity [see Table 5.8.1-8].

Table 5.8.1-8: Mouse micronucleus test: clinical findings

Clinical observations	Number of animals with signs (number examined: 5)											
Dose:	500 mg/kg bw				1000 mg/kg bw				2000 mg/kg bw			
Time point of observation (after first administration):	1h	2h	4h	1d	1h	2h	4h	1d	1h	2h	4h	1d
Poor general state	-	-	-	-	-	-	-	-	5	5	-	-
Squatting posture	-	-	-	-	5	5	5	-	5	5	5	-
Time point of observation (after first administration):	25h	26h	28h	2d	25h	26h	28h	2d	25h	26h	28h	2d
Poor general state	-	-	-	-	-	-	-	-	5	5	-	-
Squatting posture	-	-	-	-	5	5	5	-	5	5	5	-

Neither the single administration of the positive control substance cyclophosphamide in a dose of 20 mg/kg body weight nor that of vincristine in a dose of 0.15 mg/kg body weight caused any evident signs of toxicity.

Micronucleus test results

The two intraperitoneal administrations of a 0.5% CMC formulation in a volume of 10 mL/kg bw led to 1.9‰ polychromatic erythrocytes containing micronuclei after the 24-hour sacrifice interval.

After two administrations of the highest dose of 2000 mg/kg body weight, 2.2‰ polychromatic erythrocytes containing micronuclei were found after 24 hours. In the two lower dose groups, rates of micronuclei of about 1.8‰ (1000 mg/kg group) and 1.1‰ (500 mg/kg group) were detected.

With 15.9‰, the positive control substance cyclophosphamide for clastogenicity, led to the expected increase in the number of polychromatic erythrocytes containing exclusively small micronuclei.

With 47.2‰, the positive control vincristine for induction of spindle poison effects also led to a clearly enhanced number of polychromatic erythrocytes containing micronuclei with the expected amount of large micronuclei, i.e. 9.7‰.

The number of normochromatic erythrocytes containing micronuclei did not differ to any appreciable extent in the negative control or in the various dose groups.

Thus, the test substance Reg. No. 391572 did not lead to any increase in the rate of micronuclei. The number of normochromatic or polychromatic erythrocytes containing small micronuclei (d < D/4) did not deviate from the vehicle control value and was within the historical control range. Nor were large micronuclei (d > D/4) observed either in the negative control group or in the three dose groups of Reg. No. 391572.

A slight inhibition of erythropoiesis, induced by the treatment of Mice with Reg. No. 391572, was occasionally detected in individual animals at doses of 1000 mg/kg and 2000 mg/kg body weight.

Table 5.8.1-9: Induction of micronuclei in bone marrow cells

		PCE				NCE		PCE / NCE ratio
			With MN				With MN	
Sampling: 24 h post-dosing		Scored	Total [‰]	Small [‰]	Large [‰]	No.	[‰]	
CMC (0.5%)	0 mg/kg bw	10000	1.9	1.9	0.0	3219	0.9	3.11
Test sub.:	500 mg/kg bw	10000	1.1	1.1	0.0	3165	2.2	3.16
Test sub.:	1000 mg/kg bw	10000	1.8	1.8	0.0	4723	2.1	2.12
Test sub.:	2000 mg/kg bw	10000	2.2	2.2	0.0	4989	1.8	2.00
CPP:	20 mg/kg bw	10000	15.9**	15.9**	0.0	2568	0.0	3.89
Vincristine:	0.15 mg/kg bw	10000	47.2**	37.5**	9.7**	4883	2.5	2.05

Statistical analysis: ** = $p \leq 0.01$ (Wilcoxon-test, 1-sided); MN = Micronucleated cells;
PCE = Polychromatic erythrocytes; NCE = normochromatic erythrocytes;
CPP = Cyclophosphamide

III. CONCLUSION

Based on the result of this study Reg.No. 391572 did not induce micronuclei in mouse polychromatic erythrocytes under the conditions of the study, i.e. is devoid of clastogenic and aneugenic activity in vivo.

Report:	CA 5.8.1/6 Schulz M.,Landsiedel R., 2014 a Reg.No. 391572 - In vitro gene mutation test in CHO-cells (HPRT locus assay) 2014/1190533
Guidelines:	OECD 476, (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.17, EPA 870.5300
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Note: This study report has **not** been part of the documentation for the first Annex I inclusion process and a full evaluation is therefore presented here to assist in the discussion on relevant metabolites in groundwater

EXECUTIVE SUMMARY

Reg. No. 391572 (Batch: L71-12, Purity: 99.7%) was tested in vitro for its ability to induce forward mutations in mammalian cells by assessing the mutation of the HPRT locus in Chinese Hamster CHO cells. Two independent experiments were conducted in the presence and absence of metabolic activation. In the preliminary cytotoxicity assay concentrations of up to 3300 µg/mL (about 10 mM) were tested. The treatment intervals for both experiments in the presence (experiment 1 and 2) and absence of metabolic activation (experiment 1) were 4 hours. In addition, a 24 h treatment interval was applied in the second experiment in the absence of metabolic activation. EMS and DMBA served as positive controls in the experiments without and with metabolic activation, respectively. After the incubation period treatment media were replaced by culture medium in both experiments and the cells were incubated for one week for expression of mutant cells. This was followed by incubation of cells in selection medium containing 6-thioguanine for about 1 week.

No cytotoxic effects, as indicated by clearly reduced cloning efficiencies of about or below 20% of the respective negative control values were observed in all experiments under all test conditions up to the highest applied concentration. Only in the 2nd Experiment in the absence of metabolic activation after 24 hours exposure the cell densities were distinctly reduced at 1st passage at 50 and 100 µg/mL, each. These concentrations were close to the limit of test substance solubility in culture medium. At both test groups the cloning efficiencies were only weakly affected. Cell morphology was not influenced by treatment. The test substance did not cause any relevant increase in the mutant frequencies either without S9 mix or after the addition of a metabolizing system in two experiments performed independently.

Based on the results of the study it is concluded that under the conditions of this test Reg. No. 391572 does not induce forward mutations in mammalian cells in vitro.

(BASF DocID 2014/1190533)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material Reg. No. 391572
Description: Solid, beige
Lot/Batch #: L71-12
Purity: 99.7%
Stability of test compound: The stability of the test substance under storage conditions was guaranteed by the sponsor (expiry date: May 2016). The homogeneity of the test substance was guaranteed on account of the high purity and ensured by mixing prior to preparation of test substance solutions.
Solvent used: Dimethylsulfoxide (DMSO), 1% final concentration

2. Control Materials:
Negative control: A negative control was not employed in this study
Vehicle control: 1% (v/v) DMSO in culture medium
Positive control -S9: Ethylmethanesulfonate (EMS) 400 µg/mL
Positive control +S9: 7,12-Dimethylbenz[a]anthracene (DMBA) 1.25 µg/mL

3. Activation: S9 was produced from the livers of induced male Wistar rats. The rats received 80 mg/kg bw phenobarbital i.p. and β-naphthoflavone per gavage on 3 consecutive days. The S9-mix was prepared freshly prior to each experiment. For this purpose, a sufficient amount of S9-fraction is thawed at room temperature and 1 volume of S9-fraction is mixed with 9 volumes of S9-supplement (cofactors). This preparation, the so-called S9-mix, was kept on ice until used.
The S9-mix was prepared immediately before use and had the following composition:

Component	Concentration
Phosphate buffer (pH 7.4)	15 mM
Glucose 6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM

4. Test organism:	Chinese hamster ovary (CHO) cells were used. CHO cells have a high proliferation rate (doubling time about 12-16 h), high plating efficiency (about 90%) and karyotype with a modal number of 20 chromosomes. Stocks of the CHO cell line were maintained at -196°C in liquid nitrogen. Each batch used for mutagenicity testing was checked for mycoplasma contamination. The week prior to treatment, spontaneous HPRT-deficient mutants were eliminated from the stock cultures by growing the cells for 3 to 4 days in pretreatment medium (see below).
5. Culture media:	
Culture medium:	Ham's F12 medium with L-glutamine and hypoxanthine supplemented with 10% (v/v) fetal calf serum (FCS).
Pretreatment medium:	("HAT" medium): FCS-supplemented Ham's F12 medium with L-glutamine and hypoxanthine containing per mL 13.6 µg hypoxanthine, 0.18 µg aminopterin and 3.88 µg thymidine.
Selection medium:	("TG" medium): L-Glutamine- and FCS-supplemented, hypoxanthine-free Ham's F12 medium with 6-thioguanine at a final concentration of 10 µg/mL All media were supplemented with - 1% (v/v) penicillin/streptomycin (10000 IU / 10000 µg/mL) - 1% (v/v) amphotericine B (250 µg/mL)
6. Locus examined:	hypoxanthine-guanine-phosphoribosyl transferase (H(G)PRT)
7. Test concentrations:	
a) Preliminary toxicity assay:	Nine concentrations ranging from 12.9 to 3300 µg/mL
b) Mutation assay:	
1st experiment	
(4-h exposure):	6.3, 12.5, 25, 50, 100 µg/mL with/without metabolic activation
2nd experiment	
(24-h exposure):	12.5, 25, 50, 100, 412.5, 825, 1650, 3300 µg/mL without metabolic activation
2nd experiment	
(4-h exposure):	10, 20, 40, 80, 100 µg/mL with metabolic activation

B. TEST PERFORMANCE:

1. Dates of experimental work: 12-June-2014 to 25-Sep-2014

2. Preliminary cytotoxicity assay:

Cytotoxicity was assessed by determination of the cloning efficiency. About 200 cells were incubated in 25-cm² flasks with various test substance concentrations in serum-free Ham's F12 medium for about 4 hours (with and without metabolic activation) after an attachment period of 24 hours. At the end of the exposure period, the cells were washed with Hanks' balanced salt solution (HBSS), covered with Ham's F12 and incubated for a further 6 to 8 days. After this incubation period, colonies were fixed, stained and counted. In addition to the cloning efficiency the following parameters were measured: pH, osmolarity and the determination of precipitates (solubility).

3. Mutation Assay:

Pretreatment of Cells: Cells with a passage number ≥ 2 after thawing from the frozen cells stock were seeded into 75 cm^2-flasks and incubated for 3-4 days with "HAT" medium during the week prior to treatment to eliminate spontaneous HPRT-deficient mutants. Afterwards, a passage into culture medium followed and the cells were incubated for further 3-4 days.

Cell treatment: For each test group, about $1x10^6$ cells per flask (175 cm²) were seeded into flasks containing about 20 mL Ham's F12 medium supplemented with 10% FCS and incubated for about 20 – 24 hours with 5% CO_2 at 37°C and ≥ 90% humidity for cell attachment. 2x2 flasks (A/B) were used for each test group.

After the cell attachment period the medium was replaced by fresh medium. The test article, dissolved in 200 µL DMSO, was added to the culture medium. Without S9 mix 20 mL Ham’s F12 medium without FCS and 200 µL test substance preparation were used. With S9 mix 16 mL Ham’s F12 medium without FCS, 200 µL test substance preparation and 4 mL S9 mix were used. Concurrent negative and positive controls were tested in parallel. The cells were exposed for 4 hours both with and without S9-mix at 5% CO_2, 37°C and ≥ 90% humidity.

Expression: After the exposure period, the treatment medium was replaced by 20 mL Ham's F12 medium with 10% FCS after having been rinsed twice with Hanks' balanced salt solution (HBSS). Subsequently, the flasks were incubated for another 2 or 3 days (24 h or 4 h exposure) and then subcultured (1st passage). After an entire expression period of 7-9 days, the cells were transferred into selection medium ("TG" medium) at the 2nd passage.

Selection: For the mutant selection, six 75-cm^2 flasks each were seeded with $3x10^5$ cells from each treatment group in selection medium (TG medium) and incubated for about 6 to 7 days. At the end of the selection period, colonies were fixed with methanol, stained with Giemsa and counted.

Determination of Cytotoxicity: Cloning efficiency 1 (survival):
The survival (cloning efficiency 1; CE_1) was determined in parallel to the mutagenicity test. Approximately 200 cells per dose group were seeded into duplicate 25 cm^2 flasks using 5 mL Ham`s F12 medium with 10% FCS. After about 24 hour attachment period the cells were incubated with vehicle, test substance or the positive control for 4 hours as described above. Following exposure, cells were rinsed several times with HBSS. Finally, cells were cultured in 5 mL Ham's F12 medium incl. 10% (v/v) FCS.

Cloning efficiency 2 (viability):
The viability (cloning efficiency 2; CE_2) was determined after the expression period. About 200 cells were separated during the transfer into selection medium and seeded in two flasks (25 cm^2) containing 5 mL Ham's F12 medium incl. 10% (v/v) FCS each.
After seeding of the cells, the flasks were incubated for 5 – 8 days to form colonies. These colonies were fixed, stained and counted.

Calculations:

Mutant frequency: Uncorrected mutant frequency:

$$MF_{uncorr} = \frac{\text{total number of mutant colonies}}{\text{number of seeded cells}} \times 10^6$$

Corrected mutant frequency:

$$MF_{corr} = \frac{MF_{uncorr}}{CE_{2\,absolute}} \times 100$$

Cloning efficiency (CE,%) absolute:

$$CE_{absolute} = \frac{total\ number\ of\ colonies\ in\ the\ test\ group}{total\ number\ of\ seeded\ cells\ in\ the\ test\ group} x100$$

relative, in comparison to control:

$$CE_{relative} = \frac{CE_{absolute}\ of\ the\ test\ group}{CE_{absolute}\ of\ the\ vehicle\ control} x100$$

4. Check for further parameters:

pH was measured at least for the top concentrations and vehicle control with and without S9 mix. Osmolarity was measured at least for the top concentrations and for vehicle control with and without S9 mix. Possible test substance precipitation was checked immediately after treatment of the test cultures. The test cultures of all test groups were examined microscopically at the end of the exposure period with regard to cell morphology, which allows conclusions to be drawn about the attachment of the cells.

5. Statistics:

An appropriate statistical trend test (MS EXCEL function RGP) was performed to assess a dose-related increase of mutant frequencies. The number of mutant colonies obtained for the test substance treated groups was compared with that of the respective vehicle control groups. A trend is judged as statistically significant whenever the one-sided p-value (probability value) is below 0.05 and the slope is greater than 0. However, both, biological and statistical significance has been considered together.

6. Evaluation criteria:

The test chemical is considered positive in this assay if the following criteria are met:

- Increases of the corrected mutation frequencies ($MF_{corr.}$) both above the concurrent vehicle control values and the historical negative control range.
- Evidence of reproducibility of any increase in mutant frequencies.
- A statistically significant increase in mutant frequencies and the evidence of a dose-response relationship.

Isolated increases of mutant frequencies above the historical negative control range (i.e. 15 mutants per 10^6 clonable cells) or isolated statistically significant increases without a dose-response relationship may indicate a biological effect but are not regarded as sufficient evidence of mutagenicity.

A test substance is generally considered negative in this test system if:

- The corrected mutation frequency ($MF_{corr.}$) in all dose groups is within the historical control range and is not significantly above the concurrent negative control.

II. RESULTS AND DISCUSSION

A. MUTANT FREQUENCY

No increase in the number of mutant colonies was observed either with or without S9 mix. The mutant frequencies at any dose were close to the range of that of the concurrent negative control values and within the range of the historical control data.
Treatment with the positive controls EMS and DMBA resulted in a marked increase in the number of mutant colonies as well as of mutant frequencies in all experiments, thus demonstrating the sensitivity of the test.

B. CYTOTOXICITY

No cytotoxic effects, as indicated by clearly reduced cloning efficiencies of about or below 20% of the respective negative control values were observed in all experiments under all test conditions up to the highest applied concentration.
Only in the 2nd Experiment in the absence of metabolic activation after 24 hours exposure the cell densities were distinctly reduced at 1st passage at 50 and 100 µg/mL, each. These concentrations were close to the limit of test substance solubility in culture medium. At both test groups the cloning efficiencies were only weakly affected.

C. CELL MORPHOLOGY

Cell attachment was not influenced at any dose evaluated. The cell morphology was “Fibroblast-like cells” at any test substance concentration.

D. TREATMENT CONDITIONS

The pH value of the test substance preparation was adjusted by adding small amounts of HCl only in the 2nd Experiment without S9 mix. However, osmolarity and pH values were not influenced by test substance treatment at the end of exposure period.
The test substance was poorly soluble in commonly used vehicles at high concentrations. In this study, in the absence of S9 mix, test substance precipitation was observed in culture medium at the end of treatment at 50 µg/mL and above in the 1st Experiment and at 25 µg/mL and above in the 2nd Experiment, respectively. In the presence of S9 mix precipitation was observed at about 50 µg/mL and above in both main experiments.

Table 5.8.1-10: Gene mutation in mammalian cells - 1st experiment

Test group	Number of mutant colonies (A/B)[a]	Mutant frequency (per 10^6 cells)		CE_1 (survival) (4h after treatment; approx. 200 cells/flask seeded		CE_2 (viability) (at the end of the expression period; approx. 200 cells/flask seeded)	
				Cloning efficiency (%)		Cloning efficiency (%)	
		Non corrected	Corrected [b]	absolute	relative	absolute	relative
Without metabolic activation; 4-hour exposure period							
Vehicle (DMSO)	5/5	2.78	3.48	80.4	100	80.1	100
Test item							
6.3 µg/mL	4/1	1.39	1.90	96.1	119.6	74.5	93.0
12.5 µg/mL	3/3	1.67	2.28	92.4	114.9	73.3	91.4
25 µg/mL	1/3	1.11	1.25	94.9	118.0	86.4	107.8
50 µg/mL	3/6	2.50	2.93	43.8	54.4	84.9	105.9
100 µg/mL	7/2	2.50	3.07	81.5	101.4	78.1	97.5
Positive control EMS							
400.0 µg/mL	92/90	50.56	75.31	94.8	117.9	67.3	83.9
With metabolic activation; 4-hour exposure period							
Vehicle (DMSO)	9/0	2.50	3.47	94.4	100	72.9	100
Test item							
6.3 µg/mL	n.c.	n.c.	n.c.	98.1	104.0	n.c.	n.c.
12.5 µg/mL	0/3	0.83	0.95	94.8	100.4	81.6	112.0
25 µg/mL	9/5	3.89	5.07	96.5	102.3	76.9	105.5
50 µg/mL	0/11	3.06	3.86	85.8	90.9	82.4	113.0
100 µg/mL	3/3	1.67	1.90	91.4	96.8	88.3	121.1
Positive control DMBA							
1.25 µg/mL	157/216	103.61	171.40	97.4	103.2	60.3	82.7

[a] number of colonies 7 days after seeding 3 x 10^5 cells/flask into selection medium

[b] correction on the basis of absolute cloning efficiency 2 (viability) at the end of the expression period

n.c. culture was not continued since a minimum of four analyzable concentrations is required

Table 5.8.1-11: Gene mutation in mammalian cells – 2nd experiment

Test group	Number of mutant colonies (A/B)[a]	Mutant frequency (per 10^6 cells)		CE_1 (survival) (4h after treatment; approx. 200 cells/flask seeded		CE_2 (viability) (at the end of the expression period; approx. 200 cells/flask seeded)	
				Cloning efficiency (%)		Cloning efficiency (%)	
		Non corrected	Corrected[b]	absolute	relative	absolute	relative
Without metabolic activation; 24-hour exposure period							
Vehicle (DMSO)	0/0	0.0	0.0	81.9	100	87.0	100
Test item							
12.5 µg/mL	15/2	4.72	5.74	85.4	104.3	83.1	95.5
25 µg/mL	1/8	2.50	2.68	66.8	81.5	88.8	102.0
50 µg/mL	0/0	0.0	0.0	48.0	58.6	78.9	90.7
100 µg/mL	0/0	0.0	0.0	46.9	57.3	85.3	98.0
Positive control EMS							
400.0 µg/mL	558/459	282.50	495.98	66.4	81.1	57.1	65.7
With metabolic activation; 4-hour exposure period							
Vehicle (DMSO)	0/0	0.0	0.0	81.9	100	80.8	100
Test item							
10 µg/mL	0/1	0.28	0.32	84.8	103.5	81.3	100.6
20 µg/mL	6/8	3.89	4.84	84.0	102.6	80.3	99.4
40 µg/mL	4/1	1.39	1.97	87.9	107.3	72.9	90.2
80 µg/mL	0/3	0.83	0.91	86.0	105.0	88.1	109.1
100 µg/mL	n.c.	n.c.	n.c.	80.0	97.7	n.c.	n.c.
Positive control DMBA							
1.25 µg/mL	244/196	122.22	184.17	75.6	92.4	66.3	82.0

[a] number of colonies 7 days after seeding 3 x 10^5 cells/flask into selection medium

[b] correction on the basis of absolute cloning efficiency 2 (viability) at the end of the expression period

n.c. culture was not continued since a minimum of four analyzable concentrations is required

III. CONCLUSION

Based on the results of the study it is concluded that under the conditions of this test Reg.No. 391572 does not induce forward mutations in the HPRT locus in CHO cells under the experimental conditions chosen.

Report:	CA 5.8.1/7 [redacted]., 2015 a Reg.No. 391572 - Repeated dose 90-day oral toxicity study in Wistar rats - Administration via the diet 2015/1204964
Guidelines:	(EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. L 142, OECD 408, EPA 870.3100, JMAFF No 12 Nosan No 8147
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Note: This study report has **not** been part of the documentation for the first Annex I inclusion process and a full evaluation is therefore presented here to assist in the discussion on relevant metabolites in groundwater

EXECUTIVE SUMMARY

Administration of Reg.No. 391572 (Batch: L85-64; Purity: 99.2%) to Wistar rats at dietary dose levels of 0, 150, 1500 and 15000 ppm for at least 90 days did not result in impaired body weight development in both sexes or decreased food consumption at any dose level. Hematology, clinical chemistry and urinalysis were unaffected by the treatment.

There were no findings in organs other than decreased uterus weights (absolute and relative) in the mid dose group identified to be statistically significantly decreased. This finding has been regarded as incidental and not treatment-related as the ten-fold higher dose level in the high dose group was comparable to controls. All other mean absolute and relative organs weights did not show significant differences when compared to the control group.

In conclusion the administration of Reg.No. 391572 via the diet to male and female Wistar rats for 3 months caused no test substance-related adverse signs of systemic toxicity even at a concentration of 15000 ppm in male and female Wistar rats.

Therefore, under the conditions of the present study the no observed adverse effect level (NOAEL) was 15000 ppm in male (968 mg/kg bw/d) and in female (1082 mg/kg bw/d) Wistar rats.

A. MATERIALS

1. Test Material:	Reg.No. 391572
Description:	solid / beige
Lot/Batch #:	L85-64
Purity:	99.2%
Stability of test compound:	The stability of the test substance under storage conditions over the test period was guaranteed by the sponsor, up to 01 August 2016.
2. Vehicle and/or positive control:	Rodent feed
3. Test animals:	
Species:	Rat
Strain:	Wistar, Crl:WI(Han)
Sex:	Male and female
Age:	30 ± 1 days when supplied, 42 ± 1 days at the start of test substance administration
Weight at dosing (mean):	Males: 151.4 – 153.5 g (means of groups) at start of dosing Females: 116.0 – 1119.5 g (means of groups) at start of dosing
Source:	Charles River Laboratories, Research Models and Services GmbH, Sulzfeld, Germany
Acclimation period:	12 days
Diet:	Kliba maintenance diet mouse/rat "GLP", meal, supplied by Provimi Kliba SA, Kaiseraugst, Switzerland
Water:	Tap water, ad libitum
Housing:	The animals were housed together (5 animals per cage) in H-Temp polysulfonate cages supplied by TECNIPLAST, Hohenpeißenberg, Germany (floor area about 2065 cm^2). Motor activity measurements were conducted in polycarbonate cages (floor area about 800 cm^2) supplied by TECNIPLAST, Hohenpeißenberg, Germany, with small amounts of bedding. The cages were closed with wire covers from Ehret, Emmendingen, Germany.
Environmental conditions:	
Temperature:	Fully air-conditioned rooms in which central air conditioning guaranteed a range of temperature of 20-24°C
Humidity:	30-70%
Air changes:	15/hour
Photo period:	Day/night cycle of 12 hours (12 hours light from 06.00 h-18.00 h, 12 hours dark from 18.00 h-06.00 h)

STUDY DESIGN AND METHODS

1. Dates of experimental work: 07-Jan-2015 to 07-Dec2015
(In life dates: 25-Jan-2015 (start of test substance administration) to 28-Apr-2015 (necropsy))

2. Animal assignment and treatment:
Reg. No. 391 572 was administered to groups of 10 male and 10 female rats at dietary concentrations of 0, 150 (low dose), 1500 (intermediate dose), and 15000 ppm (top dose) for at least 90 days. The animals were assigned to the treatment groups by means of computer generated randomization lists based on body weights.

3. Test substance preparation and analysis:
The diets were prepared by mixing weighed amounts of test substance with a small amount of feed. Subsequently, appropriate amounts of feed were added to obtain the intended dietary concentrations and mixed in a laboratory mixer. The test substance preparations were prepared weekly.

The analyses of the test-substance preparations were carried out at the Analytical Chemistry Laboratory of [redacted].
The stability of the test substance in the diet for a period of 9 days in the freezer followed by 4 days at room temperature was demonstrated before the start of the administration period in another batch (BASF project No. 01Y0609/00Y020; see PART III, Supplement of the study report).
Homogeneity and concentration analyses were verified in 3 samples in the lowest (150 ppm) and highest concentration (15000 ppm) at the beginning of the study and additional concentration control analysis was done in the mid concentration. The samples were taken from the specific feed containers by staff of the Central Food Mixing unit. No test-article was determined in control diets.
Samples for the concentration control analyses were taken once at the beginning of the administration period. The determined values of Reg.No. 391572 in Ground Kliba maintance diet mouse/rat “GLP” meal were found to be in the range of 90-110% of the nominal concentration. These results demonstrated the correctness of the concentrations of Reg.No. 391572 in ground Kliba maintenance diet mouse/rat “GLP” meal.
According to the stability analyses of the test substance preparations, the test item was mixed in feed every 5 to 9 days, starting on 23 Jan 2015 until 23 Apr 2015. Each mixing process of the dietary concentrations followed the exactly same procedures and was performed by the same staff. The weighed amounts of test substance and feed are documented and archived in the raw data.

Analysis of diet preparations for homogeneity and test-item content

Dose level [ppm]	Sampling date	Analysis date	Concentration [ppm][#]	% of nominal concentration	Relative standard deviation [%]
150 ppm	23.01.2015	27.01.2015	153.334	102.2	
	23.01.2015	27.01.2015	148.936	99.3	
	23.01.2015	27.01.2015	155.025	103.4	
Average ± SD			**152.432**	**101.6**	**2.1**
1500 ppm	23.01.2015	27.01.2015	1481.392	98.8	
Average ± SD			n.a.	n.a.	n.a.
15000 ppm	23.01.2015	29.01.2015	14171.636	94.5	
	23.01.2015	29.01.2015	13512.828	90.1	
	23.01.2015	29.01.2015	14318.288	95.5	
Average ± SD			**14000.917**	**93.3**	**3.1**

n.a.: not applicable; [#] based on mean values of the three individual samples
Values may not calculate exactly due to rounding of values

Relative standard deviations of the homogeneity samples were in the range of 2.1 to 3.1% indicate the homogenous distribution of Reg.No. 391 572 in the diet preparations. The actual (mean) average test-substance concentrations were in the range of 93.3 to 101.6% of the nominal concentrations.

4. Statistics:

Means and standard deviations of each test group were calculated for several parameters. Further statistical analyses were performed according to following tables:

Statistics of clinical examinations

Parameter	Statistical test
body weight, body weight change	A comparison of each group with the control group was performed using DUNNETT's test (two-sided) for the hypothesis of equal means
Rearing, grip strength forelimbs, grip strength hindlimbs, foot-splay test, motor activity	Non-parametric one-way analysis using KRUSKAL-WALLIS test (two-sided). If the resulting p-value was equal or less than 0.05, a pairwise comparison of each dose group with the control group was performed using Wilcoxon-test (two-sided) for the equal medians

Statistics of clinical pathology

Parameter	Statistical test
Blood 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. Pairwise comparison of each dose group with the control group using the WILCOXON-test (one-sided) **with Bonferroni-Holm adjustment** for the hypothesis of equal medians
Urinalysis, except pH, volume, color, turbidity and specific gravity	Pairwise comparison of each dose group with the control group using the WILCOXON-test (one-sided) for the hypothesis of equal medians
Urine pH, volume, specific gravity, color and turbidity	Non-parametric one-way analysis using KRUSKAL-WALLIS test. If the resulting p-value was equal or less than 0.05, a pairwise comparison of each dose group with the control group was performed using WILCOXON-test (two-sided) for the hypothesis of equal medians. Urine color and turbidity are not evaluated statistically

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. Animals in moribund stage were sacrificed and necropsied. Observations for overt clinical signs of toxicity were performed at least once daily and documented daily for each affected animal.
Detailed clinical observations were performed in all animals prior to the start of the administration period and at weekly intervals thereafter. For this, 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. The clinical examination included, but was not limited, to the following parameters/organs:

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. Body weight:

The body weight of the animals was determined before the start of the administration period (in order to randomize the animals), at the start of the treatment (day 0), and once weekly thereafter.

3. Food consumption and test substance intake:

Food consumption was determined once weekly on a cage basis and calculated as mean food consumption in grams per animal and 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/day) on day x, C as the dose in ppm and BW_x as body weight on day x of the study (in g).

4. Water consumption:

Water consumption was monitored daily by visual inspection of water bottles.

5. Ophthalmoscopy:

Prior to the start of the administration period the eyes of all animals, and on day 91 the eyes of the control and high dose animals were examined for any changes using an ophthalmoscope after administration of a mydriatic.

6. Functional observation battery (FOB):

A functional observational battery (FOB) was performed in all animals at the end of the administration period starting at about 10:00 h. At least one hour before the start of the FOB the animals were transferred to single-animal polycarbonate cages. Drinking water was provided ad libitum, but no food was offered during the measurements. The FOB started with passive observations without disturbing the animals, followed by removal from the home cage, open field observations in a standard arena and sensory motor tests as well as reflex tests. The findings were ranked according to the degree of severity, if applicable. The observations were performed at random. A detailed description of the methods, the ranking and documentation system can be found in PART III (Supplement).

During the home cage observation, special attention was paid to posture, tremors, convulsions, abnormal movements and impairment of gait.

For open field observation, the animals were transferred to a standard arena (50 x 50 cm with sides of 25 cm high) and observed for at least 2 minutes. The following parameters were assessed:

1. behavior when removed from cage	10. respiration
2. fur	11. tremors
3. skin	12. convulsions
4. salivation	13. abnormal movements
5. nasal discharge	14. impairment of gait
6. lacrimation	15. activity/arousal level
7. eyes/pupil size	16. feces (number of fecal pellets/appearance/ consistency) within two minutes
8. posture	17. urine (appearance/quantity) within two minutes
9. palpebral closure	18. number of rearings within two minutes

For sensorimotor tests and reflexes the animals were removed from the open field. The following tests were performed:

1. approach response	8. behavior during "handling"
2. touch sensitivity/response	9. vocalization
3. vision ("visual placing response")	10. pain perception ("tail pinch")
4. pupillary reflex	11. grip strength of forelimbs
5. pinna reflex	12. grip strength of hind limbs
6. audition ("startle response")	13. landing foot-splay test
7. coordination of movements ("righting response")	14. other findings

7. Motor activity measurement:

Motor activity (MA) was also measured from 14:00 h onwards on the same day as the FOB was performed. The examinations were performed using the TSE Labmaster System supplied by TSE Systems GmbH, Bad Homburg, Germany. For this purpose, the animals were placed in new clean polycarbonate cages with a small amount of bedding for the duration of the measurement. Eighteen beams were allocated per cage. The number of beam interrupts was counted over 12 intervals for 5 minutes per interval. The sequence in which the animals were placed in the cages was selected at random. On account of the time needed to place the animals in the cages, the starting time was "staggered" for each animal. The measurement period began when the 1st beam was interrupted and finished exactly 1 hour later. No food or water was offered to the animals during these measurements and the measurement room was darkened after the transfer of the last animal

8. Clinical pathology:

Blood was withdrawn in the morning from fasted, isoflurane-anesthetized animals from the retro-orbital plexus. The blood sampling procedure and the subsequent analysis of the blood and serum samples were carried out in a randomized sequence.

The following hematological and clinical chemistry parameters were determined for all animals:

Hematology:					
	Red blood cells		*White blood cells*		*Clotting Potential*
✓	Erythrocyte count (RBC)	✓	Total leukocyte count (WBC)	✓	Prothrombin time (Hepato Quick)
✓	Hemoglobin (Hb)	✓	Neutrophils (differential)	✓	Thrombocyte count
✓	Hematocrit (Hct)	✓	Eosinophils (differential)		Activated partial thromboplastin time (APPT)
✓	Mean corp. volume (MCV)	✓	Basophils (differential)		
✓	Mean corp. hemoglobin (MCH)	✓	Lymphocytes (differential)		
✓	Mean corp. Hb. conc. (MCHC)	✓	Monocytes (differential)		
✓	Reticulocytes	✓	Large unstained cells		
Clinical chemistry:					
	Electrolytes		*Metabolites and Proteins*		*Enzymes:*
✓	Calcium	✓	Albumin	✓	Alanine aminotransferase (ALT)
✓	Chloride	✓	Bilirubin (total)	✓	Aspartate aminotransferase (AST)
✓	Phosphorus (inorganic)	✓	Cholesterol	✓	Alkaline phosphatase (ALP)
✓	Potassium	✓	Creatinine	✓	γ-glutamyl transpeptidase (γ-GT)
✓	Sodium	✓	Globulin (by calculation)		
		✓	Glucose		
		✓	Protein (total)		
		✓	Triglycerides		
		✓	Urea		

9. Urinalysis:

For urinalysis, the individual animals were transferred to metabolism cages and urine was collected overnight. No food or water was supplied during urine collection. The samples were analyzed in a randomized order.

The following parameters were determined for all animals:

Urinalysis					
	Quantitative parameters:		*Semiquantitative parameters*		
✓	Urine volume	✓	Bilirubin	✓	Protein
✓	Specific gravity	✓	Blood	✓	pH-value
		✓	Color and turbidity	✓	Urobilirubin
		✓	Glucose	✓	Sediment (microscopical exam.)
		✓	Ketones		

10. Sacrifice and pathology:

The animals were sacrificed by decapitation under Isoflurane anesthesia. The exsanguinated animals were necropsied and assessed by gross pathology. The following organs were sampled, weighed and subjected to histopathological evaluation as follows:

Pathology:

The following organs were collected (column C), weighed (W) and examined histopathologically (H, ✓: all groups, #: control and top dose).

C	W	H		C	W	H		C	W	H	
✓	✓	#	adrenals	✓	✓	#	kidneys[&]	✓		#	seminal vesicles
✓		#	aorta	✓			lachrymal glands[%]	✓		#	skin
✓		#	bone marrow[§]	✓		#	larynx	✓		#	spinal cord (3 levels)[@]
✓	✓	#	brain	✓	✓	#	liver	✓	✓	#	spleen
✓		#	caecum	✓		#	lung	✓			sternum w. marrow
✓		#	colon	✓		#	lymph nodes[#]	✓		#	stomach (fore- & glandular)
✓		#	duodenum	✓		#	mammary gland (♀)	✓	✓	#	testes
✓	✓	#	epididymides	✓		#	muscle, skeletal	✓	✓	#	thymus
✓		#	esophagus	✓		#	nerve, peripheral (sciatic n.)	✓	✓	#	thyroid/parathyroid
✓		#	eyes (with optic nerve)	✓		#	nose/nasal cavity[‡]	✓		#	trachea
✓			femur (with joint)	✓	✓	#	ovaries and oviduct**	✓		#	urinary bladder
			gall bladder	✓		#	pancreas	✓	✓	#	uterus
✓		✓	gross lesions	✓		#	pharynx	✓		#	vagina
✓		#	Harderian gland	✓		#	pituitary				
✓	✓	#	heart	✓		#	prostate		✓		body (anesthetized animals)
✓		#	ileum	✓		#	rectum				
✓		#	jejunum (w. Payer's plaque)	✓		#	salivary glands*				

[§] from femur; [#] axillary and mesenteric; [@] cervical, thoracic, lumbar; *mandibular and sublingual, ** oviduct not weighed; [%] extraorbital, [‡] histopathology at level III, [&] histopathological evaluation of all treatment groups for females only

II. RESULTS AND DISCUSSION

A. TEST SUBSTANCE ANALYSES

See Section B 3, above.

B. OBSERVATIONS

1. Clinical signs of toxicity

No test substance-related effects were observed in male and female animals.

2. Mortality

No animal died prematurely in the study.

3. Food consumption and test substance intake

No test substance-related, adverse changes with regard to food consumption were observed.
The mean daily test substance intake in mg/kg body weight/day (mg/kg bw/d) over the entire study period was calculated and is shown below.

Test group	Concentration in the vehicle (ppm)	Mean daily test substance intake (mg/kg bw/d)	
		Males	Females
1	150	9.7	11
2	1500	95	110
3	15000	968	1082

4. Water consumption

No test substance-related changes with regard to water consumption were observed.

5. Ophthalmoscopy

No treatment-related ophthalmologic findings were noted. The only findings observed at the end of the treatment period were corneal stipplings. The incidence of these findings was identical between control and high dose groups. Furthermore, corneal stipplings were already observed in these groups prior to initiation of treatment at a similar incidence level.

C. FUNTIONAL OBSERVATION BATTERY AND MOTOR ACTIVITY

Neither home cage nor open field observations revealed any indication of treatment-related effects. The same holds true for the sensorimotor tests and reflexes. All deviations from "zero values" were equally distributed between treated groups and controls or occurred in single animals only and thus were considered to be incidental.
Motor activity measurements gave no differences between control and treated animals which were considered test substance related. In regard to individual observation intervals, a statistically significant difference was observed in males of the high dose group at interval 9. This isolated occurrence has been considered incidental.

Figure 5.8.1-1 90-day feeding rat administered Reg No 391572: motor activity measurements in male animals

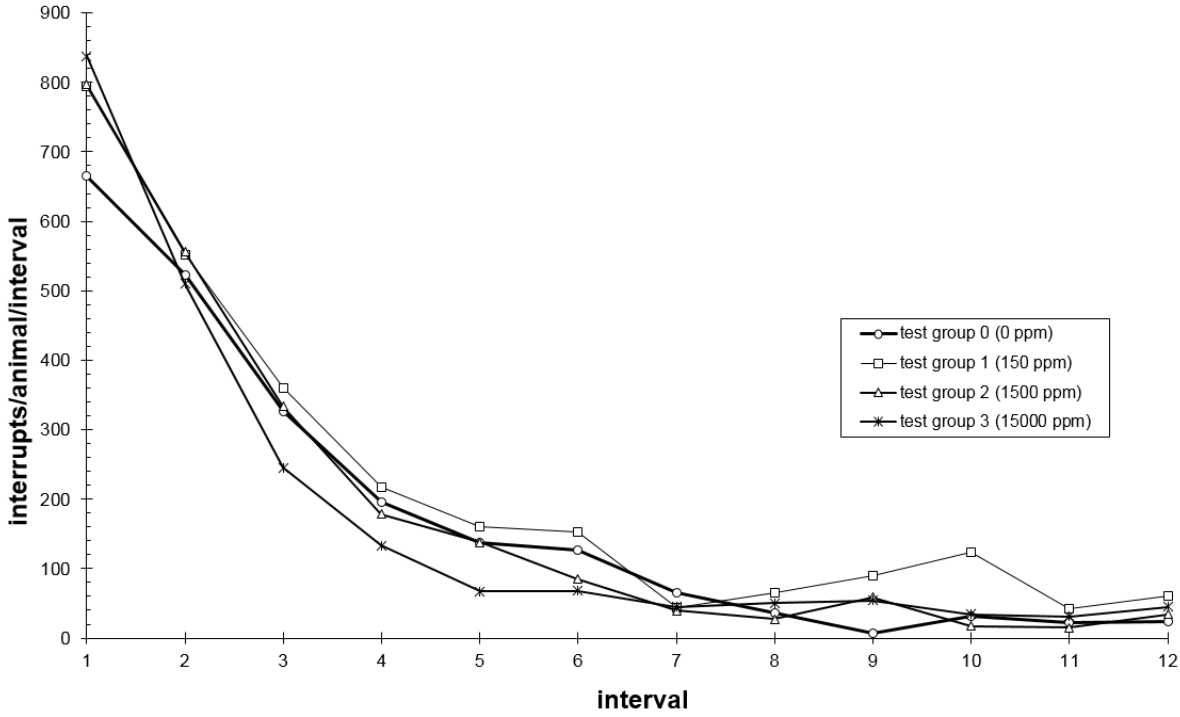
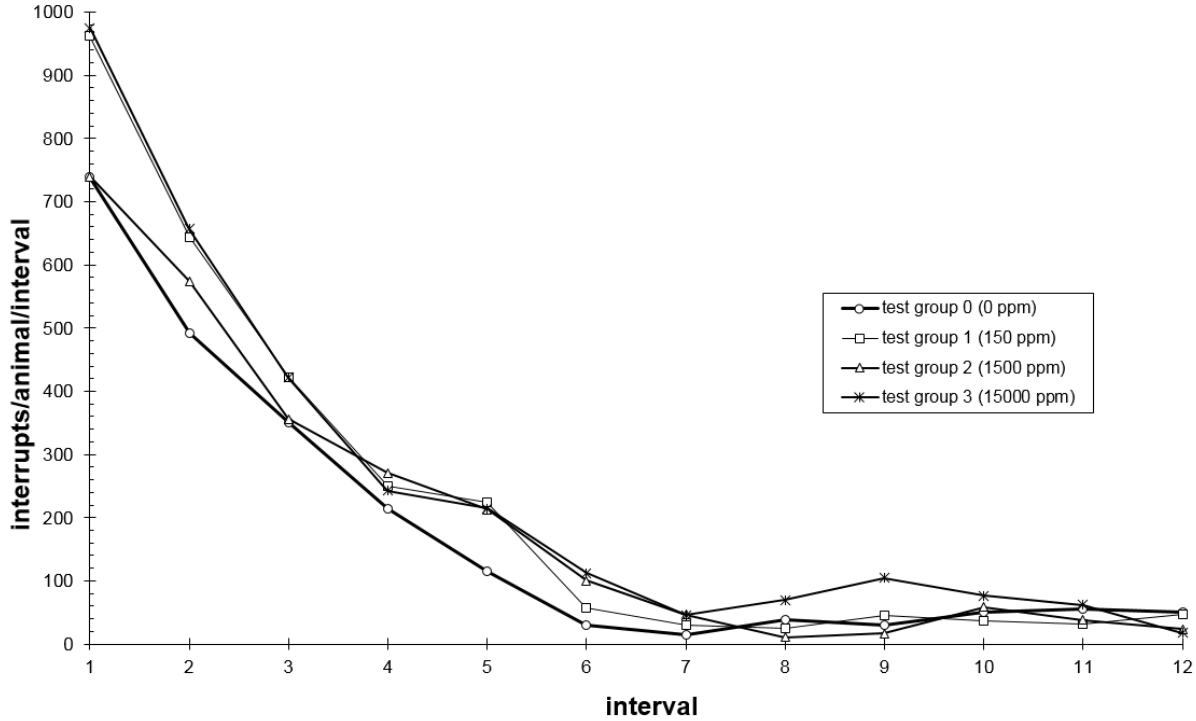


Figure 5.8.1-2 90-day feeding rat administered Reg No 391572: motor activity measurements in female animals



D. Body weight and body weight gain

Body weight development was not changed by the test substance administration up to the high dose level. For female animals of test group 2 (1500 ppm), a higher body weight change was observed on study day 14. The change was assessed not to be related to treatment.

Figure 5.8.1-3 90-day feeding rat administered Reg No 391572: mean body weights of male animals

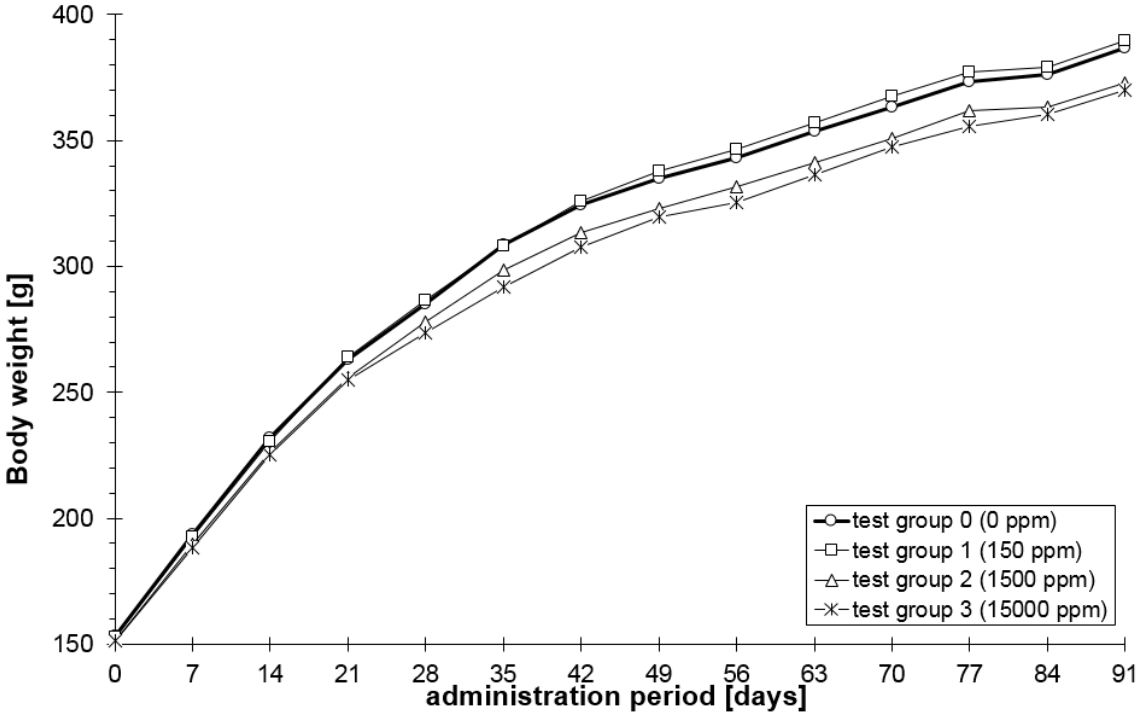
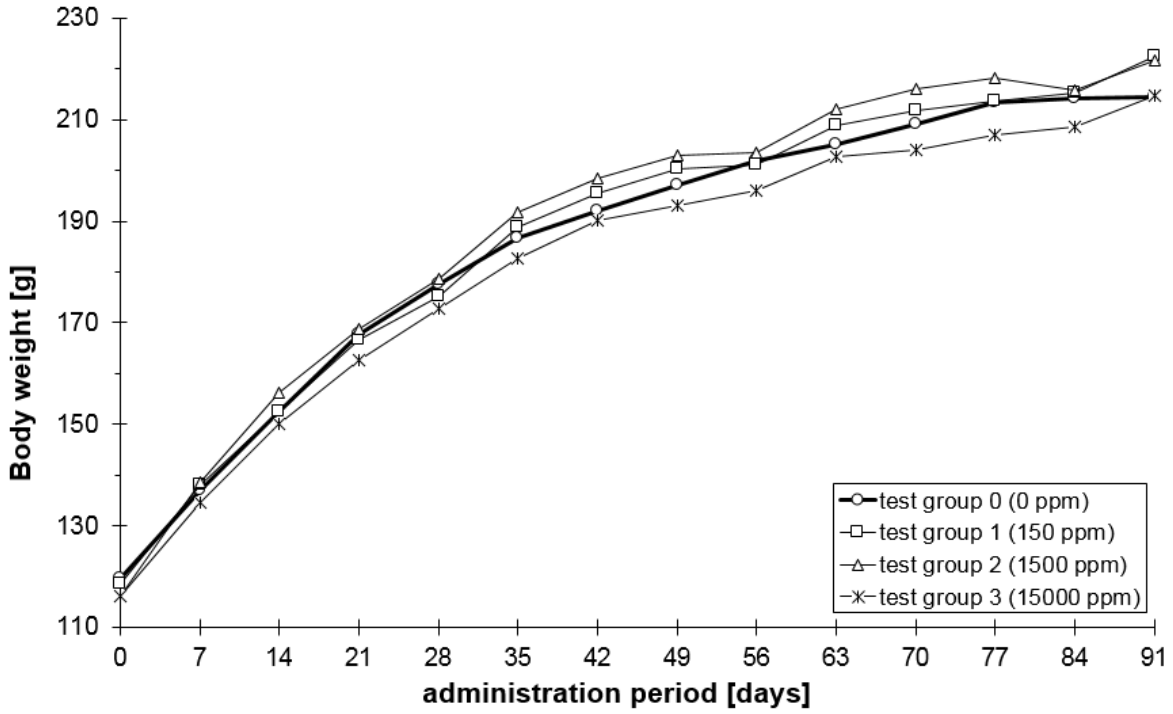


Figure 5.8.1-4 90-day feeding rat administered Reg No 391572: mean body weights of female animals



Detailed information on body weight and body weight gain is presented in the table below

Table 5.8.1-12: 90-day feeding rat administered Reg No 391572: Body weight and body weight gain

	Males				Females			
Dose level [ppm]	**0**	**150**	**1500**	**15000**	**0**	**150**	**1500**	**15000**
Body weight [g]								
- Day 0	153.5	152.6	151.5	151.4	119.5	118.5	116.0	116.2
- Day 91	386.7	389.6	372.9	369.9	214.6	222.6	221.8	214.7
Δ% (compared to control) [#]	-	0.7	-3.6	-4.4	-	3.7	3.4	0.0
Overall body weight gain [g]	233.2	237.0	221.3	218.5	95.2	104.1	105.8	98.5
Δ% (compared to control) [#]	-	1.6	-5,1	-6.3	-	9.4	11.2	3.5

Values may not calculate exactly due to rounding of mean values

E. Clinical pathology

1. Hematology

No changes in hematology attributable to treatment with the test substance could be observed.

2. Clinical chemistry

No treatment-related changes among clinical chemistry parameters were observed.

After 3 months of administration, in females of test group 1 (150 ppm) urea levels were significantly lower compared to controls, however without indication of dose-dependency given the higher dose levels to be in the range of the control value. Therefore, it was regarded as incidental and not treatment-related.

Table 5.8.1-13: 90-day feeding rat administered Reg No 391572: Selected findings in clinical chemistry

	Males				Females			
Dose level [ppm]	**0**	**150**	**1500**	**15000**	**0**	**150**	**1500**	**15000**
Clinical chemistry								
Urea [mmol/L]	5.13	5.07	4.98	5.37	6.39	5.68**	6.24	7.08

*P ≤ 0.05, **P ≤ 0.01 Kruskall-Wallis + Wilcoxon test (two-sided)

F. Urinalysis

No treatment-related changes were observed.

G. Necropsy

1. Organ weights

There were no findings other than decreased uterus weights (absolute and relative) in the mid dose group identified to be statistically significantly different. This finding has been regarded as incidental and not treatment-related as the ten-fold higher dose level in the high dose group was comparable to controls.

All other mean absolute and relative organs weights did not show significant differences when compared to the control group. This does also apply to the target organs identified for the parent compound Boscalid (i.e. liver and thyroids) and does also apply to sexual organs and accessory glands or to organs of the endocrine system. More detailed information is presented in the table below.

Table 5.8.1-14: 90-day feeding rat administered Reg No 391572: Organ weights of uterus, thyroids and liver

	Males				Females			
Dose level [ppm]	**0**	**150**	**1500**	**15000**	**0**	**150**	**1500**	**15000**
Organ weight absolute								
Uterus [g]	-	-	-	-	0.829	0.754 (91%)	0.584* (70%)	0.837 (101%)
Liver [g]	7.983	8.004	7.686	8.017	4.722	5.015	4.933	4.908
Thyroids [mg]	22.3	22.8	21.2	22.5	15.9	16.3	15.7	16.1
Organ weight relative								
Uterus	-	-	-	-	0.412	0.371	0.282**	0.419
Liver	2.212	2.202	2.210	2.322	2.373	2.456	2.399	2.473
Thyroids	0.006	0.006	0.006	0.007	0.008	0.008	0.008	0.008

*P $\leq$ 0.05, **P $\leq$ 0.01 Kruskall-Wallis + Wilcoxon test (two-sided)

Table 5.8.1-15: 90-day feeding rat administered Reg No 391572: Organ weights of the sexual and endocrine system

	Males				Females			
Dose level [ppm]	**0**	**150**	**1500**	**15000**	**0**	**150**	**1500**	**15000**
Organ weight absolute								
Testes [g]	3.552	3.502	3.549	3.584	-	-	-	-
Epididymides [g]	1.204	1.158	1.155	1.168	-	-	-	-
Ovaries [mg]	-	-	-	-	82.5	91.9	93.7	88.6
Adrenals [mg]	56.7	54.6	54.0	56.1	58.6	61.8	64.5	60.9
Organ weight relative								
Testes	0.987	0.965	1.027	1.043	-	-	-	-
Epididymides	0.336	0.319	0.334	0.339	-	-	-	-
Ovaries	-	-	-	-	0.041	0.045	0.045	0.045
Adrenals	0.016	0.015	0.016	0.016	0.030	0.030	0.031	0.031

*P $\leq$ 0.05, **P $\leq$ 0.01 Kruskall-Wallis + Wilcoxon test (two-sided)

2. Gross lesions and histopathology

All gross lesion findings and histopathological findings occurred either individually or were biologically equally distributed over control and treatment groups. They were considered to be incidental or spontaneous in origin and without any relation to treatment.

Table 5.8.1-16: 90-day feeding rat administered Reg No 391572: Macroscopic findings in the sexual organs and of the endocrine system

	Males				Females			
Dose level [ppm]	**0**	**150**	**1500**	**15000**	**0**	**150**	**1500**	**15000**
Macroscopic findings								
Uterus: Dilation					1 / 10		1 / 10	
Epididymides: Focus	1 / 10							

Table 5.8.1-17: 90-day feeding rat administered Reg No 391572: Microscopic findings in the sexual organs and of the endocrine system

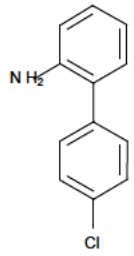
	Males				Females			
Dose level [ppm]	**0**	**150**	**1500**	**15000**	**0**	**150**	**1500**	**15000**
Macroscopic findings								
Adrenal cortex: Accessory cortical tissue				1 / 10				1 / 10
Epididymides: Granuloma. Spermatogenic Debris	1 / 10 1 / 10							
Ovaries: Mineralization, focal					1 / 10			
Prostate: Inflitration lymphoid	2 / 10							
Testes: Degeneration, tubular	1 / 10			1 / 10				
Uterus: Dilation, horn Cystic dilation, gland					1 / 10		1	1 / 10

III. CONCLUSIONS

The administration of Reg.No. 391572 via the diet to male and female Wistar rats for 3 months caused no test substance-related adverse signs of systemic toxicity even at a concentration of 15000 ppm in male and female Wistar rats.

Therefore, under the conditions of the present study the no observed adverse effect level (NOAEL) was 15000 ppm in male (968 mg/kg bw/d) and in female (1082 mg/kg bw/d) Wistar rats.

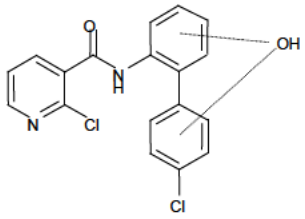
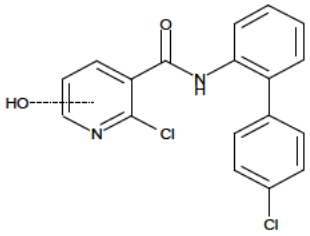
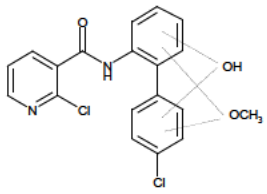
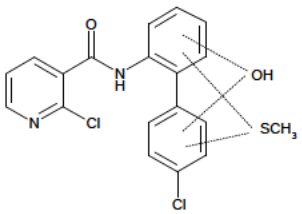
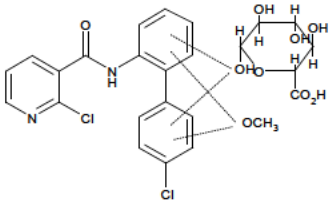
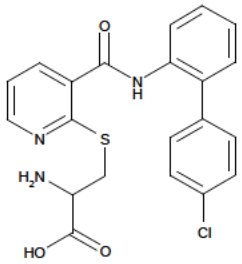
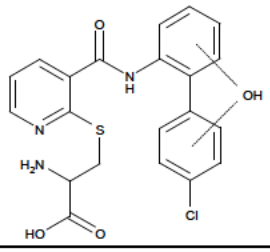
VI Metabolite M510F62

M510F62	Occurrence	TRR (mg/kg)	TRR (%)
	Bean plant	0.009	0.02
	Green bean	0.006	0.61
	Bean seed	0.001	0.71
	Bean Straw	0.61	0.6
Occurrence in animal matrices: No Occurrence in plant matrices: Impurity findings			

The metabolite M510F62 (Reg. No. 363487, 4'-chlorobiphenyl-2-amine; CAS No.: 1204-44-0) has not been detected in the rat metabolism study. In overview 5.8.1-2 classification was as 'impurity findings without relevance to consumers'. The relevance of metabolite M510F62 was not identified for consumers since the analytical findings in the bean matrices shown above have been assessed as originating from the test substance application rather than to be a true finding of plant metabolism. Further reasoning is provided in M-CA 6.2.

VII Other metabolites occurring in urine and/or faeces of goats

The following metabolites were found in the urine of goats.

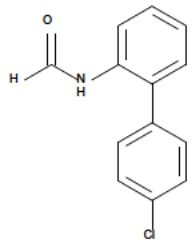
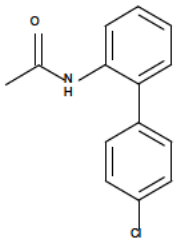
M510F59	M510F50	M510F16
		
M510F20	**M510F41**	**M510F05**
		
M510F22		
		

These metabolites were mostly detected in trace amounts in the rat metabolism, or were present at limited levels (M510F20, M510F05, see Overview 5.8.1-2). Most of these metabolites could not be individually quantified in goat matrices and were expressed as TRR combined with others of the group shown above (see Overview 5.8.1-2). Due to the rapid excretion by the goat the metabolites M510F59, M510F50, M510F16, M510F20, M510F41, M510F05 and M510F22 were only found in urine and/or faeces of goats with no findings in edible matrices and have therefore not been considered as relevant for the consumer risk assessment.

In the overall conclusion the toxicological relevance of metabolites M510F59, M510F50, M510F16, M510F20, M510F41, M510F05 and M510F22 has been adequately addressed. Given the non-existence in edible matrices of animal origin and the absence in plant matrices at all no toxicological concern has been identified.

VIII Metabolites M510F52 and M510F53 found in liver and milk

The following metabolites were found in the liver of the hen and goat (M510F52) and the liver and milk of the goat, respectively (M510F53).

M510F52	M510F53
	

Both compounds were not detected in the rat metabolism. Metabolites M510F52 and M510F53 have been considered as artefacts generated under harsh conditions of the extraction procedure applied and do therefore not represent actual metabolites occurring in these matrices. Therefore, from the toxicological and consumer exposure perspective these metabolites are considered to be not relevant.

In the overall conclusion the toxicological relevance of metabolites M510F52, and M510F53 has been adequately addressed. Both compounds have been considered to be artefacts of specific extraction procedures chosen for bound residues in the matrices liver and milk. Thus, they do not represent virtual metabolites. No toxicological concern has been identified.

2-parachlorobenzoic acid (metabolite M510F64)

Within the submission of the original dossier for Annex I inclusion information on para-chlorobenzoic acid (syn. 4-chlorobenzoic acid, CAS No. 74-11-3) was provided which was found to be a degradation product of Boscalid in the aquatic environment**. It has thus been considered not to be relevant in regard to human risk assessment.** Therefore the information shown below gives essentially the same review as the Monograph of November 08, 2002 provided.

A literature review as done at this time resulted in a number of findings regarding the acute toxicity in several species, studies with repeated administration and information on mutagenicity. A useful summary is provided in the German MAK documentation on chlorobenzoic acid and its isomers [TOX2001-737; MAK "Chlorbenzoesäure und ihre Isomeren", June 1985] and the results are presented below have been excerpted from this documentation. Results from acute toxicity testing are presented below.

Table 5.8.1-18: Parachlorobenzoic acid: summary of acute toxicity studies

Species tested	LD50 (mg/kg bw)
Mouse	661 (515 - 925
Guinea pig	1050
Rat	4170 (3723 – 4670)

In a repeated dose application study the oral administration of 1500 mg/day of parachlorobenzoic acid to rabbits for a period of six months did not result in adverse effects. Likewise, the feeding of 13 or 26 mg/d over a period of five months also did not induce adverse effects. In a drinking water study the administration of 0.3 mg/kg bw/day to rats over a period of six months did not induce toxic changes in the animals.

The feeding of 13 and 26 mg/rat/day over a period of five months did not induce adverse effects on the number of offspring and did not induce developmental toxicity.

Monochlorinated benzoic acids did not induce mutagenicity in the Ames test in several strains, a negative response was observed in sister chromatid exchange tests performed in lymphocytes of rabbits.

In view of the transient nature of the compound in the aquatic environment no need for further toxicological evaluation has been identified.

CA 5.8.2 Supplementary studies on the active substance

BAS 510 F - Hepatic enzyme induction study in Wistar rats - Administration in the diet for 2 weeks (et al., 1999)
1999/10522

Guidelines:	none applicable to this study type
GLP:	yes (certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)
Acceptance:	the study has been evaluated and considered acceptable in the EU registration process for the first Annex I inclusion

Note: This study report has been part of the documentation for the first Annex I inclusion process and a detailed summary is presented here to assist in discussion of the mode of action regarding thyroid effects

Executive Summary

BAS 510 F (Boscalid) was administered to groups of 5 male and 5 female Wistar rats at dietary dose levels of 0 and 15000 ppm for 2 weeks. Food consumption and body weights were determined weekly and the animals were examined for signs of toxicity or mortality at least once a day. At the end of the study, liver were weighed and several enzyme activities were determined, including Ethoxyresorufin-O-deethylase (EROD), Pentoxyresorufin-O-dependentylase (PROD), Cytochrome P450-content (CYP), Glutathione (GSH), Cyanide-insensitive Palmitoyl-CoA-oxidation (PALCoA), Lipidperoxidation "TBA-reactive material" using liver microsomes. Furthermore, the liver was examined by the use of light and electron microscopy. Additional groups of 3 animals per sex at the same dose levels and treatment conditions were subjected to perfusion fixation.
No mortality was observed during the study period and no clinical signs were observed. Treatment with the test substance had no effect on food or water consumption or body weight. Liver weights of treated animals were statistically significantly increased by 32% and 23% in males and females, respectively. Increased CYP P450 content was observed in the livers of treated animals of both sexes. Lipidperoxidation, determined as "TBA-reactive material", was slightly increased in males but not in females. Microscopy revealed proliferation/accumulation of smooth endoplasmatic reticulum in zone 3 hepatocytes, as well as glycogen depletion in the respective hepatocytes.
In conclusion, BAS 510 F (Boscalid) can be assessed as an inducer of rat liver cytochrome P450. The structural changes observed can be seen in the context with this enzyme induction.
(DocID 1999/10522)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:	BAS 510 F
Description:	solid / white
Lot/Batch #:	N26 /Tox Charge II
Purity:	95.3%
Stability of test compound:	The stability and homogeneity was determined analytically after the in-life phase.
2. Vehicle:	none (dietary administration)
3. Test animals:	
Species:	Rat
Strain:	Wistar rats (Chbb:THOM(SPF))
Sex:	male and female
Age:	42 days at the start of the administration period
Weight at dosing:	185 - 190 g (males), 145 – 154 (females)
Source:	Dr. Karl Thomae GmbH, Biberach/Riss, Germany
Acclimation period:	9 days
Diet:	Kliba maintenance diet rat/mouse/hamster, meal, Klingentalmühle AG, Kaiseraugst, Switzerland, ad libitum
Water:	Tap water, ad libitum
Housing:	Single housing in type DK III stainless steel with wire-mesh cages (Becker&Co., Castrop-Rauxel, Germany).
Environmental conditions:	
Temperature:	20 - 24°C
Humidity:	30 - 70%
Air changes:	central air-conditioning
Photo period:	12 h light / 12 h dark (06:00 am - 06:00 pm/ 06:00 pm - 06:00 am)

B. STUDY DESIGN AND METHODS

1. Dates of experimental work: 29/10/1997 - 12/11/1997

2. Animal assignment and treatment:
On day of arrival, the animals were subjected to an acclimatization period during which they received ground diet and drinking water ad libitum. Prior to the first detailed clinical observation, the animals were distributed according to weight among the individual test groups.
The test substance was administered daily via the diet for 2 weeks. Control animals received only the ground diet without test substance addition. Groups of 5 animals per sex were administered a diet with 0 and 15000 ppm of the test item for 2 weeks (designation 00 & 01 for control and treatment groups). Two additional groups of 3 animals per sex, subjected to perfusion fixation for subsequent electron microscopy evaluation of the liver, were also administered a diet with 0 and 15000 ppm of the test item for 2 weeks (group designation 10 & 11 for control and treatment groups). After the treatment period the animals were sacrificed.

3. Test substance preparation and analysis:
The test substance was weighed out and thoroughly mixed with a small amount of food in a beaker. Subsequently a premix was prepared in a household mixer. Then food was added to the premix in order to obtain the desired concentration, and mixing was carried out for about 10 minutes in a laboratory mixer. The test substance preparation was mixed once before the start of the administration period. The food in the food hoppers was changed weekly.

4. Statistics:
Means and standard deviations of each test group were calculated for all parameters. Further statistical analysis was performed according to the following tables:

Statistics of clinical examinations

Parameter	Statistical test
Food consumption, body weight, body weight change, food efficiency	A comparison of the dose group with the control group using STUDENT´s T-TEST (two-sided) for the hypothesis of equal means.

Statistics of bioanalytics

Parameter	Statistical test
PALCoA	Comparison of each dose group with the control group was performed using the MANN-WHITNEY U- test (two-sided) for the hypothesis of equal medians
GSH, liver weight	Comparison of each dose group with the control group was performed using the WILCOXON test (two-sided) for the hypothesis of equal medians
CYP, EROD, PROD	Comparison of each dose group with the control group was performed using the WILCOXON test (one-sided) for the hypothesis of equal medians

C. METHODS

1. Clinical examinations:
Mortality
All animals were checked twice daily on weekdays or once daily on Saturday, Sunday or at public holidays for deaths and morbidity during the study.

Clinical observations
Detailed clinical observations were carried out once daily.

2. Food consumption and intake of test substance
Food consumption was determined weekly over a period of 7 days and calculated as mean food consumption in grams per animal and day. The mean daily intake of test substance (group means) was calculated based upon individual values for body weight and food consumption:

$$IT_x = \frac{FCx}{BWx}\ x\ C$$

FC_x = mean daily food consumption on study day x [g]
BW_x = body weight on study day x [g]
IT_x= intake of test substance on study day x [mg/kg bw/day]
C = concentration [mg/kg]

3. Food efficiency
Food efficiency (group means) was calculated based upon individual values for body weight and food consumption:

$$\text{Food efficiency for day x} = \frac{BWx - BWy}{FCy\ to\ x}\ x\ 100$$

BW_x = body weight on study day x [g]
BW_y = body weight on study day y (last weighing date before day x) [g]
$FC_{y\ to\ x}$ = mean food consumption from day y to day x; calculated as mean daily food consumption on day x, multiplied with the number of days from day y to day x [g]

4. Body weight data
Body weights of all animals were recorded at the day of administration and weekly thereafter.

5. Pathology and microscopy:
The test animals selected for electron microscopy were deeply anesthetized (Nembutal, 4 mL/kg bw) at the end of the study and sacrificed by perfusion fixation. Cacodylate buffer served as rinsing solution and a solution of 5%. Glutaraldehyde in cacodylate buffer served as fixative.
For the light and electron microscopic examination, the liver was removed.
All perfused male and female animals were plastic embedded (Epoxy resin), semithin sectioned and stained with Azure-II-methylene blue-basic Fuchsin (AMbF). In addition all perfused female animals were ultrathin sectioned and stained.

Semithin sections were examined light-microscopically and assessed. The ultrathin sections were examined by electron microscopy and assessed. Selected regions of ultrathin sections were photographed.

6. Bioanalytics
The animals were killed by decapitation under CO_2 anesthesia. The liver was taken from the carcass and weighed. The liver was perfused with about 20 mL of a 0.9% NaCl solution via the portal vein. Liver microsomes and S9-fraction were prepared by (ultra)centrifugation procedures. The following enzymatic parameters were examined in 5 animals per test group and sex on day 14 after beginning of test substance administration:

- Cyanide-insensitive Palmitoyl-CoA-oxidation (PALCoA)
- Cytochrome P450-content (CYP)
- Ethoxyresorufin-O-deethylase (EROD)
- Pentoxyresorufin-O-dependentylase (PROD)
- Glutathione (GSH)
- Lipidperoxidation (“Thiobarbituric acid (TBA)-reactive material”)

PALCoA-oxidation and total protein concentrations were measured with an automatic enzyme analyzer. The content of CYP in liver microsomes as well as lipidperoxidation and GSH concentration was measured photometrically. Activities of EROD and PROD in the S9-fraction were determined fluorimetrically.

II. RESULTS AND DISCUSSION

A. TEST SUBSTANCE ANALYSES

The stability of the test substance in the diet was proven over a period of 32 days at room temperature prior to the study. As the mixtures were stored no longer than this time period, the stability was guaranteed. The concentration-control analyses for the 15000 ppm dose group showed a recovery of 103.5% of the target concentration.

B. OBSERVATIONS

1. Clinical signs of toxicity

No abnormal clinical signs of toxicity were observed.

2. Mortality

No animal died prematurely during the study.

C. BODY WEIGHT

There were no significant differences in body weights between treated and control groups during the study.

Table 5.8.2-1: Body weight data of male and female animals on days 0, 7, and 14

Dose group	Males			Females		
	Day 0	Day 7	Day 14	Day 0	Day 7	Day 14
Group 00 (0 ppm)	186.7 ± 7.0	237.2 ± 10.1	286.7 ± 15.0	148.4 ± 3.7	169.9 ± 4.1	191.7 ± 5.7
Group 01 (15000 ppm)	189.6 ± 5.6	241.7 ± 8.2	290.9 ± 7.3	154.2 ± 9.3	178.2 ± 12.5	197.8 ± 16.6
Group 10 (0 ppm)	189.0 ± 2.2	238.1 ± 6.1	284.5 ± 7.3	153.3 ± 0.5	165.8 ± 3.9	183.7 ± 3.2
Group 11 (15000 ppm)	185.1 ± 3.9	235.3 ± 5.9	280.2 ± 11.6	145.1 ± 9.5	174.2 ± 5.6	199.2 ± 11.8

D. FOOD/WATER CONSUMPTION AND COMPOUND INTAKE

There were no significant differences in food or water consumption as well as food efficiency between treated and control groups during the study.

The test substance intake (mg/kg/day) for each group is given in the following table:

Table 5.8.2-2: Calculated mean daily test substance intake

Concentration in the vehicle (ppm)	Mean daily test-substance intake (mg/kg bw/day)	
	Males	Females
15000, test group 01	1507	1494
15000, test group 11	1405	1556

E. CLINICAL PATHOLOGY AND BIOANALYTICS

Liver examinations

No treatment-related changes were determined in the activity of Cyanide-insensitive Palmitoyl-CoA-oxidation in both sexes.

Liver cytochrome P450 contents were statistically significantly increased by 124% in treated males and 74% in females.

EROD, PROD, and GSH activities were not significantly altered by treatment in either sex. However, in the treated animals a tendency to increased activities is obvious and was more pronounced in male animals. Concentrations of TBA-reactive material in liver showed a slight, statistically significant increase in males but no difference between treated and non-treated females.

Table 5.8.2-3: Results of bioanalytics performed in both sexes of both dose groups

	Males		Females	
Group	**00**	**01**	**00**	**01**
Dose [ppm]	**0**	**15000**	**0**	**15000**
CYP [nmol/mg protein]	0.145 ± 0.01	**0.325 ± 0.114****	0.084 ± 0.019	**0.146 ± 0.017****
EROD [pmol/min/mg protein]	2.18 ± 1.85	3.30 ± 1.79	3.10 ± 0.56	3.26 ± 2.37
PROD [pmol/min/mg protein]	4.33 ± 1.46	8.02 ± 2.26	3.25 ± 0.70	4.12 ± 1.46
GSH [µmol/g]	4.97 ± 1.52	5.60 ± 0.82	5.66 ± 0.18	6.08 ± 0.50
TBA-reactive material [nmol/g tissue]	9.8 ± 6.7	**20.1 ± 5.4****	1.0 ± 0.2	0.8 ± 0.9

** $p \leq 0.01$ Wilcoxon test (two sided)

F. PATHOLOGY

1. Gross lesions

There were no gross lesions noted.

2. Liver weights

After 14 days of treatment, liver weights were statistically significantly increased by 32% in males and 23% in females [see Table 5.8.2-4].

Table 5.8.2-4: Liver weights of male and female animals of all dose groups

Dose group	Liver Weights [g]	
	Males	**Females**
Group 00 (0 ppm)	13.54 ± 1.21	8.77 ± 0.73
Group 01 (15000 ppm)	**17.91 ± 0.77****	**10.81 ± 0.82****

** $p \leq 0.01$ Wilcoxon test (two sided)

3. Light microscopy

There were only spontaneous lesions noted (microgranuloma, graded with 1 or 2).

4. Electron microscopy

Zone 3 hepatocytes of the treated liver showed an increased amount of smooth endoplasmic reticulum (SER) in their cytoplasm, graded with low up to high (+/+++). The accumulation of the SER is seen to be caused by the treatment. Glycogen content was also moderately (++) decreased in the treated animals. Other cell organelles were present (P) but not pathologically changed.

III. CONCLUSIONS

The administration of Boscalid to male and female Wistar rats at a dose level of 15000 ppm led to an induction of rat liver cytochrome P450 enzyme family. This finding has been considered to be supported by the significant increase in liver weight and the structural changes observed

Report:	CA 5.8.2/1 [redacted] et al., 2003 c BAS 510 F - Study on enzyme induction in the liver and determination of thyroid hormones in Wistar rats - Administration in the diet for 28 days 2003/1012736
Guidelines:	none
GLP:	yes (certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)
Acceptance:	the study has been evaluated and considered acceptable in the EU registration process for the first Annex I inclusion

Note: This study report has **not** been part of the documentation for the first Annex I inclusion process and a full evaluation is therefore presented here to assist in discussion of the mode of action regarding thyroid effects

Executive Summary

BAS 510 F (Boscalid) was administered to groups of 10 male and 10 female Wistar rats at dietary dose levels of 500, 2000, and 5000 ppm for 4 weeks. Food consumption and body weights were determined weekly and the animals were examined for signs of toxicity or mortality at least once a day. Thyroid hormones (T3, T4, TSH) in the serum were determined on day 7, 14, and 28. At the end of the study, liver and thyroids were weighed and several phase I (EROD, PROD, BROD, CYP) and phase II (pNP-GT, MUF-GT, HOBI-GT) enzymes were determined using liver microsomes.

No mortality was observed during the study period and no clinical signs were observed. Treatment with the test substance had no effect on food or water consumption or body weight. Decreased T4 levels were observed at least in the males of the mid and high dose group. Increased TSH levels were observed in the males of the mid and high dose group. In the high dose females TSH levels were consistently increased (18 -32%), although no statistically significance was observed. Increased absolute liver (up to 32%) and thyroid (up to 46.7%) weights were observed in treated animals of both sexes. Similar results were observed for relative weights. In general, the liver effect was more pronounced in male animals. Most of the phase I and phase II enzyme activities were increased in treated animals of both sexes at least at the mid and high dose.

Overall, treatment with Boscalid led to decreases in thyroxine (T4), distinct increases in TSH, increased liver and thyroid weights as well as increased activities of phase I and II enzymes. It can be concluded that the mild imbalance in thyroid hormone levels caused by Boscalid was due to the induction of hepatic microsomal enzyme system.

(DocID 2003/1012736)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:	BAS 510 F
Description:	solid / white
Lot/Batch #:	N46
Purity:	95.7%
Stability of test compound:	The stability of the test substance under storage conditions over the test period was guaranteed (expiry date October 2004).
2. Vehicle:	none (dietary administration)
3. Test animals:	
Species:	Rat
Strain:	CrlGlxBrlHan:WI
Sex:	male and female
Age:	54-58 days
Weight at dosing:	288.4 - 359.8 g (males), 184.2 – 243.0 (females)
Source:	Charles River, Sulzfeld, Germany
Acclimation period:	approximately 4 weeks
Diet:	Kliba maintenance diet rat/mouse/hamster, meal, Provimi Kliba SA, Kaiseraugst, Switzerland, ad libitum
Water:	Tap water, ad libitum
Housing:	Single housing in type DK III stainless steel with wire-mesh cages (Becker&Co., Castrop-Rauxel, Germany).
Environmental conditions:	
Temperature:	20 - 24°C
Humidity:	30 - 70%
Air changes:	central air-conditioning
Photo period:	12 h light / 12 h dark (06:00 am - 06:00 pm/ 06:00 pm - 06:00 am)

B. STUDY DESIGN AND METHODS

1. Dates of experimental work: 03/13/2003 - 06/16/2003

2. Animal assignment and treatment:
On day of arrival, the animals were subjected to an acclimatization period during which they received ground diet and drinking water ad libitum. Prior to the first detailed clinical observation, the animals were distributed according to weight among the individual test groups. The weight variation of the animals used did not exceed 20 percent of the mean weight.
The test substance was administered daily via the diet for 4 weeks. Control animals received only the ground diet without test substance addition. Groups of 10 animals per sex were administered a diet with 0, 500, 2000, and 5000 ppm of the test item for 4 weeks. After the treatment period the animals were sacrificed without fasting period.

3. Test substance preparation and analysis:
Prior to the present study, a stability test was performed on test diet samples and it was confirmed that the test substance was stable in the diet over a period of 32 days at room temperature. No further analyses were performed within the study.

4. Statistics:
Means and standard deviations of each test group were calculated for all parameters. Further statistical analysis was performed according to the following tables:

Statistics of clinical examinations

Parameter	Statistical test
Food consumption, body weight	A comparison of each group with the control group was performed using DUNNETT's test (two-sided) for the hypothesis of equal means

Statistics of clinical pathology

Parameter	Statistical test
Hormone parameters	Non-parametric one-way analysis using KRUSKAL-WALLIS test. If the resulting p-value was equal or less than 0.05, a pairwise comparison of each dose group with the control group was performed using WILCOXON-test (two-sided) for the hypothesis of equal medians.

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

Statistics of bioanalytics

Parameter	Statistical test
Phase I and II enzyme activities, CYP content	Comparison of each dose group with the control group was performed using the WILCOXON test (one-sided) for the hypothesis of equal medians

C. METHODS

1. Clinical examinations:
Mortality
All animals were checked twice daily on weekdays or once daily on Saturday, Sunday or at public holidays for deaths and morbidity during the study.

Clinical observations
Detailed clinical observations were carried out once daily.

2. Food consumption and intake of test substance
Food consumption was determined weekly over a period of 7 days and calculated as mean food consumption in grams per animal and day. The mean daily intake of test substance (group means) was calculated based upon individual values for body weight and food consumption:

$$IT_x = \frac{FCx}{BWx}\ x\ C$$

FC_x = mean daily food consumption on study day x [g]
BW_x = body weight on study day x [g]
IT_x= intake of test substance on study day x [mg/kg bw/day]
C = concentration [mg/kg]

3. Body weight data
Body weights of all animals were recorded at the day of administration and weekly thereafter.

4. Clinical pathology
Blood was taken from the retroorbital venous plexus in the morning from non-fasted animals without anesthesia for hormone examinations. Hormone levels were determined by radioimmunoassay, using commercially available RIA test kits and a Gamma-counter. Levels of total triiodothyronine (T3), total thyroxine (T4) and thyroid stimulating hormone (TSH) were determined.

5. Sacrifice and pathology:
Weight parameters
Terminal body weight, liver weight and weight of thyroid glands (with parathyroid glands) were measured for all animals killed on schedule.

Histopathology
No microscopic investigations were performed.

6. Bioanalytics

The animals were killed by cervical dislocation and decapitation. The liver was taken from the carcass and weighed. The liver was perfused with about 15 mL of a 0.9% NaCl solution via the portal vein. Liver microsomes and S9-fraction were prepared by (ultra)centrifugation procedures. The following enzymatic parameters were examined:

Phase I enzymes

- Cytochrome P450-content (CYP)
- Ethoxyresorufin-O-deethylase (EROD)
- Pentoxyresorufin-O-dependentylase (PROD)
- Benzyloxyresorufin-O-debenzylase (BROD)

Phase II enzymes

- P-Nitrophenol-glucuronyltransferase (pNP-GT)
- 4-Methylumbelliferone-glucuronyltransferase (MUF-GT)
- 4-Hydroxybiphenyl-glucuronyltransferase (HOBI-GT)

The content of CYP in liver microsomes was measured photometrically. Activities of EROD, PROD and BROD in the S9-fraction were determined fluorimetrically. pNP-GT in liver microsomes was measured photometrically. MUF- and HOBI-GT in liver microsomes were measured fluorimetrically. Liver S9-fraction from Aroclor 1254 treated rats served as positive control for verification of assay conditions. The examinations were carried out in 10 animals per dose level and sex, which were split for technical reasons of liver processing into two groups of 5 animals each (Group A and B) which were evaluated separately.

II. RESULTS AND DISCUSSION

A. TEST SUBSTANCE ANALYSES

The stability of the test substance in the diet was proven over a period of 32 days at room temperature prior to the study. As the mixtures were stored no longer than this time period, the stability was guaranteed. During the study, no analyses of the test substance preparations were carried out.

B. OBSERVATIONS

1. Clinical signs of toxicity

No abnormal clinical signs of toxicity were observed.

2. Mortality

No animal died prematurely during the study.

C. BODY WEIGHT

There were no significant differences in body weights between treated and control groups during the study.

D. FOOD/WATER CONSUMPTION AND COMPOUND INTAKE

There were no significant differences in food or water consumption between treated and control groups during the study.

The test substance intake (mg/kg/day) for each group is given in the following table:

Table 5.8.2-5: Calculated mean daily test substance intake

Concentration in the vehicle (ppm)	Mean daily test-substance intake (mg/kg bw/day)	
	Males	Females
500	29.6	34.6
2000	117.3	141.9
5000	294.3	355.3

E. CLINICAL PATHOLOGY

Hormone levels

Total triiodothyronine (T3)

There were no treatment-related changes in serum concentrations of total triiodothyronine (T3) in both sexes after 7, 14 and 28 days of test substance administration.

Table 5.8.2-6: Total triiodothyronine (T3) [nmol/L] in the serum of male and female rats

		Males			Females		
		Day 7	Day 14	Day 28	Day 7	Day 14	Day 28
Group 0	Mean	1.60	1.78	1.51	1.70	1.80	1.64
0 ppm	-	-	-	-	-	-	-
Group 1	Mean	1.65	1.88	1.49	1.72	1.79	1.63
500 ppm	%dev.	3	5	-1	1	-1	-1
Group 2	Mean	1.62	1.86	1.39	1.51	1.63	1.63
2000 ppm	%dev.	1	4	-8	-12	-10	-1
Group 3	Mean	1.52	1.84	1.43	1.69	1.72	1.58
5000 ppm	%dev.	-5	3	-5	-1	-5	-4

%dev. = percent deviation compared to vehicle control

Total thyroxine (T4)

Total thyroxine (T4) determinations in the serum of the treated males revealed a tendency towards decreased values in the animals of the mid- and high-dose groups on day 7 and in the animals of the high-dose group on day 14. Although the decreases thyroxine levels were not statistically significant, they are regarded as treatment-related. No test substance-related findings were observed in the peripheral blood of treated females at all time intervals.

Table 5.8.2-7: Total thyroxine (T4) [nmol/L] in the serum of male and female rats

		Males			Females		
		Day 7	Day 14	Day 28	Day 7	Day 14	Day 28
Group 0	Mean	58.80	57.08	61.98	46.95	44.54	44.88
0 ppm	-	-	-	-	-	-	-
Group 1	Mean	59.40	57.78	65.15	45.10	41.97	49.64
500 ppm	%dev.	1	1	5	-4	-6	11
Group 2	Mean	56.04	56.89	61.67	41.99	43.60	50.27
2000 ppm	%dev.	-5	0	-1	-11	-2	12
Group 3	Mean	52.57	53.33	61.33	48.63	50.20	45.25
5000 ppm	%dev.	-11	-7	-1	4	13	1

%dev. = percent deviation compared to vehicle control

Thyroid stimulating hormone (TSH)
TSH levels were slightly increased in the serum of the males of the 2000 ppm and 5000 ppm dose groups at all time intervals. The increases on day 7 and the increase in the high-dose males on day 14 were statistically significantly different compared to the concurrent controls. A tendency towards increased TSH concentrations was also found in the serum of the high dose females throughout the entire course of the study. The increased TSH levels are considered to be treatment-related.

Table 5.8.2-8: TSH [µg/L] in the serum of male and female rats

		Males			Females		
		Day 7	Day 14	Day 28	Day 7	Day 14	Day 28
Group 0	Mean	9.50	10.16	8.11	6.26	6.02	6.51
0 ppm	-	-	-	-	-	-	-
Group 1	Mean	9.10	9.97	9.05	6.85	6.25	7.29
500 ppm	%dev.	-4	-2	12	9	4	12
Group 2	Mean	**14.27***	**13.66**	**10.87**	6.72	6.08	7.56
2000 ppm	%dev.	**50**	**34**	**34**	7	1	16
Group 3	Mean	**14.64****	**16.47****	**11.00**	**7.41**	**7.11**	**8.59**
5000 ppm	%dev.	**54**	**62**	**36**	**18**	**18**	**32**

*: $p \leq 0.05$; **: $p \leq 0.01$ Kruskal-Wallis and Wilcoxon test (two sided)
%dev. = percent deviation compared to vehicle control

F. PATHOLOGY

1. Absolute weights

The mean terminal body weights of the animals treated with Boscalid did not differ significantly from controls. The mean liver weight were increased in males of all treatment groups by 7.7%, 21% or 32%, respectively. Differences in absolute liver weight were statistically significantly increased at dose levels of 2000 ppm and 5000 ppm and are shown in the table below. In the females of the high dose group the liver weight was slightly, but not statistically significantly above the control values (+10.5%).
The mean weight of the thyroid glands were increased in males of all treatment groups by 14.3%, 8.2% or 25.3%, respectively, with no dose-response relationship or statistical significance. The mean weight of the thyroid glands were also increased in females of all treatment groups by 17%, 16.3% or 46.7%, with statistical significance gained in the mid and high dose group but without a clear dose-response relationship.
The liver and thyroid weight parameters that were significantly increased were regarded to be treatment-related.

Table 5.8.2-9: Absolute body and organ weights of liver and thyroids (Mean ± SD)

	Males				Females			
Dose [ppm]	**0**	**500**	**2000**	**5000**	**0**	**500**	**2000**	**5000**
Body weight [g]	366 ± 20	369 ± 20	377 ± 17	377 ± 23	224 ± 17	220 ± 14	217 ± 10	220 ± 11
Liver [g]	12.1 ± 1.2	13.1 ± 1.3	**14.7 ± 1.1****	**16.0 ± 1.7****	8.4 ± 0.9	8.1 ± 0.7	8.4 ± 0.6	9.2 ± 1.2
Thyroid gland [mg]	18.2 ± 2.6	20.8 ± 1.3	19.7 ± 3.5	22.8 ± 4.9	13.5 ± 2.7	15.8 ± 2.3	**15.7 ± 2.3***	**19.8 ± 4.4****

*: $p \leq 0.05$; **: $p \leq 0.01$ Kruskal-Wallis and Wilcoxon test (two sided)

2. Relative weights

The mean relative liver weights were increased in males of all treatment groups by 6.8%, 17.6% or 28.3%, respectively, showing a dose response relationship and statistical significance in all dose groups. In the females of the high dose group the relative liver weight was slightly but statistically significantly above the control values (+12.3%).

The mean relative weight of the thyroid glands were increased in males of the low and high dose groups by 20% with no dose-response relationship or statistical significance. The mean relative weight of the thyroid glands were increased in females of all treatment groups by 17%, 17% and 50%, with statistical significance in the high dose group.

The liver and thyroid weight parameters that were significantly increased were regarded to be treatment-related.

Table 5.8.2-10: Relative organ weights of liver and thyroids (Mean ± SD)

	Males				Females			
Dose [ppm]	**0**	**500**	**2000**	**5000**	**0**	**500**	**2000**	**5000**
Liver [%]	3.31 ± 0.20	**3.54 ± 0.21***	**3.89 ± 0.21****	**4.25 ± 0.30****	3.73 ± 0.35	3.67 ± 0.22	3.86 ± 0.26	**4.19 ± 0.39***
Thyroid gland [%]	0.005 ± 0.001	0.006 ± 0.001	0.005 ± 0.001	0.006 ± 0.001	0.006 ± 0.001	0.007 ± 0.001	0.007 ± 0.001	**0.009 ± 0.002****

*: $p \leq 0.05$; **: $p \leq 0.01$ Kruskal-Wallis and Wilcoxon test (two sided)

G. BIOANALYTICS

Cytochrome P450-content (CYP)
There was a slight trend to increased microsomal CYP contents ion the liver with increasing dose levels in both sexes, which gained statistical significance in one female group at the mid dose level only. However, this trend was not consistently reflected in all groups.

Ethoxyresorufin-O-deethylase (EROD)
EROD activities in the S9-fraction of liver homogenates were slightly and in general significantly increased at the high and mid dose level in both sexes. This increase in EROD activity indicates slight unspecific induction.

Pentoxyresorufin-O-depentylase (PROD)
PROD activities in the S9-fraction of liver homogenates showed a slight increase at the high and mid dose level in both sexes, which in most cases gained statistical significance. This slight increase in PROD activity indicates unspecific induction.

Benzyloxyresorufin-O-debenzylase (BROD)
BROD activities in the S9-fraction of liver homogenates were moderately and significantly increased at the high and mid dose level in both sexes.

p-Nitrophenol-glucuronyltransferase (pNP-GT)
pNP-GT activities in liver microsomes were moderately and significantly increased at all three dose levels in both sexes. There was indication of a dose response relationship in both sexes.

4-Methylumbelliferone-glucuronyltransferase (MUF-GT)
MUF-GT activities in liver microsomes were moderately and significantly increased at all three dose levels in both sexes, but were less pronounced in females. There was indication of a dose response relationship in both sexes.

4-Hydroxybiphenyl-glucuronyltransferase (HOBI-GT)
HOBI-GT activities in liver microsomes were moderately and significantly increased at all dose levels in males and at the mid and high dose in females. The increases were less pronounced in females. There was indication of a dose response relationship in both sexes.

Table 5.8.2-11: Results of enzyme assays performed in the first and second sacrifice group (Mean ± SD of 5 animals each)

		Males				Females			
	Group	**0**	**500**	**2000**	**5000**	**0**	**500**	**2000**	**5000**
CYP [nmol/mg protein]	A	5.37 ± 0.47	5.62 ± 1.10	6.07 ± 1.06	7.02 ± 1.96	5.72 ± 0.19	6.14 ± 0.56	**6.99 ± 0.17****	6.47 ± 0.96
	B	5.12 ± 0.45	5.10 ± 0.24	4.62 ± 0.39	4.75 ± 1.36	5.04 ± 1.18	5.03 ± 0.34	5.36 ± 0.22	5.48 ± 0.51
EROD [pmol/min/mg protein]	A	3.06 ± 0.19	3.18 ± 0.53	**4.18 ±0.90****	**4.51 ±0.83****	1.85 ± 1.15	1.85 ± 1.33	**5.06 ± 1.52****	**4.86 ±1.04****
	B	26.86 ± 7.87	34.09 ± 4.49	36.48 ± 6.24	**36.51 ±1.83****	3.89 ± 1.47	5.48 ± 1.99	**6.11 ± 1.62***	5.13 ± 1.16
PROD [pmol/min/mg protein]	A	4.43 ± 0.52	**5.22 ± 0.53***	5.24 ± 1.10	4.92 ± 0.68	14.95 ± 2.87	**18.46 ±1.45***	**22.96 ± 3.96****	**25.67 ±3.61****
	B	14.67 ± 4.75	14.88 ± 2.55	**23.03 ± 3.82***	**22.56 ±1.70****	1.72 ± 0.53	2.15 ± 1.04	2.65 ± 0.96	**2.82 ± 0.87***
BROD [pmol/min/mg protein]	A	2.73 ± 1.47	4.93 ± 2.33	**7.23 ±1.72****	**8.24 ±3.12****	1.50 ± 0.87	1.72 ± 0.91	**16.09 ±10.99****	**30.09 ±15.31****
	B	51.51 ± 12.97	57.46 ± 9.92	65.72 ± 9.21	**71.55 ±2.31****	2.41 ± 0.89	4.07± 1.79	**6.83 ± 2.82****	**5.97 ± 3.99***
pNP-GT [µmol/min/mg protein]	A	2.04 ± 0.31	**3.32 ±0.48****	**4.29 ±0.34****	**4.68 ±0.45****	1.03 ± 0.50	1.21 ± 0.53	1.30 ± 0.49	**1.66 ± 0.31***
	B	1.18 ± 0.14	1.34 ± 0.14	**1.53 ± 0.21***	**1.49 ±0.05****	0.88 ± 0.13	**1.15 ±0.14***	**1.75 ± 0.44****	**2.20 ± 0.06****
MUF-GT [FU/min/mg protein]	A	799 ± 67	**1243 ± 279***	**1953 ± 196****	**2793 ±1497****	282 ± 42	349 ± 102	**399 ± 57***	**452 ± 86****
	B	461 ± 61	**740 ± 51****	**834 ± 74****	**1152 ± 249****	127 ± 20	**153 ± 18***	**243 ± 37****	**272 ±17****
HOBI-GT [FU/min/mg protein]	A	101 ± 20	**177 ± 56***	**342 ± 55****	**294 ± 60****	106 ± 10	118 ± 12	**150 ± 7****	**182 ± 21****
	B	82.7 ± 9	**166 ± 16****	**218 ± 47****	**235 ± 47****	78 ± 8	85 ± 7	**117 ± 11****	**130 ± 13****

*: $p \leq 0.05$; **: $p \leq 0.01$ Wilcoxon test (one sided)

III. CONCLUSIONS

The administration of Boscalid to male and female Wistar rats at dose levels of 500, 1000, and 2000 ppm led to decreases in thyroxine (T4), distinct increases in TSH, increased liver and thyroid weights as well as increased activities of phase I and II enzymes. It can be concluded that the mild imbalance in thyroid hormone levels caused by Boscalid was due to the induction of hepatic microsomal enzyme system.

BAS 510 F - Hormone and enzyme induction study in Wistar rats - Administration in the diet for 4 weeks ([redacted] et al., 2001)
2001/1000141

Guidelines:	none applicable to this study type
GLP:	yes (certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)
Acceptance:	the study has been evaluated and considered acceptable in the EU registration process for the first Annex I inclusion

Note: This study report has been part of the documentation for the first Annex I inclusion process and a detailed summary is presented here to assist in discussion of the mode of action regarding thyroid effects

Executive Summary

Boscalid was administered to groups of 5 male and 5 female Wistar rats at dietary dose levels of 0 and 15000 ppm for 4 weeks. Food consumption and body weights were determined weekly and the animals were examined for signs of toxicity or mortality at least once a day. Thyroid hormones (T3, T4, TSH) in the serum were determined on day-3, 2, 4, 7, 14, 21 and 28. At the end of the study, liver were weighed and several phase II (pNP-GT, MUF-GT, HOBI-GT) enzymes were determined using liver microsomes.
No mortality was observed during the study period and no clinical signs were observed. Treatment with the test substance had no effect on food or water consumption or body weight. Liver weights of treated animals were statistically significantly increased by 25% and 22% in males and females, respectively. Decreased T3 and T4 concentrations were observed in male and female animals. TSH levels were increased up to 283% or 277% in male or females, respectively. The phase II enzyme activities investigated (pNP-GT, MUF-GT, HOBI-GT) were all increased in male and female animals between 1.25-fold to 3-fold. In conclusion, the administration of Boscalid caused changes in thyroid hormones as early as 2 days after beginning of the test substance administration, with concomitant induction of phase II enzyme activities.

(DocID 2001/1000141)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:	BAS 510 F
Description:	solid / white
Lot/Batch #:	N46
Purity:	96.3%
Stability of test compound:	The stability was determined by reanalysis
2. Vehicle:	none (dietary administration)

3. Test animals:

Species:	Rat
Strain:	Wistar rats (Chbb:THOM(SPF))
Sex:	male and female
Age:	About 3 months at the start of the administration period
Weight at dosing:	394.6 – 452.2 g (males), 237.7 – 257.5 (females)
Source:	Boehringer Ingelheim Pharma KG, Biberach/Riss, Germany
Acclimation period:	20 days
Diet:	Maintenance diet rat/mouse, 9433 LL Meal, Eberle Nafag AG Gossau Switzerland, ad libitum
Water:	Tap water, ad libitum
Housing:	Single housing in type DK III stainless steel with wire-mesh cages (Becker&Co., Castrop-Rauxel, Germany).

Environmental conditions:

Temperature:	20 - 24°C
Humidity:	30 - 70%
Air changes:	central air-conditioning
Photo period:	12 h light / 12 h dark (06:00 am - 06:00 pm/ 06:00 pm - 06:00 am)

B. STUDY DESIGN AND METHODS

1. Dates of experimental work: 03/07/2000 - 02/08/2000

2. Animal assignment and treatment:
On day of arrival, the animals were subjected to an acclimatization period during and received ground diet and drinking water ad libitum. Prior to the first detailed clinical observation, the animals were distributed according to weight among the individual test groups in randomized form.
The test substance was administered daily via the diet for 4 weeks. Control animals received only the ground diet without test substance addition. Groups of 5 animals per sex were administered a diet with 0 and 15000 ppm of the test item for 2 weeks. After the treatment period the animals were sacrificed.

3. Test substance preparation and analysis:
The test substance was weighed out and thoroughly mixed with a small amount of food in a beaker. Subsequently a premix was prepared in a household mixer. Then food was added to the premix in order to obtain the desired concentration, and mixing was carried out for about 10 minutes in a laboratory mixer. The test substance preparation was mixed once before the start of the administration period.

4. Statistics:
Means and standard deviations of each test group were calculated for all parameters. Further statistical analysis was performed according to the following tables:

Statistics of clinical examinations

Parameter	Statistical test
Food consumption, body weight, body weight change, food efficiency	A comparison of the dose group with the control group using WELCH T-TEST (two-sided) for the hypothesis of equal means.

Statistics of clinical pathology

Parameter	Statistical test
Hormone parameters	Comparison of each dose group with the control group was performed using the MANN-WHITNEY U- test (two-sided) for the hypothesis of equal medians

Statistics of clinical pathology

Parameter	Statistical test
Hormone parameters	Comparison of the dose group with the control group using MAN-WHITNEY U-test (two sided) for the equal medians

Statistics of pathology

Parameter	Statistical test
Weight parameters	Comparison of each dose group with the control group was performed using the WILCOXON test (one-sided) for the hypothesis of equal medians

Statistics of bioanalytics

Parameter	Statistical test
Phase II enzyme activities	Comparison of each dose group with the control group was performed using the WILCOXON test (one-sided) for the hypothesis of equal medians

C. METHODS

1. Clinical examinations:

Mortality

All animals were checked twice daily on weekdays or once daily on Saturday, Sunday or at public holidays for deaths and morbidity during the study.

Clinical observations

Detailed clinical observations were carried out once daily.

2. Food consumption and intake of test substance

Food consumption was determined weekly over a period of 7 days and calculated as mean food consumption in grams per animal and day. The mean daily intake of test substance (group means) was calculated based upon individual values for body weight and food consumption:

$$IT_x = \frac{FCx}{BWx}\ x\ C$$

FC_x = mean daily food consumption on study day x [g]
BW_x = body weight on study day x [g]
IT_x= intake of test substance on study day x [mg/kg bw/day]
C = concentration [mg/kg]

3. Food efficiency

Food efficiency (group means) was calculated based upon individual values for body weight and food consumption:

$$\text{Food efficiency for day x} = \frac{BWx - BWy}{FCy\ to\ x}\ x\ 100$$

BW_x = body weight on study day x [g]
BW_y = body weight on study day y (last weighing date before day x) [g]
$FC_{y\ to\ x}$ = mean food consumption from day y to day x; calculated as mean daily food consumption on day x, multiplied with the number of days from day y to day x [g]

4. Body weight data
Body weights of all animals were recorded at the day of administration and weekly thereafter.

5. Clinical pathology
Blood was taken from the retroorbital venous plexus in the morning from non-fasted animals without anesthesia for hormone examinations. Hormone levels were determined by radioimmunoassay, using commercially available RIA test kits and a Gamma-counter. Levels of total triiodothyronine (T3), total thyroxine (T4) and thyroid stimulating hormone (TSH) were determined.

6. Bioanalytics
The animals were killed by CO_2 anesthesia and decapitation. The liver was taken from the carcass and weighed. Liver microsomes and S9-fraction were prepared by (ultra)centrifugation procedures. The following enzymatic parameters were examined:

Phase II enzymes
- P-Nitrophenol-glucuronyltransferase (pNP-GT)
- 4-Methylumbelliferone-glucuronyltransferase (MUF-GT)
- 4-Hydroxybiphenyl-glucuronyltransferase (HOBI-GT)

pNP-GT in liver microsomes was measured photometrically. MUF- and HOBI-GT in liver microsomes were measured fluorimetrically.

II. RESULTS AND DISCUSSION

A. TEST SUBSTANCE ANALYSES

The stability of the test substance in the diet was proven over a period of 32 days at room temperature prior to the study. As the mixtures were stored no longer than this time period, the stability was guaranteed. The homogeneity and correctness of the concentration was verified (102.2 ± 0.2% of nominal concentration). The nominal content of the preparation was 100 mg/kg. At days 0, 13, and 32 the following percentages of initial content were found, namely 100%, 97.5%, and 97.9%, respectively.

B. OBSERVATIONS

1. Clinical signs of toxicity

No abnormal clinical signs of toxicity were observed.

2. Mortality

No animal died prematurely during the study.

C. BODY WEIGHT

There were no significant differences in body weights between treated and control groups during the study.

Table 5.8.2-12: Body weight data from male and female animals on days 0, 14 and 28

Dose group	Males			Females		
	Day 0	Day 14	Day 28	Day 0	Day 14	Day 28
0 ppm	424.4±18.3	447.3±12.3	459.1 ±17.8	245.2 ± 7.3	249.3 ± 9.4	256.5 ± 8.1
15000 ppm	439.3±9.0	459.4 ±8.9	477.1±10.7	249.8 ± 5.5	251.5 ± 5.1	264.5 ± 5.9

D. FOOD/WATER CONSUMPTION AND COMPOUND INTAKE

There were no significant differences in food or water consumption between treated and control groups during the study. Food efficiency was statistically significantly increased in males of group1 on day 28. Due to the isolated occurrence and the lack of effects on food consumption and body weight, this was assessed as being incidental.

The test substance intake (mg/kg bw/day) for each group is given in the following table:

Table 5.8.2-13: Calculated mean daily test substance intake

Concentration in the vehicle (ppm)	Mean daily test-substance intake (mg/kg bw/day)	
	Males	Females
15000	957	1196.7

E. CLINICAL PATHOLOGY AND BIOANALYTICS

Hormone levels

Total triiodothyronine (T3)

In the treated males decreased serum T3 concentrations (84% to 69% of control) were observed throughout the study. With the exception of day 4 all these changes were seen as a trend toward reduced values. In the females, slight but not statistically significant decreases (99% to 76% of control) in serum T3 levels were found from day 4 onward.

Table 5.8.2-14: Total triiodothyronine (T3) [nmol/L] in the serum of male and female rats

Males							
Dose group	**Day -3**	**Day 2**	**Day 4**	**Day 7**	**Day 14**	**Day 21**	**Day 28**
0 ppm	1.3 ± 0.2	1.4 ± 0.21	1.38 ± 0.26	1.44 ± 0.33	1.44 ± 0.24	1.0 ± 0.20	1.13 ± 0.26
15000 ppm	1.12 ± 0.14	1.18 ± 0.20	**0.96 ± 0.17***	1.13 ± 0.30	1.11 ± 0.27	0.74 ± 0.21	0.79 ± 0.24
% control	86	84	69	79	77	70	71
Females							
Dose group	**Day -3**	**Day 2**	**Day 4**	**Day 7**	**Day 14**	**Day 21**	**Day 28**
0 ppm	1.57 ± 0.14	1.67 ± 0.32	1.63 ± 0.32	1.64 ± 0.26	1.62 ± 0.20	1.14 ± 0.29	1.15 ± 0.26
15000 ppm	1.65 ± 0.11	1.67 ± 0.32	1.35 + 0.11	**1.26 ± 0.08****	1.55 ± 0.28	1.13 ± 0.10	1.06 ± 0.13
% control	105	101	83	76	96	99	92

* $p \leq 0.05$; ** $p \leq 0.02$ Mann_Whitney u-test (two-sided)

Total thyroxine (T4)

In the treated males decreased serum T4 concentrations were observed from day 4 onward. With the exception of day 4 all these changes were seen as a trend toward reduced values. On day 2 this finding was also seen as a trend toward reduced values (87% to 73% of control). In the females, slight but not statistically significant decreases (97% to 87% of control) in serum T4 levels were found from day 7 onward.

Table 5.8.2-15: Total thyroxine (T4) [nmol/L] in the serum of male rats

Dose group	**Males**						
	Day -3	**Day 2**	**Day 4**	**Day 7**	**Day 14**	**Day 21**	**Day 28**
0 ppm	64.16 ± 7.18	66.18 ± 5.77	63.36 ± 4.68	67.46 ± 7.51	65.98 ± 7.00	73.00 ± 6.01	72.73 ± 8.19
15000 ppm	61.98 ± 3.70	57.72 ± 6.13	**51.04 ± 6.84****	**50.49 ± 2.02****	**48.25 ± 4.55****	**56.47 ± 4.58****	**59.88 ± 4.22***
% control	97	87	81	75	73	77	82

* $p \leq 0.05$; ** $p \leq 0.02$ Mann_Whitney u-test (two-sided)

Table 5.8.2-16: Total thyroxine (T4) [nmol/L] in the serum of female rats

Dose group	Males						
	Day -3	**Day 2**	**Day 4**	**Day 7**	**Day 14**	**Day 21**	**Day 28**
0 ppm	50.50 ± 7.32	46.52 ± 8.82	48.92 ± 8.24	51.79 ± 8.87	51.8 ± 11.19	55.65 ± 11.67	51.94 ± 9.40
15000 ppm	56.21 ± 5.34	55.16 ± 1.94	49.82 ± 6.37	45.05 ± 7.47	48.45 ± 6.66	51.54 ± 6.41	50.22 ± 11.05
% control	111	119	102	87	94	93	97

Thyroid stimulating hormone (TSH)

TSH levels were statistically significantly increased in the serum of the males (168% to 283% of control) from day 14 onward. From day 2 through day 7 this finding was also seen as a trend toward increased values. With the exception of day 7 statistically significantly increased TSH concentrations (180% to 277% of control) were found in the sera of the treated females throughout the study.

Table 5.8.2-17: TSH [µg/L] in the serum of male rats

Dose group	Males						
	Day -3	**Day 2**	**Day 4**	**Day 7**	**Day 14**	**Day 21**	**Day 28**
0 ppm (nmol/l)	19.38 ± 9.15	22.21 ± 9.64	20.59 ± 11.60	20.12 ± 12.83	19.32 ± 4.89	14.50 ± 4.40	23.17 ± 8.83
15000 ppm (nmol/l)	19.28 ± 6.06	28.63 ± 12.05	26.20 ± 12.09	32.76 ± 9.98	**36.53 ± 13.75****	**41.02 ± 11.46****	**38.88 ± 14.39***
% control	100	129	127	163	189	283	168

* $p \leq 0.05$; ** $p \leq 0.02$ Mann_Whitney u-test (two-sided)

Table 5.8.2-18: TSH [µg/L] in the serum of female rats

Dose group	Males						
	Day -3	**Day 2**	**Day 4**	**Day 7**	**Day 14**	**Day 21**	**Day 28**
0 ppm (nmol/l)	9.70 ± 4.03	9.31 ± 1.70	9.15 ± 2.09	12.00 ± 7.35	11.70 ± 2.34	9.09 ± 2.87	10.28 ± 1.59
15000 ppm (nmol/l)	10.77 ± 3.94	**16.77 ± 8.39***	**18.21 ± 6.29****	24.80 ± 8.79	**32.39 ± 8.89****	**19.27 ± 5.53****	**22.32 ± 5.45****
% control	111	180	199	207	277	212	217

* $p \leq 0.05$; ** $p \leq 0.02$ Mann_Whitney u-test (two-sided)

Bioanalytics

Liver weights

Liver weights were statistically significantly increased by 25% in males and 22% in females [see Table 5.8.2-19].

Table 5.8.2-19: Liver weights of male and female animals of all dose groups

Dose group	Liver Weights [g]	
	Males	**Females**
Group 00 (0 ppm)	18.08 ± 1.76	9.55 ± 0.69
Group 01 (15000 ppm)	**22.62 ± 1.11***	**11.70 ± 0.53***

* $p \leq 0.05$

p-Nitrophenol-glucuronyltransferase (pNP-GT)

pNP-GT activities were statistically significantly increased in both sexes (about 2-fold in males and 1.25-fold in females).

4-Methylumbelliferone-glucuronyltransferase (MUF-GT)

MUF-GT activities were statistically significantly increased in treated males and females. The increase was 2-fold in males and 2.4-fold in females.

4-Hydroxybiphenyl-glucuronyltransferase (HOBI-GT)

HOBI-GT activities were statistically significantly increased in treated males and females. The increase was about 3-fold in males and females.

Table 5.8.2-20: Results of bioanalytics performed in both sexes of both dose groups

	Males		**Females**	
Group	**0**	**1**	**0**	**1**
Dose [ppm]	**0**	**15000**	**0**	**15000**
pNP-GT [nmol/min/mg protein]	1.47 ± 0.36	**2.84 ± 0.48***	5.27 ± 0.32	**6.59 ± 0.29***
MUF-GT [FU/min/mg protein]	894 ± 180	**1789 ± 333***	462 ± 133	**1111 ± 204***
HOBI-GT [FU/min/mg protein]	56.7 ± 10.4	**175.2 ± 39.8***	45.6 ± 25.8	**131.8 ± 23.3***

* $p \leq 0.05$ Wilcoxon test (one sided)

III. CONCLUSIONS

In conclusion, the administration of Boscalid caused changes in thyroid hormones as early as 2 days after beginning of the test substance administration, with concomitant induction of phase II enzyme activities.

Report: CA 5.8.2/2
[redacted] et al., 2004 c
BAS 510 F - Thyroid function test in Wistar rats using perchlorate discharge as a diagnostic test - Administration in the diet over 14 days
2004/1013467
Guidelines: none
GLP: yes
(certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Note: This study report has **not** been part of the documentation for the first Annex I inclusion process and a full evaluation is therefore presented here to assist in discussion of the mode of action regarding thyroid effects

Executive Summary

Boscalid was investigated on the ability of affecting the thyroid glands either be directly inhibiting organification of iodine in the glands or by an indirect mechanism. Boscalid, propylthiouracil (PTU) and phenobarbital (PB) were each administered to 2 groups of 6 male Wistar rats over a period of 2 weeks. Treatment groups received 5000 ppm Boscalid (groups 2/3), 2000 ppm Propylthiouracil (groups 4/5), or 1000 ppm Phenobarbital (groups 6/7) via the diet. PTU and PB were used as positive controls for substances directly inhibiting organification of iodine (PTU) and acting indirectly by secondary mechanism via hepatic enzyme induction (PB).

After Boscalid administration thyroid weights of rats were increased. ^{125}Iodide uptake in the thyroid of animals was significantly higher compared to the concurrent controls and no significant discharge occurred after perchlorate administration.

Phenobarbital administration also caused a significant increase in thyroid weights of rats. ^{125}Iodide uptake in the thyroid was significantly higher compared to the concurrent controls and similar to Boscalid no discharge of ^{125}iodide occurred after perchlorate administration.

Propylthiouracil administration caused a significant increase in thyroid weights of rats. ^{125}Iodide uptake in the thyroid was markedly decreased compared to the concurrent controls. A significant discharge of ^{125}iodide occurred after perchlorate administration.

Considering the increase of iodide organification and the lack of ^{125}iodide discharge after co-administration with perchlorate, it can be concluded that Boscalid has the potential to promote indirectly thyroid toxicity, similar to Phenobarbital.

(DocID 2004/1013467)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:	BAS 510 F **(**Boscalid)
Description:	Solid / white
Lot/Batch #:	N46
Purity:	95.7%
Stability of test compound:	The test substance was stable over the study period (until October 2004).
2. Vehicle and/or positive control:	
Vehicle control:	Control diet was fed
Positive control 1:	Propylthiouracil (PTU; batch: 032K2526),
Positive control 2:	Phenobarbital (PB; batch: 052K2513),
3. Test animals:	
Species:	Rat
Strain:	CrlGlxBrlHan:WI
Sex:	Male
Age:	42 - 44 days (at administration)
Weight at dosing:	168.4 – 172.3 g
Source:	Charles River Laboratories, Research Models and Services, Germany GmbH, Sulzfeld, Germany
Acclimation period:	9 days
Diet:	Ground Kliba maintenance diet mouse/rat “GLP”, meal, Provimi Kliba SA, Kaiseraugst, Switzerland, ad libitum
Water:	Drinking water, ad libitum
Housing:	Single housing in type DK III stainless steel wire mesh cages (Becker&Co., Castrop-Rauxel, Germany)
Environmental conditions:	
Temperature:	20 - 24°C
Humidity:	30 – 70%
Air changes:	fully air-conditioned
Photo period:	12 hours light (6 a.m./6 p.m.) / 12 hours dark cycle (6 p.m./6 a.m.)

B. STUDY DESIGN

1. Dates of experimental work: 28-Oct-2003 - Apr-2003

2. Animal assignment and treatment:
On the day of arrival, the animals were subjected to an acclimatization period during which they received ground diet and drinking water ad libitum. Prior to the start of the administration period, the animals were distributed according to weight among the individual test groups. The weight variation of the animals used did not exceed 20 percent of the mean weight. The list of randomization instructions was compiled with a computer. At the start of the administration period (day 0) the rats were 41-43 days old. The test substances were administered daily for 4 weeks. Boscalid, propylthiouracil (PTU) and phenobarbital (PB) were each administered to 2 groups of 6 male Wistar rats over a period of 4 weeks via the diet:

- Untreated control: diet only, groups 0 and 1
- Boscalid: 5000 ppm, groups 2 and 3
- Propylthiouracil (PTU): 2000 ppm, groups 4 and 5
- Phenobarbital (PB): 1000 ppm, groups 6 and 7

Control animals (groups 0 and 1) were fed with ground diet during the test period. At the end of the administration period the perchlorate discharge assay was performed. The dose level for Boscalid was selected on the basis of previous oral toxicity in rats, where thyroid effects were observed at 5000 ppm.
On study day 14 the rats received an intraperitoneal dose of 0.5 mL (1 µCurie) of radio labeled NaI (^{125}iodide). Six hours after ^{125}iodide administration, groups 0, 2, 4, and 6 were treated with 0.9% saline solution (10 ml/kg bw) and groups 1, 3, 5, and 7 with 10 mg/kg bw potassium perchlorate ($KClO_4$) in 0.9% w/v saline solution by i.p. injection. After 2.5 minutes, the animals were sacrificed by cervical dislocation. Following cervical dislocation at necropsy, blood sample (approximately 1 mL) from the cervical vessels was collected from each non-fasted animal at decapitation and weighed. The thyroid glands were removed immediately, trimmed and weighed. All blood samples and thyroid glands were then counted in a gamma-radiation counter over 1 minute. The amount of ^{125}iodide in each tube was expressed as counts per minute (cpm). The total thyroid count, blood count, thyroid weight and blood weight were used to calculate thyroid count per gram tissue, blood count per gram blood and thyroid to blood ^{125}iodide ratio.

3. Test substance preparation and analysis:
For each preparation, the test substance was weighed out and mixed with a small amount of food. Then corresponding amounts of food, depending on dose group, were added to this premix in order to obtain the desired concentrations. Mixing was carried out for about 10 minutes in a laboratory mixer. Details of the mixers used are retained with the raw data. The mixtures were prepared once before the start of the study.
No analyses of the test substance preparations were carried out for this study.

4. Statistics:
Means and standard deviations of each test group were calculated for several parameters. Further statistical analyses were performed according to following table:

Parameters	Statistical test	Markers in the table	References
Food consumption, body weight	A comparison of each group with the control group was performed using DUNNETT test (two sided) for the hypothesis of equal means	* for $p \leq 0.05$ ** for $p \leq 0.01$	DUNNETT, C.W. (1955): A multiple comparison procedure for comparing several treatments with a control. JASA, Vol. 50, 1096 – 1121 DUNNETT, C.W. (1964): New tables for multiple comparisons with a control. Biometrics, Vol. 20, 482 - 491
Clinical pathology	Comparison of the dose group with the control group using Wilcoxon-test (two-sided) for the equal medians	* for $p \leq 0.05$ ** for $p \leq 0.01$	SIEGEL, S. (1956): Non-parametric statistics for the behavioural sciences. McGraw-Hill New York

C. METHODS

1. Observations:
The animals were examined for overt signs of toxicity or mortality twice a day (in the morning and in the late afternoon) from Mondays to Fridays and once a day (in the morning) on Saturdays, Sundays and public holidays. Additionally, further general clinical examinations were carried out daily.

2. Body weight:
Body weight was determined before the start of the administration period in order to randomize the animals. During the administration period the body weight was determined weekly (day 0, 7, and 14).

3. Food and water consumption:
Individual food consumption was determined weekly over a period of 1 day and calculated as mean food consumption grams per animal and day.

4. Intake of test substance:
The mean daily intake of each test substance was calculated based upon individual values for body weight and food consumption.

$$\frac{FC_x \times C}{BW_x} = \text{Substance intake for day x}$$

BW_x = body weight on day x [g]
FC_x = mean daily food consumption on day x [g]
C = concentration in the diet on day x [mg/kg]

II. RESULTS AND DISCUSSION

A. ANALYSES OF THE TEST ITEM
No analyses of the test substance preparations were carried out for this study. Boscalid has been shown to be stable in the food from previous studies.

B. OBSERVATIONS
1. Clinical signs of toxicity
No abnormal clinical signs were observed.

2. Mortality
No animal died during the study.

C. BODY WEIGHT AND BODY WEIGHT GAIN
Statistically reduced body weights were observed in PTU-treated dose groups (4 and 5) on study days 7 and 14. At the end of the study (day 14) the reduction was about -24% in both group. All other groups showed no significant deviations in comparison to the control (group 0).

Table 5.8.2-21: Body weight (g/animal)

	Group							
	Control	Control	Boscalid	Boscalid	PTU	PTU	PB	PB
Day	+NaCl	$+KClO_4$	+NaCl	$+KClO_4$	+NaCl	$+KClO_4$	+NaCl	$+KClO_4$
0	169.7	168.6	169.0	172.3	169.6	170.4	170.9	168.4
7	208.7	209.0	207.7	217.0	169.4**	169.3**	213.9	210.3
14	242.4	246.1	240.3	254.1	184.9**	185.0**	250.4	247.7

*: $p \leq 0.05$; **: $p \leq 0.01$ Dunnett's test (two-sided)

D. FOOD CONSUMPTION AND TEST ITEM INTAKE

Statistically reduced food consumption was observed in PTU-treated dose groups (4 and 5) on study days 7 and 14. At the end of the study (day 14) the reduction was about -35% in both group. All other groups showed no significant deviations in comparison to the control (group 0).

Table 5.8.2-22: Food consumption (g/animal/day)

		Group							
		Control	Control	Boscalid	Boscalid	PTU	PTU	PB	PB
Day		+NaCl	+$KClO_4$	+NaCl	+$KClO_4$	+NaCl	+$KClO_4$	+NaCl	+$KClO_4$
7		22.1	20.8	20.8	21.4	10.8**	11.1**	22.6	22.3
14		23.7	22.4	22.7	23.8	15.5**	15.4**	23.8	24.1

*: $p \leq 0.05$; **: $p \leq 0.01$ Dunnett's test (two-sided)

The mean daily test substance intake in mg/kg bw/d over the entire study period was calculated where possible and is shown in the following table:

Test substance	Concentration in the diet (ppm)	Mean daily test substance intake (mg/kg bw/d)
Boscalid	5000	484
Propylthiouracil	2000	148
Phenobarbital	1000	101

E. BIOANALYTICS

^{125}Iodide level after 0.9% saline solution administration (6 hours after ^{125}iodide injection)

On day 14 ^{125}Iodide was determined 6 hours after ^{125}iodide injection.

Thyroid weights were observed significantly increased in rats treated with PTU (+277%) and PB (+105%) as compared to the concurrent controls. A small increase of thyroid weights was observed for the Boscalid-treated animals (+21%). For animals treated with PTU ^{125}iodide levels in the blood and per gram of blood were significantly increased (+78% and +74%, respectively). No significant change in the concentration of ^{125}iodide in the blood and ^{125}iodide per gram of blood was found for Boscalid (-9% and – 7%) and PB (+9% and +7%) groups.

Markedly reduced ^{125}iodide uptake per gram of thyroid was observed in PTU (-80%) treated animals whereas a slight decrease of ^{125}iodide concentration in the thyroid was found after PTU treatment (-24%).

The ^{125}iodide uptake in the thyroid of animals treated with Boscalid and PB was found significantly increased (+65% and +173%, respectively). Consequently, an increase in the ^{125}iodide per gram of thyroid was observed in animals treated with Boscalid (+37%; statistically significant) and PB (+33%) as compared to the control group. Thyroid to blood ratio was found 45% and 22% higher in groups of animals treated with Boscalid and PB, respectively, while a significant reduction of 89% in the ratio was observed after PTU treatment.

Table 5.8.2-23: ^{125}Iodide count in various compartments after saline administration on day 14

Dose group		Control +NaCl	Boscalid +NaCl	PTU +NaCl	PB +NaCl
Thyroid weight [g]	Mean %dev.	0.011	0.014 +21	**0.043**** +277	**0.023**** +105
Blood count[#] [cpm]	Mean %dev.	3.262	2.981 -9	**5.796**** +78	3.550 +9
Thyroid count[#] [cpm]	Mean %dev.	105	**173*** +65	80 -24	**286**** +173
Blood count/blood weight[#] [cpm/g]	Mean %dev.	3.207	2.984 -7	**5.588**** +74	3.432 +7
Thyroid count/thyroid weight[#] [cpm/g]	Mean %dev.	9266	**12710*** +37	**1884**** -80	**12337*** +33
Ratio thyroid/blood	Mean %dev.	3009	4356 +45	**343**** -89	3669 +22

*: p≤0.05; **: p≤0.01 (Wilcoxon test two-sided; comparison to Group 1)
[cpm] = counts per minute; [g] = gram; %dev. = deviation from control, [#] = x1000
PTU = Propylthiouracil; PB = Phenobarbital

^{125}Iodide level after potassium perchlorate administration (6 hours after ^{125}iodide injection)

On day 14 ^{125}Iodide was investigated after potassium perchlorate administration 6 hours after ^{125}iodide injection.

Thyroid weights were significantly increased in rats treated with Boscalid (+71%), PTU (+290%) and PB (+68%) as compared to the concurrent controls. ^{125}iodide level in the blood and ^{125}iodide per gram of blood were significantly increased in the animals treated with PTU (128% and 130%) and PB (47% and 48%). A slight decrease in the concentration of ^{125}iodide in the blood and ^{125}iodide per gram of blood was found for the Boscalid group (-17% and -15%). Markedly reduced ^{125}iodide uptake in the thyroid and ^{125}iodide per gram of thyroid were observed in PTU (-56% and -88%) treated animals. ^{125}Iodide uptake in the thyroid and ^{125}iodide per gram of thyroid were significantly increased in the animals treated with Boscalid (+132% and +35%) and PB (+161% and +56%) compared to the control group.

Thyroid to blood ratio was 58% and 4% higher in the animals treated with Boscalid and PB, respectively, while a significant reduction of 95% in the ratio was observed after PTU treatment.

Table 5.8.2-24: ^{125}Iodide count in various compartments after potassium perchlorate administration on day 14

Dose group		Control +$KClO_4$	Boscalid +$KClO_4$	PTU +$KClO_4$	PB +$KClO_4$
Thyroid weight [g]	Mean %dev.	0.012 -	**0.020**** +71	**0.045**** +290	**0.019**** +68
Blood count[#] [cpm]	Mean %dev.	3.233 -	**2.683*** -17	**7.365**** +128	4.767** +47
Thyroid count[#] [cpm]	Mean %dev.	100 -	**231**** +132	44** -56	**260**** +161
Blood count/blood weight[#] [cpm/g]	Mean %dev.	3.189 -	2.697 -15	**7.325**** +130	**4.732**** +48
Thyroid count/thyroid weight[#] [cpm/g]	Mean %dev.	8633 -	**11686**** +35	**1003**** -88	**13464**** +56
Ratio thyroid/blood	Mean %dev.	2772 -	**4374**** +58	**135**** -95	2890 +4

*: $p \leq 0.05$; **: $p \leq 0.01$ (Wilcoxon test two-sided; comparison to Group 1)
[cpm] = counts per minute; [g] = gram; %dev. = deviation from control, [#] = x1000
PTU = Propylthiouracil; PB = Phenobarbital

Discharge of ^{125}iodide after potassium perchlorate administration on study day 14

A significant discharge of ^{125}iodide per gram of thyroid in the animals treated with PTU (-47%) was observed after potassium perchlorate administration. No discharge was found in the group of animals, which received Boscalid (-8%) and PB (+9%).

Table 5.8.2-25: Discharge of ^{125}Iodide uptake after potassium perchlorate administration on day 14

Dose group		Boscalid +NaCl	Boscalid +$KClO_4$	PTU +NaCl	PTU +$KClO_4$	PB +NaCl	PB +$KClO_4$
Thyroid count/thyroid weight[#] [cpm/g]	Mean	12710	11686	1884	**1003****	12337	13464**
	%dev.		-8		-47		+9

*: p≤0.05; **: p≤0.01 (Wilcoxon test two-sided; comparison to Group 1)
[cpm] = counts per minute; [g] = gram; %dev. = deviation from control, [#] = x1000
PTU = Propylthiouracil; PB = Phenobarbital

III. CONCLUSIONS

In conclusion, after 2 weeks of administration Boscalid showed the potential to promote indirectly thyroid toxicity similar to phenobarbital. A direct thyroid toxicity of Boscalid comparable to propylthiouracil (PTU) was not observed.

Report: CA 5.8.2/3
[redacted] et al., 2001 b
BAS 510 F - Reversibility study in Wistar rats - Administration in the diet for 4 weeks followed by recovery periods of 4 weeks and 3 months
2001/1017611
Guidelines: none
GLP: yes
(certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Note: This study report has **not** been part of the documentation for the first Annex I inclusion process and a full evaluation is therefore presented here to assist in discussion of the mode of action regarding thyroid effects

Executive Summary

BAS 510 F (Boscalid, batch: N46, purity: 94.1%) was administered to groups of 5 male Wistar rats at dietary dose levels of 0, 100, 2500, and 15000 ppm, equivalent to 7.7, 190.3 and 1137.4 mg/kg bw/day, for 4 weeks. Control animals received only the ground diet without test substance addition. The administration period was followed by a treatment-free recovery period for 0 (treatment group, TG), 28 (recovery group 1, RG 1) or 91 days (RG 2). Food consumption and body weights were determined weekly and the animals were examined for signs of toxicity or mortality at least once a day. Thyroid hormones (T3, T4, TSH) in the serum were determined prior to start of dosing, at the end of the application period as well as at the end of each recovery period. At termination, all animals were subjected to gross pathological evaluation, followed by organ weight determination as well as histopathological evaluation of liver and thyroids.

No mortality was observed during the study period and no clinical signs were observed. Treatment with the test substance had no effect on food or water consumption or body weight. No impairment of T3 and T4 levels were observed at any dose group tested. Increased TSH levels were observed in the mid and high dose group, directly at the end of the administration period but were reversible within 4 weeks.

Correspondingly, at the end of the administration period thyroid weight with corroborating histopathological changes as evidenced by hypertrophy of follicular epithelium and diffuse hyperplasia of follicular cells were observed at ≥ 2500 ppm. Morphologically, the effects were reversible within 4 weeks. Additionally, increased liver weight corroborated by histopathological changes were evident as shown by centrilobular hypertrophy of hepatocytes (zone 3) and portal (zone 1) fatty change at the end of the administration period in the same dose groups (≥ 2500 ppm). All liver effects were fully reversible within 4 weeks.

Overall, due to induction of hepatic enzymes, dietary administration of Boscalid for 4 weeks gave distinct, however, reversible effects on liver and thyroid at dose levels of ≥ 2500 ppm in the feed.

(DocID 2001/1017611)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: BAS 510 F
Description: solid / white
Lot/Batch #: N46
Purity: 94.1%
Stability of test compound: The stability of the test substance under storage conditions (room temperature) over the test period was verified by re-analysis after the in-life phase of the study, revealing purity of 95.7%.

2. Vehicle: none (dietary administration)

3. Test animals:
Species: Rat
Strain: CrlGlxBrlHan:WI
Sex: male
Age at dosing: 56 ± 2 days
Weight at dosing: 208.0 - 245.6 g (group mean: 226.4 g)
Source: Charles River, Sulzfeld, Germany
Acclimation period: at least 7 days
Diet: Ground Kliba maintenance diet rat/mouse/hamster, meal (Provimi Kliba SA, Kaiseraugst, Switzerland), ad libitum
Water: Drinking water from bottles, ad libitum
Housing: Single housing in type DK III stainless steel with wire-mesh cages (Becker&Co., Castrop-Rauxel, Germany) with a floor area of about 800 cm^2. Underneath the cages, waste trays were fixed containing type 3/4 dust-free embedding as absorbent material (SSNIFF, Soest, Germany).

Environmental conditions:
Temperature: 20 - 24°C
Humidity: 30 - 70%
Air changes: central air-conditioning
Photo period: 12 h light / 12 h dark
(06:00 am - 06:00 pm/ 06:00 pm - 06:00 am)

B. STUDY DESIGN AND METHODS

1. Dates of experimental work: in-life phase: 27-Mar-2001 (start of administration) to 30-Jul-2001 (last necropsy)

2. Animal assignment and treatment:
On day of arrival, the animals were subjected to an acclimatization period during which they received ground diet and drinking water ad libitum. Prior to the starting of the administration period, the animals were distributed according to their weight among the individual test groups.
Several groups of 5 males were administered a diet with 0, 100, 2500, and 15000 ppm of the test item for 4 weeks. Control animals received only the ground diet without test substance. The administration period was followed by a treatment-free recovery period for 0 (treatment group, TG), 28 (recovery group 1, RG 1) or 91 days (RG 2). All animals were sacrificed after a fasting period (withdrawal of food) for about 16 -20 hours.

3. Test substance preparation and analysis:
For each concentration, the test substance was weighted out and mixed with a small amount of food. Thereafter, corresponding amounts of food, depending on the dose group were added to this pre-mix in order to obtain the desired concentrations, and mixed for about 10 minutes in a laboratory mixer (Ruberg Mischtechnik KG, Paderborn, Germany). All mixtures were prepared once at the start of the study.

The stability of the test substance in the diet preparation was proven over a period of 32 days at room temperature.
Homogeneity and concentration control analyses were performed at the start of the administration period.

4. Statistics:
Means and standard deviations of each test group were calculated for all parameters. Further statistical analysis was performed according to the following tables:

Statistics of clinical examinations

Parameter	Statistical test
Food consumption, body weight, body weight gain, food efficiency	Parametric one-way analysis using the F-test (ANOVA, two sided). If the resulting p-values was equal or less 0.05, a comparison of each group with the control group was performed using DUNNETT's test (two-sided) for the hypothesis of equal means

Statistics of clinical pathology

Parameter	Statistical test
Hormone 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 pairwise comparison of each dose group with the control group was performed using WILCOXON-test (two-sided) for the hypothesis of equal medians.

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 pairwise comparison of each dose group with the control group was performed using the WILCOXON test for the hypothesis of equal medians

C. METHODS

1. Clinical examinations:
Mortality
All animals were checked twice daily on weekdays or once daily on weekends or at public holidays for deaths and morbidity during the study.

Clinical observations
Detailed clinical observations were carried out once daily.

2. Food consumption, food efficiency and intake of test substance
Food consumption was determined weekly over a period of 7 days and calculated as mean food consumption in grams per animal and day. The mean daily intake of test substance (group means) as well as food efficiency (group mean) were calculated based upon individual values for body weight and food consumption:

$$IT_x = \frac{FCx}{BWx} \text{ x } C$$

IT_x= intake of test substance on study day x [mg/kg bw/day]
FC_x = mean daily food consumption on study day x [g]
BW_x = body weight on study day x [g]
C = concentration [mg/kg]

$$FE_x = \frac{BWx - BWy}{FCy \text{ to } x} \text{ x } 100$$

FE_x= food efficiency on study day x [%]
BW_x = body weight on study day x [g]
BW_y = body weight on study day y [g] (last weighing date before day x)
$FC_{x \text{ to } y}$ = mean food consumption from day x [g] (calculated as mean daily food consumption on day x, multiplied with the number of days from day y to day x)

3. Body weight data

Body weight was determined 4 days before the start of the administration period in order to randomise the animals.

During the study period, body weights of all animals was determined at administration start (day 0), and weekly thereafter. The difference between the body weight of the respective day of weighing and the body weight at day 0 was calculated as body weight gain.

4. Clinical pathology

Blood was taken from the retro-orbital venous plexus in the morning from non-fasted animals without anaesthesia for thyroid hormone examinations.

Determination of total triiodothyronine (T3), total thyroxine (T4) and thyroid stimulating hormone (TSH) levels was performed by radioimmunoassay, using commercially available RIA test kits and a Gamma-counter (LB 2111, Berthold, Germany).

5. Sacrifice and pathology:

All animals were sacrificed by decapitation under CO_2-anaesthesia. The exsanguinated animals were necropsied and assessed by gross pathology.

Weight parameters

Terminal body weight, liver weight and weight of thyroid glands (with parathyroid glands) were measured for all animals killed on schedule.

Histopathology

Liver and thyroid glands (with parathyroid glands) were subjected for histopathological investigations. The haematoxylin-eosin stained slides were examined by light microscopy and assessed for all animals per treatment groups.

II. RESULTS AND DISCUSSION

A. TEST SUBSTANCE ANALYSES

The stability of the test substance in the diet over a period of 32 days at room temperature was demonstrated by 97.9% of initial content measured by HPLC/UV. As the food mixtures were stored no longer than this time period, the stability over the study period was guaranteed.
Homogeneous distribution of the test item in food was demonstrated by the low relative standard deviation of 2.5% and 1.7% at 100 and 15000 ppm, respectively. The correctness of the applied concentrations was demonstrated as the actual measured concentrations were from 92.4% to 97.6% of the nominal concentrations. No test item was detected in the control samples of the diet.

B. OBSERVATIONS

1. Clinical signs of toxicity

No substance-related clinical signs of toxicity were observed. One control animal of the RG 1 group as well as one male of the RG 2 group receiving 2500 ppm had alopecia that was clearly incidental in nature.

2. Mortality

No animal died prematurely.

C. BODY WEIGHT AND BODY WEIGHT GAIN

No substance-related impact on body weight development was observed.

The significantly increased body weight gain of TG 0-males at the dose level of 2500 ppm and decreased body weight gain of RG 2-males at the 15000 ppm dose level (on day 7 each) were assessed being incidental in nature, due to isolated occurrence and the lack of dose-response relationship of these findings.

D. FOOD/WATER CONSUMPTION, FOOD EFFICIENCY AND COMPOUND INTAKE

There was no substance-related impact on food or water consumption as well as on food efficiency.

The significantly increased food efficiency of RG 2-males at the 2500 ppm dose level on day 7 was assessed being incidental in nature, due to isolated occurrence and the lack of dose-response relationship of that finding.

The test substance intake [mg/kg bw/day] for each dose group of each treatment group as well as the mean over all treatment groups is given in the following table:

Table 5.8.2-26: Calculated mean daily test substance intake

Concentration in the food [ppm]	Mean daily test-substance intake [mg/kg bw/day]			
	TG	RG 1	RG 2	Mean
100	7.8	7.6	7.8	**7.7**
2500	187.7	191.2	192.0	**190.3**
15000	1144.6	1140.8	1126.8	**1137.4**

E. CLINICAL PATHOLOGY

Total triiodothyronine (T3)

There were no substance-related changes in serum concentrations of total triiodothyronine (T3) after 4-week test substance administration. No delayed effects were observed either 4 or 13 weeks after the end of the administration period.

Table 5.8.2-27: Triiodothyronine level in the serum of male rats after test substance administration for 4 weeks

Dose groups		T3 [nmol/L]			
		Day -1	4 weeks treatment	4 weeks treatment + 4 weeks recovery	4 weeks treatment + 13 weeks recovery
Number of animals per dose group		15	15	10	5
0 ppm	Mean	1.80	1.95	1.62	1.63
	%	100	100	100	100
100 ppm	Mean	1.73	1.83	1.74	1.76
	%	96	94	108	108
2500 ppm	Mean	1.64	1.89	1.60	1.75
	%	91	97	99	108
15000 ppm	Mean	1.64	1.98	1.79	1.86
	%	91	101	111	114

Total thyroxine (T4)

There were no substance-related changes in serum concentrations of total thyroxine (T4) after 4-week test substance administration. No delayed effects were observed either 4 or 13 weeks after the end of the administration period.

Table 5.8.2-28: Thyroxine level in the serum of male rats after test substance administration for 4 weeks

Dose groups		T4 [nmol/L]			
		Day -1	4 weeks treatment	4 weeks treatment + 4 weeks recovery	4 weeks treatment + 13 weeks recovery
Number of animals per dose group		15	15	10	5
0 ppm	Mean	51.98	58.35	56.26	46.42
	%	100	100	100	100
100 ppm	Mean	52.57	58.89	58.66	46.25
	%	101	101	104	100
2500 ppm	Mean	52.18	62.12	62.46	58.18
	%	100	107	111	125
15000 ppm	Mean	51.44	55.21	59.70	52.20
	%	99	95	106	112

Thyroid stimulating hormone (TSH)

After 4-week administration of the test substance, serum TSH levels were significantly increased in rats at dose levels of 2500 and 15000 ppm by +68% and +87%, respectively, but returned to normal values within 4 weeks and retained at this level.

Table 5.8.2-29: Thyroid stimulating hormone level in the serum of male rats after test substance administration for 4 weeks

Dose groups		TSH [µg/L]			
		Day -1	4 weeks treatment	4 weeks treatment + 4 weeks recovery	4 weeks treatment + 13 weeks recovery
Number of animals per dose group		15	15	10	5
0 ppm	Mean	7.84	9.72	8.59	7.21
	%	100	100	100	100
100 ppm	Mean	7.24	9.83	7.09	7.57
	%	92	101	83	105
2500 ppm	Mean	7.79	**16.36****	6.60	7.19
	%	99	168	77	100
15000 ppm	Mean	7.88	**18.20*****	7.90	6.68
	%	101	187	92	93

F. PATHOLOGY

1. Organ weights

The mean terminal body weights of the animals treated with Boscalid did not differ significantly from controls either directly after the end of the administration period or after the respective recovery phase.

No effect on liver weight was observed at the dose level of 100 ppm. A dose-dependent increase of the absolute and relative liver weights was observed in rats at dose levels of 2500 and 15000 ppm of the test substance by ~ 20% and ~ 50%, respectively, directly after the end of the treatment phase. This effect was considered being test-substance related. At both dose levels the increase in liver weight was completely reversible within 4 weeks.

Regarding thyroid glands, absolute and relative weight increase at dose levels ≥ 2500 ppm were observed directly after the end of the administration period. The dose level of 100 ppm gave some marginal weight increase as evidenced by statistically significantly increased relative organ weight. At dose levels ≥ 2500 ppm reversibility of organ weight increase was not complete, however, the decrease in relative organ weight is indicative of the organs capability to adjust to decreased demands within this period. Within the application phase, the thyroid effect at ≥ 2500 ppm can be assessed being substance-related. At the dose level of 100 ppm thyroid weights were not different to control after 4 weeks of recovery, but absolute weights were statistically significantly increased after 13 weeks of recovery. For the evaluation of this finding further information from the histopathological evaluation is considered necessary and has been used below.

Table 5.8.2-30: Organ weights of male rats after test substance administration for 4 weeks

Dose groups		**TG**		**RG 1**		**RG 2**	
Parameter		**absolute**	**relative**	**absolute**	**relative**	**absolute**	**relative**
Terminal body weight [g]							
0 ppm	Mean	291.84		351.74		389.76	
	SD	*20.57*		*31.75*		*34.68*	
100 ppm	Mean	286.92		341.88		418.41	
	SD	*12.22*		*28.31*		*52.78*	
2500 ppm	Mean	299.52		343.42		418.64	
	SD	*14.57*		*24.06*		*22.76*	
15000 ppm	Mean	285.8		337.84		371.98	
	SD	*19.34*		*28.662*		*14.94*	
Liver weight							
		Absol [g]	Rel [%]	Absol [g]	Rel [%]	Absol [g]	Rel [%]
0 ppm	Mean	7.71	2.64	9.14	2.60	8.76	2.25
	SD	*0.8*	*0.15*	*0.93*	*0.23*	*1.11*	*0.22*
	%	100	100	100	100	100	100
100 ppm	Mean	7.89	2.75	8.28	2.42	9.62	2.31
	SD	*0.37*	*0.14*	*0.69*	*0.13*	*0.99*	*0.19*
	%	102	104	91	93	110	103

Table 5.8.2-30: Organ weights of male rats after test substance administration for 4 weeks

Dose groups		TG		RG 1		RG 2	
2500 ppm	Mean	**9.63***	**3.21***	8.89	2.59	9.26	2.21
	SD	*0.97*	*0.27*	*0.54*	*0.07*	*0.61*	*0.08*
	%	125	122	97	100	106	98
15000 ppm	Mean	**11.16****	**3.90****	8.70	2.57	8.40	2.26
	SD	*1.07*	*0.19*	*0.96*	*0.16*	*0.53*	*0.06*
	%	145	148	95	99	96	100
Thyroid weight							
		Absol [mg]	Rel [%]	Absol [mg]	Rel [%]	Absol [mg]	Rel [%]
0 ppm	Mean	16.6	0.006	17.0	0.005	17.8	0.005
	SD	*1.51*	*0.001*	*0.71*	*0.00*	*2.86*	*0.001*
	%	100	100	100	100	100	100
100 ppm	Mean	19.0	**0.007****	17.6	0.005	**23.2***	0.006
	SD	*1.58*	*0.00*	*1.95*	*0.001*	*2.49*	*0.001*
	%	114	117	104	100	130	120
2500 ppm	Mean	**24.8****	**0.008****	**20.4****	**0.006***	**23.4***	0.006
	SD	*2.86*	*0.001*	*2.19*	*0.001*	*2.79*	*0.00*
	%	149	133	120	120	131	120
15000 ppm	Mean	**24.4****	**0.009****	20.6	**0.006***	**23.6***	0.006
	SD	*5.32*	*0.002*	*3.27*	*0.001*	*2.97*	*0.001*
	%	147	150	121	120	133	120

2. Gross pathology

In the liver, size enlargement was observed in all males at the dose level of 15000 ppm directly at the end of the 4-week administration period. No enlarged liver size was noticed in animals of lower dose groups and in all dose groups after both recovery periods.

In the second target organ the thyroid glands, no gross lesions were observed.

All other gross lesions observed, were regarded being not of toxicological relevance, due to their individual occurrence and/or lack of dose-response relationship.

2. Histopathology

In **liver**, centrilobular hypertrophy and a portal (zone 1) fatty change were found in 4/5 and 5/5 TG-males receiving 2500 and 15000 ppm, respectively. This findings were considered being substance-related, however reversible within 4 weeks. All other liver findings noted were considered being incidental in nature and thus not treatment related, due to either isolated occurrence or lack of dose-response relationship [see Table 5.8.2-31].
The histopathological findings corroborate the macroscopic finding at 15000 ppm and correlate with the increased liver weight at ≥ 2500 ppm, directly at the end of the 4-week exposure period to Boscalid. All pathological liver findings (macro- and microscopic) were reversible within 4 weeks.

Table 5.8.2-31: Histopathological liver and thyroid findings of male rats received Boscalid for 4 weeks

Organ	Grade	Dose group [ppm]											
		0			100			2500			15000		
		TG	RG1	RG2	TG	RG1	RG2	TG	RG1	RG2	TG	RG1	RG2
Liver - examined		5	5	5	5	5	5	5	5	5	5	5	5
- Granuloma, Kupff.	1	3	1	4	5	3	4	4	3	4	4	3	2
	2	2	4	1	-	2	-	1	2	1	1	2	3
	3	-	-	-	-	-	1	-	-	-	-	-	-
- Fatty change, portal	1	-	-	-	-	-	-	3	-	-	1	-	-
	2	-	-	-	-	-	-	1	-	-	2	-	-
	3	-	-	-	-	-	-	-	-	-	2	-	-
- Hypertrophy, centrilobular	1	-	-	-	-	-	-	2	-	-	-	-	-
	2	-	-	-	-	-	-	2	-	-	4	-	-
	3	-	-	-	-	-	-	-	-	-	1	-	-
- Congestion		-	-	-	-	-	-	-	-	1	-	-	-
Thyroids - examined		5	5	5	5	5	5	5	5	5	5	5	5
- Hyperplasia, follicular	1	-	2	1	-	-	-	1	-	-	-	-	-
	2	-	-	-	-	-	-	1	-	-	3	-	-
	3	-	-	-	-	-	-	1	-	-	2	-	-
- Hypertrophy, follicular cells	1	-	-	1	-	1	1	-	-	1	-	1	-
	2	-	2	-	-	-	-	2	1	-	1	1	-
	3	-	-	-	-	-	-	2	-	-	2	-	-
	4	-	-	-	-	-	-	-	-	-	2	-	-

Regarding **thyroid glands**, directly at the end of the exposure period, hypertrophy of follicular epithelial cells and a diffuse hyperplasia were observed in some animals at the dose level of 2500 ppm and all animals at the dose level of 15000 ppm Boscalid. Additionally, the severity i.e. number of animals with same grade of the finding was dose-dependently increased, corroborating the assessment of these findings being substance-related. These observation are consistent with the increase of thyroid weight at ≥ 2500 ppm.
At the dose level of 100 ppm no follicular hyperplasia and isolated findings of follicular in one animal of each recovery group was observed. No hypertrophy was found in the treatment group sacrificed that day after treatment (TG).

III. DISCUSSION

Boscalid dietary administration to male Wistar rats for a period of 4 weeks resulted in adverse effects on the liver and thyroid at dose levels ≥ 2500 ppm as evidenced by organ weight increase and corresponding histopathological findings such as fatty change and centrilobular hypertrophy in liver as well as hyperplasia and follicular hypertrophy in thyroids with dose dependent increase in severity The liver effects were clearly reversible within 4 weeks following the treatment period. The thyroid effects were reversible within 4 weeks from the morphological point of view. The reversibility of the thyroid weight effects was also evident as indicated by reduction of the relative organ weights to the level of untreated control animals after 13 weeks of recovery but may have been not fully completed by this time as evidenced by the absolute weights still increased for the dose levels of 2500 ppm and 15000 ppm.

At the 100 ppm dose level no histopathological effects were observed which were attributable to the test substance administration. Overall, after 4 weeks and 13 weeks of recovery thyroid weights were comparable to the untreated control based on the relative organ weights and the absence of histopathological findings. The statistically significant increase in absolute thyroid weight after 13 weeks of recovery needs to be interpreted in the light of the full recovery of organ weight already after 4 weeks of test substance withdrawal and is therefore considered to be spontaneous in nature.

After 4-week administration of the test substance, serum TSH levels were significantly increased in rats at dose levels of 2500 and 15000 ppm by +68% and +87%, respectively, but returned to normal values within 4 weeks and retained at this level whereas T4 and T3 levels were kept at comparable levels as untreated controls. As known from other mechanistic studies, Boscalid induces the hepatic enzyme system of the endoplasmatic reticulum in the rat that lead to increased glucuronidation of T4, which is excreted into the bile as T4-glucuronid. The increased rate of biliary T4 excretion is generally followed by an initial reduction of circulating T4 levels in blood. Following the negative feedback mechanism, the ensuing decrease of serum T4 levels leads to a compensatory response by marked increase in TSH secretion that for its part leads to the stimulation of thyroid activity and ultimately results in the increase in thyroid gland weight.

As hormone synthesis is increased by TSH stimulation, T4 levels return to normal in the course of the 4-week treatment period and a new status quo can be measured at the end of the Boscalid administration period. After cessation of test item administration, serum TSH levels returned to the control levels within 4 weeks. Thus, the test substance-related effects on thyroid hormone concentrations are assessed being reversible in nature.

IV. CONCLUSIONS

The administration of Boscalid in the feed of male Wistar rats did not results in impairment of T3 and T4 levels at any dose group tested. Increased TSH levels were observed in dose groups of 2500 ppm and 15000 ppm directly at the end of the administration period but were reversible within 4 weeks.

Correspondingly, at the end of the administration period thyroid weight with corroborating histopathological changes as evidenced by hypertrophy of follicular epithelium and diffuse hyperplasia of follicular cells were observed at ≥ 2500 ppm. Morphologically, the effects were reversible within 4 weeks. Additionally, increased liver weight corroborated by histopathological changes were evident as shown by centrilobular hypertrophy of hepatocytes (zone 3) and portal (zone 1) fatty change at the end of the administration period in the same dose groups (≥ 2500 ppm). All liver effects were fully reversible within 4 weeks.

Overall, due to induction of hepatic enzymes, dietary administration of Boscalid for 4 weeks gave distinct, however, reversible effects on liver and thyroid at dose levels of ≥ 2500 ppm in the feed.

Report: CA 5.8.2/4
[redacted] 2002 c
BAS 510 F - Peak effect study in Wistar rats - Single administration by gavage and observation for following 3 days
2002/1006331
Guidelines: none
GLP: yes
(certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Note: This study report has **not** been part of the documentation for the first Annex I inclusion process and a full evaluation is therefore presented here

Executive Summary

Single doses of 1000 and 2000 mg/kg bw of Boscalid preparations in 0.5% CMC-solution were applied to each 3 male and 3 female animals by oral gavage. The respective control groups only received the vehicle 0.5% CMC solution at the dose volume of 20 mL/kg bw. Mortality and general clinical signs were recorded and detailed examination for clinical signs was performed directly after test substance administration, 15 min, 30 min, and hourly up to 6 h after administration of the test substance. On days 1 to 3 the detailed examination was performed once daily. At day 6 the animals were sacrificed without further examination.

No mortality occurred and no clinical signs were observed in any animal of any dose group throughout the study period. In conclusion, Boscalid did not provoke any acute effects in rats after single oral administration of up to 2000 mg/kg bw.

DocID (2002/1006331)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: BAS 510 F

Description:	Solid / white
Lot/Batch #:	N37
Purity/content:	94.4%
Stability of test compound:	The stability of the test substance and test substance preparation (over 4 days) was proven analytically.

2. Vehicle: aqueous 0.5% CMC-solution (carboxymethylcellulose)

3. Test animals:

Species:	Rat
Strain:	Wistar / Chbb:THOM (SPF)
Sex:	Male and female
Age:	Not reported
Weight at dosing:	Not reported
Source:	Boehringer Ingelheim, Pharma KG, Biberach/Riss, Germany
Acclimation period:	2 weeks
Diet:	Kliba-Labordiät (rats/mice/hamsters maintenance diet), 343 meal, Klingentalmühle AG, Kaiseraugst, Switzerland, ad libitum
Water:	Tap water, ad libitum
Housing:	Single housing in stainless steel wire mesh cages, type DK-III (Becker & Co., Castrop-Rauxel, FRG)

Environmental conditions:

Temperature:	20 - 24 °C
Humidity:	30 - 70 %
Air changes:	Central air-conditioning
Photo period:	Alternating 12-hour light and dark cycles, artificial light from 6 am to 6 pm

B. STUDY DESIGN AND METHODS

1. Dates of work: 27-Jan-1998 test substance administration) - 02-Feb-1998 (necropsy)

2. Animal assignment and treatment:

Single doses of 1000 and 2000 mg/kg bw of test material preparations in 0.5% CMC-solution were applied to each 3 male and 3 female animals by oral gavage. The respective control groups only received the vehicle at the dose volume of 20 mL/kg bw. Mortality and general clinical signs were recorded twice a day on each workday and once a day at Saturdays, Sundays or public holidays. Detailed examination for clinical signs was performed directly after test substance administration, 15 min, 30 min, and hourly up to 6 h after administration of the test substance. On days 1 to 3 the detailed examination was performed once daily.
Body weight was determined on the day of test substance administration, but was not included in the report.
On day 6, the animals were sacrificed using CO_2 without further examinations.

II. RESULTS AND DISCUSSION

A. MORTALITY

No mortality occurred in the administration groups.

B. CLINICAL OBSERVATIONS

No abnormal clinical signs were observed in any animal and any dose group.

C. BODY WEIGHT

Body weight was not determined throughout the study duration.

D. NECROPSY

No necropsy was performed

III. CONCLUSION

Under the conditions of this study, no clinical signs or mortalities were observed in male and female Wistar rats after administration of 1000 and 2000 mg/kg bw Boscalid by oral gavage.

Additional information on immunotoxicity is presented below which has already been included in the documentation of Boscalid for the first Annex I inclusion process at a later stage and was thus not incorporated in the Monograph of November 08, 2002.

BAS 510 F: 4-week oral feeding immunotoxicity study in rats (2003) 2003/1025755

Guidelines: none applicable to this study type
GLP: yes
Acceptance: the study has been evaluated and considered acceptable in the EU registration process for the first Annex I inclusion

Note: This study report has been part of the documentation for the first Annex I inclusion process and a detailed summary is presented here

Executive Summary

The immunotoxic potential of Boscalid (Batch: N37; Purity: 94.4%) was investigated in groups of 16 male Wistar rats using dietary dose levels of 0, 100, 1000 and 10000 ppm (corresponding to mean intake levels of 7.45, 73.1 and 736.2 mg/kg bw/day, respectively) for a period of 4 weeks. Additionally, a positive control and vehicle control groups were exposed by gavage to 3 mg/kg bw/day cyclophosphamide monohydrate (CPA) and 0.5% methyl cellulose solution, respectively, for the period of 4 weeks. The parameters used for evaluation of potential test substance related alterations in the morphology of the immune system included a) the determination of lymphoid organ weights (spleen and thymus), b) thymic and splenic lymphocyte subset analysis by flow cytometry and c) the analysis of the primary humoral (IgM response) immune response to sheep red blood cells (SRBC).
The administration of Boscalid did not result in systemic toxicity or mortality and did not affect food consumption or body weight development in rats, up to the highest dose tested (736.2 mg/kg bw/day). Furthermore, Boscalid did not induce any toxic effects on immune-toxicological parameters, like spleen and thymus weights and cellularity, splenic and thymic lymphocyte subsets and SRBC IgM antibody titres. The treatment with the positive control substance, cyclophosphamide monohydrate (CPA, 3 mg/kg bw/day) induced clear signs of immunotoxicity as regards these parameters, demonstrating the reliability of the test system under the study conditions employed.

Based on the obtained results it can be concluded that Boscalid does not bear an immune-modulatory/immunotoxic potential under the conditions of this study. The NOAEL for the immune-toxicologically relevant endpoints as well as for systemic toxicity was determined to be 10000 ppm, corresponding to a substance intake of 736.2 mg/kg bw/day by male Wistar rats.

(BASF DocID 2003/1025755)

I. MATERIAL AND METHODS

1. Test Material:	Boscalid (BAS 510 F)
Description:	solid (powder) / white
Lot/Batch #:	N37
Purity:	94.4%
Stability of test compound:	The test substance was stable over the study period
2. Vehicle control:	Rodent diet (Boscalid) 0.5% methyl cellulose solution (for CPA)
3. Positive control:	Cyclophosphamide monohydrate (CPA)
Description:	Solid (powder) / white
Lot/Batch #:	91K1176
Purity:	99.5%
Stability of test compound:	The test substance was stable over the study period
Vehicle for CPA:	0.5% methyl cellulose solution (Wake Pure Chemical Industries, Ltd., Osaka, Japan)
4. Test animals:	
Species:	Rat
Strain:	Crj:Wistar, SPF
Sex:	Male
Age at dosing:	6 weeks
Reason for the selection:	This mechanistic study is conducted to investigate the effects on thymus and spleen in rats of this strain observed in a previously performed 2-generation reproduction toxicity study.
Weight at dosing:	188 - 207 g
Source:	from Charles River Japan, Inc, (Yokohama, Kanagawa, Japan).
Acclimation period:	7 days
Diet:	Certified diet MF Mash (Oriental Yeast Co., Ltd., Tokyo), ad libitum
Water:	Filtered and sterilized (sodium hypochloride and UV-light) water in bottles, ad libitum
Housing:	4 animals per wire-mesh stainless steel cage (width 310 mm x depth 440 mm x high 230 mm).
Environmental conditions:	
Temperature:	22 ± 3°C
Humidity:	55 ± 20%
Air changes:	>10/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: 22-Apr-2003 - 10-Jul-2003
In life date: 29-Apr-2003 (start of administration)

2. Animal assignment and treatment:
The animals were assigned to the treatment groups by means of computer generated randomization list based on body weights.
Boscalid was administered to groups of 16 male rats at dietary concentrations of 0, 100, 1000 and 10000 ppm over a period of 4 weeks. Control animals received the ground diet only. Additionally, two groups of male rats were treated orally (gavage) either with 3 mg/kg bw/day CPA or aqueous 0.5% Methyl Cellulose preparation (vehicle used).
Six days before necropsy (day 23), one half of the animals (8 male animals per group) received a single injection into tail vein of 0.5 mL sheep red blood cell (SRBC)-suspension containing 2 x 10^8 cells/mL for immunization.

3. Test substance preparation and analysis:
The diets were prepared every two week by mixing weighed amounts of test substance with a small amount of food in a mortar. Subsequently, appropriate amounts of food were added to obtain the intended dietary concentrations and mixed in a laboratory mixer (HP-60, Kanto Kongoki Industrial Co., Ltd., Tokyo, Japan). Formulated diets were sealed in plastic bags and stored in aluminium containers in a dark and cold room (4°C) until use. These test diets were used within 8 days after opened in an animal room.
The stability of Boscalid in the diet at room temperature over a period of up to 32 days was already verified for previous studies. Nevertheless, the stability of the current diet preparations after storage at 4°C for 14 days and then at room temperature for another 12 days in aluminium container was verified analytically. Homogeneity and concentration analyses of the diet preparations were performed at the beginning of the administration period for all concentrations.

Table 5.8.2-32: Results of homogeneity and concentration control analysis of Boscalid in rodent diet

Nominal Dose level [ppm]	Concentration Mean ± SD# [ppm]	Mean of nominal concentration [%]	Relative standard deviation [%]
100	99 ± 2.6	99	2.6
1000	974 ± 11.2	97	1.1
10000	9682 ± 24.4	97	0.3

Relative standard deviations of the homogeneity of the Boscalid samples were quite low in the range of 0.3 to 2.6%, which indicates the homogenous distribution of Boscalid in the diet preparations. The actual (mean) average test-substance concentrations were in the range of 97 to 99% of the nominal concentrations confirming the correctness of the concentrations. No test substance amount above the LOD of the applied method was found in the control samples.

The positive control substance preparation (CPA in 0.5% Methyl Cellulose solution, corrected for purity) was prepared weekly for a dosing volume of 5 mL/kg bw by ultrasonic exposure for 5 minutes and stored at 4°C.
Stability analysis of the CPA solutions was performed prior to administration start for 4 h at room temperature, 3 days at 4°C in the dark, and 7 days at 4°C in the dark and then at room temperature for 4 hours.

Table 5.8.2-33: Results of storage control analysis of CPA preparations

Nominal Concentration [x 10 mg/L]	Storage period	Analytical concentration [x 10 mg/L]	Mean of nominal concentration [%]
60	0	65	108
60	4 h at room temperature (RT)	65	108
60	3 d at 4°C in the dark	61	102
60	7 d at 4°C in the dark, 4 h at RT	63	105

The stability of the CPA preparations for every storage condition described above was indicated by the measured actual CPA concentrations in the range of 102 to 108% of the nominal concentration, also confirming the correctness of the applied concentration.

4. Statistics:

Statistical significance of the difference between the control and treated groups was be estimated at 5 and 1% levels of probability.
The data of body weight, organ weights, number of cells, population of lymphocytes, and Ig M titres were evaluated by Bartlett's test for equality of variance. When group variances are homogeneous, a parametric analysis of variance of a one way layout type was conducted to determine if any statistical differences exist among groups. When the analysis of variance is significant, Dunnett's multiple comparison test was applied. When the group variances are heterogeneous, the data were evaluated by Kruskal-Wallis non-parametric analysis of variance. When significant, Dunnett type mean rank sum test was applied.
Concerning to the statistical evaluation of the positive control substance treatment, Student's t-test was applied for the data of body weight, organ weights, number of cel1s, population of lymphocytes, and IgM titres.
Fisher's exact probability test (one-tail analysis) was used to analyse the incidences of gross lesions.

C. METHODS

1. Observations:

Mortality, morbidity and clinical signs of toxicity were observed daily. Detailed physical examination including palpation of the body was performed at least once a week.

2. Food consumption and compound intake:

Food consumption was determined for a period of 3 days by 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.

$$\text{Substance intake} = \frac{FC \times C}{BW}$$

Parameters were FC as the mean daily food consumption (in g), C as the nominal concentration in the food (in mg/kg) and BW as mean body weight (in g).

3. Body weight:

The body weight of the animals was determined at the start of the treatment (day 0), and twice weekly thereafter. Additionally, final body weight at study termination was recorded.

4. Immunological examinations:

After 4 weeks of treatment, 8 males in each group were sacrificed under a deep anaesthesia, necropsied, weighed for the thymus and spleen, and subjected to the immunological examinations including cellularity and flow cytometry analysis for the lymphocytes of the thymus and spleen.
Remaining 8 males in each group which is immunized with Sheep red blood cells 6 days before terminal sacrifice were bled through the posterior vena cava for collection of serum and subjected to the immunoglobulin (IgM) measurement

Cell suspension:
After weighing the thymus and spleen, a half of either tissue was placed in PBS with 5% FCS (Foetal calf serum, Life Technologies Co., MD, USA) and kept on ice. The thymus and spleen were individually mashed in a petri dish using a stainless steel mesh. Then, for spleen cells, single cell suspension was suspended in a 0.85% ammonium chloride aqueous solution and incubated for 10 min at room temperature for erythrocyte lysing. The thymus cells without lysing step and spleen cells were further washed twice with 5% FCS PBS.

Cellularity:
Cell number was determined by a Coulter counter Z2 (Beckman Coulter Col, Ltd, Tokyo, Japan). The number of cells per mg unit organ and per organ were calculated.

Flow cytometry:
An aliquot of cell suspension ($1x10^7$) of the thymus and spleen cell was incubated for 10 min at 4°C in 20% goat serum PBS for blocking of non-specific binding. After centrifugation, cells were suspended in 5% FCS PBS. Then cells ($1x10^6$) were incubated for 30 rain at 4°C with following antibodies (AB, BD PharMingen Co., San Diego, USA):
FITC (Fluorescein isothiocyanate)-labelled anti-CD^3 AB, PE (Phycoerythrin)-labelled anti-CD^8 AB, and Cy-Chrom-labelled anti-CD^4 AB for T cell subset analysis; Cy-Chrom-labelled anti-CD^{45}RA AB for B cell analysis and PE-labelled anti-NKR-P1A AB for NK cell analysis.
After staining with fluorescence, the cell were washed with PBS, and analysed on a FACS Calibur (Nippon Becton Dickinson Co., Ltd, Tokyo, Japan). Flow cytometry analyses of the thymic and splenic lymphocyte were performed on the following phenotype populations:
For thymic lymphocyte, immature cell of CD^4/CD^8 double positive cell (DP; $CD^{4+}CD^{8+}$) and CD^4/CD^8 double negative cell (DN; $CD^{4-}CD^{8-}$), and the mature cell of Helper-T cell ($CD^{4+}CD^{8-}$) and Cytotoxic-T cell ($CD^{4-}CD^{8+}$); as well as for splenic lymphocyte, Pan-T cell (CD^{3+}), Pan-B cell ($CD45RA^+$), Helper-T cell ($CD^{4+}CD^{8-}$), Cytotoxic-T cell ($CD^{4-}CD^{8+}$), and NK cell ($NKR\text{-}P1A^+$).
The number of thymic and splenic lymphocyte was evaluated by multiplying the phenotype populations and the number of cells

IgM measurement:
Six days prior to sacrifice at 4 weeks of the treatment, 8 males in each group were immunized via tail vein with 0.5 ml SRBC ($2x10^8$ of Sheep Red Blood Cell; Nippon Bio-supp. Center, Tokyo, Japan) in sterile saline. Trunk blood was collected from the posterior vena cava under an ether anaesthesia and serum sample was obtained. The serum was stored in a deep freezer (below -70°C) until assay. The serum titres of SRBC specific immunoglobulin (Ig) M were determined by an ELISA method. SRBC membrane antigen was extracted with 0.1% of SDS (Sodium n-dodecyl-sulphate) after lysing of cells for carting agent. First, 96-well microplates were coated with 100 µL/well of SRBC membrane antigen, which is prepared at a concentration of 10 mg/mL (in 0.1 M sodium carbonate buffer, pH 9.5), and incubated for 1 hour at room temperature and for about 18 hours (overnight) at 4°C. Microplates were blocked by 200 µL/well of 1% powdered milk solution (Block Ace; Dainippon Pharmaceutical Co. Ltd., Osaka, Japan) for 2 h at room temperature. Then a 100 µL of adequately diluted serum sample (7 dilutions of a 1:4 to 1:16384 dilution), which was diluted with 1% powdered milk solution was added to each well. After a 2-h incubation, peroxidase conjugated affinity purified anti-rat IgM (1:15000 dilution; Rochland Inc. PA, USA) was added to the plate and incubated for another 2 hours, followed by addition of 100 µL/well of the substrate for the enzyme (o-phenylenediamine, OPD). Incubations were carded out at room temperature. The reaction was allowed to proceed for 20 minutes and terminated by addition of 100 µL/well 2 N sulphuric acid. The colour in each well was determined with a plate reader at 492 nm. Prior to each step from coating step to OPD colouring step, microplates were washed 0.01% Tween 20 PBS. Antibody titres were expressed as log values of serum dilution to reach the optical density (OD) of 1.0.

5. Necropsy and pathology:

The animals were sacrificed by decapitation under light aether anaesthesia. The animals killed at schedule as well as animals killed in extremis were necropsied and assessed by gross pathology.

After necropsy the organs and tissues listed below were removed from 8 males in each group which is subjected to the flow cytometry analysis and preserved in neutral buffered 10% formalin for possible future histopathological examination. Thymus and spleen weights were recorded before fixation.

1. Gross lesions
2. Lymph nodes (axillary, cervical and mesenteric)
3. Spleen (half, also weighed)
4. Thymus (left portion)

II. RESULTS AND DISCUSSION

A. OBSERVATIONS

1. Clinical signs of toxicity

No Boscalid-induced clinical signs of toxicity were observed in this study.

One animal of the 10000 ppm group exhibited wound in the ear auricle, pale coloured skin, discolouration of eyes, decreased spontaneous motor activity, hypothermia, and lacrimation and was killed in extremis at week 3. However, due to necropsy observations these signs can be linked to the wound lesions and haemorrhage in the ear auricle, and thus assessed being not induced by the Boscalid exposure.

No clinical signs of toxicity were observed in animals of any other treatment groups, including the positive control.

2. Mortality

No Boscalid-induced mortality was observed during the study.

One animal of the 10000 ppm group was found moribund at week 3 and was killed in extremis, however necropsy evaluation revealed no effects that could be attributed to the Boscalid exposure.

No mortality or morbidity occurred in other treatment groups, including the positive control.

B. FOOD CONSUMPTION AND COMPOUND INTAKE

No test or positive substance-related findings were observed for the food 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 5.8.2-34: Calculated intake of Boscalid via diet

Test group	Concentration in the diet (ppm)	Mean daily test-substance intake (mg/kg bw/day)
		Males
1	100	7.45
2	1000	73.1
3	10000	736.2

C. BODY WEIGHT

Body weight development was not affected either by Boscalid or CPA treatment.

D. IMMUNOLOGICAL ANALYSES

1. Lymphocyte analysis of thymus and spleen

Weight and cellularity of the organs

The thymic and splenic cell number was not affected by Boscalid treatment. In contrast, CPA induced a statistically significant reduction of the cell number in the spleen and to some extend in the thymus, as compared to the control group.

Table 5.8.2-35: Spleen and thymus organ weight and cellularity of male rats treated with Boscalid or Cyclophosphamide for 28 days

	Boscalid				CPA	
Dose [ppm]	**0**	**100**	**1000**	**10000**		
[mg/kg bw/day]		**7.45**	**73.1**	**736.2**	**0**	**3.0**
Spleen						
[cell number ($x10^7$) /mg organ weight]	0.196	0.202	0.194	0.193	0.190	**0.144****
SD	0.018	0.016	0.023	0.026	0.020	0.012
[% of control]	100	103	99	99	100	76
[cell number ($x10^7$) /rat]	168.0	200.5	158.9	181.5	169.6	**97.9****
SD	21.7	28.0	27.8	43.7	34.3	11.4
[% of control]	100	119	95	108	100	58
Thymus						
[cell number ($x10^7$) /mg organ weight]	0.370	0.362	0.385	0.386	0.381	0.364
SD	0.044	0.023	0.029	0.033	0.023	0.050
[% of control]	100	98	104	104	100	96
[cell number ($x10^7$) /rat]	217.3	219.8	211.0	256.1	240.1	**187.6***
SD	47.4	42.6	36.5	69.3	40.3	50.2
[% of control]	100	101	97	118	100	78

* $p \leq 0.05$; ** $p \leq 0.01$ (Student's t-test)

Flow cytometry

Thymic and splenic lymphocyte subsets were not affected by the Boscalid treatment.

In contrast, CPA induced statistically significant decrease in the CD^4/CD^8 double positive cell population of the immature thymic cells and Cytotoxic-T cell population, as compared to the respective control.

Table 5.8.2-36: Splenic and thymic lymphocyte subsets in male rats treated with Boscalid or Cyclophosphamide for 28 days

		Boscalid				CPA	
Dose [ppm]		**0**	**100**	**1000**	**10000**		
[mg/kg bw/day]			**7.45**	**73.1**	**736.2**	**0**	**3.0**
Splenic lymphocyte [$x10^7$/rat]							
Pan-T cell	[mean]	24.2	**34.5***	27.1	30.2	29.2	**15.3****
	SD	7.0	5.5	8.9	5.8	9.4	4.4
	[% of control]	100	143	112	125	100	52
Pan-B cell	[mean]	32.5	43.4	33.5	41.5	38.6	**15.6****
	SD	5.5	11.2	8.8	13.0	9.7	4.6
	[% of control]	100	134	103	128	100	40
Helper-T cell	[mean]	22.6	30.3	23.4	26.6	24.6	**11.2****
	SD	6.7	4.6	6.8	5.0	7.2	3.8
	[% of control]	100	134	104	118	100	46
Cytotoxic-T cell	[mean]	8.9	11.6	9.6	9.8	10.3	**6.7***
	SD	2.3	1.7	2.9	2.2	3.6	1.6
	[% of control]	100	130	108	110	100	65
NK cell	[mean]	4.0	4.2	3.5	3.8	4.0	**2.0****
	SD	1.0	1.0	0.7	1.0	1.3	0.5
	[% of control]	100	105	88	95	100	50
Thymic lymphocyte [$x10^7$/rat]							
Immature cells							
DN cell	[mean]	4.9	4.2	4.0	4.9	4.4	3.5
	SD	2.1	1.1	1.5	1.2	1.3	1.3
	[% of control]	100	86	82	100	100	80
DP cell	[mean]	120.2	117.0	112.8	124.8	118.9	**86.5***
	SD	24.3	26.7	19.7	38.2	22.3	26.3
	[% of control]	100	97	94	104	100	73
Mature cells							
Helper-T cell	[mean]	15.3	17.6	14.6	20.6	19.3	15.3
	SD	4.8	4.4	4.6	6.0	4.1	6.6
	[% of control]	100	115	95	135	100	79
Cytotoxic-T cell	[mean]	4.6	4.8	3.9	5.2	5.1	**3.5***
	SD	1.7	1.5	1.0	1.4	1.2	1.2
	[% of control]	100	104	85	113	100	69

* $p \leq 0.05$; ** $p \leq 0.01$ (Dunnett's test or Student's t-test)

In the lymphocyte subset of the spleen, statistically significant increased Pan-T cell population was observed in males receiving 100 ppm Boscalid. However, since no elevation was observed in the both groups with up to 100-fold higher doses, this finding was considered to be incidental in nature due to the lack of the dose-response. In contrast, CPA induced statistically significant decrease in all splenic lymphocyte subsets as compared to the respective control.

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 male rats treated with Boscalid, whereas the SRBC titres were significantly lower in rats exposed to the positive control CPA [see Table 5.8.2-37].

Table 5.8.2-37: Analysis of the specific primary (IgM) immune response to SRBC in rats treated with Boscalid or Cyclophosphamide for 28 days

Treatment	**Boscalid**				**CPA**	
Dose [ppm]	**0**	**100**	**1000**	**10000**		
[mg/kg bw/day]		**7.45**	**73.1**	**736.2**	**0**	**3.0**
Specific IgM Titre [serum dilution to reach OD of 0.5]						
Mean	472.9	384.5	431.4	280.9	459.9	**60.9****
SD	256.7	172.8	321.6	144.7	458.3	24.8

** $p \leq 0.01$ (Student's t-test)

G. NECROPSY

1. Terminal body weight and organ weights

Analogously to the body weight development, terminal body weight was not affected either by Boscalid or CPA treatment.

Spleen and thymus weights were not affected by Boscalid treatment. In contrast, CPA induced statistically significant decrease of the absolute and relative thymus and spleen weights, as compared to the respective controls.

Table 5.8.2-38: Mean absolute and relative organ weights of male rats treated with Boscalid or Cyclophosphamide for 28 days

	Boscalid				CPA	
Dose [ppm]	**0**	**100**	**1000**	**10000**		
[mg/kg bw/day]		**7.45**	**73.1**	**736.2**	**0**	**3.0**
Terminal bodyweight [g]	382	381	383	384	365	356
SD	31	29	27	16	19	21
[% of control]	100	100	100	101	100	98
Spleen, absolute [mg]	857	991	816	940	889	**682****
SD	59	103	70	177	124	90
[% of control]	100	116	95	110	100	77
Spleen, relative [%]	0.226	0.260	0.213	0.245	0.243	**0.192****
SD	0.025	0.017	0.013	0.048	0.029	0.025
[% of control	100	115	94	108	100	79
Thymus, absolute [mg]	584	609	551	657	628	**509***
SD	95	120	93	134	84	78
[% of control]	100	104	94	113	100	81
Thymus, relative [%]	0.153	0.159	0.144	0.171	0.173	**0.143***
SD	0.022	0.027	0.018	0.034	0.026	0.023
[% of control]	100	104	94	112	100	83

* $p \leq 0.05$; ** $p \leq 0.01$ (Student's t-test)

2. Gross pathology

No treatment-related gross lesions were observed.

The one animal of the 10000 ppm group that was moribund after 3 weeks of Boscalid application and had to be killed in extremis revealed enlargement of the spleen and kidney, red contents in the stomach, distended bladder with urine, and erosion and/or ulcer on the ear auricle and dorsal neck skin (with haemorrhage). This finding was considered to be in line with the observed clinical signs of anaemia might as being associated with the wound lesions and haemorrhage in the ear auricle, and thus suggesting to be not test substance related.

III. CONCLUSIONS

Under the conditions of the study, Boscalid did not reveal any signs of immunotoxicity when administered via the diet to male Wistar rats over a period of 4 weeks. The NOAEL for the immunotoxicity as well as for systemic toxicity was determined to be 10000 ppm (736.2 mg/kg bw/day; highest dose tested).

The oral administration of the positive control substance Cyclophosphamide (3.0 mg/kg bw/day) led to findings indicative of immunotoxicity. This was represented by significantly lower SRBC IgM antibody titres, reduced spleen and thymus weights and cellularity, as well as decreased splenic and thymic lymphocyte subsets. Thus, assay sensitivity was shown in the present immunotoxicity study performed in male Wistar rats.

Literature data

Report: CA 5.8.2/5
Montoya G.A. et al., 2014 a
Mechanistic analysis of metabolomics patterns in rat plasma during administration of direct thyroid hormone synthesis inhibitors or compounds increasing thyroid hormone clearance
2014/1327472
Guidelines: none
GLP: no

Report: CA 5.8.2/6
Kamp H. et al., 2012 a
Reproducibility and robustness of metabolome analysis in rat plasma of 28-day repeated dose toxicity studies
2012/1368184
Guidelines: none
GLP: no

Executive Summary of the Literature

In these publications plasma metabolome investigations were performed and a database was established (MetaMap®Tox) to identify toxicological modes of action (MoAs) such as direct and indirect thyroid toxicity. To establish patterns predictive effects on the thyroid, animals have been treated with reference compounds directly acting on the thyroid hormone formation (such as methimazole, ethylenethiourea) as well as liver enzyme inducers leading to an increased excretion of thyroid hormones and therewith to a secondary response of the thyroid (such as Aroclor 1254 and Boscalid). In the experiments, groups of five Wistar rats per sex were treated with the test substances over a period of 28 days. Boscalid was applied at dietary levels of 15000 ppm. At study days 7, 14, and 28 blood samples from fasted rats were taken and metabolite profiling was performed by the means of mass spectrometry from K-EDTA plasma samples. All animals were checked daily for any clinically abnormal signs and mortalities. Food consumption was determined on study days 7, 14, 21 and 28. Body weight was determined before the start of the administration period in order to randomize the animals and on study days 0, 4, 7, 14, 21 and 28. At the end of the treatment period, the animals were sacrificed by decapitation under Isoflurane anesthesia.

Sets of common metabolite level changes (metabolite patterns) were arranged to characterize thyroid toxicological MoAs. Within the resulting direct and indirect thyroid toxicity pattern some key markers were found. In the direct thyroid toxicity pattern the key metabolite T4 was decreased and tricosanoic acid was increased in both sexes. Overall, a whole range of metabolites were altered by direct-acting thyroid modulators, of which 70% are complex lipids, fatty acids and related and 18% are amino acids and related. For indirect-acting thyroid modulators the majority (78.3 for females and 90.9% for males) of altered metabolites also belongs to complex lipids, fatty acids and related group. In contrast to the direct pattern, thyroxine levels were not consistently decreased but liver lipid metabolism was consistently enhanced (increased fatty acid levels). This profile fits well with typical metabolite changes in profiles correlated to liver toxicity.

The metabolite pattern after treatment with Boscalid is comparable to other indirect-acting thyroid modulators like Aroclor 1254, Fipronil or Pendimethalin. Kamp et al. (2012) evaluated the robustness and reproducibility of the applied methodology, indicating that Boscalid, tested three times under the exact identical experimental and analytical conditions, but at different time points, showed a perfect to good match.

Conclusion of the applicant:
Boscalid, a known indirect-acting thyroid modulator, was used as a model compound for the development of a metabolom project that could be used for screening purposes to identify several MoAs. The experiments with Boscalid were reproducible and indicated that Boscalid produces comparable metabolite patterns as other known indirect-acting thyroid modulators. These results support the fact that Boscalid is an indirect-acting thyroid modulator and provides information regarding the metabolite pattern, which could help to interpret clinical chemistry parameters from other in vivo studies.

Classification of study: Supplementary information

Report: CA 5.8.2/7
Reif D.M. et al., 2010 a
Endocrine profiling and prioritization of environmental chemicals using ToxCast data
2010/1231552
Guidelines: none
GLP: no

Executive Summary of the Literature

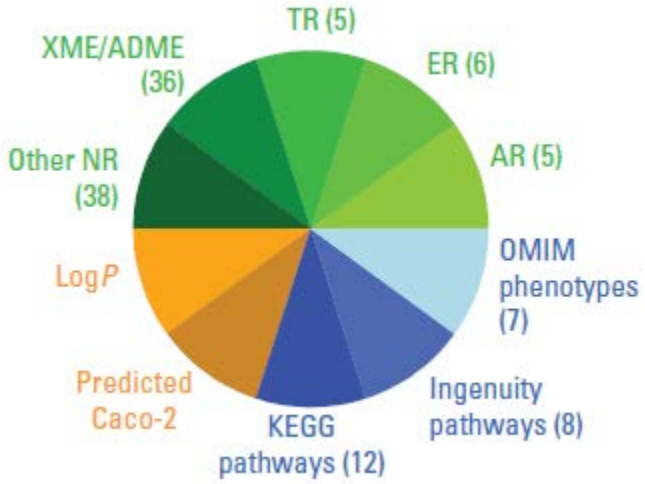
This publication describes a profiling tool developed on the ToxCast database to prioritize chemicals with regard to endocrine disruption evaluation/testing as a decision support tool. Thus this prioritization tool was applied also to Boscalid being part of the ToxCast program. The prioritization tool 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 current evaluation of the results can be seen on EPA's dashboard (http://actor.epa.gov/dashboard/).

The overall ToxPi score sums up the normalised summed potencies of the individual endpoints (ToxPi = f(in vitro assays + chemical properties + pathways). This overall ToxPi score indicates the overall activity of a substance relative to the other chemicals of the underlying database, here the 309 chemicals tested. The range of the overall ToxPi scores within these 309 chemicals was from 0.11 to 5.79. Boscalid showed an overall ToxPi score of 2.67, indicating that it had a medium overall activity compared to the other chemicals tested.

When considering the individual ToxPi scores for the respective assays/endpoints it is obvious that the chemical properties ToxPi score for Boscalid (1.17) contributed to a large extend to the overall score, indicating medium bioactivity when compared to the other chemicals (0.3 – 1.8). Similarly, a low to medium pathway activation was observed for Boscalid with a specific ToxPi of 0.5 in comparison to the other chemicals (0.04 – 1.9).

When evaluating the AR, ER and TR activity profiles it was shown that Boscalid (ToxPi AR = 0) did not affect AR signalling (total range from 0 – 1). Specific ToxPi score for ER signalling was 0.13 for Boscalid (total range 0 – 1), indicating minimal activity in respective ER-related assays. When reassessing the underlying raw data it can be concluded that no activity for ER-signalling may be contributed to Boscalid, as all tests regarding ER-related signalling were checked inactive in the iCSS dashboard application. Specific ToxPi score for TR signalling was 0.47 for Boscalid (total range 0 – 1), indicating moderate activity in respective TR-related assays. The battery of TR-signalling assays consisted of four direct TR-receptor related signalling assays and one assay determining the gene expression of UGT1A1 responsible for T_4 glucuronidation and thus affecting the thyroid pathway by an indirect receptor-independent mode of action. The four receptor-related assays were all negative for Boscalid, whereby Boscalid affected UGT1A1 gene expression.

The summary information (ToxPi) for Boscalid is provided below:



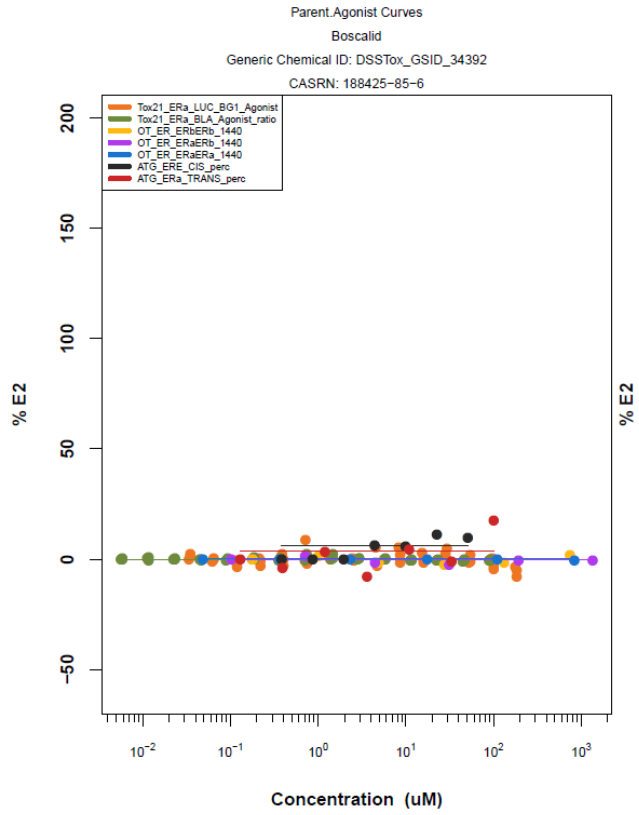
Conclusion of the applicant:
The available ToxPi scores can be used as an indicator for possible effects regarding special endpoints and are based on the ToxCast screening information. The individual test results of the underlying database are discussed in more detail in CA 5.8.3 focussing on the AR, ER and TR signalling. The results from this publication indicate that Boscalid exerts no effect on AR and ER signalling, which is in concordance with further screening information as well as in vivo studies.
The observed medium activity on the thyroid receptor is also in concordance with screening information as well as mechanistic and in vivo studies. The pattern of the underlying database clearly indicates that the mechanism leading to altered thyroid-related parameters is not related to direct TR-mediated signalling rather to altered phase-II metabolism of thyroid hormones.

Classification of study: Supplementary information

Report: CA 5.8.2/8
Rotroff D.M. et al., 2014 a
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 Boscalid is relevant and thus the results are described in the following figures. In summary, Boscalid is negative for estrogen receptor (ER) signaling endpoints namely agonist, antagonist and cell growth responses. The ER Interaction Score was found to be 0 for Boscalid. Thus, Boscalid is one of the 82% chemicals that did not display indications of interacting with the ER signaling pathway.


Parent.Agonist Curves
Boscalid
Generic Chemical ID: DSSTox_GSID_34392
CASRN: 188425-85-6
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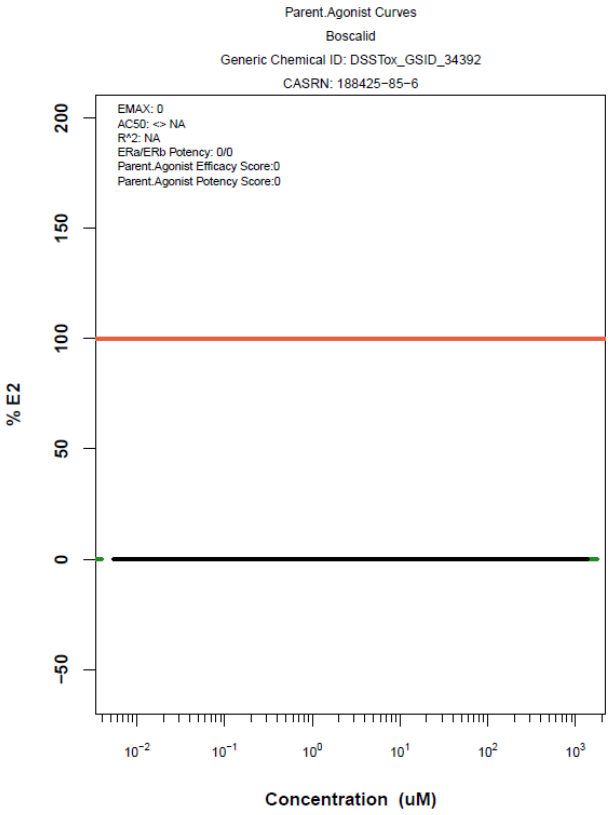
Tox21_ERa_BLA_Agonist_ratio
OT_ER_ERbERb_1440
OT_ER_ERaERb_1440
OT_ER_ERaERa_1440
ATG_ERE_CIS_perc
ATG_ERa_TRANS_perc
% E2
Concentration (uM)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Boscalid
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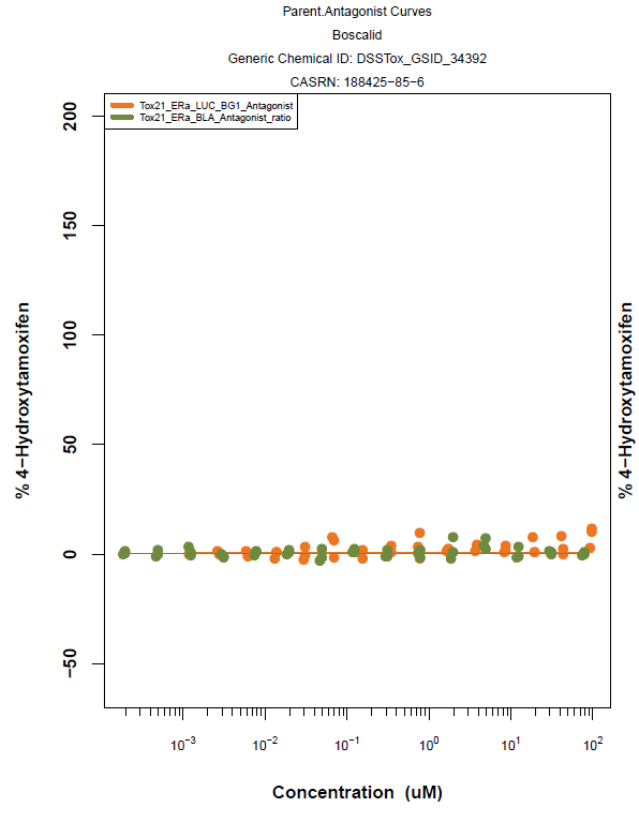
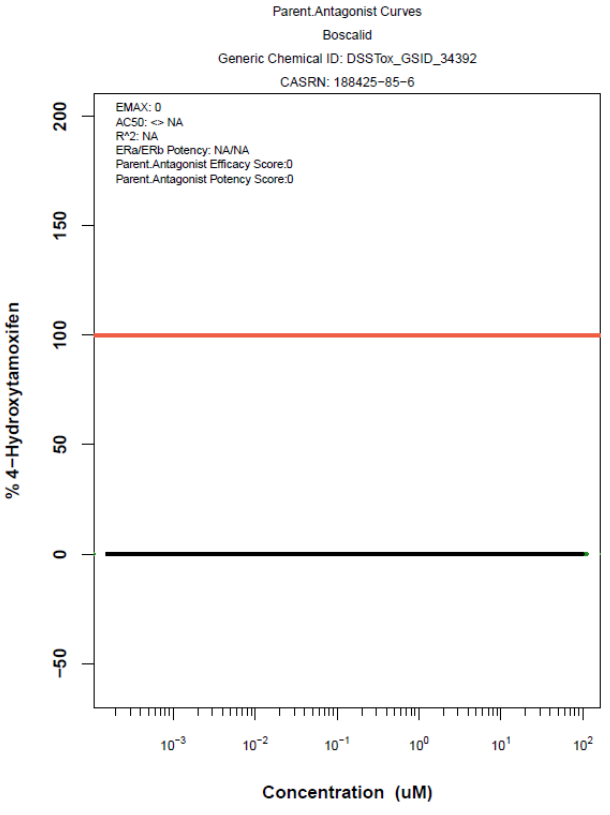
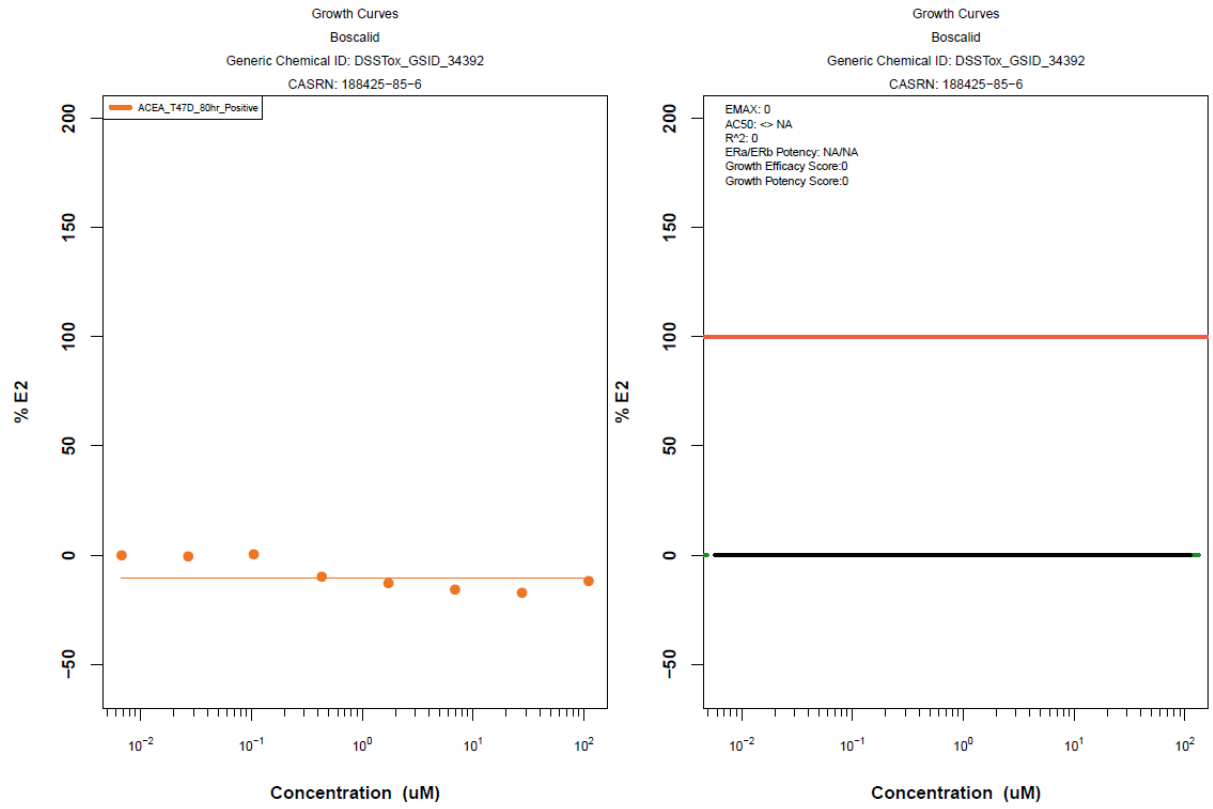
CASRN: 188425-85-6
EMAX: 0
AC50: <> NA
R^2: NA
ERa/ERb Potency: 0/0
Parent.Agonist Efficacy Score:0
Parent.Agonist Potency Score:0
% E2
Concentration (uM)


Parent.Antagonist Curves
Boscalid
Generic Chemical ID: DSSTox_GSID_34392
CASRN: 188425-85-6
Tox21_ERa_LUC_BG1_Antagonist
Tox21_ERa_BLA_Antagonist_ratio
% 4-Hydroxytamoxifen
Concentration (uM)


Parent.Antagonist Curves
Boscalid
Generic Chemical ID: DSSTox_GSID_34392
CASRN: 188425-85-6
EMAX: 0
AC50: <> NA
R^2: NA
ERa/ERb Potency: NA/NA
Parent.Antagonist Efficacy Score:0
Parent.Antagonist Potency Score:0
% 4-Hydroxytamoxifen
Concentration (uM)



Conclusion of the author:
An ER Interaction Score was developed by aggregating data from 13 different in vitro ER assays based on the known cellular ER 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. Boscalid was one of them and found to be zero for the 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 5.8.2/9
Sipes N.S. et al., 2013 a
Profiling 976 toxcast chemicals across 331 enzymatic and receptor signaling assays
2013/1371960
Guidelines: none
GLP: no

Executive Summary of the Literature
Summary report on the ToxCast program. The publication makes some general statements on the progress of the ToxCast program. The data provided for Boscalid is the same as in Shah et al. (see CA 5.8.2/11). No linkage between Boscalid and other endpoints is provided.

Classification of study: Supplementary information

Report: CA 5.8.2/10
Medjakovic S. et al., 2013 a
Effect of nonpersistent pesticides on estrogen receptor, androgen receptor, and aryl hydrocarbon receptor
2013/1419983
Guidelines: none
GLP: no

Executive Summary of the Literature
The aim of the present study was to determine the transactivation potential of Boscalid and other pesticidal active ingredients when tested on human estrogen receptor a (ERα), androgen receptor (AR) and arylhydrocarbon receptor (AhR) in vitro. Moreover pesticidal products containing the considered active ingredient were tested for transactivation potential, one of which was Cantus, a water dispersible granular preparation containing 50% (w/w) of the active substance Boscalid. Relative binding affinities of the pure pesticide constituents for AR and their proliferative effect on human breast cancer and prostate cancer cell lines were evaluated.

Boscalid showed no binding or transactivation potential to the AhR. No activity regarding EC_{50} for AR or ERα or IC_{50} regarding AR was detectable. No ERα transactivating potential of Boscalid was observed when tested in the range of 10 nM to 100 µM in the yeast estrogen assay α (yESα). Furthermore, no AR transactivating potential of Boscalid was observed when tested in the range of 10 nM to 100 µM in the yeast androgen assay (yAS). Boscalid had no effect on the cell growth of LNCaP (androgen sensitive), DU-145 (androgen insensitive), MCF-7 (ERα positive), and MDA-MB-231 cells (ERα negative). In conclusion, Boscalid showed no AhR binding potential and no effects on AR or ERα regulated transactivation or cell growth was observed.

Classification of study: Supplementary information

Report: CA 5.8.2/11
Shah I. et al., 2011 a
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.
Boscalid, was grouped into category VI E; group E indicated, that the compound can activate the nuclear receptors AhR, CAR and PXR. The rationale for this grouping is largely based on the activation of the reporter genes.

Boscalid was tested active in the following assays: ATG_PPARg_TRANS and ATG_PXRE_CIS as well as CLZD_CYP1A1_24, CLZD_CYP1A2_24, CLZD_CYP2B6_24, and CLZD_CYP3A4_24. Boscalid was not associated with the activation in other assays targeting estrogen receptor and/or androgen receptor.

Classification of study: Supplementary information

Report: CA 5.8.2/12
Rotroff D.M. et al., 2010 a
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 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.
Boscalid induced gene expression of following genes: CYP1A2, UGT1A1, CYP3A4, CYP1A1, CYP2B6, SULT2A1, GSTA2, CYP2C9, ABCB1.
Expression of genes that were induced by Boscalid were linked to AhR, CAR, and PXR. In conclusion, these results together with the results of the transactivation studies (Shah et al., 2011) should be used as supporting screening information only.

Classification of study: Supplementary information

CA 5.8.3 Endocrine disrupting properties

The most widely used definition of an endocrine disruptor is based on the WHO/IPCS (2002): 'An endocrine disrupter is an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations'. This definition is purely based on hazard identification, looking at whether the adverse effects reported are regarded to be ED-related and supported by mechanistic information.

According to Regulation (EC) No 1107/2009, Annex II, Point 3.6.5 'an active substance shall only be approved if, […], it is not considered to have endocrine disrupting properties that may cause adverse effect in humans.'
Nevertheless, there is no regulatory guidance available yet on how to address endocrine disruption (ED) and no final criteria are established.
Pending the adoption of the final scientific criteria for the determination of ED properties, currently the so called Interim criteria are applied. There are two interim criteria defined within Regulation (EC) No 1107/2009, Annex II, Point 3.6.5:

1) '[…] substances that are or have to be classified, in accordance with the provisions of Regulation (EC) No 1272/2008, as carcinogenic category 2 and toxic for reproduction category 2, shall be considered to have endocrine disrupting properties.'

2) 'Substances such as those that are or have to be classified, in accordance with the provisions of Regulation (EC) No 1272/2008, as toxic for reproduction category 2 and which have toxic effects on the endocrine organs, may be considered to have such endocrine disrupting properties.'

There are no commonly accepted scientific criteria defined to assess adversity and what specifically is considered to be 'toxic effects on endocrine organs', which leaves space for interpretation and does not give a guidance. Additionally, no clear advice is given in the Interim Criteria as to which organs are to be regarded as endocrine.

All studies for the evaluation of carcinogenic effects and reproduction toxicity have already been peer-reviewed by the Rapporteur Member State Germany and national competent authorities following submission of the original Annex II Dossier (2000). The endpoints were fixed in the European Commission Review Report for the active substance Boscalid (SANCO/3919/2007 –Rev 5, 21 January 2008).

Regarding the classification of Boscalid it is at present neither classified for carcinogenicity nor for reproductive toxicity in the EU, nor does the review of the data made within this AIR3 renewal process come to a different conclusion (see M-CA 5.5 and M-CA 5.6). Thus, the conditions of the Interim Criterion 1 are not met for Boscalid. Likewise, Boscalid is not classified for reproduction toxicity with concomitant toxic effects on endocrine organs and thus, the conditions of the Interim Criterion 2 are also not met for Boscalid.

The evaluation of potential endocrine disruption was not a data-requirement at the time of Annex I inclusion of Boscalid. Therefore, all relevant pivotal in vivo studies as well as mechanistic studies already evaluated for the purpose of the first Annex I inclusion were reviewed within this supplemental dossier with regard to the identification of possible endocrine disrupting effects and did not provide indication of such.
Besides the effects on the liver, including increased liver weights and cell hypertrophy as the predominant cellular alteration, the thyroids were identified as a second target organ as evidenced by weight increases (rats and dogs) and concomitant histopathological alterations, notably follicular cell hypertrophy/hyperplasia (rats only). With regard to the identification of endocrine disrupting properties, the available toxicological database of Boscalid was in addition screened for possible endocrine-mediated effects, which are further discussed below. For this, mechanistic data and results of scientific literature were considered.

Estrogenic/anti-estrogenic effects

In the following all available data are evaluated regarding estrogenic or anti-estrogenic effects. Available information includes a 2-generation study in rats, developmental toxicity studies in rats and rabbits, and screening studies from public literature (see Table 5.8.3-1)

Table 5.8.3-1: Overview on available information from studies/literature regarding estrogenicity.

Study type	Endpoint (Conclusion)	Main effects/target organ	Reference
Level 5 screening studies (according to OECD guidance on EDC, GD150)			
2-Generation, Wistar rat Oral, feed: 0, 100, 1000 & 10000 ppm (see CA 5.6)	Parental toxicity Fertility Offspring toxicity	Increased liver weights with histopathological correlates (hypertrophy) No effects observed regarding fertility or offspring toxicity that were related to estrogenic/anti-estrogenic effects	2001/1000117
Level 4 screening studies (according to OECD guidance on EDC, GD150)			
Developmental toxicity, Wistar rat Gavage: 0, 100, 300 & 1000 mg(kg bw (day 6-19) (see CA 5.6)	Maternal toxicity Developmental toxicity	No effects observed regarding maternal or offspring toxicity that were related to estrogenic/anti-estrogenic effects	2000/1015001
Developmental toxicity, Himalayan rabbits Gavage: 0, 100, 300 & 1000 mg(kg bw (day 7-28) (see CA 5.6)	Maternal toxicity Developmental toxicity	No effects observed regarding maternal or offspring toxicity that were related to estrogenic/anti-estrogenic effects	2000/1013425
Level 2 screening studies (according to OECD guidance on EDC, GD150)			
Protein-fragment complementation assays on estrogen receptor alpha (ERα) and beta (ERβ) homo- and heterodimerization (ERα-ERα; OT_ERaERa_1440,	ER Receptor dimerization (no effect)	Inactive: No dimerization activity	Rotroff et al., 2014 2014/1323273 ToxCast Data accessible via iCSSDashboard http://actor.epa.gov/actor/faces/CSSDashboardLaunch.jsp

Table 5.8.3-1: Overview on available information from studies/literature regarding estrogenicity.

Study type	Endpoint (Conclusion)	Main effects/target organ	Reference
ERα-ERβ; OT_ERaERb_1440, ERβ-ERβ; OT_ERbERb_1440)			
yESα	ERα-transactivation (no effect)	not estrogenic (10 nM-100 µM)	Medjakovic et al., 2013 2013/1419983
mRNA transcription ATG_ERE_CIS and ATG_ERa_TRANS ATG_ERRa_TRANS_up ATG_ERRg_TRANS_up	ER transcription factor mRNA transcription (no effect)	Inactive: No mRNA transcription	Rotroff et al., 2014 2014/1323273 ToxCast Data accessible via iCSSDashboard http://actor.epa.gov/actor/faces/CSSDashboardLaunch.jsp
Transactivation assay Tox21_ERa_BLA_agonist, Tox21_ERa_BLA_antagonist,	ERa-transactivation (no effect)	Inactive: No receptor agonist or antagonist	Rotroff et al., 2014 2014/1323273 ToxCast Data accessible via iCSSDashboard http://actor.epa.gov/actor/faces/CSSDashboardLaunch.jsp
Transactivation assay Tox21_ERa_LUC_BG1_agonist Tox21_ERa_LUC_BG1_antagonist	ERa-transactivation (no effect)	Inactive: No receptor agonist or antagonist	Rotroff et al., 2014 2014/1323273 ToxCast Data accessible via iCSSDashboard http://actor.epa.gov/actor/faces/CSSDashboardLaunch.jsp
Cell proliferation T47D cell growth assay (ACEA_T47D_80hr_Positive; ACEA_T47D_80hr_Negative)	Cell proliferation (no effect)	Inactive: No estrogen sensitive cell proliferation	Rotroff et al., 2014 2014/1323273 ToxCast Data accessible via iCSSDashboard http://actor.epa.gov/actor/faces/CSSDashboardLaunch.jsp
Estrogen sensitive cell proliferation assay (MCF-7)	Hormone dependent cell proliferation (no effect)	Not estrogenic (10 µM and 100 µM)	Medjakovic et al., 2013 2013/1419983
Level 1 screening studies (according to OECD guidance on EDC, GD150)			
QSAR Profiling Tool	Activity on ER pathway	ER pathway activation (minimum)	Reif et al., 2010 DocID 2010/1231552

The available screening studies indicate that Boscalid is not a potent estrogen or anti-estrogen. The yeast-assay and ERα-transactivation assays performed within the EPA ToxCast program were clearly negative. The E-screen was reported to be negative in the recent publication of Medjakovic et al. 2013 [see CA 5.8.2/10]. In the available in vivo studies no indication of an estrogenic or anti-estrogenic effect was observed.

No effects on gestation, gestation length, implantation loss, sex distribution and sexual maturation data, malformations or histopathological abnormalities have been observed in the 2-generation study in rats, and the developmental toxicity studies in rats and rabbits.

In conclusion, Boscalid is considered to have no estrogenic or anti-estrogenic properties based on the available information.

Androgenic/anti-androgenic effects

In the following all available data are evaluated regarding androgenic or anti-androgenic effects. Available information includes several screening assays as well as subchronic, chronic, and carcinogenicity studies in mice, rats, or dogs (see Table 5.8.3-2).

Table 5.8.3-2: Overview on available information from studies/literature regarding androgenicity.

Study type	Endpoint (Conclusion)	Results	Reference
Level 5 screening studies (according to OECD guidance on EDC, GD150)			
2-Generation, Wistar rat Oral, feed: 0, 100, 1000 & 10000 ppm (see CA 5.6)	Parental toxicity Fertility Offspring toxicity	Increased liver weights with histopathological correlates (hypertrophy) No effects observed regarding fertility or offspring toxicity that were related to androgenic/anti-androgenic effects	2001/1000117
Level 4 screening studies (according to OECD guidance on EDC, GD150)			
Developmental toxicity, Wistar rat Gavage: 0, 100, 300 & 1000 mg(kg bw (day 6-19) (see CA 5.6)	Maternal toxicity Developmental toxicity	No effects observed regarding maternal or offspring toxicity that were related to androgenic/anti-androgenic effects	2000/1015001
Developmental toxicity, Himalayan rabbits Gavage: 0, 100, 300 & 1000 mg(kg bw (day 7-28) (see CA 5.6)	Maternal toxicity Developmental toxicity	No effects observed regarding maternal or offspring toxicity that were related to androgenic/anti-androgenic effects	2000/1013425
Level 2 screening studies (according to OECD guidance on EDC, GD150)			
Receptor mediated pathway activation OT_AR_ARELUC_ AG_1440	Gene expression regulation (no effect)	Inactive: No activation of AR pathway	ToxCast Data accessible via iCSSDashboard http://actor.epa.gov/actor/faces/CSSDashboardLaunch.jsp
Pathway specific protein stabilization OT_AR_ARSRC1_0480 OT_AR_ARSRC1_0960	Gene expression regulation (no effect)	Inactive: No protein stabilization	ToxCast Data accessible via iCSSDashboard http://actor.epa.gov/actor/faces/CSSDashboardLaunch.jsp

Table 5.8.3-2: Overview on available information from studies/literature regarding androgenicity.

Study type	Endpoint (Conclusion)	Results	Reference
mRNA transcription ATG_AR_trans_up	AR transcription factor mRNA transcription (no effect)	Inactive: no mRNA transcription	ToxCast Data accessible via iCSSDashboard http://actor.epa.gov/actor/faces/CSSDashboardLaunch.jsp
Transactivation assay Tox21_AR_BLA_agonist Tox21_AR_BLA_ antagonist	AR-transactivation (no effect)	Inactive: Not a receptor agonist or antagonist	Shah et al., 2011 2011/1295091 ToxCast Data accessible via iCSSDashboard http://actor.epa.gov/actor/faces/CSSDashboardLaunch.jsp
Transactivation assay Tox21_AR_LUC_ MDAKB2_agonist Tox21_AR_LUC_ MDAKB2_antagonist	AR-transactivation (no effect)	Inactive: Not a receptor agonist or antagonist	Shah et al., 2011 2011/1295091 ToxCast Data accessible via iCSSDashboard http://actor.epa.gov/actor/faces/CSSDashboardLaunch.jsp
Testing for androgenic and anti-androgenic potential in yeast (YAS)	AR- transactivation (no effect)	not androgenic (10 nM-100 µM)	Medjakovic et al., 2013 2013/1419983
Androgen sensitive Proliferation assay (LN CAP)	Hormone dependent cell proliferation (no effect)	No proliferative nor proliferation inhibition effect in androgen sensitive cell line LNCaP	Medjakovic et al., 2013 2013/1419983

The available screening studies indicate that Boscalid is not a potent androgen or anti-androgen. No AR-binding or induction of AR-mediated gene expression was observed after incubation with Boscalid. A YAS-assay and a cell proliferation assay in androgen-sensitive cells showed no evidence of androgenic or anti-androgenic effects. This conclusion is supported by the various in vivo studies of the underlying toxicology data base were no effects related to a potential androgenic/anti-androgenic mode of action were observed.

No effects on male sexual organs were noticed in any of the repeated dose toxicity studies conducted. No effects on gestation, gestation length, implantation loss, sex distribution and sexual maturation data, malformations or histopathological abnormalities have been observed in the 2-generation study in rats, and the developmental toxicity studies in rats and rabbits.

In conclusion, Boscalid is considered to have no androgenic or anti-androgenic properties based on the available information.

Thyroid effects

In the following all available data are evaluated regarding effects on the thyroid. Available information includes several screening assays, mechanistic studies as well as subchronic, chronic, and carcinogenicity studies in mice, rats, or dogs and reprotoxicity in rats and rabbits (see Table 5.8.3-3).

Table 5.8.3-3: Overview on available information from studies/literature regarding effects on the thyroid.

Study type	Endpoint	Results	Reference
Level 5 studies (according to OECD guidance on EDC, GD150)			
2-Generation, Wistar rat Oral, feed: 0,100, 1000 & 10000 ppm (see CA 5.6)	Parental toxicity Fertility Offspring toxicity Sexual maturation	Increased liver weights with histopathological correlates (hypertrophy), thyroids not investigated No effects observed regarding fertility or offspring toxicity considered to be related to thyroid-linked parameters, no effects on sexual maturation	2001/1000117
Level 4 studies (according to OECD guidance on EDC, GD150)			
Developmental toxicity, Wistar rat Gavage: 0, 100, 300 & 1000 mg(kg bw (day 6-19) (see CA 5.6)	Maternal toxicity Developmental toxicity	No effects observed regarding maternal or offspring toxicity considered to be related to thyroid-linked parameters	2000/1015001
Developmental toxicity, Himalayan rabbits Gavage: 0, 100, 300 & 1000 mg(kg bw (day 7-28) (see CA 5.6)	Maternal toxicity Developmental toxicity	No effects observed regarding maternal or offspring toxicity considered to be related to thyroid-linked parameters	2000/1013425
Rat: 28-day dermal 0, 100, 250 & 1000 mg/kg bw/day (see CA 5.3)	Clinical signs, Pathology, Histology	No effects observed related to thyroid-linked parameters or tissues	2000/1013240
Rat: 90-day oral 0, 100, 500, 2000, 5000 & 15000 ppm (see CA 5.3)	Clinical signs, Pathology, Histology	Increased liver and thyroid weights with histopathological correlates (hypertrophy)	2000/1012190
Mouse: 90-day oral 0, 150, 1000, 4000 & 8000 ppm (see CA 5.3)	Clinical signs, Pathology, Histology	Increased liver weights (fatty liver) No effects observed related to thyroid-linked parameters or tissues	2000/1000188
Dog: 90-day oral 0, 250, 2500 & 25000 ppm (see CA 5.3)	Clinical signs, Pathology, Histology	Increased liver and thyroid weights without histopathological correlates	2000/1012306

Table 5.8.3-3: Overview on available information from studies/literature regarding effects on the thyroid.

Study type	Endpoint	Results	Reference
Dog: 12-month oral 0, 200, 800, 2000 & 20000 ppm (see CA 5.3)	Clinical signs, Pathology, Histology	Increased liver and thyroid weights without histopathological correlates	2000/1016881
24-month oral chronic toxicity in Wistar rats 0, 100, 500, 2500, 15000 ppm (see CA 5.5)	Clinical signs Pathology Histology	Increased liver and thyroid weights with histopathological correlates (hypertrophy)	2001/1000114 (2002/1004026)
24-month oral carcinogenicity study in Wistar rats 0, 100, 500, 2500, 15000 ppm (see CA 5.5)	Clinical signs Pathology Histology	Increased liver and thyroid weights with histopathological correlates (hypertrophy) Slightly increased incidence of thyroid follicular cell adenomas (not considered relevant for humans)	2001/1000115
18-month carcinogenicity C57BL mouse 0, 80, 400, 2000, 8000 ppm (see CA 5.5)	Clinical signs Pathology Histology	Increased liver weights with histopathological correlates (hypertrophy) No effects observed related to thyroid-linked parameters or tissues	2001/1000116
Level 3 studies (according to OECD guidance on EDC, GD150)			
14-day feeding study in Wistar rats – perchlorate discharge assay	Iodine uptake into the thyroid and discharge after perchlorate administration	Increased thyroid weights Significant higher iodine uptake No discharge of iodine after perchlorate administration	2004/1013467
14-day feeding study in Wistar rats	Hepatic enzyme induction study	Increased liver weights Increased CYP P450 content Proliferation/accumulation of smooth ER in zone 3 hepatocytes Glycogen depletion	1999/10522
28-day feeding study in Wistar rats	Hepatic enzyme induction study including determination of thyroid hormones	Increased liver weights (thyroid weight not determined) Increased Phase II enzyme activities Increased TSH levels Decreased T_3/T_4 levels	2001/1000141
28-day feeding study in Wistar rats (supplementary study with additional dose levels)	Hepatic enzyme induction study including determination of thyroid hormones	Increased liver and thyroid weights Increased Phase I/II enzyme activities Increased TSH levels Decreased T_4 levels	2003/1012736
28-day feeding study in Wistar rats with 4-weeks and 13-weeks recovery period	Determination of thyroid hormones, liver and thyroid weights	Increased liver and thyroid weights, increased TSH levels. Recovery of organ weight increase, histological changes and TSH levels	2001/1017611

Table 5.8.3-3: Overview on available information from studies/literature regarding effects on the thyroid.

Study type	Endpoint	Results	Reference
Montoya et al., 2014 Kamp et al, 2012	Metabolome analysis	Metabolome pattern of Boscalid was similar to several known indirect-acting thyroid modulators	2014/1327472 2012/1368184.
Level 2 studies (according to OECD guidance on EDC, GD150)			
mRNA transcription ATG_THRa1_ trans_up	TR mRNA transcription factor transcription	Inactive: TR transcription factor mRNA transcription	ToxCast Data accessible via iCSSDashboard http://actor.epa.gov/actor/faces/CSSDashboardLaunch.jsp
Transactivation assay TOX21_TR_LUC_ GHR_Agonist TOX21_TR_LUC_GHR _Antagonist	TR transactivation	Inactive. No receptor agonist or antagonist	ToxCast Data accessible via iCSSDashboard http://actor.epa.gov/actor/faces/CSSDashboardLaunch.jsp
Level 1 studies (according to OECD guidance on EDC, GD150)			
QSAR Profiling Tool (based on results from Shah et al, 2011)	Activity on TR pathway	TR pathway activation (negative), increase of T_4 glucuronidation	Reif et al., 2010 DocID 2010/1231552

Summary evaluation on ED assessment

All relevant pivotal in vivo studies as well as mechanistic studies already evaluated for the purpose of the first Annex I inclusion were reviewed within this supplemental dossier with regard to the identification of possible endocrine disrupting properties. No findings attributable to possible endocrine disrupting properties were made in the 2-generation study in rats (DocID 2001/1000117), in the prenatal toxicity studies in rats (Doc ID 2000/1015001) and rabbits (DocID 2000/1013425), the 28-day dermal toxicity study in rats (Doc ID 2000/1013240) and the 90-day feeding study in mice (DocID 2000/1000188). In further studies the following findings were made:

90-day feeding rat (DocID 2000/1012190)

Increased thyroid weights (≥ 2000 ppm, i.e. ≥137 mg/kg bw/day) with correlating histopathological changes (follicular cell hypertrophy / hyperplasia) were observed in males. Minimal up to slight hypertrophy of the follicular epithelium and slight diffuse hyperplasia of follicular cells were observed with increased incidence in dose groups of 2000 ppm onwards, albeit without a clear dose response. In female rats statistically significantly increased thyroid weights were observed at dose levels of 5000 ppm and 15000 ppm (i.e. ≥395 mg/kg bw/day). Although no corresponding histomorphological changes were seen at these dose levels the organ weight changes suggest a dose related response in female rats. Adrenal weights (absolute and relative) were decreased at 5000 ppm and 15000 ppm dose levels. However, there was no histological finding correlated to the decreased weights observed as evidenced by histopathological investigation of the control and top dose group.

90-day feeding dog (DocID 2000/1012306)
Relative thyroid weight of females were increased at the top dose level of 25000 ppm (i.e. 825 mg/kg bw/day), however, without dose dependent histological correlate. The only finding was C-cell hyperplasia which occurred evenly distributed in a single animal in each of the female dose groups including the control group and one isolated finding each in the low and intermediate dose group of males. There were no findings in other organs of the endocrine system that could be related to the test substance administration

360-day feeding dog (DocID 2000/1016881)
Increase in thyroids weights were observed at dose levels of 2000 (i.e. 57.4 mg/kg bw/day in males and 58.3 mg/kg bw/day in females) and 20000 ppm (i.e. 544 mg/kg bw/day in males and 593 mg/kg bw/day in females), albeit not corroborated by dose dependent macro- and microscopic findings. Absolute and relative weights of other organs of the endocrine system were not adversely affected. Some isolated findings were made in testes, prostrate, adrenals and pituitary glands such as reduced size or enlargement, foci or cysts with the top dose group mostly having no findings at all. Notably all findings of these organs (except thyroids) were without dose-response relationship and thus considered of spontaneous nature.

24-month chronic feeding study rats (DocID 2001/1000114)
Increased absolute thyroid weights (2500 ppm, males) with correlating histopathological changes (diffuse follicular cell hypertrophy and focal follicular cell hyperplasia in both sexes at 2500 ppm corresponding to 110 mg/kg bw/day in males and 150 mg/kg bw/day in females) were seen. Absolute and relative weights of other organs of the endocrine system were not adversely affected. Since the study was performed over the whole life span of the test animals various findings were observed in the macroscopic and microscopic examination of endocrine related organs. Macroscopically there was increased number of cystic degenerations of in top-dose males which correlated histopathologically mainly with diffuse tubular degenerations or Leydig cell adenomas. Both, the incidence of Leydig cell hyperplasia and Leydig cell adenoma gave no indication of a dose response relationship and these findings were thus considered to be not test substance-related. All other morphological changes detected showed either a single occurrence or no dose-response relationship and were seen to be of incidental or spontaneous nature and were not induced by the test substance.

24-month carcinogenicity study rats (DocID 2001/1000115)
Treatment-related findings included increased incidences of thyroid follicular cell adenomas (2500 ppm, both sexes corresponding to 116.1 mg/kg bw/day in males and 155.6 mg/kg bw/day in females) in combination with increased thyroid weights (2500 ppm, males) and correlating histopathological changes (diffuse follicular cell hypertrophy and focal follicular cell hyperplasia in both sexes). Since the study was performed over the whole life span of the test animals various findings were observed in the macroscopic and microscopic examination of endocrine related organs. Male animals of the mid dose group a statistically significant increase in absolute testes weight of 20.2% and decrease in relative adrenal weight of 14.3% as compared to the controls. Absolute and relative weights of other organs of the endocrine system were not adversely affected. Since the study was performed over the whole life span of the test animals various findings were observed in the macroscopic and microscopic examination of the sexual organs. No particular findings were made that were considered attributable to a specific endocrine related effect of Boscalid.

18-month carcinogenicity study mouse (DocID 2001/1000116)
No effects on thyroids were observed. Since the study was performed over the whole life span of the test animals various findings were observed in the macroscopic and microscopic examination of endocrine related organs. Males at all dose levels showed a statistically significant increase in relative testes weights. This effect was, however, was lacking a clear dose-response relationship and there were no differences in absolute testes weights. This finding has thus been associated with the slightly lower body weights and this conclusion has been confirmed by the absence of correlates in the macroscopic and microscopic examination of the organ. This effect was considered to be incidental in nature and not treatment related. All males showed a statistically significant increase of absolute and relative adrenal weights. However, this has been associated with the fact that both, the absolute and relative adrenal weights in control animals were the lowest in the set of historical control animals, and all absolute as well as relative adrenal weight values of this study were within the range of historical control data. This effect was therefore considered to be devoid of a dose response. Therefore, this effect was assessed to be incidental in nature and not treatment-related. Absolute and relative weights of other organs of the endocrine system were not adversely affected

In **supplementary mechanistic studies**, effects of Boscalid on hepatic enzymes in rats and, in particular, on the thyroid in the rat have been investigated. It was demonstrated that Boscalid administered at 5000 ppm in the diet to rats for 14 days did not show a thyroid blocking activity. There were no indications of inhibition of iodine organification in the thyroid (i.e. tyrosine iodination) or of a significant discharge of [^{125}I] after perchlorate challenge by co-administration. Results after treatment with Boscalid were similar to those obtained with phenobarbital which modifies also thyroid homeostasis by an indirect mechanism via increased hepatic clearance of thyroid hormones. Conversely, PTU known to directly block the thyroid function by inhibiting the iodination in the thyroid gland gave the expected effect with decrease of iodide uptake in the thyroid and significant discharge of [^{125}I] after perchlorate co-administration.
Furthermore, additional mechanistic studies with Boscalid have shown that the test substance induces liver weight increase with concomitant histopathological changes in zone 3 hepatocytes (proliferation/accumulation of smooth endoplasmatic reticulum (SER) and glycogen depletion). These changes are considered to be related to the induction of hepatic metabolizing enzymes.

Boscalid induced both phase I (oxidative) enzymes, as demonstrated by increased cytochrome P450 content and increased EROD, PROD and BROD activities, as well as phase II (conjugation) enzymes. The latter was shown by increased activities of p-nitrophenol-glucuronyltransferase (pNP-GT), 4-methylumbelliferoneglucuronyltransferase (MUF-GT), and 4-hydroxybiphenyl-glucuronyltransferase (HOBI-GT) as well as glutathione-S-tranferase. The increases in phase II (conjugation) hepatic activity were accompanied by the reduction of thyroid hormone T3 and T4 levels via increased hepatic clearance and by a concomitant increase in TSH. While the decrease in serum T3 and T4 concentrations appear to have resulted from increased elimination via Boscalid-mediated induction of phase II hepatic enzyme activities, the increased TSH levels were obviously the result of a feedback mechanism induced by decreased T3 and T4 serum levels [Hurley et al. 1998; K-CA 5.8.2/13, 1998/1009835]. Withdrawal of Boscalid from the feed after 4 weeks of exposure leads to reversibility of both, increased liver and thyroid weights including the histopathological changes seen as well as TSH serum concentrations to levels of untreated controls within 4 weeks and 13 weeks, respectively.

These data are in line with screening information from QSAR and transactivation assays were no thyroid receptor-dependent transactivation was observed with Boscalid.

Relevance assessment of animal thyroid findings for human risk evaluation

In comparison to laboratory rodents, humans are considered to be less sensitive to the mode of action underlying the effects seen in the rat studies. Reasons are the existence of the thyroxine-binding globulin, a serum protein that is missing or detected at very low levels in laboratory rodents. Humans have a substantial reserve supply of thyroid hormone, much of which is carried in thyroxine-binding globulin, a serum protein that is missing in laboratory rodents [Odell W. D. et al.; 1967; Studies of thyrotropin physiology by means of radioimmunoassay; Proceedings of the 1966 Laurentian Hormone Conference; edited by G. Pincus; Academic Press, Volume 23: 47 – 85 K-CA 5.8.2/14, 1967/10025]. Therefore, release of stored thyroid hormones causes serum hormone levels to stay normal for weeks in euthyroid humans [Martindale W. B.; 1993; Carbimazole and other antithyroid agents. In the Extra Pharmacopoeia (N. W. Blacow, Ed.) 26th ed., 379 – 385, Pharmaceutical Press, London K-CA 5.8.2/15, 1993/11283] and for weeks to several months in hyperthyroid individuals [Odell W. D. et al.; 1967] despite daily doses of anti-thyroid drugs, sufficient to completely block synthesis. Under conditions of prolonged thyroid insufficiency, caused for example by dietary iodine deficiency, the primary human response resulting from increased TSH levels, is goiter rather than neoplasia [Costigan M.; 1998; The relevance of rat thyroid gland tumours to humans; HSE Toxicology Unit, Bootle K-CA 5.8.2/16, 1998/1001262 and Choksi et al., 2003; K-CA 5.8.2/17, 2003/1034659].
Furthermore, the half-lives of thyroid hormones are much longer in humans than in rats and dogs. Another important point for risk assessment is the fact that development of thyroid cancer in humans is rather rare compared to laboratory animals.

The following table clearly indicates that several fundamental parameters of the thyroid system vary between laboratory animals and humans explaining the effects observed in the animal studies and supporting the conclusion that the effects are considered not relevant for humans. In summary, species can be grouped in sensitive (e.g. rat, dog) and resistant (e.g. human) species in regard to xenobiotic increased hepatic clearance of thyroid hormones. Half-life of T4 and T3 in humans is distinctly greater than in rat and dog which is associated with the thyroid hormone storage capacity via thyroxine-binding globulin levels being high in humans but low in the rat and dog. In regard to the dog half-life of T4 and T3 as well as thyroxine-binding levels [Daminet S., Ferguson D.; C. K-CA 5.8.2/18, 2003/1034759] is considered comparable to the rat.

Parameter	**Human***	**Rat***	**Dog****
Half-life of T4	5-9 days	0.5-1 day	0.5-0.75 day
Half-life of T3	1 day	0.25 day	0.25 day
Thyroxine-binding globulin levels	High	Very low	Low (15% of humans)

***** Choksi et al., 2003; K-CA 5.8.2/17, 2003/1034659
** Daminet S., Ferguson D.; C. K-CA 5.8.2/18, 2003/1034759

The available screening, mechanistic, and in vivo studies support the conclusion that Boscalid has thyroidal relevance for rats. In dogs, thyroid effects suggest to be less pronounced, given the fact that the increase in thyroid weights were marginal in the 90-day study with statistically identified differences for relative organ weight in females of the top dose (25000 ppm, 825 mg/kg bw/day) only, and at high dose levels (2000 ppm, ≥57.4 mg/kg bw/day in males, and 20000 ppm, 592.9 mg/kg bw/day in females) in the 12-months study. There was no histological correlate associated with this finding in any case in both studies. Increase in liver weight was associated with thyroid findings in these studies supporting the conclusion of above that the dog is likely to have the same underlying principles of mode of action as the rat.

Based on the available information regarding the mode of action no relevance for humans has been identified. This lack of relevance for humans is strongly supported by the mechanistic studies performed with Boscalid and already discussed in the conclusion on the peer review of Boscalid. The conclusion has also been supported by the HSE-CRD review document and impact assessment for endocrine disrupting substances [Ewence A. et al, 2013; K-CA 5.8.2/19, 2013/1420780] suggesting that Boscalid should not be considered as an endocrine disrupter for mammalian toxicology and that the level of information available does not require enhancement.
In conclusion, Boscalid is considered to have no thyroidal effects that are relevant for humans.

Final conclusion on ED assessment

Boscalid overall showed no effects in several screening assays for androgenicity/anti-androgenicity or estrogenicity/anti-estrogenicity. Furthermore, no indications of androgenic/estrogenic effects were observed in in-vivo studies of the underlying toxicological data base. There were no alerts for endocrine disruption from the evaluation of reproductive organs, mating performance and fertility or from pre- or post-natal development of the offspring. Pathology and histopathology of subchronic, chronic or carcinogenicity studies in mice, rats, and dogs were not indicative of adverse effects related to androgenicity or estrogenicity.

Effects of Boscalid on the thyroid pathology/histopathology were observed in several in vivo studies. In rats chronic exposure to Boscalid resulted in a slightly increased incidence of follicular cell adenoma in thyroids, but the non-relevance for has been in detail discussed and has widely found acceptance at EU level. Mechanistic studies and the results of additional literature evaluation clearly indicated that Boscalid elicits its effects on the thyroid via a reversible and extra-thyroidal, indirect mechanism. These data are supported by Level 1 and 2 studies according to OECD GD 150, where no thyroid receptor-dependent pathway-activation, gene expression or transactivation was observed with Boscalid. In comparison to laboratory animals humans are expected to be less sensitive to thyroid inhibitors due to several reasons:

- Humans possess the thyroxine-binding globulin, a serum protein that is missing in laboratory animals or is available at low levels, and which prevents the glucuronidation and thus excretion of thyroid hormones.
- The half-lives of thyroid hormones are much longer in humans as compared to rats and dogs.
- Humans do develop goiter rather than neoplasms in case of thyroid insufficiency, which is also an important factor for human risk assessment.

The available screening, mechanistic, and in vivo studies indicate that Boscalid has thyroidal relevance in rats and limited relevance in dogs, but following a mechanism that is not relevant for humans. This lack of relevance for humans is strongly supported by the mechanistic studies performed with Boscalid and was already discussed in the Monograph of Boscalid (November 08, 2002). The additional screening and mechanistic studies performed confirm this conclusion.

In conclusion, Boscalid is considered to have no endocrine related effects that are relevant for humans.

Thus, the conclusion for relevant endpoints for the current renewal was amended as follows:

Other toxicological studies (Regulation (EU) N° 283/2013, Annex Part A, point 5.8)

Endocrine disrupting properties	No relevant endocrine effects on the oestrogen, androgen hormone system is observed. Effects observed on the thyroid hormone system are based on an indirect mechanism not related to endocrine disruption and not considered of relevance for humans. Not classifiable for endocrine disruption according to interim criteria

CA 5.9 Medical Data

A search in the databases listed below - restricted to “pps=human” and “ct d human” - has been performed on September 23rd, 2015 via DIMDI-host for the following terms:

- **Boscalid**
- **CAS 188425-85-6**
-

ME66	MEDLINE	NLM
ME0A	MEDLINE Alert	NLM
EM74	EMBASE	2005 Elsevier B.V.
EA08	EMBASE Alert	2005 Elsevier B.V.
CL63	CancerLit	NCI
CCTR93	Cochrane Library - Central	Cochrane

2. Crosscheck via ChemIDplus (http://chem2.sis.nlm.nih.gov/chemidplus/chemidlite.jsp)

3. Crosscheck via PubMed (http://www.ncbi.nlm.nih.gov/sites/entrez)

4. GUA-internal literature database “FAUST”

5. Regarding the databases HSDB (NLM) and GESTIS (BGIA)

6. Register of the internal medical ward

CA 5.9.1 Medical surveillance on manufacturing plant personnel and monitoring studies

All persons handling crop protection products are surveyed by regular medical examinations. There are no specific parameters available for effect monitoring of Boscalid. 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 Boscalid exposure have not been observed.

CA 5.9.2 Data collected on humans

One case of slight skin irritation has been registered in the BASF-internal clinical incident log in employees accidently exposed to Boscalid in combination with another product and it is not clear whether Boscalid was the cause for this irritation.

CA 5.9.3 Direct observations

Some cases of slight irritation of the eyes and skin have been reported to BASF in persons exposed to Boscalid in combination with other products. These reports could not be verified, and it is not clear whether Boscalid was the cause for these irritations.

CA 5.9.4 Epidemiological studies

Neither data on exposure of the general public nor epidemiologic studies are available for BASF SE, nor is BASF SE aware on any epidemiologic studies performed by third parties.

As such, no observations regarding health effects after exposure of the general public are known to us.

CA 5.9.5 Diagnosis of poisoning (determination of active substance, metabolites), specific signs of poisoning, clinical tests

Analytical methods in biological matrices are not established. Clinical tests are not known. No specific symptoms of poisoning are seen.

CA 5.9.6 Proposed treatment: first aid measures, antidotes, medical treatment

See safety data sheet / precautions; symptomatic and supportive treatment, no specific antidote known.

CA 5.9.7 Expected effects of poisoning

Expected effects were derived for acute and subacute studies in animals.

We create chemistry

Boscalid

Document M-CA, Section 6

RESIDUES IN OR ON TREATED PRODUCTS, FOOD AND FEED AND PLANT METABOLISM

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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 6 RESIDUES IN OR ON TREATED PRODUCTS, FOOD AND FEED AND PLANT METABOLISM

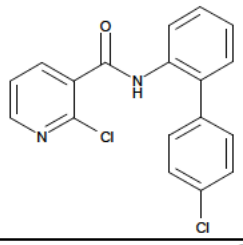
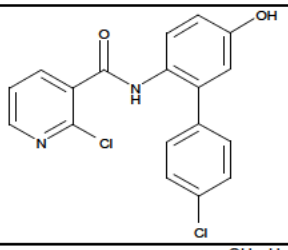
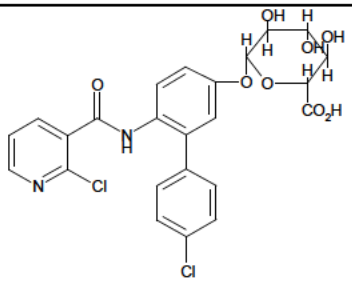
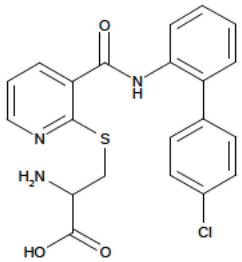
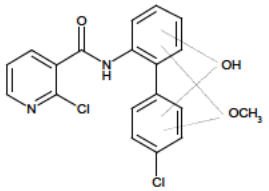
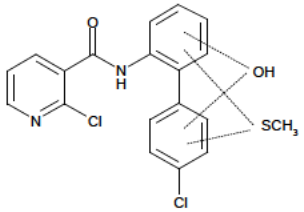
Boscalid (BAS 510 F) is a fungicide for the use in a wide range of crops including various fruits and vegetables as well as several other crops. It is registered in Europe since many years. Boscalid was reviewed under Directive 91/414/EEC. It has been included into Annex I of Commission Directive 91/414 EEC by Commission Directive (EU) No 2008/44/EC of 04 April 2008 and is approved according to Commission Implementing Regulation (EU) No 540/2011 under Regulation (EC) No 1107/2009.

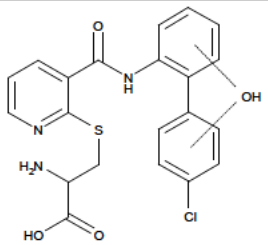
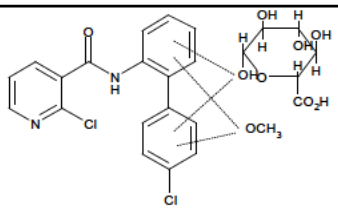
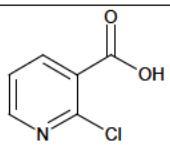
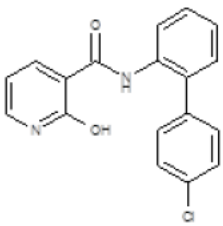
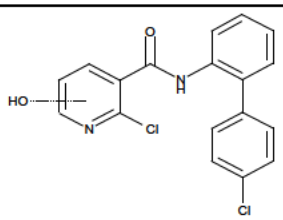
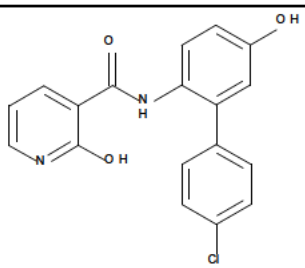
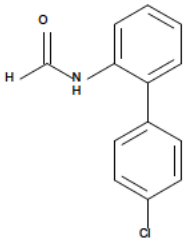
All relevant information on the first Annex I review and the endpoints used in consumer dietary assessments can be found in the Monograph of boscalid (Draft assessment report on the active substance boscalid prepared by the rapporteur Member State Germany in the framework of Council Directive 91/414/EEC, November 2002), in the Addendum (Addendum to the draft assessment report on the active substance boscalid prepared by the rapporteur Member State Germany in the framework of Council Directive 91/414/EEC, May 2006) and in the review of the existing MRLs for boscalid (EFSA Reasoned opinion on the review of the existing maximum residue levels (MRLs) for boscalid according to Article 12 of Regulation (EC) No 396/2005, 2014) and in SANCO/3919/2007 – rev. 5 (EU Review Report finalized in January 2008).

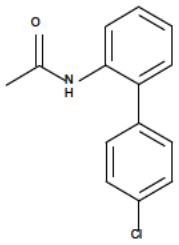
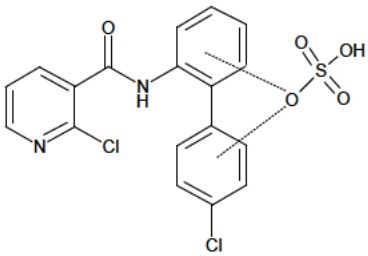
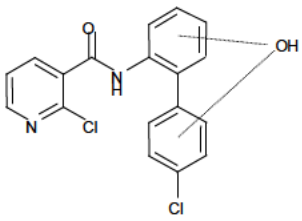
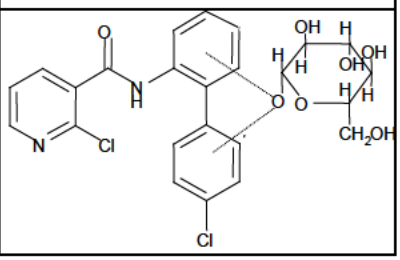
For the current renewal of approval under Regulation (EC) No 1107/2009, a data gap analysis according to new guidelines and new guidance documents was performed and new studies or evaluations were initiated where considered necessary. All new data are provided in this section or in the respective sections of the dossier for the representative formulation.

Furthermore, a literature search was performed and scientific publications were evaluated for their endpoint relevance and quality. Although title and abstract of several publications indicated a potential connection to respective consumer safety chapters of this dossier, the detailed evaluation of these publications showed no endpoint of sufficient reliability which could be used for the required risk assessments. Consequently, for consumer safety, no summaries of public literature data on boscalid are provided in this section. Further information on the literature assessment and respective justifications can be found in M-CA 9.

An overview of metabolites identified during consumer safety studies is given below. The list of metabolites occurring in rats is not complete; as metabolites that are rapidly excreted and only detected in feces and urine are not considered relevant for consumer exposure. The complete list can be found in M-CA 5.1. The information in the table allows a comparison between the pathways in different test systems.

Code Numbers			CAS-No	Compound found in	Structure
Substance Code	Reg. No.	Synonyms			
BAS 510 F	300355	Boscalid M510F00	2-chloro-N-(4'-chlorobiphenyl-2-yl)nicotinamide 188425-85-6	Rat, goat (fat, muscle, liver, milk, kidney) Hen (liver, fat, muscle, eggs), plant	
M510F01	398794		2-chloro-N-(4'-chloro-5-hydroxybiphenyl-2-yl)nicotinamide 661463-87-2	Rat, goat (muscle, fat, liver, milk, kidney) Hen (eggs, liver)	
M510F02	Not assigned		4'-chloro-6-{[(2-chloro-3-pyridinyl)carbonyl]amino}biphenyl-3-yl glycopyranosiduronic acid 661463-88-3	Rat, goat (kidney, muscle, milk) Hen (eggs, muscle)	
M510F05	Not assigned		(3-{[(4'-chlorobiphenyl-2-yl)amino]carbonyl}-2-pyridinyl)cysteine	Rat, goat	
M510F16	Not assigned		2-chloro-N-(4'-chloro-?-hydroxy-?-methoxybiphenyl-2-yl)nicotinamide	Rat, goat	
M510F20	Not assigned		2-chloro-N-(4'-chloro-?-hydroxy-?-methylsulfanylbiphenyl-2-yl)nicotinamide	Rat, goat	

Code Numbers			CAS-No	Compound found in	Structure
Substance Code	Reg. No.	Synonyms			
M510F22	Not assigned		(3-{[(4'-chloro-?-hydroxybiphenyl-2-yl)amino]carbonyl}-2-pyridinyl)cysteine	Rat, goat	
M510F41	Not assigned		2'-{[(2-chloro-3-pyridinyl)carbonyl]amino}-4-chloro-?-methoxybiphenyl-?-yl glycopyranosiduronic acid	Rat, goat	
M510F47	107371		2-chloronicotinic acid 2942-59-8	Rat, plant, soil	
M510F49	391572		N-(4'-chlorobiphenyl-2-yl)-2-hydroxynicotinamide	Hen, soil	
M510F50	Not assigned		2-chloro-N-(4'-chlorobiphenyl-2-yl)-hydroxynicotinamide	Rat, goat, plant, soil	
M510F51	4035208		N-(4'-chloro-5-hydroxybiphenyl-2-yl)-2-hydroxynicotinamide	Goat, hen	
M510F52	4035211		4'-chlorobiphenyl-2-ylformamide	Goat (liver), hen (liver) marker for bound residues)	

Code Numbers			CAS-No	Compound found in	Structure
Substance Code	Reg. No.	Synonyms			
M510F53	4035210		N-(4 '-chlorobiphenyl-2-yl)acetamide 705561-17-7	Goat (liver) (marker for bound residues)	
M510F54	Not assigned		2-chloro-N-(4'-chloro-?-sulfooxybiphenyl-2-yl)nicotinamide	Goat, hen	
M510F59	Not assigned		2-chloro-N-(4'-chloro-?-hydroxybiphenyl-2-yl)nicotinamide	Goat, plant	
M510F61	Not assigned		4'-chloro-6-{[(2-chloro-3-pyridinyl)carbonyl]amino}biphenyl-?-yl glycopyranosiduronic acid	Plant	

CA 6.1 Storage stability of residues

The stability of residues was reviewed during the previous Annex I inclusion process (Annex II, section 4, point 6.1) and no further data was requested.

The storage stability of boscalid has been fully evaluated during the re-registration process.

The following conclusion was taken from the Monograph (Appendix 3 – List of Endpoints, Chapter 4 (residues)) prepared by RMS Germany in the framework of Council Directive 91/414/EEC, 2002:

Stability of residues (Annex IIA, point 6 Introduction, Annex IIIA, point 8 Introduction)

Food of animal origin: (milk, muscle, liver) Nicobifen[1] and metabolite M510F01 stable for 5 months Food of plant origin (wheat plant, wheat grain, wheat straw, oilrape seed, sugar beet, white cabbage, peach, pea): Nicobifen[1] stable for 24 months.

This was recently confirmed by EFSA in the "Reasoned opinion on the review of the existing maximum residue levels (MRLs) for boscalid according to Article 12 of Regulation (EC) No 396/2005" (EFSA Journal 2014;12(7):3799):
Storage stability of boscalid was demonstrated for a period of 16 months at -18 °C in commodities with high acid content (grape) and 24 months at -18 °C in commodities with high water content (cabbage, peach, pea), high oil content (rape seed), dry commodities (wheat grain) and cereal straw. Degradation of residues during storage of the trial samples is therefore not expected. Storage stability of boscalid and M510F01 in milk, muscle, fat, liver, kidney and egg for up to 5 months was demonstrated, when stored deep frozen.

[1] Former name of boscalid

CA 6.2 Metabolism, distribution and expression of residues

During the previous Annex I review process of the active substance boscalid, the metabolism of boscalid has been studied in grapes, lettuce and beans as well as in goat and hens (Monograph 2002). These studies have been part of the previous evaluation and are still scientifically valid and therefore not submitted again in this supplementary dossier. During Article 12 review reported in the EFSA Reasoned Opinion 2014 (EFSA Journal 2014;12(7):3799) a short overview of the main conclusions was given and is summarized below.

Study type	Title	Test system	Results	Reference (BASF DocID)
Study according to OECD 501	Metabolism of 14C-BAS 510 F in grapevine	Grape	The investigation of the metabolism of ^{14}C-BAS 510 F in grapevine leads to the conclusion that the parent compound BAS 510 F itself is the only relevant residue in grapes, grape stalks and leaves of grapevines.	2000/1014860
Study according to OECD 501	Metabolism of BAS 510 F in lettuce	Lettuce	The investigation of the metabolism of ^{14}C-BAS 510 F in lettuce leads to the conclusion that the parent compound BAS 510 F itself is the only relevant residue in this matrix.	1999/11240
Study according to OECD 501	Metabolism of 14C-BAS 510 F in beans	Beans	The investigation of the metabolism of ^{14}C-BAS 510 F in beans leads to the conclusion that the parent compound BAS 510 F itself is the dominant and only relevant residue.	2000/1014861
Study according to OECD 503	14C-BAS 510 F - Absorption, distribution and excretion after repeated oral administration in lactating goats	Goat	^{14}C-BAS 510 F was rapidly absorbed and completely excreted. There was no indication of accumulation of ^{14}C-BAS 510 F in goat tissues, organs and milk. The parent compound and the hydroxylated metabolite M510F01, including its glucuronic acid conjugate were the main residues in milk and tissues of goats.	2000/1012353
Study according to OECD 503	The metabolism of 14C-BAS 510 F in lactating goat	Goat	The investigation of the metabolism of ^{14}C-BAS 510 F in goats leads to the conclusion that the parent compound and the hydroxylated metabolite M510F01, including its glucuronic acid conjugate were the main residues in milk and tissues of goats. As in liver the remaining radioactive residue (RRR) was high, M510F52 and M510F53 were used as markers for bound residues.	2000/1017221
Study according to OECD 503	Nature of residues of 14C-BAS 510 F in laying hens	Hen	^{14}C-BAS 510 F was rapidly absorbed and completely excreted. There was no indication of accumulation of ^{14}C-BAS 510 F in hen tissues, organs and eggs. The parent compound and the hydroxylated metabolite M510F01, including its glucuronic acid conjugate were the main residue. As in liver the remaining radioactive residue (RRR) was high, M510F52 was used as a marker for bound residues.	2000/5154

CA 6.2.1 Metabolism, distribution and expression of residues in plants

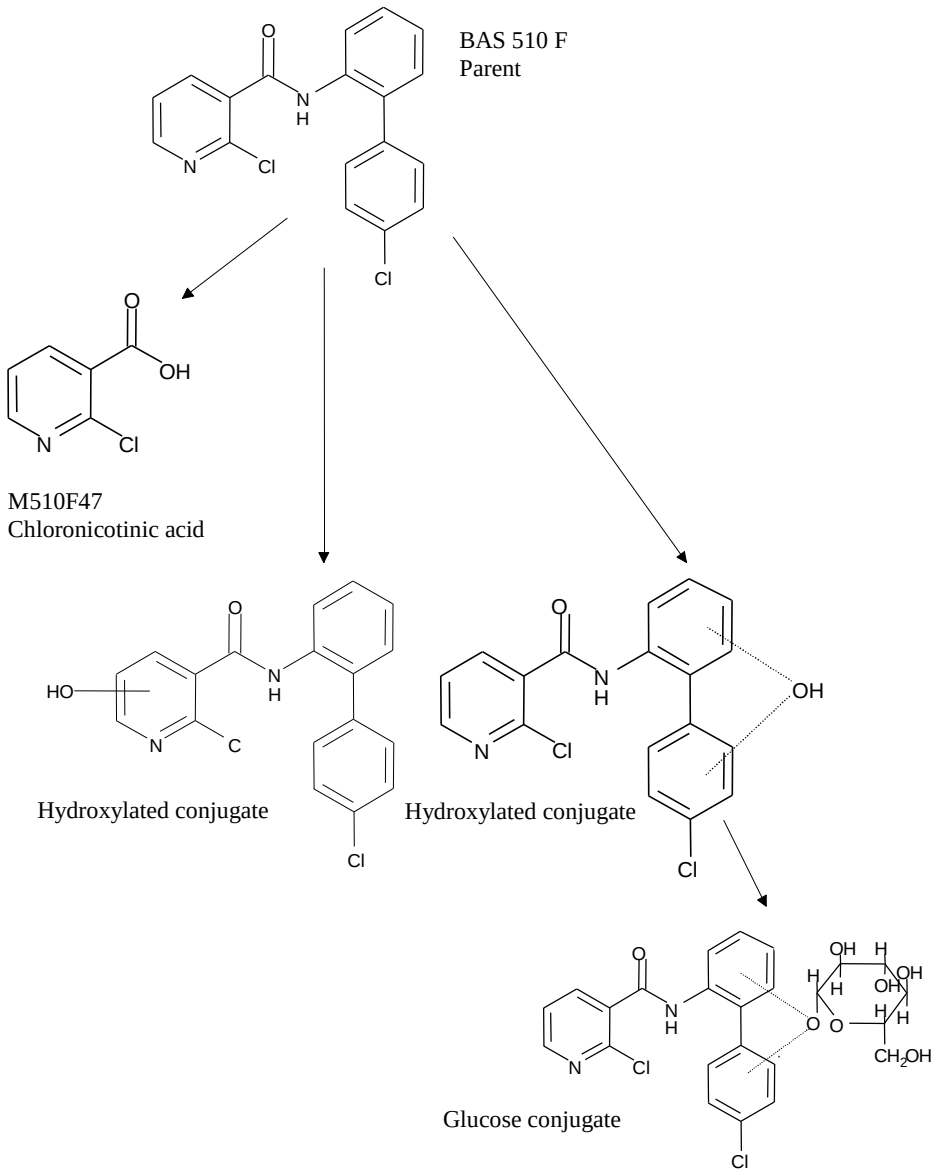
In context of the previous submission of boscalid for EU review, plant metabolism studies of boscalid were submitted and evaluated for foliar application on fruits and fruiting vegetables (grapes), on pulses and oilseeds (beans) and on leafy vegetables (lettuce), using U-^{14}C-diphenyl and 3-^{14}C-pyridine labelled boscalid. Additionally, this was confirmed in the Article 12 review reported in the EFSA Reasoned Opinion 2014 (EFSA Journal 2014;12(7):3799). For the sake of completeness the results of the study is summarized briefly in the following and the proposed metabolic pathway is shown below (Figure 6.2.1-1).

In grapes, the highest TRR was identified in leaves and stalks (63.4 and 19.6 mg/kg respectively), whereas only 2 mg/kg was found in grapes (fruits). Unchanged parent boscalid was the main component of the TRR in all plant parts, ranging from 92.7% in grape fruits to 96.4% in stalks. In lettuce, the unchanged parent compound formed the major part of the residue with 99.3% of the TRR. The residues in beans (edible part) were much lower compared to the rest of the plant. When separating green beans into pods and seeds, the major part of radioactivity was found in pods (0.9 mg/kg) rather than in seeds (0.2 mg/kg). Residue levels were also higher in dry pods (6.1 mg/kg) than in dry seeds (0.2 mg/kg). Parent boscalid was identified as the major compound of the TRR in bean leaves and forage (>98%), in green beans and green pods (97%), in bean straw (≥94%), in dry pods (80-95%) and in dry seeds (72%). 2-Chloronicotinic acid (M510F47) was also identified in green beans and seeds but only in very low concentrations (<0.01 mg/kg).
A glucose conjugate and two hydroxyl derivatives of BAS 510 F were identified in bean straw, although they were not present in amounts greater than 0.61% TRR each and were not further quantified. The compound M510F62 was found in the following bean matrices: Plant (PHI 0), green beans and seeds (both PHI 14), as well as straw (PHI 53). The occurrence of the metabolite at day 0 and the decrease at the later sampling points indicates that it was already present in the application formulation and is not the result of a metabolic reaction.
According to the results of these three studies, the metabolic pathways in these three crops were comparable and the unchanged parent compound formed the major part of the residue. In addition, hydroxylation in the diphenyl and the pyridine rings was observed, but were less pronounced. The hydroxylation reaction is followed by glucosylation.

Considering these results parent BAS 510 F the residue for enforcement and risk assessment in all plant commodities is defined as **boscalid** only.

This assessment was recently confirmed by EFSA during the re-evaluation of the established MRLs according to Regulation (EC) No 396/2005, Art. 12. (EFSA Journal 2014;12(7):3799).

Figure 6.2.1-1: Proposed metabolic pathway in plants



CA 6.2.2 Poultry

The nature of boscalid residues in commodities of animal origin was investigated in the framework of Directive 91/414/EEC (Germany, 2002) and was confirmed in the Article 12 review reported in the EFSA Reasoned Opinion 2014 (EFSA Journal 2014;12(7):3799). Reported metabolism studies include one study in laying hens using [U-^{14}C-diphenyl] labelled boscalid. For the sake of completeness the results of the study is summarized briefly in the following and the proposed metabolic pathway is shown below (Figure 6.2.2-1).

Laying hens were dosed with 0.80–1.14 mg/kg bw/d of boscalid. Including uptake of residues in crops from previously treated soil, these dose levels represent at least 1.6 times the maximum dietary burden of poultry.

Boscalid is extensively excreted (97.7% of the applied dose (AD)), with a relatively low transfer of residues to tissues (0.04% AD in liver, 0.003-0.004% AD for muscle and fat) and eggs (0.12% AD). The highest TRR in edible matrices was found in liver (0.17 mg/kg). Other TRR values were 0.058 mg/kg in eggs (with a maximum of 0.08 mg/kg), 0.025 mg/kg in fat and 0.003 mg/kg in muscle. A plateau is reached in eggs at day 6 (0.07 mg/kg).

A summary of metabolite identities and quantities in edible matrices of laying hens after dosing with ^{14}C-BAS 510 F is given in Table 6.2.2-1. Because of low radioactivity levels, residues in muscle were not characterized.

Table 6.2.2-1: Summary of metabolite identities and quantities in edible matrices of laying hens after dosing with ^{14}C-BAS 510 F

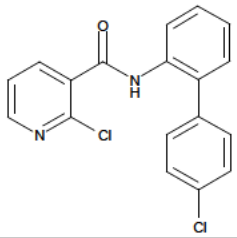
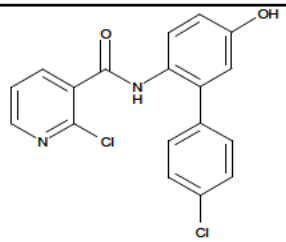
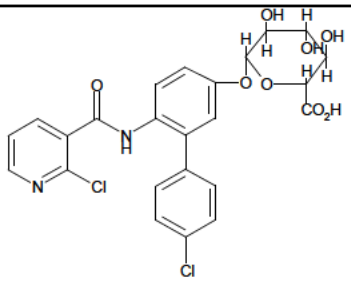
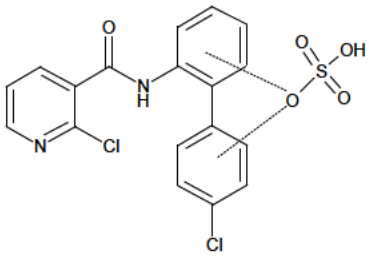
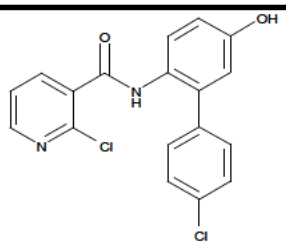
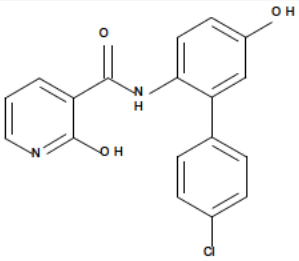
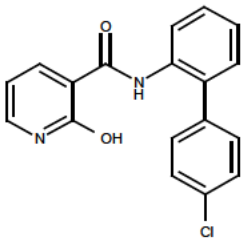
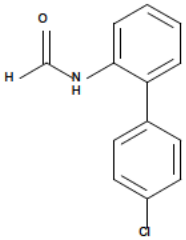
Metabolite Code	Structure	Eggs, average 2-10d [mg/kg] (%TRR)	Fat [mg/kg] (%TRR)
BAS 510 F		0.020 (35.5)	0.023 (93.3)
M510F01		0.015 (26.9)	- -
M510F02		0.011 (17.3)	- -
M510F54		0.001 (1.89)	- -

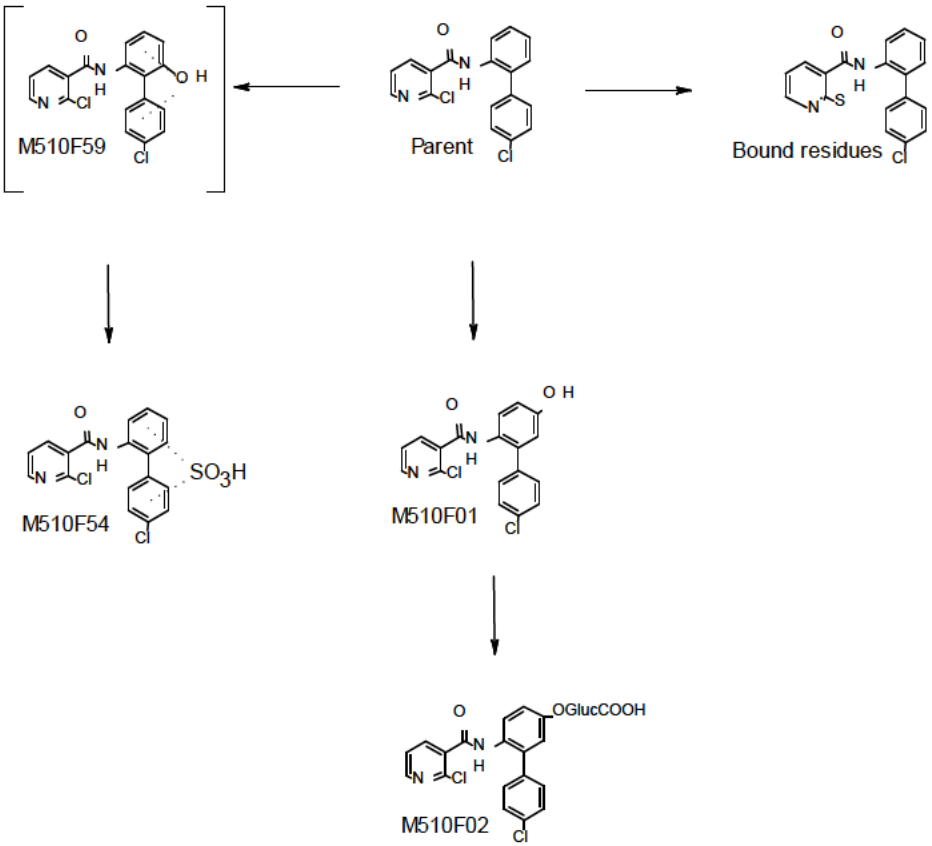
Table 6.2.2-2: Summary of metabolite identities and quantities in liver of laying hens after microwave treatment

Metabolite Code	Structure	Liver [mg/kg] (%TRR)
M510F01		0.009 (5.6)
M510F51		0.037 (21.7)
M510F49		0.021 (12.7)
M510F52		0.071 (42.1)

Taken together, in hens dosed with 12.5 mg/kg feed, the residues in eggs, fat, and muscle mainly consisted of unchanged parent. In eggs, beside the parent compound also its hydroxy metabolite M510F01 including conjugates were present. Low extractability could be observed in liver due to a high level of bound residues. By application of a specially developed microwave method under harsh conditions it was possible to differentiate between extractable and bound residues of BAS 510 F by the formation of M510F01, M510F49 and M510F51 (originating from extractable residues) and M510F52 (originating from bound residues), respectively. The bound parent (measured as cleavage product M510F52) is therefore the main compound in the liver. The bound residues mainly resulted from a substitution of the chlorine of the pyridine system by thiol groups of liver proteins and binding parent to the protein.

In the Article 12 review reported in the EFSA Reasoned Opinion 2014 (EFSA Journal 2014;12(7):3799) it was noted that there is a possibility that bound residues are released during cooking and a cleavage on the amine bound of boscalid cannot be excluded, as only a diphenyl label was applied. However, in BASFs' opinion the metabolite M510F52 was not formed under biotic conditions, the harsh extraction conditions used to release liver bound residues do not apply under normal conditions in food processing. Additionally, the amide bound of BAS 510 F was very stable under metabolic conditions in hens. The found cleavage product M510F52 is therefore considered a marker for bound residues in liver.

Figure 6.2.2-1: Proposed metabolic pathway of boscalid in laying hen



CA 6.2.3 Lactating ruminants

The nature of boscalid residues in commodities of animal origin was investigated in the context of the previous submission of boscalid for EU review and was confirmed in the Article 12 review reported in the EFSA Reasoned Opinion 2014 (EFSA Journal 2014;12(7):3799). Reported metabolism studies include a study in lactating goats using [U-^{14}C-diphenyl] labelled boscalid. For the sake of completeness, a brief summary of the former metabolism study is given here and a figure of the proposed metabolic pathway is shown below (Figure 6.2.3-1).

Lactating goats were dosed with 1.41-1.80 mg/kg bw/d of boscalid. Including the uptake of residues in crops from previously treated soil, these dose levels represent at least 3.3 times the maximum dietary burden of meat ruminant (0.422 mg/kg bw/d), 1.9 times of dairy ruminants (0.751 mg/kg bw/d) and 1.3 times of sheep (1.089 mg/kg bw/d).

Boscalid is extensively excreted (89-93% of the applied dose (AD)), with a relatively low transfer of residues to tissues (0.4-0.6% AD in liver, 0.01-0.02% AD for muscle, fat and kidney) and milk (0.06-0.15% AD). The highest TRR was found in liver (2.59 mg/kg). Other TRR values were 0.27 mg/kg in kidney, 0.04 mg/kg in milk, 0.036 mg/kg in fat and 0.012 mg/kg in muscle.

A summary of metabolite identities and quantities in edible matrices of lactating goats after dosing with ^{14}C-BAS 510 F is given in Table 6.2.3-1.

Table 6.2.3-1: Summary of metabolite identities and quantities in edible matrices of lactating goats after dosing with ^{14}C-BAS 510 F

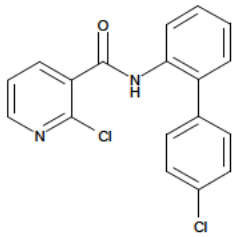
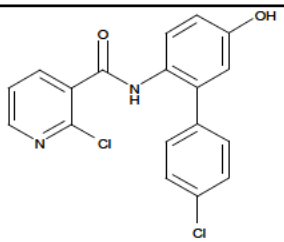
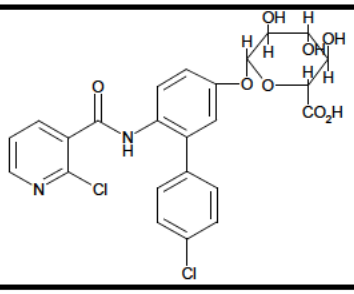
Metabolite Code	Structure	Milk (pool) [mg/kg] (%TRR)	Muscle [mg/kg] (%TRR)	Fat [mg/kg] (%TRR)	Kidney [mg/kg] (%TRR)	Liver [mg/kg] (%TRR)
BAS 510 F		0.001 (3.2)	0.002 (20.4)	0.012 (34.6)	0.007 (2.5)	0.129 (5.0)
M510F01		0.006 (14.9)	0.003 (20.6)	0.009 (26.3)	0.023 (8.6)	0.074 (2.9)

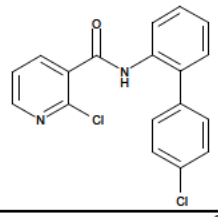
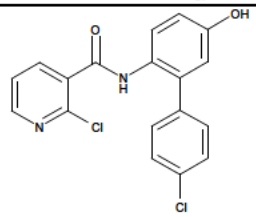
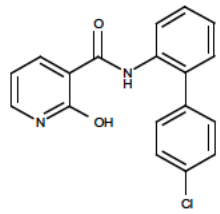
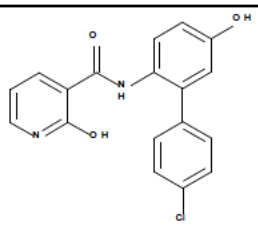
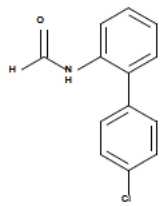
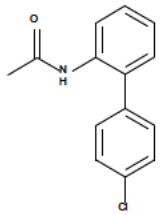
Table 6.2.3-1: Summary of metabolite identities and quantities in edible matrices of lactating goats after dosing with ^{14}C-BAS 510 F

Metabolite Code	Structure	Milk (pool) [mg/kg] (%TRR)	Muscle [mg/kg] (%TRR)	Fat [mg/kg] (%TRR)	Kidney [mg/kg] (%TRR)	Liver [mg/kg] (%TRR)
M510F02		0.002 (6.4)	0.001 (11.9)	- -	0.136 (50.3)	- -

Boscalid was the most abundant compound in fat and represented a major part of the residue in muscle. It was also detected in liver, milk and kidney. The metabolite M510F01 was the most abundant compound in muscle and represented a major part of the residue in fat. It was also detected in liver, milk and kidney. M510F02, the glucuronide conjugate of M510F01, is the most abundant compound in kidney and was also detected in muscle and milk.

Non extractable residues accounted for 85% TRR (2.2 mg/kg) in liver. The same microwave extraction method used in the metabolism study on hen was applied using harsh conditions with either a mixture of acetonitrile and acetic acid or with formic acid. This microwave method allowed again the differentiation between extractable and bound residues of BAS 510 F by the formation of M510F01, M510F49 and M510F51 (originating from extractable residues) and either M510F53 (43.6% TRR) or M510F52 (35.4% TRR) for the respective solvent. The bound parent (measured as cleavage product M510F53 and M510F52) is therefore the main compound in the liver. Again, according to BASFs' opinion, the metabolites M510F52 and M510F53 were not formed under biotic conditions and are used as a marker for bound residues.

Table 6.2.3-2: Summary of metabolite identities and quantities in edible matrices of lactating goats after microwave treatment

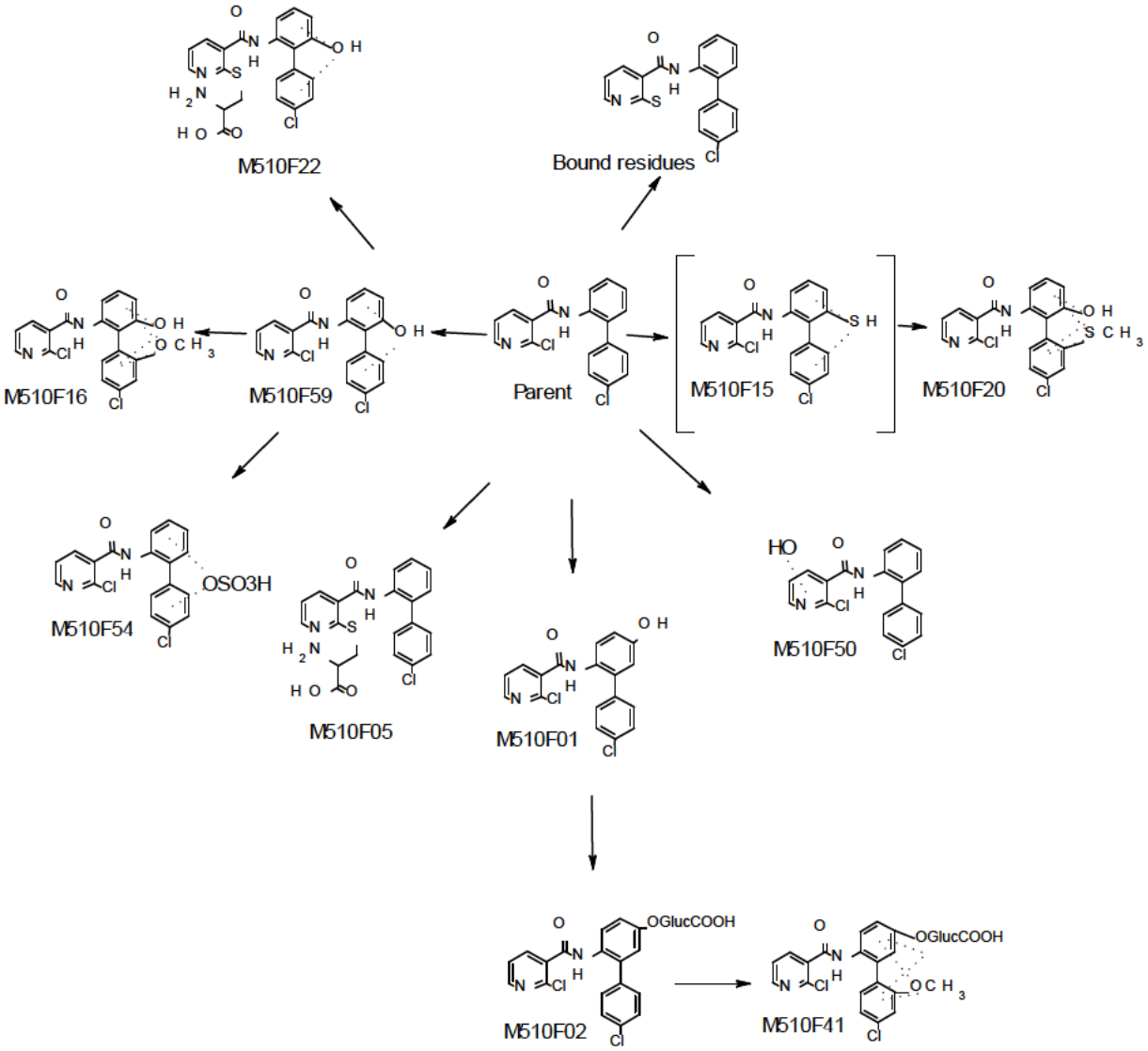
Metabolite Code	Structure	Milk (pool) [mg/kg] (%TRR)	Liver [mg/kg] (%TRR)	Liver [mg/kg] (%TRR)
Specific Microwave treatment:		**Acetonitrile and acetic acid, 170°C, 0.5 h**		**Acetonitrile and formic acid, 170°C, 0.5 h**
BAS 510 F		0.003 (7.9)	-	0.148 (5.7)
M510F01		0.007 (19.0)	0.166 (6.4)	0.109 (4.2)
M510F49		0.003 (7.7)	0.285 (11.0)	0.296 (11.4)
M510F51		0.005 (12.2)	0.062 (2.4)	0.171 (6.6)
M510F52		-	-	0.918 (35.4)
M510F53		0.004 (11.2)	1.130 (43.6)	-

The metabolism studies on both ruminant and poultry show that parent compound, its hydroxy metabolite M510F01 and its conjugate are the main components of the residue in animal tissues and products, except in liver where the bound parent (measured as cleavage product M510F53 and M510F52) were found to be the main component of the residue.

During the Member States' consultation, it was agreed the relevant residue for enforcement is defined as **boscalid** in muscle, fat, milk and eggs and as **the sum of boscalid and its hydroxy metabolite M510F01 including its conjugates expressed as boscalid in liver and kidney**. For risk assessment in liver, bound residues (measured as M510F53 and M510F52, but expressed as boscalid) should also be included, but data is sufficient to derive a conversion factor for ruminant and pig livers only.

This assessment was recently confirmed by EFSA during the re-evaluation of the established MRLs according to Regulation (EC) No 396/2005, Art. 12. (EFSA Journal 2014;12(7):3799).

Figure 6.2.3-1: Proposed metabolic pathway of boscalid in ruminants



CA 6.2.4 Pigs

No separate metabolism study for pigs is required since the metabolic pathways in rodents (rats) and ruminants (goats) are comparable. Metabolism data for pigs can be extrapolated from ruminants.

CA 6.2.5 Fish

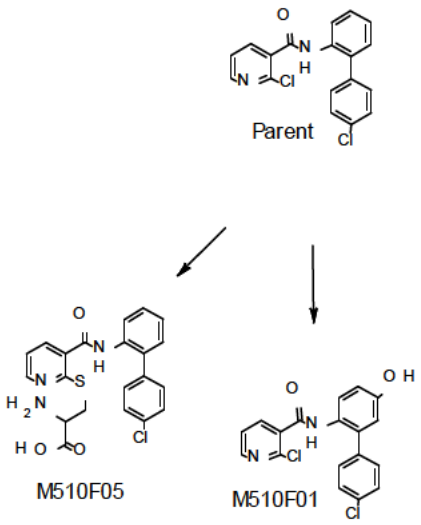
A bioaccumulation study (including identification of the nature of the residue) has been performed in 2000 and was peer-reviewed in the Monograph (2002). For the sake of completeness the results of the study are summarized briefly in the following and the proposed metabolic pathway is shown below (Figure 6.2.5-1). Boscalid has a log Po/w of 2.96 indicating a medium liability to bioaccumulation. Results from the bioconcentration study with *Oncorhynchus mykiss* showed a maximum BCF (whole fish) of 125 after 35 days of exposure. However, the substance was eliminated with a half-life of 1.0 day. After 3.3 days 90% of the accumulated net total radioactivity had been eliminated. Therefore the overall risk of bioaccumulation is considered to be acceptable. Furthermore during this study the metabolism in fish was investigated and the proposed metabolic pathway is presented below.

Table 6.2.5-1: Summary of identified metabolites in fish tissues sampled at Day 28. Values in mg/kg tissue and % of total tissue radioactivity (in parenthesis).

	Exposure level 20 µg a.s./L			**Exposure level 200 µg a.s./L**		
Metabolite	**Edibles**	**Inedibles**	**Whole fish**	**Edibles**	**Inedibles**	**Whole fish**
BAS 510 F	0.899 (95.4)	1.717 (86.4)	1.269 (89.4)	9.657 (97.0)	21.931 (84.9)	15.017 (88.9)
M510F01	0.016 (1.7)	0.177 (8.9)	0.089 (6.3)	ND	1.967 (7.6)	0.859 (5.1)
M510F05	ND	0.031 (1.6)	0.014 (1.0)	ND	0.955 (3.7)	0.417 (2.5)

ND = not detectable

Figure 6.2.5-1: Proposed metabolic pathway in fish



As presented above the metabolism in fish was investigated during the bioaccumulation study and boscalid was only metabolized in small amounts to M510F01 and M510F05.

Although the Log Po/w of boscalid is close to 3 (Monograph 2002) there is no risk of accumulation in fish or other aquatic organisms because of the rapid excretion of the parent compound and its metabolites.

CA 6.3 Magnitude of residues trials in plants

Boscalid is registered in several crops belonging to different EU crop groups. Within this dossier residue data are only provided for the representative uses in grapes, beans, peas and oilseed rape supporting the renewal of approval. Several magnitude of residue studies for oilseed rape and beans which were not peer-reviewed during the previous Annex I inclusion process of boscalid are presented in this dossier. Relevant peer-reviewed studies are highlighted in the table below. In these studies, the WG formulation BAS 510 01 F (containing boscalid, 50%) has been used as representative formulation.

Table 6.3-1: Overview of residue trials included in the dossier

Crop	Formulation	Applied dose (kg a.s./ha)	Application timing	PHI* (days)	Residues** (mg/kg)	Reference
Data already peer-reviewed						
Grapes	BAS 510 01 F (WG)	0.600	79-83 BBCH	21	0.19-1.26 1.47 (35 DALA)	2003/1001357
	BAS 510 01 F (WG)	0.600	79-85 BBCH	21	1.03	2004/1015915
Peas	BAS 510 01 F (WG)	2 x 0.500	1st appl: 71-75 BBCH 2nd appl: 73-81 BBCH	7	<0.05 (seed)	2000/1014848
	BAS 510 01 F (WG)	2 x 0.500	1st appl: 63-65 BBCH 2nd appl: 69-72 BBCH	7	<0.05 (seed)	2000/1014878
	BAS 510 01 F (WG)	2 x 0.500	1st appl: 65-77 BBCH 2nd appl: 69-79 BBCH	7	0.07 (seed) <0.05 (14 DALA)	2000/1014879
	BAS 510 01 F (WG)	2 x 0.500	1st appl: 65-75 BBCH 2nd appl: 73-77 BBCH	7	<0.05-0.09 (seed)	2000/1014852
OSR	BAS 510 01 F (WG)	2 x 0.250	1st appl: 53-55 BBCH 2nd appl: 65 BBCH	Maturity	<0.05 (seed)	2000/1014851
	BAS 510 01 F (WG)	2 x 0.250	1st appl: 65 BBCH 2nd appl: 69 BBCH	Maturity	<0.05 (seed)	2000/1014877
New data						
Beans	BAS 510 01 F (WG)	2 x 0.5	1st appl: 14±1 DBH 2nd appl: 7±1 DBH	7	0.12-1.59 (beans with pods)	2008/1028266
	BAS 510 01 F (WG)	2 x 0.5	1st appl: 14±1 DBH 2nd appl: 7±1 DBH	7	0.97-3.62 (N) 0.23-1.34 (S) (beans with pods)	2010/1165744
	BAS 510 01 F (WG)	2 x 0.5	1st appl: 14±1 DBH 2nd appl: 7±1 DBH	7	0.41 (N) 0.29-1.1 (S) (pods with seed)	2011/1251203
OSR	BAS 510 01 F (WG)	2 x 0.5	1st appl: 51-55 BBCH 2nd appl: 75 BBCH	35	<0.05-0.088 (pods with seed) 0.058 (seeds)	2005/1004971
	BAS 510 01 F (WG)	2 x 0.25	1st appl: 51 55 BBCH 2nd appl: 75 DBH	35	0.25 (N) – 0.11 (S) (seeds)	2007/1007952
	BAS 664 AS F (SC)/ BAS 510 01 F (WG)	0.133/ 0.25	1st appl: 49-55 BBCH 2nd appl: 71-75 BBCH	42	0.03 (N) – 0.02 (S) (seeds)	2008/1074165

* Intended pre-harvest interval
** Residues at PHI. If higher residues were found at later harvest times these are indicated.
*** Defined by growth stage at last application
DBH Days before harvest

CA 6.3.1 Grapes

The use in grapes was part of the previous active substance inclusion process. For the sake of completeness, data supporting the corresponding GAP are given in Table 6.3.1-2.
Sufficient data supporting the representative GAP were submitted and were evaluated on EU level. Both, the current GAP and the one given during Article 12 review reported in the EFSA Reasoned Opinion 2014 (EFSA Journal 2014;12(7):3799), as well as the one given in the Monograph 2002 are presented in Table 6.3.1-1. The current GAP has changes compared to the peer-reviewed one with regards to the PHI.

Table 6.3.1-1: Representative GAP for the use of boscalid (BAS 510 F) on grapes

Crop	Maximum applied dose (kg a.s./ha)	Water volume (L/ha)	PHI (days)	Application method	Application timing
Grapes (current GAP)	1 x 0.6	200-1000	21	Foliar spray	BBCH 60-81
Grapes (peer-reviewed GAP, Monograph 2002)	1 x 0.6	1000-1600	28	Foliar spray	BBCH 68-81
Grapes (GAP, EFSA, Reasoned Opinion 2014)	1 x 0.6	n.r.	21	Foliar spray	BBCH 68-81

n.r. not reported
PHI Pre-harvest interval

Report:	Beck J. et al. 2003 Study on the residue behaviour of Boscalid (BAS 510 F) in grapes (wine) after application of BAS 510 01 F under field conditions in Germany, France, Italy and Spain, 2002 BASF AG, Agrarzentrum Limburgerhof; Limburgerhof; Germany Fed.Rep. unpublished BASF DocID 2003/1001357
Guidelines:	EEC 1607/VI/97 rev. 2 10.06.1999; EEC 7029/VI/95 rev. 5; EEC 7525/VI/95 rev. 7; EEC 91/414 Annex II (Part A Section 6); EEC 91/414 Annex III (Part A Section 8)
GLP:	Yes (laboratory certified by Landesamt fuer Umweltschutz und Gewerbeaufsicht, Mainz, Germany)

Report:	Schulz H. 2004 Study on the residue behaviour of BAS 510 F in vines after application of BAS 510 01 F under field conditions in France (N & S), Spain, Italy and Germany, 2003 Fresenius, Chem. und biolog. Laboratorien; Taunusstein-Neuhof; Germany Fed.Rep. unpublished BASF DocID 2004/1015915
Guidelines:	EEC 1607/VI/97 rev. 2 10.06.1999; EEC 7029/VI/95 rev. 5; EEC 7525/VI/95 rev. 7; EEC 91/414 Annex II (Part A Section 6); EEC 91/414 Annex III (Part A Section 8)
GLP:	Yes (laboratory certified by Hessisches Ministerium fuer Umwelt, Laendlichen Raum und Verbraucherschutz)

Table 6.3.1-2: Results in grape from EU trials (peer-reviewed)

Study details	Crop	Country year (variety)	Formulation, appl. rate (g a.s./ha)	GS[1]	PHI (days)	Matrix	Boscalid (mg/kg)	Recovery data
Study code: 144235 **Doc ID:** 2003/1001357 **Author:** Beck J. **GLP:** Yes **Year:** 2003	grape	Germany 2002 (Spät-burgunder)	BAS 510 01 F 1 x 600	83	0	fruit	0.38	**Method no:** 445/0 (L0076/01) **analysed as:** boscalid **matrix:** grape, fruit **spiking level (mg/kg):** 0.05 **n:** 6 **mean (%):** 83 **RSD (%):** 9.3 **(mg/kg):** 5.0 **n:** 2 **mean (%):** 80 **RSD (%):** 7.1
					21	fruit	0.33	
					28	fruit	0.35	
					35	fruit	<u>0.41</u>	
	grape	Germany 2002 (Riesling)	BAS 510 01 F 1 x 600	79	0	fruit	0.85	
					22	fruit	<u>0.71</u>	
					28	fruit	0.48	
					35	fruit	0.45	
	grape	France (N) 2002 (Chardonnay)	BAS 510 01 F 1 x 600	79	0	fruit	1.26	
					21	fruit	<u>1.12</u>	
					29	fruit	0.79	
					35	fruit	0.91	
	grape	France (N) 2002 (Grolleau)	BAS 510 01 F 1 x 600	83	0	fruit	0.26	
					23	fruit	0.24	
					28	fruit	0.13	
					35	fruit	<u>0.26</u>	
	grape	France (S) 2002 (Negrette)	BAS 510 01 F 1 x 600	81	0	fruit	1.14	
					21	fruit	<u>0.34</u>	
					28	fruit	0.32	
					35	fruit	0.30	
	grape	Spain 2002 (Cardenal)	BAS 510 01 F 1 x 600	79	0	fruit	0.53	
					21	fruit	0.19	
					28	fruit	<u>0.23</u>	
					35	fruit	0.12	
	grape	Spain 2002 (Palomino)	BAS 510 01 F 1 x 600	81	0	fruit	0.23	
					21	fruit	<u>0.24</u>	
					28	fruit	0.20	
					35	fruit	0.21	
	grape	Italy 2002 (Barbera)	BAS 510 01 F 1 x 600	83	0	fruit	1.96	
					22	fruit	1.26	
					28	fruit	1.35	
					35	fruit	<u>1.47</u>	

Table 6.3.1-2: Results in grape from EU trials (peer-reviewed)

Study details	Crop	Country year (variety)	Formulation, appl. rate (g a.s./ha)	GS[1]	PHI (days)	Matrix	Boscalid (mg/kg)	Recovery data
Study code: 02/02/PF **Doc ID:** 2003/1001279 **Author:** Moreno, S. **GLP:** Yes **Year:** 2003 **Amendment:** 2003/1009789	grape	Spain 2002 (Palomino)	BAS 510 01 F 1 x 600	79-81	0 20 27 34	fruit fruit fruit fruit	0.18 0.12 <u>0.19</u> 0.11	**Method no:** 445/0 (L0076/01) **analysed as:** boscalid **matrix:** grape, fruit **spiking level (mg/kg):** 0.05 - 5 **n:** 2 **mean (%):** 90 **RSD (%):** -
Study code: 144241 **Doc ID:** 2004/1015915 **Author:** Schulz H. **GLP:** Yes **Year:** 2004	grape	Germany 2003 (Riesling)	BAS 510 01 F 1 x 600	85	0 21 28 35	fruit fruit fruit fruit	0.19 <u>0.24</u> 0.23 0.20	**Method no:** 445/0 (L0076/01) **analysed as:** boscalid **matrix:** grape, fruit **spiking level (mg/kg):** 0.05 - 0.5 **n:** 4 **mean (%):** 98 **RSD (%):** 11.6
	grape	Germany 2003 (Spät-burgunder)	BAS 510 01 F 1 x 600	83	0 21 28 35	fruit fruit fruit fruit	0.79 <u>1.03</u> 0.43 0.51	
	grape	France (N) 2003 (Palomino)	BAS 510 01 F 1 x 600	83	0 21 28 35	fruit fruit fruit fruit	0.65 0.50 <u>0.78</u> 0.61	
	grape	France (N) 2003 (Chenin)	BAS 510 01 F 1 x 600	83	0 21 28 35	fruit fruit fruit fruit	0.80 0.36 <u>0.39</u> 0.35	
	grape	France (S) 2003 (Syrah)	BAS 510 01 F 1 x 600	85	0 21 28 35	fruit fruit fruit fruit	0.69 <u>0.78</u> 0.58 0.34	
	grape	Spain 2003 (Cardenal)	BAS 510 01 F 1 x 600	79	0 21 28 35	fruit fruit fruit fruit	0.40 0.47 <u>0.50</u> 0.34	
	grape	Spain 2003 (Airen)	BAS 510 01 F 1 x 600	81	0 21 28 35	fruit fruit fruit fruit	0.22 0.16 <u>0.28</u> 0.09	
	grape	Italy 2003 (Barbera)	BAS 510 01 F 1 x 600	83	0 21 28 35	fruit fruit fruit fruit	0.98 0.76 <u>0.88</u> 0.42	

1 Growth stage (BBCH) at application
_ Underlined values used for MRL calculation

CA 6.3.2 Beans

The use in beans was part of the previous Annex I inclusion process for boscalid. Data were submitted to the designated Rapporteur Member State and were evaluated on EU level. Since those residue trials are not fully GAP compliant (the number of applications was 3 instead of 2), they are not considered here even though they were considered acceptable during the peer-review process. Further studies have been conducted and will be submitted in this dossier. Both, the current GAP and the ones used during Article 12 review reported in the EFSA Reasoned Opinion 2014 (EFSA Journal 2014;12(7):3799), as well as the one given in EFSA Reasoned Opinion 2015 (EFSA Journal 2015;13(3):4045) are presented in Table 6.3.2-1.

Table 6.3.2-1: Representative GAP for the use of boscalid (BAS 510 F) on beans

Crop	Maximum applied dose (kg a.s./ha)	Water volume (L/ha)	PHI (days)	Application method	Application timing
Beans (current GAP)	2 x 0.5	150-600	7	Foliar spray	BBCH 60-69 (7 day interval)
Beans (peer-reviewed GAP, Monograph 2002)	2 x 0.5	300	7	Foliar spray	BBCH 60-69 (7-10 day interval)
Beans (GAP, EFSA, Reasoned opinion 2014)	2 x 0.5	n.r.	7	Foliar spray	BBCH 60-69 (7-15 day interval)
Beans with pods (GAP, EFSA, Reasoned opinion 2015)	2 x 0.4	400-1000	7	Foliar spray	BBCH 15-89 (10 day interval)

n.r. not reported
PHI Pre-harvest interval

Report:	CA 6.3.2/1 Klaas P., 2008 a Study on the residue behaviour of BAS 510 F in green beans after treatment with BAS 510 01 F under field conditions in Germany, The Netherlands, United Kingdom and Northern France, 2007 2008/1028266
Guidelines:	EEC 91/414 Annex II (Part A Section 6), EEC 91/414 Annex III (Part A Section 8), EEC 1607/VI/97 rev. 2 10.06.1999, EEC 7029/VI/95 rev. 5, EEC 7525/VI/95 rev. 7, SANCO/825/00 rev. 7 (17 March 2004), EEC 94/46
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Laendlichen Raum und Verbraucherschutz)

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test Material:** Boscalid (BAS 510 F),
Description: BAS 510 01 F (WD)
Lot/Batch #: 1453 (50.0% nominal)
Purity: Not reported
CAS#: 188425-85-6
Development code: Not reported
Spiking levels: 0.01 and 1.0 mg/kg

2. **Test Commodity:**
Crop: Green beans
Type: Legume vegetables
Variety: Cantara, Sopra, Phantheon, White Apollo
Botanical name: *Phaseolus vulgaris*
Crop parts(s) or processed Commodity: Rest of plant without roots, seeds
Sample size: 12-24 units, 0.5 kg nominal

B. STUDY DESIGN AND METHODS

1. Test procedure

During the 2007 growing season in total 4 residue field trials on green beans were conducted in the northern part of the EU to determine the residue levels of BAS 510 F (boscalid). BAS 510 01 F (WG, 50% nominal) was applied twice at individual rates equivalent to 0.50 kg boscalid/ha in spray volumes of about 300 L/ha. The applications were performed 13-14 and 7 days before harvest. Immediately after the last application (0 DALA; BBCH 75-83) as well as after 2-4 DALA (BBCH 76-84), 7 DALA (7=PHI, BBCH 78-86) and 13-15 DALA (BBCH 79-88) rest of plant specimens without roots and beans with pods specimens were collected. Samples were frozen within 24 hours and stored below -18°C until analysis. The maximum storage interval (-18°C) from sampling until extraction was 181 days.

Table 6.3.2-2: Target application rates and timings for green beans

Year	No of trials	No of appl.	F P or G	Test item	Active substance	Appl. rate (kg a.s./ha)	Water volume (L/ha)	Target date / timing [DBH[1]]
2007	4	2	F	BAS 510 01 F (WG)	Boscalid	0.50 0.50	300	1st appl: 14±1 2nd appl: 7±1

1 Days before harvest

2. Description of analytical procedures

The specimens were analyzed for boscalid (BAS 510 F) using BASF method SOP-PA.0243, based on BASF method No 445/0 and 535/1. Residues of boscalid were extracted using a mixture of methanol, water and hydrochloric acid. An aliquot was centrifuged and partitioned against cyclohexane. The final determination of the analytes was performed by LC-MS/MS. The limit of quantitation (LOQ) of the method is 0.01 mg/kg for all sample matrices.

Table 6.3.2-3: Summary of procedural recoveries for boscalid in green beans

Matrix	Fortification level (mg/kg)	Summary recoveries		
		n	Mean (%)	RSD (%)
BASF method SOP-PA.0243, LOQ=0.01 mg/kg		**Boscalid (BAS 510 F)**		
Rest of plant without roots	0.01/1.0	7	79.9	7.7

II. RESULTS AND DISCUSSION

Directly after the last application, residues of BAS 510 F ranged between 0.76 and 2.80 mg/kg in beans with pods specimens and between 12.74 and 20.19 mg/kg in specimens of plants without roots.
At 2-4 DALA, residues of BAS 510 F were found in the range between 0.29 and 1.92 mg/kg in beans with pods specimens and between 6.40 and 14.49 mg/kg in rest of plants without roots specimens.
At the PHI of 7 days after the last application, residues of BAS 510 F were between 0.12 and 1.59 mg/kg in beans with pods specimens and between 1.90 and 15.11 mg/kg in rest of plants without roots specimens.
In beans with pods harvested at growth stage 79-88, 13-15 days after the last application BAS 510 F ranged between 0.10 and 0.77 mg/kg in beans with pods specimens and between 1.52 and 11.49 mg/kg in rest of plants without roots specimens.

In the control samples no residues of boscalid at or above the LOQ were found.

The residue ranges for the different trials are shown in Table 6.3.2-4, detailed residue levels are shown in Table 6.3.2-5.

Table 6.3.2-4: Summary of boscalid residues in green beans

Region	Year	Application	DALA[1]	Growth stage (BBCH)[2]	Range of residues (mg/kg)	
					Matrix	BAS 510 F
EU North	2008	BAS 510 01 F (WG)	0	75-83	Beans with Pods	0.76-2.80
					Rest of plants without roots	12.74-20.19
			2-4	76-84	Beans with Pods	0.29-1.92
					Rest of plants without roots	6.40-14.49
			7	78-86	Beans with Pods	0.12-1.59
					Rest of plants without roots	1.90-15.11
			13-15	79-88	Beans with Pods	0.10-0.77
					Rest of plants without roots	1.52-11.49

1 Days after last application
2 At sampling

III. CONCLUSION

After two applications of BAS 510 01 F, the residue levels of boscalid (BAS 510 F) in beans with pods at the intended PHI (7 DALA) were in the range of 0.12-1.59 mg/kg.

Table 6.3.2-5: Residues of boscalid in green beans after two applications of BAS 510 01 F in the EU North

Study Details		Crop	Country	Formulation, Application rate[0] (kg a.s./ha)	GS[2] BBCH	DALA[1]	Residues found (mg/kg)	
							Matrix	BAS 510 F
Study code:	309368	Green beans	Germany	BAS 510 01 F BAS 510 F: 2 x 0.50	83	0	Beans with pods	0.98
Doc ID:	2008/1028266					0	Rest of plant*	19.73
Trial No:	L070848					3	Beans with pods	0.29
GLP:	Yes					3	Rest of plant*	6.40
Year:	2008					7	Beans with pods	<u>0.12</u>
						7	Rest of plant*	1.90
						15	Beans with pods	0.10
						15	Rest of plant*	1.52
Study code:	309368	Green beans	France	BAS 510 01 F BAS 510 F: 2 x 0.50	79	0	Beans with pods	0.76
Doc ID:	2008/1028266					0	Rest of plant*	12.86
Trial No:	L070849					3	Beans with pods	0.50
GLP:	Yes					3	Rest of plant*	6.94
Year:	2008					7	Beans with pods	0.42
						7	Rest of plant*	7.78
						14	Beans with pods	<u>0.77</u>
						14	Rest of plant*	6.60
Study code:	309368	Green beans	Germany	BAS 510 01 F BAS 510 F: 2 x 0.50	75	0	Beans with pods	1.14
Doc ID:	2008/1028266					0	Rest of plant*	12.74
Trial No:	L070850					4	Beans with pods	0.49
GLP:	Yes					4	Rest of plant*	11.73
Year:	2008					7	Beans with pods	<u>0.61</u>
						7	Rest of plant*	7.05
						14	Beans with pods	0.32
						14	Rest of plant*	7.28
Study code:	309368	Green beans	United Kingdom	BAS 510 01 F BAS 510 F: 2 x 0.50	79	0	Beans with pods	2.80
Doc ID:	2008/1028266					0	Rest of plant*	20.19
Trial No:	L070851					2	Beans with pods	1.92
GLP:	Yes					2	Rest of plant*	14.49
Year:	2008					7	Beans with pods	<u>1.59</u>
						7	Rest of plant*	15.11
						13	Beans with pods	0.76
						13	Rest of plant*	11.49

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
* Without roots
_ underlined values used for MRL calculation

Report:	CA 6.3.2/2 Schulz H., 2011 a Study on the residue behaviour of Boscalid in green beans after treatment with BAS 510 01 F under field conditions in Germany, the Netherlands, Northern France, Belgium, Southern France, Greece, Italy and Spain, 2009 2010/1165744
Guidelines:	EEC 91/414 Annex II (Part A Section 6), EEC 91/414 Annex III (Part A Section 8), EEC 1607/VI/97 rev. 2 10.06.1999, EEC 7029/VI/95 rev. 5, EEC 7525/VI/95 rev. 7
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test Material:** Boscalid (BAS 510 F),
Description: BAS 510 01 F (WD)
Lot/Batch #: 1453 (50.0% nominal)
Purity: Not reported
CAS#: 188425-85-6
Development code: Not reported
Spiking levels: 0.01 and 50.0 mg/kg

2. **Test Commodity:**
Crop: Green beans
Type: Legume vegetables
Variety: Tamara, Nagano, Flagrano, Etna, Flavert, Marconi
Botanical name: *Phaseolus vulgaris*
Crop parts(s) or processed Commodity: Rest of plant without roots, pods with seeds, pods without seeds, seeds
Sample size: 12-24 units, 0.5-1.0 kg nominal

B. STUDY DESIGN AND METHODS

1. Test procedure

During the 2009 growing season in total 8 residue field trials on green beans (field conditions) were conducted in the northern part of the EU (4 trials) and in the southern part of the EU (4 trials), to determine the residue levels of BAS 510 F (boscalid) in or on green beans. The fungicidal test item BAS 510 01 F (WG, 50% nominal) was applied twice at individual rates equivalent to 0.50 kg boscalid/ha in spray volumes of about 300 L/ha. The applications were performed 12-14 and 6-8 days before harvest. Pre-harvest interval (PHI) was 7 days. For the analysis beans with pods specimens, rest of plant specimens without roots, pods without seeds, and seed specimens were collected immediately after the last application (0 DALA; BBCH 75-83) as well as after 2-3 DALA (BBCH 77-86), 6-8 DALA (BBCH 78-89) and 13-14 DALA (BBCH 79-89). Samples were frozen within 12 hours and stored below -18°C until analysis. The maximum storage interval (-18°C) from sampling until extraction was 338 days.

Table 6.3.2-6: Target application rates and timings for green beans

Year	No of trials	No of appl.	F P or G	Test item	Active substance	Appl. rate (kg a.s./ha)	Water volume (L/ha)	Target date / timing [DBH[1]]
2009	8	2	F	BAS 510 01 F (WG)	Boscalid	0.50 0.50	300	1st appl: 14±1 2nd appl: 7±1

1 Days before harvest

2. Description of analytical procedures

The specimens were analyzed for boscalid (BAS 510 F) using BASF method No 535/1 (L0076/01). Residues of boscalid were extracted using a mixture of methanol, water and hydrochloric acid. An aliquot was centrifuged and partitioned against cyclohexane. The final determination of the analytes was performed by LC-MS/MS. The limit of quantitation (LOQ) of the method is 0.01 mg/kg for all sample matrices.

Table 6.3.2-7: Summary of procedural recoveries for boscalid in green beans

Matrix	Fortification level (mg/kg)	Summary recoveries		
		n	Mean (%)	RSD (%)
BASF method No 535/1 (L0076/01), LOQ=0.01 mg/kg		**Boscalid (BAS 510 F)**		
Seeds	0.01, 0.1, 1.0 and 10	10	82.3	8.1
Beans with pods	0.01, 0.1 and 10	11	79.8	9.1
Rest of plant[1]	0.01, 0.1, 1.0 and 10	21	78.0	10.2
Beans with pods	0.01, 0.1, and 10	24	79.2	8.1
Overall		66	79.4	8.9

1 Without root

II. RESULTS AND DISCUSSION

The residue ranges for the different trials are shown in Table 6.3.2-8 detailed residue levels are shown in Table 6.3.2-9.

After two applications of BAS 510 01 F, the residue levels of BAS 510 F in beans with pods ranged between 0.23 and 3.62 mg/kg and in seed specimens between <0.01 and 1.46 mg/kg collected at 6-8 DALA (intended PHI).
In the control samples no residues of boscalid at or above the LOQ were found, with the exception of two trials. In trial L090152 one sample of rest of plant specimens and one of seed specimens showed both residues at the LOQ of 0.01 mg/kg. As boscalid is known to be taken up in succeeding crops, this is most likely due to uptake from soil (for detail see chapter 6.6). Therefore the trial is valid. In trial L090154 the whole set of untreated samples was obviously contaminated with boscalid residues between 0.06 and 16.8 mg/kg. Due to the very high contamination in untreated samples with boscalid, trial L090154 will not be taken into account.

Table 6.3.2-8: Summary of boscalid residues in green beans

Region	Year	Application	DALA[1]	Growth stage (BBCH)[2]	Range of residues (mg/kg)	
					Matrix	BAS 510 F
EU North	2009	BAS 510 01 F (WG)	0	79-83	Beans with pods Rest of plants*	0.76-3.81 30.7-47.0
			2-3	78-86	Beans with pods Rest of plants*	0.58-2.42 20.0-45.5
			6-7	82-89	Beans with pods Rest of plants* Beans without pods Seeds	0.97-3.62 18.3-44.9 1.18-1.72 0.09-1.46
			13-14	85-89	Beans with pods Rest of plants* Beans without pods Seeds	1.40-1.86 10.7-34.6 1.57-8.15 0.09-0.59
EU South	2009	BAS 510 01 F (WG)	0	75-79	Beans with pods Rest of plants*	0.45-4.61 25.3-35.0
			2-3	77-81	Beans with pods Rest of plants*	0.37-3.36 6.10-48.8
			7-8	78-82	Beans with pods Rest of plants* Beans without pods Seeds	0.23-1.34 4.54-46.5 0.25-31.2 <0.01-0.07
			14	79-82	Beans with pods Rest of plants* Beans without pods Seeds	0.15-0.37 1.84-14.5 0.18-35.0 <0.01-0.05

1 Days after last application
2 At sampling
* Without roots

III. CONCLUSION

After two applications of BAS 510 01 F, the residue levels of boscalid (BAS 510 F) in beans with pods at the intended PHI (7 DALA, BBCH 79-89) were in the range of 0.23-3.62 mg/kg.

Table 6.3.2-9: Residues of boscalid in green beans after two applications of BAS 510 01 F in the EU North

Study Details		Crop	Country	Formulation, application rate[0] (kg a.s./ha)	GS[2] BBCH	DALA[1]	Residues found (mg/kg)	
							Matrix	BAS 510 F
Study code:	309349	Green beans	Germany	BAS 510 01 F BAS 510 F: 2 x 0.50	78	0	Pods with seeds	2.03
Doc ID:	2010/1165744					0	Rest of plant*	32.40
Trial No:	L090151					2	Pods with seeds	1.18
GLP:	Yes					2	Rest of plant*	31.40
Year:	2009					7	Pods with seeds	1.44
						7	Rest of plant*	37.10
						7	Pods without seeds	1.72
						7	Seeds	0.25
						14	Pods with seeds	<u>1.62</u>
						14	Rest of plant*	34.60
						14	Pods without seeds	2.41
						14	Seeds	0.09
Study code:	309349	Green beans	The Netherlands	BAS 510 01 F BAS 510 F: 2 x 0.50	83	0	Pods with seeds	0.76
Doc ID:	2010/1165744					0	Rest of plant*	30.70
Trial No:	L090152					3	Pods with seeds	0.58
GLP:	Yes					3	Rest of plant*	20.00
Year:	2009					6	Pods with seeds	0.97
						6	Rest of plant*	18.30
						6	Pods without seeds	1.18
						6	Seeds	0.09
						14	Pods with seeds	<u>1.40</u>
						14	Rest of plant*	22.30
						14	Pods without seeds	1.57
						14	Seeds	0.12
Study code:	309349	Green beans	Northern France	BAS 510 01 F BAS 510 F: 2 x 0.50	79	0	Pods with seeds	3.81
Doc ID:	2010/1165744					0	Rest of plant*	32.20
Trial No:	L090153					2	Pods with seeds	2.42
GLP:	Yes					2	Rest of plant*	26.20
Year:	2009					6	Pods with seeds	<u>3.62</u>
						6	Rest of plant*	23.00
						6	Pods without seeds	5.00
						6	Seeds	1.46
						13	Pods with seeds	1.86
						13	Rest of plant*	10.70
						13	Pods without seeds	8.15
						13	Seeds	0.59

Table 6.3.2-9: Residues of boscalid in green beans after two applications of BAS 510 01 F in the EU North

Study Details		Crop	Country	Formulation, application rate[0] (kg a.s./ha)	GS[2] BBCH	DALA[1]	Residues found (mg/kg)	
							Matrix	BAS 510 F
Study code:	309349	Green beans	Belgium	BAS 510 01 F BAS 510 F: 2 x 0.50	79	0	Pods with seeds	1.90
Doc ID:	2010/1165744					0	Rest of plant*	47.00
Trial No:	L090154[3]					2	Pods with seeds	1.20
GLP:	Yes					2	Rest of plant*	45.50
Year:	2009					6	Pods with seeds	1.28
						6	Rest of plant*	44.90
						6	Pods without seeds	2.01
						6	Seeds	0.07
						13	Pods with seeds	1.12
						13	Rest of plant*	28.70
						13	Pods without seeds	2.37
						13	Seeds	0.03

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
3 Due to contamination in untreated samples with boscalid, trial L090154 will not be taken into account.
* Without roots
_ Underlined values used for MRL calculation

Table 6.3.2-10: Residues of boscalid in green beans after two applications of BAS 510 01 F in the EU South

Study Details		Crop	Country	Formulation, application rate[0] (kg a.s./ha)	GS[2] BBCH	DALA[1]	Residues found (mg/kg)	
							Matrix	BAS 510 F
Study code:	309349	Green beans	Southern France	BAS 510 01 F BAS 510 F: 2 x 0.50	79	0	Pods with seeds	1.01
Doc ID:	2010/1165744					0	Rest of plant*	35.00
Trial No:	L090155					2	Pods with seeds	0.56
GLP:	Yes					2	Rest of plant*	12.20
Year:	2009					8	Pods with seeds	0.33
						8	Rest of plant*	4.54
						8	Pods without seeds	0.56
						8	Seeds	<0.01
						14	Pods with seeds	0.15
						14	Rest of plant*	1.84
						14	Pods without seeds	0.23
						14	Seeds	<0.01
Study code:	309349	Green beans	Greece	BAS 510 01 F BAS 510 F: 2 x 0.50	77	0	Pods with seeds	0.45
Doc ID:	2010/1165744					0	Rest of plant*	25.30
Trial No:	L090156					3	Pods with seeds	0.62
GLP:	Yes					3	Rest of plant*	19.40
Year:	2009					7	Pods with seeds	0.23
						7	Rest of plant*	7.55
						7	Pods without seeds	0.25
						7	Seeds	0.02
						14	Pods with seeds	0.15
						14	Rest of plant*	4.77
						14	Pods without seeds	0.18
						14	Seeds	0.03
Study code:	309349	Green beans	Italy	BAS 510 01 F BAS 510 F: 2 x 0.50	75-77	0	Pods with seeds	1.15
Doc ID:	2010/1165744					0	Rest of plant*	30.20
Trial No:	L090157					3	Pods with seeds	0.37
GLP:	Yes					3	Rest of plant*	6.10
Year:	2009					7	Pods with seeds	0.28
						7	Rest of plant*	4.55
						7	Pods without seeds	0.39
						7	Seeds	0.07
						14	Pods with seeds	0.37
						14	Rest of plant*	2.31
						14	Pods without seeds	0.36
						14	Seeds	0.05

Table 6.3.2-10: Residues of boscalid in green beans after two applications of BAS 510 01 F in the EU South

Study Details		Crop	Country	Formulation, application rate[0] (kg a.s./ha)	GS[2] BBCH	DALA[1]	Residues found (mg/kg)	
							Matrix	BAS 510 F
Study code:	309349	Green beans	Spain	BAS 510 01 F BAS 510 F: 2 x 0.50	79	0	Pods with seeds	4.61
Doc ID:	2010/1165744					0	Rest of plant*	34.90
Trial No:	L090158					3	Pods with seeds	3.36
GLP:	Yes					3	Rest of plant*	48.80
Year:	2009					7	Pods with seeds	<u>1.34</u>
						7	Rest of plant*	46.50
						7	Pods without seeds	31.20
						7	Seeds	0.02
						14	Pods with seeds	0.15
						14	Rest of plant*	14.50
						14	Pods without seeds	35.00
						14	Seeds	0.03

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
* Without roots
_ Underlined values used for MRL calculation

Report:	CA 6.3.2/3 Meyer M., 2011 a Study on the residue behaviour of Boscalid in green beans after treatment with BAS 510 01 F under field conditions in Southern Europe (Southern France, Greece, Italy and Spain) and Northern Europe (Belgium), 2010 2011/1251203
Guidelines:	EEC 91/414 Annex II (Part A Section 6), EEC 91/414 Annex III (Part A Section 8), EEC 1607/VI/97 rev. 2 10.06.1999, EEC 7029/VI/95 rev. 5, EEC 7525/VI/95 rev. 7
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test Material:** Boscalid (BAS 510 F),
Description: BAS 510 01 F (WD)
Lot/Batch #: 1453 (50.0% nominal)
Purity: Not reported
CAS#: 188425-85-6
Development code: Not reported
Spiking levels: 0.01; 0.10; 1.0 and 10.0 mg/kg

2. **Test Commodity:**
Crop: Green beans
Type: Legume vegetables
Variety: Pedra, Etna, Flavert, Marconi, Flagrano,
Botanical name: *Phaseolus vulgaris*
Crop parts(s) or processed Commodity: Rest of plant without roots, pods with seeds, pods without seeds, seeds
Sample size: 12-24 units, 0.2-1.0 kg nominal

B. STUDY DESIGN AND METHODS

1. Test procedure

During the growing season of 2010 in total 5 field trials on green beans (field conditions) were conducted in the southern part of the EU (4 trials) and in the northern part of the EU (1 trial) in order to determine the residue level of BAS 510 F (boscalid) in or on green beans. The fungicidal test item BAS 510 01 F (WG, 50% nominal) was applied twice at individual rates equivalent to 0.50 kg boscalid/ha in spray volumes of about 300 L/ha. The applications were performed 14 and 7 days before harvest. Pre-harvest interval (PHI) was 7 days. With the exception of two trials (L100342, L100345) rest of plant specimens without roots, pods without seeds, and seed specimens were collected immediately after the last application (0 DALA; BBCH 71-81) as well as after 3-4 DALA (BBCH 75-82), at 7 DALA (BBCH 77-82) and 13-15 DALA (BBCH 79-89). In trial L100342, the pods with seeds and rest of plant without roots were not taken at 7 DALA and in trial L100345 no specimens of seed and pods without seed were taken, due to misunderstanding of the study plan.
Samples were frozen within 12 hours and stored below -18°C until analysis. The maximum storage interval (-18°C) from sampling until extraction was 299 days.

Table 6.3.2-11: Target application rates and timings for green beans

Year	No of trials	No of appl.	F P or G	Test item	Active substance	Appl. rate (kg a.s./ha)	Water volume (L/ha)	Target date / timing [DBH[1]]
2010	5	2	F	BAS 510 01 F (WG)	Boscalid	0.50 0.50	300	1st appl: 14±1 2nd appl: 7±1

1 Days before harvest

2. Description of analytical procedures

The specimens were analyzed for boscalid (BAS 510 F) using BASF method No 535/1 (L0076/01). Residues of boscalid were extracted using a mixture of methanol, water and hydrochloric acid. An aliquot was centrifuged and partitioned against cyclohexane. The final determination of the analytes was performed by LC-MS/MS. The limit of quantitation (LOQ) of the method is 0.01 mg/kg for all sample matrices.

Table 6.3.2-12: Summary of procedural recoveries for boscalid in green beans

Matrix	Fortification level (mg/kg)	Summary recoveries		
		n	Mean (%)	RSD (%)
BASF method No 535/1 (L0076/01), LOQ=0.01 mg/kg		**Boscalid (BAS 510 F)**		
Seeds	0.01, 0.1 and 1.0	3	84.2	2.9
Pods with seeds	0.01, 0.1, 1.0 and 10	6	85.0	6.9
Rest of plant[1]		6	87.6	7.8
Pods without seeds		4	84.1	4.1
Overall		19	85.5	6.1

1 Without root

II. RESULTS AND DISCUSSION

The residue ranges for the different trials are shown in Table 6.3.2-13, detailed residue levels are shown in Table 6.3.2-14 and Table 6.3.2-15.

After two applications of BAS 510 01 F, the residue levels of boscalid (BAS 510 F) at 0 DALA ranged from 0.46 to 1.7 mg/kg in pods with seeds specimens and were in the range of 8.0 and 38 mg/kg in rest of plant (no roots) specimens.

At the PHI sampling (7 days after last application) the residues of BAS 510 F declined to 0.010-0.12 mg/kg for seed specimens and to 0.29-1.1 mg/kg for pods with seeds specimens.

No residues at or above LOQ of 0.01 mg/kg were present in control specimens except for the rest of plant without root specimens from Italy and Belgium where a residue with a maximum value of 0.023 mg/kg was detected. As boscalid is known to be taken up in succeeding crops, this is most likely due to uptake from soil (for detail see chapter 6.6). Therefore both trials are valid.

Table 6.3.2-13: Summary of boscalid residues in green beans

Region	Year	Application	Growth stage (BBCH)[2]	DALA[1]	Range of residues (mg/kg)	
					Matrix	BAS 510 F
EU North	2009	BAS 510 01 F (WG)	78	0	Pods with seeds	0.46
				0	Rest of plant*	20
				4	Pods with seeds	0.39
				4	Rest of plant*	11
				7	Pods with seeds	0.41
				7	Rest of plant*	8.7
				13	Pods with seeds	0.62
				13	Rest of plant*	11
				13	Pods without seeds	0.59
				13	Seeds	0.032
EU South	2009	BAS 510 01 F (WG)	71-81	0	Pods with seeds	0.59-1.7
			71-81	0	Rest of plant*	8.0-38
			75-82	3	Pods with seeds	0.55-1.6
			75-82	3	Rest of plant*	12-33
			77-79	7	Pods with seeds[3]	0.29-1.1
			77-79	7	Rest of plant*[3]	8.5-21
			77-82	7	Pods without seeds[4]	0.44-1.3
			77-82	7	Seeds[4]	0.010-0.12
			79-89	14-15	Pods with seeds[5]	0.41-1.4
			79-89	14-15	Rest of plant*[5]	6.3-40
			79-89	14-15	Pods without seeds[4, 5]	0.84-2.2
			79-89	14-15	Seeds[4, 5]	0.027-0.033

1 Days after last application
2 At sampling
3 In trial L100342 no specimens of “pods with seeds“ and “rest plant (no roots)“ were taken
4 Neither at 7 DALA nor at 14 DALA specimens of “seeds“ and “pods without seeds“ were taken in trial L100345
5 Trial L100342 was harvested prior to the 14 DALA sampling
* Without roots

III. CONCLUSION

After two applications of BAS 510 01 F, the residue levels of boscalid (BAS 510 F) at the intended PHI (7 DALA) were in the range of 0.010-0.12 mg/kg in seed specimens and 0.29-1.1 mg/kg for beans with pods.

Table 6.3.2-14: Residues of boscalid in green beans after two applications of BAS 510 01 F in the EU North

Study Details		Crop	Country	Formulation, application rate[0] (kg a.s./ha)	GS[2] BBCH	DALA[1]	Residues found (mg/kg)	
							Matrix	BAS 510 F
Study code:	309371	Green beans	Belgium	BAS 510 01 F	78	0	Beans with pods	0.46
Doc ID:	2011/1251203			BAS 510 F		0	Rest of plant*	20
Trial No:	L100613			2 x 0.50		4	Beans with pods	0.39
GLP:	Yes					4	Rest of plant*	11
Year:	2010					7	Beans with pods	0.41
						7	Rest of plant*	8.7
						13	Beans with pods	<u>0.62</u>
						13	Rest of plant*	11
						13	Pods without seeds	0.59
						13	Seeds	0.032

[0] Actual application rates varied by 10% at most
[1] Days after last application
[2] At last application
* Without roots
_ Underlined values used for MRL calculation

Table 6.3.2-15: Residues of boscalid in green beans after two applications of BAS 510 01 F in the EU South

Study Details		Crop	Country	Formulation, application rate[0] (kg a.s./ha)	GS[2] BBCH	DALA[1]	Residues found (mg/kg)	
							Matrix	BAS 510 F
Study code:	309371	Green beans	France	BAS 510 01 F BAS 510 F: 2 x 0.50	81	0	Pods with seeds	0.84
Doc ID:	2011/1251203					0	Rest of plant*	15
Trial No:	L100342					3	Pods with seeds	0.83
GLP:	Yes					3	Rest of plant*	15
Year:	2010					7	Pods without seeds	0.82
						7	Seeds	0.010
Study code:	309371	Green beans	Greece	BAS 510 01 F BAS 510 F: 2 x 0.50	77	0	Pods with seeds	0.59
Doc ID:	2011/1251203					0	Rest of plant*	20
Trial No:	L100343					3	Pods with seeds	0.55
GLP:	Yes					3	Rest of plant*	13
Year:	2010					7	Pods with seeds	0.29
						7	Rest of plant*	8.5
						7	Pods without seeds	0.44
						7	Seeds	0.024
						15	Pods with seeds	0.41
						15	Rest of plant*	7.3
						15	Pods without seeds	0.84
						15	Seeds	0.033
Study code:	309371	Green beans	Italy	BAS 510 01 F BAS 510 F: 2 x 0.50	71-75	0	Pods with seeds	1.7
Doc ID:	2011/1251203					0	Rest of plant*	38
Trial No:	L100344					3	Pods with seeds	1.6
GLP:	Yes					3	Rest of plant*	33
Year:	2010					7	Pods with seeds	1.1
						7	Rest of plant*	21
						7	Pods without seeds	1.3
						7	Seeds	0.12
						14	Pods with seeds	1.4
						14	Rest of plant*	40
						14	Pods without seeds	2.2
						14	Seeds	0.027
Study code:	309371	Green beans	Spain	BAS 510 01 F BAS 510 F: 2 x 0.50	75	0	Pods with seeds	0.81
Doc ID:	2011/1251203					0	Rest of plant*	8.0
Trial No:	L100345					3	Pods with seeds	0.67
GLP:	Yes					3	Rest of plant*	12
Year:	2010					7	Pods with seeds	0.77
						7	Rest of plant*	13
						14	Pods with seeds	0.43
						14	Rest of plant*	6.3

[0] Actual application rates varied by 10% at most
[1] Days after last application
[2] At last application
[3] In trial L100342 no specimens of “pods with seeds“ and “rest plant (no roots)“ were taken
[4] Neither at 7 DALA nor at 14 DALA specimens of “seeds“ and “pods without seeds“ were taken in trial L100345
[5] Trial L100342 was harvested prior to the 14 DALA sampling
* Without roots
_ Underlined values used for MRL calculation. As in trial L100342 no residue values at 7 DALA are available for pods with seeds, the sum of the residue values obtained for pods without seeds and seeds were taken as worse case.

CA 6.3.3 Peas

The use in peas was part of the previous Annex I inclusion process for boscalid. For the sake of completeness, corresponding data are given in Table 6.3.3-2.
Sufficient data supporting the representative GAP were submitted and were evaluated on EU level. Both, the current GAP and the one given during Article 12 review reported in the EFSA Reasoned Opinion 2014 (EFSA Journal 2014;12(7):3799), as well as the one given in EFSA Reasoned Opinion 2015 (EFSA Journal 2015;13(3):4045) are presented in Table 6.3.3-1.

Table 6.3.3-1: Representative GAP for the use of boscalid (BAS 510 F) on peas

Crop	Maximum applied dose (kg a.s./ha)	Water volume (L/ha)	PHI (days)	Application method	Application timing
Peas (current GAP)	2 x 0.5	150-600	7	Foliar spray	BBCH 60-69 (7 day interval)
Peas (peer-reviewed GAP, Monograph 2002)	2 x 0.5	300	7	Foliar spray	BBCH 60-69 (7-10 day interval)
Peas (GAP, EFSA, Reasoned opinion 2014)	2 x 0.5	n.r.	7	Foliar spray	BBCH 60-69 (7-15 day interval)
Peas with pods (GAP, EFSA, Reasoned opinion 2015)	2 x 0.4	400-1000	7	Foliar spray	BBCH 15-89 (10 day interval)

n.r. not reported
PHI Pre-harvest interval

Report:	Heck W. et al. 2000 Study on the residue behaviour of BAS 510 F in peas after treatment with BAS 510 01 F under field conditions in Germany, Denmark and Sweden, 1999 BASF DocID 2000/1014848
Guidelines:	BBA IV 3-3; IVA Guidelines for Residue Studies; Sections IA and IB; 2nd edition 1992; FAO Guidelines Rome 1990
GLP:	Yes (laboratory certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Report:	Schulz H. 2001 Determination of the residue of BAS 510 F in peas following treatment with BAS 510 01 F under field conditions in France 1999 BASF DocID 2000/1014879
Guidelines:	BBA IV 3-3; IVA Guidelines for Residue Studies; Sections IA and IB; 2nd edition 1992; FAO Guidelines Rome 1990
GLP:	Yes (laboratory certified by Hessisches Ministerium fuer Umwelt, Energie, Jugend, Familie und Gesundheit, Wiesbaden)

Report:	Heck W., Mackenroth C. 2001 Study on the residue behavior of BAS 510 F in peas after treatment with BAS 510 01 F under field conditions in Germany, Denmark, France and Sweden, 2000 BASF DocID 2000/1014852
Guidelines:	BBA IV 3-3; IVA Guidelines for Residue Studies; Sections IA and IB; 2nd edition 1992; Guidelines on Producing Pesticide Residue Data from Supervised Trials; FAO Rome; 1990
GLP:	Yes (laboratory certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Report:	Perny A. 2001(b) Residue study in green peas following treatment with the preparation BAS 510 01 F under field conditions in France in 2000 BASF DocID 2000/1014878
Guidelines:	EEC 91/414
GLP:	Yes (laboratory certified by Groupe Interministeriel des Produits Chimiques, France)

Table 6.3.3-2: Results in pea from EU trials (peer-reviewed)

Study details		Crop	Country year (variety)	Formulation, appl. rate (g a.s./ha)	GS[1]	PHI (days)	Matrix	Boscalid (mg/kg)	Recovery data
Study code:	NEU/FR/08/99	pea	Germany 1999 (Progress)	BAS 510 01 F 2x500 field	73	0	pl. w/o root	5.78	**Method No:** 445/0 (L0076/01)
Doc ID:	2000/1014848					3	rest of plant	11.49	
Author:	Heck W. et al.					3	pods [2]	1.54	**analysed as:** boscalid
GLP:	Yes					3	seed	<0.05	**crop:** pea
Report year:	2000					7	rest of plant	10.81	**matrix:** plant / pod / seed
						7	pods [2]	1.16	
						7	seed	<0.05	**Spiking level (mg/kg):** 0.05 – 5.0
						14	rest of plant	7.57	
						14	pods [2]	1.27	**n:** 20
						14	seed	<0.05	**mean (%):** 97
		pea	Denmark 1999 (Polar)	BAS 510 01 F 2x500 field	75	0	pl. w/o root	8.24	**RSD (%):** 9.2
						3	rest of plant	12.02	
						3	pods [2]	1.24	
						3	seed	0.102	
						7	rest of plant	1.07	
						7	pods [2]	10.54	
						7	seed	<0.05	
						14	rest of plant	7.60	
						14	pods [2]	1.27	
						14	seed	<0.05	
		pea	Sweden 1999 (F8)	BAS 510 01 F 2x500 field	76	0	pl. w/o root	5.83	
						3	rest of plant	1.51	
						3	pods [2]	12.08	
						3	seed	0.057	
						7	rest of plant	2.26	
						7	pods [2]	10.72	
						7	seed	<0.05	
						15	rest of plant	7.49	
						15	pods [2]	34.10*	* reanalysed
						15	seed	0.061	
Study code:	IF-99/09278-00	pea	France (S) 1999 (Alladin)	BAS 510 01 F 2x500 field	69	0	pl. w/o root	6.59	**Method no:** 445/0 (L0076/01)
Doc ID:	2000/1014879					3	rest of plant	10.01	
Author:	Schulz H.					3	pods [2]	1.19	**analysed as:** boscalid
GLP:	Yes					3	seed	0.13	**crop:** pea
Report year:	2000					8	rest of plant	11.49	**matrix:** plant / pod / green seed
						8	pods [2]	0.80	
						8	seed	0.07	**spiking level (mg/kg):** 0.05 – 5.0
						14	rest of plant	9.02	
						14	pods [2]	0.50	**n:** 8
						14	seed	<0.05	**mean (%):** 92
		pea	France (N) 1999 (Bonette)	BAS 510 01 F 2x500 field	77-79	0	pl. w/o root	8.97	**RSD (%):** 9.9
						3	rest of plant	11.98	
						3	pods [2]	1.86	
						3	seed	0.06	
						8	rest of plant	15.19	
						8	pods [2]	2.38	
						8	seed	0.07	
						14	rest of plant	22.10	
						14	pods [2]	3.88	
						14	seed	<0.05	

Table 6.3.3-2: Results in pea from EU trials (peer-reviewed)

Study details		Crop	Country year (variety)	Formulation, appl. rate (g a.s./ha)	GS[1]	PHI (days)	Matrix	Boscalid (mg/kg)	Recovery data
Study code:	NEU/FR/08/00	pea	Germany 2000 (Duell)	BAS 510 01 F 2x500 field	73	0	pl. w/o root	4.38	**Method no:** 445/0 (L0076/01)
Doc ID:	2000/1014852					3	rest of plant	3.64	
Author:	Heck W. et al.					3	pods [2]	0.504	**analysed as:** boscalid
GLP:	Yes					3	seed	0.122	**crop:** pea
Report year:	2001					7	rest of plant	2.93	**matrix:** plant / pod / seed
						7	pods [2]	0.433	
						7	seed	<0.05	**spiking level (mg/kg):** 0.05 – 5.0
						14	rest of plant	1.15	**n:** 8
						14	pods [2]	0.208	**mean (%):** 92
						14	seed	<0.05	**RSD (%):** 4.4
		pea	Denmark 2000 (Polar)	BAS 510 01 F 2x500 field	77	0	pl. w/o root	13.3	
						3	rest of plant	22.6	
						3	pods [2]	3.89	
						3	seed	0.334	
						7	rest of plant	21.6	
						7	pods [2]	3.62	
						7	seed	0.090	
						14	rest of plant	22.4	
						14	pods [2]	4.71	
						14	seed	0.230	
		pea	France (N) 2000 (Modena)	BAS 510 01 F 2x500 field	77	0	pl. w/o root	11.0	
						3	rest of plant	17.5	
						3	pods [2]	1.76	
						3	seed	0.064	
						7	rest of plant	15.9	
						7	pods [2]	2.47	
						7	seed	0.064	
						14	rest of plant	22.6	
						14	pods [2]	2.68	
						14	seed	0.067	
		pea	Sweden 2000 (40 453)	BAS 510 01 F 2x500 field	76	0	pl. w/o root	6.32	
						3	rest of plant	2.06	
						3	pods [2]	0.354	
						3	seed	<0.05	
						7	rest of plant	1.81	
						7	pods [2]	0.308	
						7	seed	<0.05	
						14	rest of plant	1.83	
						14	pods [2]	0.183	
						14	seed	<0.05	

Table 6.3.3-2: Results in pea from EU trials (peer-reviewed)

Study details		Crop	Country year (variety)	Formulation, appl. rate (g a.s./ha)	GS[1]	PHI (days)	Matrix	Boscalid (mg/kg)	Recovery data	
Study code:	A0035	pea	France (S) 2000 (Cisca)	BAS 510 01 F 2x500 field	69-72	0	pl. w/o root	5.03	**Method no:**	445/0 (L0076/01)
Doc ID:	2000/1014878					3	rest of plant	10.6		
Author:	Perny A.					3	pods [2)]	3.06	**analysed as:**	boscalid
GLP:	Yes					3	seed	0.082	**crop:**	pea
Report year:	2000					8	rest of plant	8.78	**matrix:**	green peas
						8	pods [2)]	3.86	**spiking level**	
						8	seed	<0.05	**(mg/kg):**	0.05 – 5.0
						14	rest of plant	11.9	**n:**	8
						14	pods [2)]	7.14	**mean (%):**	96
						14	seed	<0.05	**RSD (%):**	2.9
		pea	France (N) 2000 (Merveille de Kelvedon)	BAS 510 01 F 2x500 field	72	0	pl. w/o root	8.70		
						3	rest of plant	3.53		
						3	pods [2)]	0.60		
						3	seed	<0.05		
						7	rest of plant	1.60		
						7	pods [2)]	0.274		
						7	seed	<0.05		
						14	rest of plant	1.52		
						14	pods [2)]	0.231		
						14	seed	<0.05		

1 Growth stage (BBCH) at application
2 Without seeds

CA 6.3.4 Oilseed rape

The use in oilseed rape was part of the previous Annex I inclusion process for boscalid. Data supporting the corresponding GAP were submitted to the designated Rapporteur Member State and were evaluated on EU level; they are summarized in Table 6.3-1. Further studies have been conducted and will be submitted in this dossier. Both, the current GAP and the one used during Article 12 review reported in the EFSA Reasoned Opinion 2014 (EFSA Journal 2014;12(7):3799), as well as the one given in the Monograph 2002 are presented in Table 6.3.4-1.

Table 6.3.4-1: Representative GAP for the use of boscalid (BAS 510 F) on oilseed rape

Crop	Maximum applied dose (kg a.s./ha)	Water volume (L/ha)	PHI (days)	Application method	Application timing
Oilseed rape (current GAP)	2 x 0.25	100-400	N/A	Foliar spray	BBCH 13-75 (28 day interval)
Oilseed rape (peer-reviewed GAP, Monograph 2002)	2 x 0.25 (NEU)	200-400	-	Foliar spray	BBCH 30; BBCH 63-65 (4-6 week interval)
	2 x 0.20 (SEU)				
Oilseed rape (GAP, EFSA, Reasoned Opinion 2014)	2 x 0.25	n.r.	N/A	Foliar spray	BBCH 61-69 (8-12 day interval)

n.r. Not reported
PHI Pre-harvest interval;
N/A Not applicable. Defined by growth stage at latest application timing.

Report: Raunft E. 2001
Study on the residue behaviour of BAS 510 F in winter rape after treatment with BAS 510 01 F under field conditions in Germany, Sweden and Great Britain, 2000
BASF DocID 2000/1014851

Guidelines: UK Guidance on Crop Residue Data Requirements PSD October 1992, BBA IV 3-3, IVA Guidelines for Residue Studies, Sections IA and IB, 2nd edition 1992, Guidelines on Producing Pesticide Residue Data from Supervised Trials, FAO Rome, 1990

GLP: Yes
(laboratory certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Report: Perny A. 2001
Residue study in oil seed rape following treatment with the preparation BAS 510 01 F under field conditions in France in 2000
BASF DocID 2000/1014877

Guidelines: IVA Guidelines for Residue Studies, Sections IA and IB, 2nd edition 1992, BBA Guideline for the evaluation of pesticides for registration of a pesticide, Ribbesbuettel, 2nd edition

GLP: Yes
(laboratory certified by Group interministeriel des produits chemiques, Republique Francaise)

Table 6.3.4-2: Results in oilseed rape trials from EU North and South (peer-reviewed)

Study details	Crop	Country year (variety)	Formulation, appl. rate (g a.s./ha)	GS[1]	DALA[2] (days)	Matrix	Boscalid (mg/kg)	Recovery data
Study code: NEU/FR/06/00 **Doc ID:** 2000/1014851 **Author:** Raunft E. **GLP:** Yes **Year:** 2001	oilseed rape	Germany 2000 (Artus)	BAS 510 01 F 2 x 250 field	65	0 81	pl. w/o root seed	2.67 <u><0.05</u>	**Method no:** 445/0 **analysed as:** boscalid **crop:** oilrape **matrix**: plant/seed **spiking level (mg/kg):** 0.05-5.0 **n:** 4 **mean (%):** 80 **RSD (%):** 10.4
	oilseed rape	UK 2000 (Apex)	BAS 510 01 F 2 x 250 field	65	0 83	pl. w/o root seed	2.16 <u><0.05</u>	
	oilseed rape	Sweden 2000 (Capitol)	BAS 510 01 F 2 x 250 field	65	0 69	pl. w/o root seed	2.27 <u><0.05</u>	
Study code: A0034 **Doc ID:** 2000/1014877 **Author:** Perny A. **GLP:** Yes **Year:** 2001	oilseed rape	France (S) 2000 (Ebonite)	BAS 510 01 F 2 x 250 field	69	0 38	pl. w/o root seed	2.03 <u><0.05</u>	**Method no:** 445/0 **analysed as:** boscalid **crop:** oilrape **matrix**: plant/seed **spiking level (mg/kg):** 0.05-5.0 **n:** 4 **mean (%):** 93 **RSD (%):** 5.2
	oilseed rape	France (S) 2000 (Constant)	BAS 510 01 F 2 x 250 field	69	0 48	pl. w/o root seed	2.32 <u><0.05</u>	

1 Growth stage (BBCH) at application
2 Days after the last application
_ Underlined values used for MRL calculation

Report:	CA 6.3.4/1 Schulz H., 2005 a Study on the residue behaviour of BAS 510 F in spring rape after treatment with BAS 510 01 F under field conditions in Germany, England and Sweden, 2004 2005/1004971
Guidelines:	EEC 91/414 (1607/VI/97), EEC 7029/VI/95 rev. 5, EEC 7525/VI/95 rev. 7
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Laendlichen Raum und Verbraucherschutz)

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test Material:** Boscalid (BAS 510 F),
Description: BAS 510 01 F (WD)
Lot/Batch #: 2000-1 (50.0% nominal)
Purity: Not reported
CAS#: 188425-85-6
Development code: Not reported
Spiking levels: 0.05 and 0.5 mg/kg

2. **Test Commodity:**
Crop: Oilseed rape
Type: Oilseeds
Variety: Helios, Heros
Botanical name: *Brassica napus L.*
Crop parts(s) or processed Commodity: Whole plant without roots, seed
Sample size: 12 units, 0.5-1 kg nominal

B. STUDY DESIGN AND METHODS

1. Test procedure

During the growing season of 2004, in total 3 field trials were conducted on spring rape in Northern Europe in order to determine the residue level of BAS 510 F (boscalid). The fungicidal test item BAS 510 01 F (WG, 50% nominal) was applied twice at individual rates equivalent to 0.25 kg boscalid/ha in spray volumes of about 300 L/ha. The first application was made at BBCH stage 51-55 and the second at BBCH 75. Sampling was carried out on five occasions. Oilseed rape plants specimens were collected after the last application (0 DALA; BBCH 75) as well as after 20-21 DALA (BBCH 79-85), 27-28 DALA (BBCH 80-87), 35 DALA (BBCH 80-88) and 42-43 DALA (BBCH 86-89). Seeds at maturity were sampled 35-43 DALA (BBCH 86-89). Samples were frozen within 24 hours and stored below -18°C until analysis. The maximum storage interval (-18°C) from sampling until extraction was 720 days.

Table 6.3.4-3: Target application rates and timings for oilseed rape

Year	No of trials	No of appl.	F P or G	Test item	Active substance	Appl. rate (kg a.s./ha)	Water volume (L/ha)	Target date / timing [BBCH[1]]
2004	4	2	F	BAS 510 01 F (WG)	Boscalid	0.25 0.25	300	1st appl: 51-55 2nd appl: 75

1 Growth stage

2. Description of analytical procedures

The specimens were analyzed for boscalid (BAS 510 F) using BASF method No 445/0 (L0076/01). Residues of boscalid were extracted using a mixture of methanol, water and hydrochloric acid. An aliquot was centrifuged and partitioned against cyclohexane. The final determination of the analytes was performed by LC-MS/MS. The limit of quantitation (LOQ) of the method is 0.05 mg/kg for all sample matrices.

Table 6.3.4-4: Summary of procedural recoveries for boscalid

Matrix	Fortification level (mg/kg)	Summary recoveries		
		n	Mean (%)	RSD (%)
BASF method No 445/0 (L0076/01), LOQ=0.05 mg/kg		**Boscalid (BAS 510 F)**		
Rape - shoots	0.05, 5.0	2	102	N/A
Rape - seeds	0.05, 5.0	2	88.5	N/A
Rape - pods	0.05, 5.0	4	103	5.5
Overall	0.05, 5.0	8	98.9	7.6

N/A Not applicable

II. RESULTS AND DISCUSSION

The residue ranges for the different trials are shown in Table 6.3.4-5, detailed residue levels are shown in Table 6.3.4-6.

After two applications of BAS 510 01 F, the residue levels of boscalid (BAS 510 F) ranged from 3.18 to 4.39 mg/kg in shoot specimens at 0 DALA and declined to 0.083 to 0.84 mg/kg and to 0.14 to 0.79 mg/kg in pod specimens and in shoot, without pod specimens at 20-21 DALA, respectively.

At 35 DALA), the residues of BAS 510 F ranged from <0.05 to 0.088 mg/kg in pod specimens, 0.085 to 0.13 mg/kg in shoot without pod specimens and were found at 0.058 mg/kg in seed specimens. In the seed specimens at 42 DALA to 43 DALA, the residues were between <0.05 and 0.071 mg/kg.

No residues at or above LOQ of 0.05 mg/kg were present in control samples.

Table 6.3.4-5: Summary of boscalid residues in oilseed rape

Region	Year	Application	DALA[1]	Growth stage (BBCH)[2]	Range of residues (mg/kg)	
					Matrix	BAS 510 F
EU North	2004	BAS 510 01 F (WG)	0	75	Rape - shoots	3.18-4.39
			20-21	79-85	Rape - pods Rape - shoots (no pods)	0.083-0.84 0.14-0.79
			27-28	80-87	Rape - pods Rape - shoots (no pods)	<0.05-1.19 0.058-0.91
			35	80-83 88	Rape - pods Rape - shoots Rape - seeds	<0.05-0.088 0.085-0.13 0.058
			42-43	86-89	Rape - seeds	<0.05-0.071

1 Days after last application
2 At sampling

III. CONCLUSION

After two applications of BAS 510 01 F, the residue levels of boscalid (BAS 510 F) at 35 DALA (BBCH 80-88) were at 0.058 mg/kg in oilseed rape seed specimens and at DALA 42-43 in the range of <0.05-0.071 mg/kg in mature rape seed specimens.

Table 6.3.4-6: Residues of boscalid in oilseed rape after two applications of BAS 510 01 F in the EU North

Study Details		Crop	Country	Formulation, application rate[0] (kg a.s./ha)	GS[2] BBCH	DALA[1]	Residues found (mg/kg)	
							Matrix	BAS 510 F
Study code:	150307	Oilseed rape	Germany	BAS 510 F: 2 x 0.25	75	0	Shoots	3.18
Doc ID:	2005/1004971					20	Pods	0.24
Trial No:	ACK24/04					20	Shoots (no pods)	0.35
GLP:	Yes					27	Pods	0.11
Year:	2004					27	Shoots (no pods)	0.18
						35	Pods	0.09
						35	Shoots	0.13
						43	Seeds	<u>0.07</u>
Study code:	150307	Oilseed rape	Sweden	BAS 510 F: 2 x 0.25	75	0	Shoots	3.30
Doc ID:	2005/1004971					21	Pods	0.08
Trial No:	HUS/12/04					21	Shoots (no pods)	0.14
GLP:	Yes					28	Pods	<0.05
Year:	2004					28	Shoots (no pods)	0.06
						35	Pods	<0.05
						35	Shoots	0.09
						43	Seeds	<u><0.05</u>
Study code:	150307	Oilseed rape	United Kingdom	BAS 510 F: 2 x 0.25	75	0	Shoots	4.39
Doc ID:	2005/1004971					21	Pods	0.84
Trial No:	OAT/26/04					21	Shoots (no pods)	0.79
GLP:	Yes					28	Pods	1.19
Year:	2004					28	Shoots (no pods)	0.91
						35	Seeds	0.06
						42	Seeds	<u>0.06</u>

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
_ Underlined values used for MRL calculation

Report: CA 6.3.4/2
Oxspring S., 2007 a
Study on the residue behaviour of BAS 510 F in spring oilseed rape after treatment with BAS 510 01 F under field conditions in Southern Europe during 2006
2007/1007952
Guidelines: <none>
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:** Boscalid (BAS 510 F),
Description: BAS 510 01 F (WD)
Lot/Batch #: 1453 (50.0% nominal)
Purity: Not reported
CAS#: 188425-85-6
Development code: Not reported
Spiking levels: 0.01 and 0.5 mg/kg

2. **Test Commodity:**
Crop: Oilseed rape
Type: Oilseeds
Variety: Fantasio
Botanical name: *Brassica napus L.*
Crop parts(s) or processed Commodity: Whole plant without roots, seed
Sample size: 12 units, 0.5-1 kg nominal

B. STUDY DESIGN AND METHODS

1. Test procedure

During the 2006 growing season, in total 2 field trials were conducted in representative oilseed rape growing areas in Southern Europe in order to determine the residue level of BAS 510 F (boscalid). The fungicidal test item BAS 510 01 F (WG, 50% w/w) was foliar applied twice at a rate of 0.25 kg boscalid/ha to oilseed rape at growth stage 51-55 (BBCH) and 35 days before expected harvest. The nominal spray volume used was 300 L/ha. Whole oilseed rape plant specimens were collected immediately after the last application (0 DALA) and specimens of whole pods, remaining plant and seeds were collected 21 days thereafter (21 DALA), seed specimens only were taken at 28, 35 and 42 DALA.
Samples were frozen within 24 hours and stored below -18°C until analysis. The maximum storage interval (-18°C) from sampling until extraction was 231 days.

Table 6.3.4-7: Target application rates and timings for oilseed rape

Year	No of trials	No of appl.	F P or G	Test item	Active substance	Appl. rate (kg a.s./ha)	Water volume (L/ha)	Target date / timing
2006	2	2	F	BAS 510 01 F (WG)	Boscalid	0.250 0.250	300	1st appl.: 51-55 BBCH[2] 2nd appl.: 75 DBH[1]

1 Days before harvest
2 Growth stage

2. Description of analytical procedures

The specimens were analyzed for boscalid (BAS 510 F) using BASF method No 535/0. Residues of boscalid were extracted using acidified mixture of methanol and water. An aliquot was partitioned into cyclohexane under alkaline conditions and reconstituted into methanol:water. The final determination of the analytes was performed by LC-MS/MS. The limit of quantitation (LOQ) of the method is 0.01 mg/kg for all sample matrices.

Table 6.3.4-8: Summary of procedural recoveries for boscalid

Matrix	Fortification level (mg/kg)	Summary recoveries		
		n	Mean (%)	RSD (%)
BASF method No 535/0 (L0076/01), LOQ=0.01 mg/kg		**Boscalid (BAS 510 F)**		
Rape, whole plant	0.01, 0.1, 0.5, 5	4	79.9	11.4
Rape, pods	0.01, 0.1, 0.5, 5	4	79.9	13.9
Rape, remaining plant	0.01, 0.1, 0.5	3	86.0	15.2
Rape, seeds	0.05, 5.0	3	77.0	6.0
Overall	0.05, 5.0	14	80.6	11.6

II. RESULTS AND DISCUSSION

The residue ranges for the different trials are shown in Table 6.3.4-9, detailed residue levels are shown in Table 6.3.4-10.

After two applications of BAS 510 01 F, the residue levels of boscalid (BAS 510 F) ranged from 3.16 to 3.69 mg/kg, 1.10 to 3.04 mg/kg and 0.45 to 0.35 mg/kg in whole plant, whole pods and remaining plant specimens, respectively.
At 35 DALA the residues of BAS 510 F ranged from 0.25 to 0.11 mg/kg in seed specimens. In seed specimens, the residues were between 0.61 and 0.83 mg/kg and between 0.21 and 0.06 mg/kg, taken at 28 and 42 DALA, respectively.

No residues at or above LOQ of 0.05 mg/kg were present in control samples.

Table 6.3.4-9: Summary of boscalid residues in oilseed rape

Region	Year	Application	DALA[1]	Growth stage (BBCH)[2]	Range of residues (mg/kg)	
					Matrix	BAS 510 F
EU South	2006	BAS 510 01 F (WG)	0	75	Whole plant*	3.16-3.69
			21	79-85	Pods with seed	1.10-3.04
					Whole plant (no pods)*	0.45-0.35
			28	87-89	Seeds	0.61-0.83
			35	89	Seeds	0.25-0.11
			42	89	Seeds	0.21-0.06

1 Days after last application
2 At sampling
* Without roots

III. CONCLUSION

After two applications of BAS 510 01 F, the residue levels of boscalid (BAS 510 F) found in the mature oilseed rape seed specimens were in the range of 0.06-0.21 mg/kg at 35 DALA.

Table 6.3.4-10: Residues of boscalid in oilseed rape after two applications of BAS 510 01 F in the EU South

Study Details		Crop	Country	Formulation, application rate[0] (kg a.s./ha)	GS[2] BBCH	DALA[1]	Residues found (mg/kg)	
							Matrix	BAS 510 F
Study code:	241888	Oilseed rape	France	BAS 510 F: 2 x 0.25	75	0	Whole plant*	3.16
Doc ID:	2007/1007952					21	Pods with seed	1.1
Trial No:	AF/10506/BA/1					21	Whole plant*[3]	0.45
GLP:	Yes					28	Seed	0.61
Year:	2006					35	Seed	<u>0.25</u>
						42	Seed	0.21
Study code:	241888	Oilseed rape	France	BAS 510 F: 2 x 0.25	73	0	Whole plant*	3.69
Doc ID:	2007/1007952					21	Pods with seed	3.04
Trial No:	AF/10506/BA/2					21	Whole plant*[3]	0.35
GLP:	Yes					28	Seed	0.83
Year:	2006					35	Seed	<u>0.11</u>
						42	Seed	0.06

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
3 Without pods
* Without roots
_ Underlined values used for MRL calculation

Report:	CA 6.3.4/3 Schaeufele M., 2009 a Residue study (decline) with BAS 664 AS F, BAS 510 01 F and BAS 555 00 F applied to oilseed rape in Germany and Northern France in 2008 2008/1074165
Guidelines:	EEC 91/414 Annex II 6, EEC 91/414 Annex III (Part A Section 8)
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	1) BAS 664 AS F 2) BAS 510 01 F (boscalid) 3) BAS 555 00 F (metconazole)
Lot/Batch #:	1) 380004, BAS 664 AS F, boscalid: 138.7 g/L; metconazole: 58.3 g/L; SC 2) 1453, BAS 510 01 F, boscalid: 51.2%; WG 3) 1089, BAS 555 00 F, metconazole: 60.9 g/L; SL
Purity:	Not reported
CAS#:	Boscalid: 188425-85-6, metconazole: 125116-23-6
Development code:	Not reported
Spiking levels:	0.01-10 mg/kg

2. Test Commodity:

Crop:	Oilseed rape
Type:	Oilseeds
Variety:	PR 45001 D, Billy, Fidness, Exagone
Botanical name:	*Brassica napus L.*
Crop parts(s) or processed Commodity:	Whole plant without roots, pod with seed, rest of plant without pod, seed
Sample size:	>1 kg (for pod with seed > 0.5 kg)

B. STUDY DESIGN AND METHODS

1. Test procedure

During the 2008 growing season, a total of 4 bridging field trials were conducted in oilseed rape in Northern Europe in order to determine the residue levels of BAS 510 F (boscalid) in or on Raw Agricultural Commodities (RAC). The intended product BAS 510 01 F on plot 3 (50% boscalid, WG) was compared to the fungicidal combination product BAS 664 AS F on plot 2 (133 g/L of boscalid). In all cases, two applications were done as shown in the table below in a spray volume of 200 L/ha. As application of boscalid with BAS 664 AS F is not within 25% tolerance of the critical GAP, the results are not reported here.

Whole plants without root specimens were collected directly after the last application. After about 35 and 43 days, rest of plant without pods as well as pods with seed were taken (in one trial at day 43: rest of plant including empty pods and seeds). On the last sampling day, mature seed was taken. This was done as soon as BBCH 89 was reached.

Table 6.3.4-11: Target application rates and timings for oilseed rape

Year	No of trials	No of appl.	F P or G	Test item	Active substance	Appl. rate (kg a.s./ha)	Water volume (L/ha)	Target date / timing (BBCH[1])
2008	1	2	F	BAS 510 01 F (WG)	Boscalid	0.25	200	1st appl.: 49-55 2nd appl.: 71-75

1 Growth stage

2. Description of analytical procedures

The specimens were analyzed for boscalid (BAS 510 F) using BASF method No 535/1 (L0076/01). Residues of boscalid were extracted using a mixture of methanol, water and hydrochloric acid. An aliquot of the extract was centrifuged and partitioned at alkaline conditions against cyclohexane. The final determination of the analytes was performed by HPLC-MS/MS. The limit of quantitation (LOQ) of the method is 0.01 mg/kg for all sample matrices.

Table 6.3.4-12: Summary of procedural recoveries for boscalid

Matrix	Fortification level (mg/kg)	Summary recoveries		
		n	Mean (%)	RSD (%)
BASF method No 535/1 (L0076/01), LOQ=0.01 mg/kg		**Boscalid (BAS 510 F)**		
Rest of plant w/o pods	0.01, 0.1, 10.0	3	85.2	4.8
Whole plant w/o root	0.01, 0.1, 10.0	3	83.7	5.9
Pods with seeds	0.01, 0.1, 1.0	6	98.7	8.4
Seeds	0.01, 0.1, 1.0	3	84.2	5.8
Overall	0.01, 0.1, 1.0	15	87.9	9.3

II. RESULTS AND DISCUSSION

The residue ranges for the different trials are shown in the table below, detailed residue levels are shown in Table 6.3.4-13.

Directly after the last application, boscalid residues were between 1.12 and 2.49 mg/kg in oilseed rape whole plant specimens treated with BAS 510 01 F. At 43 DALA, boscalid residues slightly above the LOQ with 0.03 mg/kg were found in the seeds. In the seeds taken after about eight weeks, boscalid residues were <0.01 mg/kg.
No residues at or above LOQ of 0.01 mg/kg were present in control samples.

Table 6.3.4-13: Summary of boscalid residues in oilseed rape from EU North

Region	Year	Application	DALA[1]	Growth stage (BBCH)[2]	Range of residues (mg/kg)	
					Matrix	BAS 510 F
EU North	2008	BAS 510 01 F (WG)	0	73-77	Whole plant*	1.12-2.49
			35-36	81-86	Rest of plant[3]	0.11-0.40
					Pods with seeds	0.05-0.65
			43	83-85	Rest of plant[3]	0.07-0.15
					Pods with seeds	0.03-0.18
				89	Rest of plant[4]	0.59
					Seeds	0.03
			55-61	89	Seeds	<0.01

1 Days after last application
2 At sampling
3 Without pods
4 Incl. empty pods
* Without roots

III. CONCLUSION

After two applications of BAS 510 01 F, the residue levels of boscalid (BAS 510 F) found in the mature oilseed rape seed specimens were in the range of <0.01-0.03 mg/kg at harvest.

Table 6.3.4-14: Residues of boscalid in oilseed rape after two applications of BAS 510 01 F in the EU North

Study Details		Crop	Country	Formulation, application rate[0] (kg a.s./ha)	GS[2] BBCH	DALA[1]	Residues found (mg/kg)	
							Matrix	BAS 510 F
Study code:	334204	Oilseed rape	Germany	BAS 510 01 F	73	0	Whole plant*	1.36
Doc ID:	2008/1074165			Boscalid:		36	Rest of plant*[3]	0.11
Trial No:	L080238			2 x 0.250		36	Pods with seed	0.14
GLP:	Yes					43	Rest of plant*[3]	0.14
Year:	2008					43	Pods with seed	0.17
						55	Seeds	<u><0.01</u>
Study code:	334204	Oilseed rape	Germany	BAS 510 01 F	73	0	Whole plant*	1.60
Doc ID:	2008/1074165			Boscalid:		36	Rest of plant*[3]	0.24
Trial No:	L080239			2 x 0.250		36	Pods with seed	0.27
GLP:	Yes					43	Rest of plant*[3]	0.15
Year:	2008					43	Pods with seed	0.18
						55	Seeds	<u><0.01</u>
Study code:	334204	Oilseed rape	North France	BAS 510 01 F	77	0	Whole plant*	2.49
Doc ID:	2008/1074165			Boscalid:		35	Rest of plant*[3]	0.40
Trial No:	L080240			2 x 0.250		35	Pods with seed	0.65
GLP:	Yes					43	Rest of plant*[4]	0.59
Year:	2008					43	Seeds	<u>0.03</u>
Study code:	334204	Oilseed rape	North France	BAS 510 01 F	73	0	Whole plant*	1.12
Doc ID:	2008/1074165			Boscalid:		35	Rest of plant*[3]	0.13
Trial No:	L080241			2 x 0.250		35	Pods with seed	0.05
GLP:	Yes					43	Rest of plant*[4]	0.07
Year:	2008					43	Pods with seed	0.03
						61	Seeds	<u><0.01</u>

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
3 Without pods
4 With pods
* Without roots
_ Underlined values used for MRL calculation

CA 6.4 Feeding studies

In the context of this document, the anticipated maximum dietary burden for poultry, pigs and ruminants (dairy cattle and beef cattle) was calculated with the OECD calculator considering the representative uses on grape and oilseed rape and in addition all boscalid uses as a worst case scenario. The representative uses in fresh beans and peas are no feed items in the OECD model. Details on the calculation in regards to the proposed residue definition and the selected input values can be found in chapter M-CA 6.7 where the proposed EU MRLs for animal products are derived.

During Article 12 review of all EU MRLs (see EFSA Reasoned Opinion 2014, EFSA Journal 2014;12(7):3799), the calculated dietary burdens for all groups of livestock were found to exceed the trigger value of 0.1 mg/kg DM/day; further investigation of residues would therefore be required in all commodities of animal origin.

The results of the dietary burden calculation are reported in the table below.

Table 6.4-1: Results of the dietary burden calculation by EFSA (EFSA Reasoned Opinion 2014)*

	Median dietary burden (mg/kg bw/d)	Maximum dietary burden (mg/kg bw/d)	Highest contributing commodity	Max dietary burden (mg/kg DM)	Trigger exceeded (Y/N)
Risk assessment residue definition: boscalid					
Dairy ruminants	0.41	1.43	Grass, fresh	39.9	Y
Meat ruminants	0.99	2.04	Wheat straw	47.5	Y
Poultry	0.10	0.23	Kale	3.66	Y
Pigs	0.10	0.37	Grass silage	9.31	Y

* EFSA Journal 2014;12(7):3799

A lactating cow feeding study and a laying hen feeding study for boscalid were peer-reviewed during the Annex I inclusion process and were considered to be acceptable.

The following endpoints were taken from the Addendum for boscalid (Addendum 2, May 2006):

Residues from livestock feeding studies (Annex IIA, point 6.4, Annex IIIA, point 8.3)

Intakes by livestock ≥ 0.1 mg/kg diet/day	Ruminant: yes	Poultry: yes	Pig*: yes
Muscle	<0.05	<0.05	<0.05
Liver	0.2	0.1	0.1
Kidney	0.3	not applicable	0.1
Fat	0.3	0.1	0.1
Milk	0.05	not applicable	not applicable
Eggs	not applicable	<0.02	not applicable

* No pig feeding study conducted. Metabolism in rat and ruminant similar.

As the dose levels were insufficient to cover the calculated dietary burden for ruminants, a new feeding study was submitted during Member State consultation in the context of the Article 12 review of the existing MRLs and evaluated by EFSA (Reasoned Opinion 2014, EFSA Journal 2014;12(7):3799).

The following endpoints were obtained from the Reasoned Opinion 2014 (EFSA Journal 2014;12(7):3799).

Overview of the values derived from the livestock feeding studies (including uptake from previously treated soils)

Intakes by livestock ≥ 0.1 mg/kg diet/day	Ruminant: yes	Poultry: yes	Pig: yes
Muscle	<0.025	<0.025	<0.025
Liver	0.16	0.14	0.05
Kidney	0.16	N/A	0.05
Fat	0.23	0.07	0.07
Milk	0.01	N/A	N/A
Eggs	N/A	0.01	N/A

Highest residue value (tissues, eggs) or mean residue value (milk) value according to the enforcement residue definition (muscle, fat, milk, egg: boscalid; liver: sum of boscalid and M510F01 (free and conjugated), expressed as boscalid), derived by interpolation/extrapolation from the feeding study for the median dietary burden.
N/A: not applicable

CA 6.4.1 Poultry

Magnitude of boscalid residues in poultry was investigated in a feeding study with laying hens (Addendum 2 on the active substance boscalid prepared by the rapporteur Member State Germany in the framework of Council Directive 91/414/EEC, May 2006). The available data are considered sufficient for deriving MRLs in poultry.

This study is still scientifically valid and therefore not submitted again in this supplementary dossier. For reasons of convenience, a short overview of the main conclusions is given below.

Study type	Title	Test system	Results	Reference (BASF DocID)
Study according to OECD 505	A meat and egg magnitude of the residue study with BAS 510 F in laying hens	hen	Boscalid was administered orally to groups of hens for 29 days. The target dosage was 1, 5 and 20 mg/kg. These dose levels correspond to actual dose levels of 1.02, 5.31 and 19.63 mg/kg in the diet. At the 1.0 mg/kg (1x) and 5.0 mg/kg (5x) dose levels, all egg samples resulted in residues <0.02 mg/kg. Only the 20x treatment demonstrated enough residues to show a time dependence of the residue levels, egg residues were <0.02 mg/kg through Study Day 3, then increased at Day 5 to 7, and reached a plateau within the first two weeks of dosing. The remaining residues from this group ranged from <0.02 mg/kg (six days depuration) to 0.03 mg/kg (2 days depuration). Chicken liver, fat and muscle tissues were analysed for residues of BAS 510 F and M510F01. There were no detectable residues above the LOQ in any muscle samples from the three treatment groups. In liver, residues were <0.05 mg/kg for the 1x dose group. Residues ranged from 0.11 to 0.18 mg/kg and from 0.32 to 0.47 mg/kg for the 5x and the 20x dose group, respectively. In fat, residues were <0.05 mg/kg for the 1x dose group. In this matrix, residues ranged from 0.05 to 0.12 mg/kg and from 0.14 to 0.20 mg/kg for the 5x and the 20x dose group, respectively. The residues in all matrices were below the LOQ after a depuration period of three days.	2002/5002466

The new feed burden is covered by the dose levels used in the feeding study. Details on the calculation in regards to the proposed residue definition and the selected input values can be found in chapter M-CA 6.7 where the proposed EU MRLs for poultry products are derived.

CA 6.4.2 Ruminants

The magnitude of boscalid residues in ruminants was investigated in a feeding study with lactating cows (Monograph on the active substance boscalid prepared by the rapporteur Member State Germany in the framework of Council Directive 91/414/EEC, November 2002). A new feeding study covering the maximum dietary burden of ruminants was evaluated during Article 12 review of the existing MRLs for boscalid (EFSA Reasoned Opinion 2014, EFSA Journal 2014;12(7):3799). The available data are considered sufficient for deriving MRLs in ruminants.

These studies are still scientifically valid and are therefore not submitted again in this supplementary dossier. For reasons of convenience, a short overview of the main conclusions is given below.

Study type	Title	Test system	Results	Reference (BASF DocID)
Study according to OECD 505	Investigation of residues of BAS 510 F in tissues and milk of dairy cows	cows	During the cow feeding study with boscalid the animals were dosed with 1.5, 4.5 and 18 mg/kg feed (dry matter) equal to 30, 120 and 360 mg/animal/day for a minimum period of 28 days. In the lowest dose group, no residues could be detected in milk, meat, liver and kidney. In fat, the group average was zero though a single residue above the limit of quantitation was detected. In cream, residues were found at all dose levels. At higher dose levels, residues were detected in milk, fat, liver and kidney. No residues of non-extractable boscalid metabolite residues occurred in liver samples from the lowest and medium dose groups. In the highest group non-extractable residues could be detected.	2000/1017228
Study according to OECD 505	A meat and milk magnitude of the residue study with BAS 510 F in lactating dairy cows	cows	A new residue transfer study with boscalid was conducted in cows to cover an increased dietary burden for livestock animals due to new uses for boscalid. The animals were dosed at actual levels of 35.8 and 116.3 mg/kg feed (dry matter), equal to 1.22 and 3.49 mg/kg bodyweight per day for a period of 29 days followed by a depuration period of 14 days. In the low dose group (35 mg/kg), residues in milk were low at a mean concentration of 0.03 mg/kg but concentrated slightly in cream (0.07 mg/kg). Low residues were also found in liver and kidney (0.10 mg/kg and 0.11 mg/kg) while no residues were detected in muscle. Residues in the various fat tissues ranged from 0.16 mg/kg to 0.19 mg/kg. All residues declined fast after withdrawal of the test item and after 10 days of depuration no residues were detectable anymore demonstrating that residues do not accumulate.	2008/7015330

The new feed burden is covered by the dose levels used in the second feeding study. Details on the calculation in regards to the proposed residue definition and the selected input values can be found in chapter M-CA 6.7 where the proposed EU MRLs for ruminant products are derived.

CA 6.4.3 Pigs

A feeding study in pigs is only required, if the metabolic pathways differ significantly in pigs as compared to ruminants. This is not the case, since there was no significant difference found between the metabolic pathways in rats and cows (please refer to chapter M-CA 6.2).
The general metabolic pathways in rodents and ruminants were found to be comparable; the findings in ruminants can therefore be extrapolated to pigs.

CA 6.4.4 Fish

According to the Commission Regulations (EU) No 283/2013 (active substances) as of 1 March 2013, fish feeding studies might be required for active substances whose approval expires on 1 January 2016 or later. Furthermore, a fish feeding study may be required where residues at levels above 0.01 mg/kg may be reasonably expected in edible tissues, based on the findings of the fish metabolism study and the estimated maximum residues which might occur in fish feed. Particular attention should be laid on lipophilic substances with an intrinsic tendency for accumulation.
For boscalid, during the bioconcentration study the fish metabolism was investigated. Only small amounts of boscalid were metabolized to M510F01 and M510F05 and after 3.3 days 90% of the accumulated net total radioactivity had been eliminated. Although the Log Po/w of boscalid is close to 3 (Monograph, 2002), there is no risk of accumulation in fish or other aquatic organisms due to the rapid excretion of the parent compound and its metabolites (see chapter M-CA 6.2.5). Thus, no accumulation of residue levels in edible fish tissues is assumed.

Further a feeding study on fish is required

(1) where significant residues (≥ 0.1 mg/kg of the total diet as received, except special cases, such as active substances which accumulate) occur in crops or part of the crops fed to fish

and

(2) the log Po/w is >3.

The fish feed burden has been reviewed based on the calculation procedures described in the available working document the working document for nature of pesticide residues in fish (SANCO/11187/2013, http://ec.europa.eu/food/plant/pesticides/guidance_documents/docs/app-j_en.pdf), where the EU Commission gives guidance for the calculation of a maximum reasonably balanced diet (MRDB) approach.

In the European Commission Summary Report of the Standing Committee on Plants, Animals, Food and Feed held in Brussel on 24 November -25 November 2014, it was emphasized that the Commission working document on the nature of pesticide residues in fish was discussed in 2013 and it was concluded that it is not yet finalized and ready to be noted as a guidance document. The Commission emphasized that for the time being there are no agreed test guidelines and that hence the pertinent data requirements can be waived. This was also clarified in general at the meeting of the Committee's section on Plant Protection Products - Legislation on 09/10 October 2014, and laid down in document SANCO/10181/2013 Rev 2.1. Such test guidelines must be published in the form of an update of the respective Commission Communications (CIRCABC Link: https://circabc.europa.eu/w/browse/a6c9bbb3-ca04-4afe-9b49-4b483f9c4314).

The calculated feed burden (see chapter M-CA 6.7.2) was found to be above the trigger for fish metabolism and fish feeding studies of 0.1 mg/kg DM (0.997 mg/kg feed DM for carb and 1.270 mg/kg feed DM). Therefore, a fish feeding study would be required. Nevertheless, no fish feeding study was performed, since no suitable EU guideline/guidance document for the conduct of fish feeding studies is available.

Moreover, from a scientific perspective boscalid does not show any significant bioconcentration properties in fish (see above). Thus, residues of the active substance in fish are of no concern and no accumulation in the food chain is to be expected.

CA 6.5 Effects of Processing

Data/information on processing studies were reviewed during the Annex I inclusion process of boscalid and were considered acceptable. After inclusion of boscalid into Annex I to Directive 91/414/EEC additional studies have been performed and are summarized in the EFSA Reasoned Opinion 2014 for boscalid (EFSA Journal 2014;12(7):3799).
Data are sufficient to describe the behaviour of boscalid and no further studies are necessary.

CA 6.5.1 Nature of the residue

Data/information on the hydrolysis studies for boscalid were reviewed at EU level as part of the Annex I inclusion process and was considered to be acceptable.

The effect of processing on the nature of the boscalid residue was investigated in a standard hydrolysis study (Monograph 2002) performed at three test conditions (20 minutes at 90°C, pH 4; 60 minutes at 100°C, pH 5; 20 minutes at 120°C, pH 6).

The following conclusion was taken from the Draft assessment report (section 3, B7) prepared by RMS Germany in the framework of Council Directive 91/414/EEC, 2002:

Boscalid was not degraded neither during the simulation of pasteurization (pH 4, 90°C) nor during the simulation of baking, boiling, brewing (pH 5, 100°C) or during sterilization (pH 6, 120°C). No degradation products were observed.

Therefore, it was concluded that boscalid is stable under all conditions tested simulating processing. Thus, for processed commodities the same residue definition as for raw agricultural commodities (RAC) is applicable and confirmed by EFSA Reasoned Opinion 2014: "*The relevant residue for enforcement and risk assessment in processed commodities is therefore expected to be the same as for primary crops*." (EFSA Journal 2014;12(7):3799).

A separate high temperature hydrolysis study for M510F01 (included in proposed residue definition in animal matrices for monitoring and risk assessment) is not considered necessary for the following reason: M510F01's chemical structure differ from their parent boscalid only by hydroxyl groups (-OH) at the diphenyl moiety. As no cleavage or ring opening occurred under all tested conditions in the high temperature hydrolysis study, it is highly unlikely that M510F01 will react differently. Therefore, the metabolite and their respective conjugates can be considered as covered by the previous high temperature hydrolysis studies for parent.

CA 6.5.2 Distribution of the residue in inedible peel and pulp

All representative uses to be evaluated in this dossier (grapes, oilseed rape, beans and peas) are crops with edible peel only. Therefore, studies on the distribution between peel and pulp are not required.

CA 6.5.3 Magnitude of residues in processed commodities

For the previous evaluation for Annex I inclusion of boscalid processing studies on grapes and peas have been submitted and peer-reviewed (Monograph 2002). Further processing studies, including oilseed rape and beans were reported within the evaluation under Article 12 of Regulation (EC) No 396/2005, and have been summarized in the EFSA Reasoned Opinion 2014 for boscalid (EFSA Journal 2014;12(7):3799).

In grape products, residues of boscalid increase with calculated processing factors of 2.40 (raisins, mean value) and 2.50 (wet pomace; mean value). No concentration of boscalid could be observed during the processing of grapes in wine, where a processing factor of 0.40 (mean value) was observed (EFSA Journal 2014;12(7):3799).

For peas, four field trials using twofold application rates have been conducted to determine the residue levels of boscalid in peas and process fractions involved in canned pea production. At harvest (PHI of 7 days) residues of boscalid of 0.14 mg/kg were determined in one trial only; all other trials did not show any residues above the limit of quantification. Thus, despite the enhanced application rate, residues were not of sufficient magnitude to derive processing factors for washing, blanching and canning. Considering that processing factors of other vegetables such as tomatoes and head cabbage are <1 (EFSA Journal 2014;12(7):3799), the accumulation of residues in peas is very unlikely.

The oilseed rape processing studies were conducted using incurred residues (0.72 to 1.76 mg/kg in oilseed rape seeds) derived from two applications of 1.344 kg a.s./ha. Residues were of sufficient magnitude both before and after processing to derive processing factors for meal and refined oil. Processing factors of of 1.3 (refined oil, mean value) and of 0.5 (meal, mean value) were calculated.

For the representative uses to be evaluated in this dossier (grapes, oilseed rape, beans and peas) the following processing procedures are essential (category 1) according to OECD guideline 508 and OECD guidance document 96: production of must, wine, juice and wet pomace for grape and production of oil and meal for oilseed rape. Additionally, for peas and beans, processing data for cooked and canned products are given. A summary of the processing factors of the representative uses on grapes, oilseed rape, and peas is given in Table 6.5.3-1, which are published in the EFSA Reasoned Opinion 2014 for boscalid (EFSA Journal 2014;12(7):3799). Data are considered sufficient. Further processing studies are not required, as they are not expected to affect the outcome of the risk assessment.

The study investigating the residues in processed fractions of oilseed rape which has not been submitted during the previous Annex I process, is presented in the following.

Table 6.5.3-1: Processing studies for boscalid

Processed commodity	Number of studies	Median PF (a)	Median CF (b)	Comments
Enforcement residue definition: boscalid				
Table grapes, dried (raisins)	4	2.40	1.00	Processing study evaluated in the Monograph (2002).
Wine grapes, juice	4	0.40	1.00	
Wine grapes, wet pomace	4	2.50	1.00	
Rape seed, refined oil	4	1.26	1.00	Details shown below.
Rape seed, meal/press cake	4	0.56	1.00	
Rape seed, crude oil	2	1.11	1.00	
Peas cooked/canned	1	<0.36	1.00	Processing study evaluated in the Monograph (2002).

Oilseed rape

The following processing study on oilseed rape is summarized, as the study has not been peer-reviewed.

Report:	CA 6.5.3/2 Versoi P.L.,Abdel-Baky S., 2001 b The magnitude of BAS 510 F residues in canola seed processed fractions 2001/5001064
Guidelines:	EPA 860.1520, PMRA 98-02 Section 10
GLP:	yes (certified by United States Environmental Protection Agency)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test material:

Description:	BAS 510 UCF (WG)
Lot/batch #:	AF464-17 (70% boscalid, nominal)
CAS#:	188425-85-6

2. Test commodity:

Crop:	Oilseed rape (canola)
Type:	Oilseeds
Variety:	Hyola 401, Canterra 1867RR, Quest, Golden Ready
Botanical name:	*Brassica napus*
Crop parts(s) or processed Commodity:	Seeds, cleaned seeds, expeller crude oil, solvent extracted oil, meal, refined oil, soapstock

B. STUDY DESIGN

1. Test procedure

During the 2000 growing season, four field trial were laid out at representative oilseed rape growing areas in the USA (North Dakota, Minnesota) and Canada (Manitoba) to determine the residue level of boscalid (BAS 510 F) in oilseed rape processed fractions.
Each trial consisted of an untreated plot and one plot, where BAS 510 UCF (WG, 70% boscalid nominal) was applied two times at exaggerated target rates of 1.35 kg a.s./ha in a tank mix with an adjuvant and dimoxystrobin (BAS 505 F). The applications were made 26±1 and 21±1 days prior to the anticipated harvest. The spray volume used was 112-196 L/ha.
Oilseed rape seeds were sampled at crop maturity 20-22 days after the last application. The samples were dried in the field for 0-7 days before storage. The samples were processed to meal, refined oil, cleaned seed, expeller crude oil, solvent extracted crude oil and soapstock. The samples were stored frozen at or below -10°C for a maximum of 5 months until analysis.

2. Description of analytical procedures

Residues of boscalid were determined using BASF method D9908, which quantifies the analyte by means of LC-MS/MS. Residues of boscalid were extracted from oilseed rape seeds, meal and soapstock with a methanol water solution acidified with 2N HCL (70:25:5, v:v:v). An aliquot of the extract is cleaned by liquid-liquid partition. Residues from oil matrices were extracted by liquid-liquid partition using acetonitrile:hexane. An aliquot was cleaned by C-18 micro column chromatograpy and a Silica Gel Speedisk micro-column. The final determination was performed by LC-MS/MS. The validated limit of quantitation was 0.05 mg/kg.

II. RESULTS AND DISCUSSION

Residues of boscalid in oilseed rape seeds were in the range of 0.72 to 2.28 mg/kg. Residues in cleaned seeds, crude oil, extracted oil, meal, refined oil and soapstock ranged from 0.56-2.25 mg/kg, 1.11-2.74 mg/kg, 1.05-2.49 mg/kg, 0.26-1.40 mg/kg, 1.35-2.61 mg/kg and 0.50-1.77 mg/kg, respectively.
This results in mean processing factors of 0.8, 1.2, 1.1, 0.5, 1.3 and 0.6 for cleaned seeds, crude oil, extracted oil, meal, refined oil and soapstock.

III. CONCLUSION

It was shown that boscalid did not concentrate in cleaned seeds (0.8x), meal (0.5x) and soapstock (0.6x), while in expeller crude oil (1.2x), solvent extracted oil (1.1x) and refined oil (1.3x) a slight increase was observed.

Table 6.5.3-2: Residues of boscalid in oilseed processed fractions

Trial location Trial number Year	Processed commodity	Application rate (kg a.s./ha)	DALA[1] (days)	Residues found (mg/kg)	Processing factor[2]
				Boscalid	
Velva, NA, USA (RCN 2000111) 2000	Seeds (RAC)	2 x 1.35	21 (26)	0.72	-
	Cleaned seeds			0.56	0.78
	Expeller crude oil			1.11	1.54
	Solvent extracted oil			1.05	1.46
	Meal			0.37	0.51
	Refined oil			1.39	1.93
	Soapstock			0.50	0.69
Minto, MB, CA (RCN 2000121) 2000	Seeds (RAC)	2 x 1.35	21 (28)	1.87	-
	Cleaned seeds			1.00	0.53
	Expeller crude oil			1.51	0.81
	Solvent extracted oil			1.26	0.67
	Meal			0.26	0.14
	Refined oil			1.35	0.72
	Soapstock			0.59	0.32
Bethany, MB, CA (RCN 2000123) 2000	Seeds (RAC)	2 x 1.35	20 (25)	2.28	-
	Cleaned seeds			2.25	0.99
	Expeller crude oil			2.74	1.20
	Solvent extracted oil			2.49	1.09
	Meal			1.40	0.61
	Refined oil			2.61	1.14
	Soapstock			1.77	0.78
Dalton, MN, USA (RCN 2000131)	Seeds (RAC)	2 x 1.35	22 (22)	1.76	-
	Cleaned seeds			1.57	0.89
	Expeller crude oil			2.00	1.14
	Solvent extracted oil			1.99	1.13
	Meal			1.11	0.63
	Refined oil			2.53	1.44
	Soapstock			1.13	0.64

1 Days after last application (value in parentheses includes the drying time on the field)
2 The processing factor (PF) is calculated by dividing the residue in the processed fraction by the residue in the RAC sample

Table 6.5.3-3: Summary processing factors in oilseed processed fractions

Processed commodity	RCN 2000111	RCN 2000121	RCN 2000123	RCN 2000131	Average Processing factor
Cleaned seeds	0.78	0.53	0.99	0.89	0.8
Expeller crude oil	1.54	0.81	1.20	1.14	1.2
Solvent extracted oil	1.46	0.67	1.09	1.13	1.1
Meal	0.51	0.14	0.61	0.63	0.5
Refined oil	1.93	0.72	1.14	1.44	1.3
Soapstock	0.69	0.32	0.78	0.64	0.6

CA 6.6 Residues in Rotational Crops

During the previous EU review of the active substance boscalid, the metabolism of boscalid in rotational crops has been studied in radish, lettuce and wheat. In addition, a field study on wheat grown as succeeding crop after boscalid application was submitted. Both of these studies have been part of the previous evaluation, are still scientifically valid and are therefore not submitted again in this supplementary dossier. For reasons of convenience, a short overview of the main results is given below.

Study type	Title	Test system	Results	Reference (BASF DocID)
Study according to OECD 502	Confined rotational crop study with 14C-BAS 510 F	Radish, lettuce, wheat	A confined rotational crop study was conducted with ^{14}C-BAS 510 F (two label positions) with a single application of 2.1 kg a.s./ha to bare soil. Lettuce, radish (leaves and roots) and wheat (forage, straw, grain) were sown 30, 120, 270 and 365 days after treatment. The results of this study clearly demonstrate that the only relevant residue was BAS 510 F and a potential for accumulation of boscalid residues in crops grown in rotation is expected.	2000/1014862
Study according to OECD 504	Determination of the residues of BAS 510 F in wheat obtained from the trial year 2000	Wheat	Two trials were performed applying boscalid. In both cases, wheat was planted on these plots in the succeeding season. Boscalid residues in plant without root were 0.09-0.11 mg/kg. In straw, residues were <0.05-0.82 mg/kg. In grain no residues of boscalid above the limit of quantitation were found.	2000/1014853 2001/1000989 (addendum)

The following endpoints were given in the boscalid Monograph (2002).

Metabolism in plants (Annex IIA, point 6.1 and 6.7, Annex IIIA, point 8.1 and 8.6)

Rotational crops	Radish, lettuce, wheat

Residues in succeeding crops (Annex IIA, point 6.6, Annex IIIA, point 8.5)

30, 120, 270, 365 days plant back interval after application of 2.1 kg a.s./ha to soil. With the exception of wheat grain the major residue was parent nicobifen[1)]. Residue of parent: lettuce: 0.014-0.146 mg/kg; radish leaf: 0.09-0.30 mg/kg, radish root: 0.01-0.09 mg/kg; wheat grain: 0.005-0.028 mg/kg, wheat forage: 0.19-1.47 mg/kg, wheat straw: 0.81-7.99 mg/kg. From the results it cannot be excluded that residues above the LOQ (0.05 mg/kg) occur in succeeding crops. This is confirmed by the results of a field test with residues of nicobifen[1)] in wheat plant at 0.10 mg/kg and in wheat straw at 0.75 mg/kg.

[1)] former name of boscalid

As residues may occur in plants due to uptake from soil, field trials were conducted to assess the magnitude of residues in rotational crops under practical conditions. In addition to the peer-reviewed study, several field rotational crop studies are available which were not part of the peer-review process. They were evaluated by EFSA in the context of the Article 12 review (EFSA Reasoned Opinion 2014, EFSA Journal 2014;12(7):3799). An overview of the results is given below; more details are presented in chapter M-CA 6.6.2.
In the studies, uptake of boscalid was investigated in various crops following an annual application rate of 2.0 to 2.15 kg a.s./ha on a bare soil (or with strawberry as primary crop, in one study). Results were summarized as follows "*The median and the highest boscalid residues were respectively identified in root and tuber vegetables at 0.05 and 0.37 mg/kg, in brassica vegetables at 0.035 and 0.05 mg/kg, in sweet corn cobs at 0.05 and 0.05 mg/kg, in pulses and oilseeds at 0.05 and 0.06 mg/kg, in cereal grains at 0.05 and 0.12 mg/kg, in root crop leaves and tops at 0.05 and 0.84 mg/kg, in various legume animal feeds at 0.08 and 1.46 mg/kg and in straw and fodder of cereal grains at 0.21 and 6.8 mg/kg"* (EFSA Reasoned Opinion 2014, EFSA Journal 2014;12(7):3799)

A new OECD guidance document on residues in rotational crops is currently in preparation. A draft was recently presented, proposing a tiered approach. The draft gives a guidance of crop grouping for field trials for MRL setting:

Crop group		Crops suggested for Tier 3 studies	Extrapolation for MRL setting
I	Root and tuber, bulb and stem vegetables	1. **potatoes**, **carrots** or similar 2. **radishes**, **beets**, leek or celery	to bulb and stem vegetables, all other roots, tubers, sugar plants, potatoes
II	Cereals	1. **maize** 2. **wheat** or barley	to all other cereals
III	Leafy and brassica vegetables	1. lettuce or spinach 2. cauliflower, broccoli, **head cabbage** or kale	to all other leafy and brassica vegetables
IV	Pulses and oilseeds	1. **beans** or **peas** (dry) 2. oilseed rape or **soybean**	to all other pulses, oilseeds, legume vegetables
V	Semi-permanent fruits, fruiting vegetables	1. **strawberries** 2. cucumber, pepper or tomato	to all other fruits and fruiting vegetables (field crops)

For nearly all crop groups sufficient trials were already evaluated for boscalid (see table above; crops emboldened). The data set is completed with representative crops from leafy vegetables and fruiting vegetables (new study, see chapter M-CA 6.6.2 below). In conclusion, sufficient data are available to evaluate the residue behavior of boscalid in rotational crops.

As the DT_{90} value exceeds one year, EFSA concluded that boscalid is likely to accumulate in soils treated for several consecutive years and particular attention has to be paid to the plateau concentration expected in soil after several years of applications (EFSA Reasoned Opinion 2014, EFSA Journal 2014;12(7):3799). BASF is familiar with this issue. Consequently, a field soil accumulation study is currently in progress; interim results covering a period of about 5.5 years are presented in chapter M-CA 7.1.2. As a result, the plateau value can be expressed as accumulation factor of 1.67 of the yearly total applied compound. Under consideration of the total yearly application rate of 1.600 kg/ha in the soil accumulation study of boscalid, the plateau value at steady state of the study conducted in vegetable crops was extrapolated with 2.676 kg/ha.
Taken into account all uses in crops with a potential use in crop rotation, the maximum application rate is 1.2 kg a.s./ha. This will lead to a calculated plateau level of 2 kg/ha. Therefore, the soil application rates of 2.0-2.15 kg a.s./ha used in the field rotational crop studies cover the estimated plateau level.

Furthermore, a study on the residue behavior of boscalid in rotational crops after growing on aged boscalid-treated soil was conducted (please refer to chapter M-CA 6.6.2 for details). It was shown that aging of boscalid residues in soil leads to a reduction of the bioavailability for plant of about 50%. This effect starts about 3 months after the application. In conclusion, for the assessment of the uptake of boscalid after multi-year use of boscalid, the results of the rotational crop study should be corrected with a factor of 0.5.

During Article 12 review, EFSA performed an estimate whether or not a significant uptake of boscalid residues from the soil is expected which would contribute to the residue levels for the annual crops under consideration (EFSA Reasoned Opinion 2014, EFSA Journal 2014;12(7):3799). Where a significant residue uptake could not be excluded, the MRL proposal presented by EFSA took into account the additional residue via soil uptake. This approach was pursued by the European Commission with the currently pending EU MRLs (SANTE/10530/2015), which were taken into account for the risk assessments conducted within this dossier (see chapter M-CA 6.9).

CA 6.6.1 Metabolism in rotational crops

The metabolism of boscalid in rotational crops has been evaluated in the framework of the peer review. One confined rotational crop study investigating the nature of residues following different plant-back intervals is available. The characteristics of this study are summarized in the following table. (EFSA Reasoned Opinion 2014, EFSA Journal 2014;12(7):3799)

Table 6.6.1-1: Summary of available metabolism study in rotational crops

Crop group	Crop	Label position	Application and sampling details				
			Method, F or G[(a)]	Rate (kg a.s./ha)	Sowing intervals (DAT)	Harvest Intervals (DAT)	Remarks
Leafy vegetables	Lettuce	U-^{14}C-diphenyl and 3-^{14}C-pyridine	Bare soil, G	2.1	30, 120, 270, 365	Mature crops	-
Root and tuber vegetables	Radish						-
Cereals	Wheat						-

(a): Outdoor/field application (F) or glasshouse/protected/indoor application (G)

The highest TRR values were observed in radish leaves (0.34 mg/kg; 30 DAT, pyridine study) and in wheat straw (9.83 mg/kg, 30 DAT, diphenyl study and 4.01 mg/kg, 120 DAT, pyridine study). The highest TRR in lettuce amounted to 0.16 mg/kg (120 DAT, pyridine study), in radish root to 0.098 mg/kg (270 DAT, diphenyl study) and 0.066 mg/kg (365 DAT, pyridine study) and in wheat grain to 0.285 mg/kg (120 DAT, pyridine study) and 0.243 mg/kg (120 DAT, diphenyl study) (EFSA Journal 2014;12(7):3799).
Except in wheat grain, parent boscalid was the major component of the TRR in all crops. Levels of the parent compound ranged from 50% TRR in wheat straw (270 DAT, pyridine label) to 93% TRR in wheat forage (270 DAT, pyridine label), and in lettuce leaves from 55.6% TRR (270 DAT, diphenyl label) to 94.1% TRR (365 DAT, diphenyl label). In wheat grain, the concentration of parent was low (between 1.9% TRR at 270 DAT with the pyridine label and 16.8% TRR at 30 DAT with the diphenyl label). Most of the radioactive residues in grain were not extractable (65 to 96% TRR) and were detected in the starch fraction (36.2 to 48.4% TRR, 0.06-0.12 mg/kg, pyridine label). The metabolite M510F61 (sugar conjugate of hydroxylated boscalid) was the only metabolite identified at levels exceeding 10% TRR, in wheat forage (18.1% TRR, diphenyl label, 270 DAT) and in radish leaves (21.2% TRR for diphenyl label, 270 DAT and 11.2-15.5% TRR, 365 DAT) (EFSA Journal 2014;12(7):3799).

The proposed metabolic pathway in succeeding crops involves hydroxylation and conjugation reactions. A part of the residue was also incorporated into and/or associated with natural products, such as starch, cellulose and lignin. The parent compound is therefore the main substance of concern in rotational crops and no metabolites of concern were identified in soil. Consequently, metabolic patterns in primary and rotational crops are found to be similar and a specific residue definition for rotational crops is not deemed necessary (EFSA Reasoned Opinion 2014, EFSA Journal 2014;12(7):3799).

CA 6.6.2 Magnitude of residues in rotational crops

Two rotational crop field trials were evaluated in the framework of the previous peer review. In the first trial, boscalid was applied the first year to lettuce (2 × 0.3 kg a.s./ha – 0.75N) and green beans (3 × 0.5 kg a.s./ha – 1.5N) and the second year to carrots (3 × 0.3 kg a.s./ha – 1.7N) and cauliflower (2 × 0.4 kg a.s./ha – 1N). The third year, wheat was sown in the same field. In the second field trial, boscalid was applied on winter rape (0.5 kg a.s./ha – 1N) and, 365 days after harvest, wheat was sown on the same plot. Only wheat was analyzed for residues, indicating that boscalid residues were not found above the LOQ in wheat grain when wheat was planted as a succeeding crop after vegetables or rape. However, boscalid residues were found in wheat straw (0.75 mg/kg) when planted after vegetables (not when rape was sown as a primary crop). Moreover, residues in vegetables grown in the second year of the first trial were not investigated (EFSA Reasoned Opinion 2014, EFSA Journal 2014;12(7):3799)

In addition, as mentioned in chapter M-CA 6.6, a number of field rotational crop studies were performed in the US and the EU to provide a broader and hence clearer picture on residue levels as found in rotational crop studies conducted under natural conditions. They were already evaluated by EFSA in the context of the Article 12 review (EFSA Journal 2014;12(7):3799) and are summarized in the following.

Report:	Jeannine M. Jordan Cereal Grains and Soybean Field Rotational Crop Study for BAS 510 F 2002/5001341
Guidelines:	US, EPA Residue Chemistry Test Guidelines OPPTS 860.1900, Rotational Crop Field Trials
GLP:	yes

Abstract

Forty-nine extended field trials were conducted on field corn, sweet corn, grain sorghum, rice, wheat (spring and winter), and soybean plants grown in soil treated with a WG formulation of BAS 510 F to determine the magnitude of the residue of the fungicide in rotational crop Raw Agricultural Commodity (RAC) samples.

Each trial consisted of two plots, an untreated control plot (Treatment 1), and a treated plot (Treatment 2; Treatment 3 for winter wheat). Three sequential broadcast applications of BAS 510 UCF were made to the bare soil of the treated plot at the maximum label rate targeting 0.72 (0.8 kg/ha), 0.55 (0.62 kg/ha), and 0.55 (0.62 kg/ha) pounds active ingredient per acre (lb ai/A) for each application, respectively, resulting in a maximum seasonal target rate of 1.82 lb ai/A (2.04 kg/ha). There was a 7 (±1) day target interval between applications, beginning 28 days prior to the rotational crop planting date. The rotational crops were planted at a targeted plantback interval of 14 (±1) days after the last application (DALA) to the soil. All rotational crop RAC samples were collected at normal maturity.

A summary of residues in rotational crops grown in soil treated with a targeted seasonal rate of 1.82 lb ai/A (2.04 kg/ha) of BAS 510 F is shown below (Table 6.6.2-1).

Table 6.6.2-1: Summary of boscalid residues in succeeding crops after application of 2.04 kg/ha on bare soil

Crop	Matrix	PBI (days)[1]	N	Range of BAS 510 F residues (mg/kg) [2]
Wheat	Forage	13-15	32	<0.05 – 1.4
	Hay		34	<0.05 – 0.99
	Grain		34	<0.05 – 0.07
	Straw		34	0.10 – 2.7
Field Corn	Forage	13-19	30	<0.05 – 0.14 [<0.05 – 0.10] [3]
	K+CWHR[4]		8	[<0.05]
	Stover		36	<0.05 – 0.35 [<0.05 – 0.12]
	Grain		36	<0.05
Sweet Corn	Forage	13-14	8	<0.05 – 0.09
	K+CWHR		8	<0.05
	Stover		8	0.05 – 0.50
Grain Sorghum	Forage	13-15	23	<0.05 – 0.26
	Grain		24	<0.05
	Stover		24	<0.05 – 0.36
Rice	Grain	13-17	12	<0.05 – 0.12
	Straw		12	<0.05 – 1.32
Soybean	Forage	13-15	30	<0.05 – 0.20
	Hay		28	<0.05 – 0.54
	Seed		30	<0.05 – 0.06

1 PBI = Plantback Interval.
2 The limit of quantitation of BAS 510 F is 0.05 mg/kg in all rotational crop RACs.
3 The range of residues for field corn forage and stover samples (n=16 each) from the four sites supporting sweet corn are shown in brackets.
4 K+ CWHR = kernal plus cob with husk removed (milk stage ”fresh corn”)

Report: Jeannine M. Jordan
Field Rotational Study for BAS 510 F on Grasses, Alfalfa, and Clover As Livestock Feed Crops
2002/5002063
Guidelines: US, EPA Residue Chemistry Test Guidelines
OPPTS 860.1900, Rotational Crop Field Trials
GLP: yes

Abstract
Livestock feed items including grasses (blue, rye, bermuda, fescue, brome), alfalfa and clover were planted in a rotation after ground application of a WG formulation of BAS 510 F to determine the field accumulation of the fungicide in the rotational crop Raw Agricultural Commodity (RAC) samples. Each trial, or site, consisted of two plots, an untreated control plot (Treatment 1), and a treated plot (Treatment 2). Three sequential bareground applications of BAS 510 UCF were made to the treated plot at the maximum label rate targeting 0.72 (0.8 kg/ha), 0.55 (0.62 kg/ha), and 0.55 (0.62 kg/ha) pounds active ingredient per acre (lb ai/A) for each application, respectively, resulting in a maximum seasonal target rate of 1.82 lb ai/A (2.04 kg/ha). There was a 7 (±1) day target interval between applications, beginning 28 days prior to the rotational crop planting date. The interval from the last application to planting was targeted at 14 days which is the minimum plantback interval.

All RAC samples were targeted for collection at normal maturity.

A summary of the residues is shown in Table 6.6.2-2.

Table 6.6.2-2: Summary of boscalid residues in succeeding crops after application of 2.04 kg/ha on bare soil

Crop	Matrix	Range of BAS 510 F residues (mg/kg)
Grasses including blue, rye, brome, fescue and bermuda	Forage	0.07 – 1.9
	Hay	0.18 – 7.1
	Seed Screenings	0.06 – 0.10
	Straw	0.12 – 0.22
Alfalfa	1st cutting forage	<0.05 – 0.52
	2nd cutting forage	<0.05 – 0.5
	3rd cutting forage	<0.05 – 0.07
	1st cutting hay	<0.05 – 1.59
	2nd cutting hay	<0.05 – 1.5
	3rd cutting hay	<0.05 – 0.2
	Seed	<0.05
Clover	Forage	<0.05 – 0.57
	Hay	<0.05 – 0.52

Report: David W. Haughey, Samy Abdel-Baky
Limited Rotational Crop Study for the Use of BAS 510 F in Strawberries
2001/5000966
Guidelines: US, EPA Residue Chemistry Test Guidelines
OPPTS 860.1900, Rotational Crop Field Trials
GLP: yes

Abstract
A total of six limited field trials were conducted in order to determine the magnitude of the residue of BAS 510 F in or on Raw Agricultural Commodities (RAC) of winter wheat, cabbage, and radish planted as rotational crops, with a minimum plant-back interval (PBI) of 14 days following BAS 510 UCF Fungicide applications. The rotational crops were planted following applications of a WG formulation of BAS 510 F to strawberries at field sites in Georgia and California. Each trial, or site, consisted of two plots, an untreated control plot (Treatment 1), and an treated plot (Treatment 2). Five sequential foliar applications of BAS 510 UCF were made to the treated plot targeting 0.37 (0.42 kg/ha) pounds active ingredient per acre (lb ai/A) for each application, resulting in a maximum seasonal target rate of 1.85 lb ai/A (2.04 kg/ha). There was a 7 (+/-1) day target interval between applications, beginning 28 days prior to strawberry harvest. All sprays were applied in combination with a locally-available, non-silicone spray adjuvant.

Strawberries were harvested at normal maturity. The rotational crops were planted into the control and treated plots at 14, 30, and 45 (+/-1) day plantback intervals. Wheat, cabbage, and radish RAC samples were collected at normal crop maturity for each plot and from both sites.

Quantifiable levels of BAS 510 F were observed in rotational crops planted up to 45 days after treatment of the primary crop, with the exception of cabbage and wheat grain. There were no BAS 510 F residues detected above the limit of quantitation (LOQ, 0.05 mg/kg) in cabbage (with and without wrapper leaves) or wheat grain samples collected from the 14-day PBI; therefore, samples from the 30 and 45 day PBI were not analyzed. One wheat grain sample from the California site contained BAS 510 F residues at the LOQ, but residues were <LOQ in the replicate sample from the same plot.

A summary of residues in rotational crops following strawberries treated with a total seasonal rate of 1.85 lb ai/A (2.04 kg/ha, 1X) for each site is presented in Table 6.6.2-3.

Table 6.6.2-3: Summary of boscalid residues in succeeding crops after application of 2.04 kg/ha on strawberries

Rotational crop matrix	Maximum BAS 510 F residues (mg/kg)		
	14 day PBI	30 day PBI	45 day PBI
Radish roots	0.32	0.15	0.20
Radish tops	0.82	0.41	0.55
Cabbage	<0.05	Not Analysed	
Wheat forage	0.34	0.39	0.31
Wheat hay	1.68	1.28	1.10
Wheat grain	0.05	Not Analysed	
Wheat straw	3.18	2.54	2.42

Report:	Peter L. Versoi, Samy Abdel-Baky Magnitude of the Residue of BAS 510 F in Peas and Beans Planted As Rotational Crops and of BAS 500 F in Peas and Beans When Applied as a Foliar Spray 2001/5003311
Guidelines:	US, EPA Residue Chemistry Test Guidelines OPPTS 860.1500, Crop Field Trials OPPTS 860.1900, Rotational Crop Field Trials
GLP:	yes

Abstract

Dry field pea plants at three locations in ID, OR, and WA, and bean (cowpea) plants at three locations in AR, NC, and OK were grown at a plantback interval (PBI) of 14 days in soil treated with a WG formulation of BAS 510 F in order to determine the magnitude of residues of the fungicide in or on rotational pea and bean foliage Raw Agricultural Commodity (RAC) samples. The crops were subsequently treated foliar with a WG formulation of BAS 500 F in order to determine the magnitude of the residues of the fungicide in or on pea and bean foliage RAC samples.

Each trial, or site, consisted of two plots, an untreated control plot (Treatment 1), and one or two treated plots (Treatment 2 for the pea sites; Treatments 2 and 3 for the bean sites). BAS 510 UCF was applied three times to the bare soil surface of each treated plot targeting the proposed maximum label rate of 0.72 pounds (0.8 kg/ha) active ingredient per acre (lb ai/A) for the first application and 0.55 lb ai/A (0.62 kg/ha) for each of the two remaining applications, resulting in a maximum seasonal rate of 1.82 lb ai/A (2.04 kg/ha). The applications targeted 28, 21 & 14 (+/-1) days before planting. Field peas and beans were planted into both the treated and untreated plots at the targeted plantback interval of 14 (+/-1) days after the last application (DALA) of BAS 510 F to the soil. After crop emergence and growth, BAS 500 02 F was applied twice foliarly to the peas and beans in the same treated plots targeting 0.2 lb ai/A/Application, at a targeted 5 (+/-1) day retreatment interval, resulting in a maximum seasonal target rate of 0.4 lb ai/A. A locally-available spray adjuvant was applied with all BAS 500 F test substance applications.

Field pea (vines and hay) and bean (forage and hay) RAC samples were harvested at normal crop maturity, 0, 7, and 14 (+/-1) days after the last application of BAS 500 F (14 day PBI with respect to BAS 510 F). The plants cut for hay were allowed to dry for 2-7 days before collection.

For the extended field trials, the highest individual residues of BAS 510 F were 1.05 and 1.50 mg/kg in bean (cowpea) forage and hay from the 14 days plantback interval. BAS 510 F residues were below the limit of quantitation (<LOQ, <0.05 mg/kg) in all field pea vines and a maximum of 0.15 mg/kg in field pea hay, each from the 13-14 day PBI.

A summary of the residues from individual field samples of peas and beans grown as rotational crops in soil treated with BAS 510 F at 1X the maximum proposed seasonal rate are shown in Table 6.6.2-4.

Table 6.6.2-4: Summary of boscalid residues in succeeding crops after application of 2.04 kg/ha on bare soil

Matrix	N	PBI [1]	DAP [2]	Range of BAS 510 F residues
Field pea, vines	18	13-14	46-92	<0.05
Field pea, hay	18	13-14	46-92	<0.05-0.15
Bean, forage	18	14	30-49	<0.05-1.05
Bean, hay	18	14	49-79	<0.05-1.50

1 PBI = Plantback Interval (in days), with respect to BAS 510 F application
2 DAP = Days After Planting

Report: Raymond C. Leonard
Sugar Beet, Garden Beet and Turnip Field Rotational Crop Study for BAS 510 02 F Residues
2002/5004273
Guidelines: US, EPA Residue Chemistry Test Guidelines
OPPTS 860.1500, Crop Field Trials
OPPTS 860.1900, Rotational Crop Field Trials
GLP: yes

Abstract
Fourteen field rotational crop trials were conducted in the principal growing regions for sugar beets, garden beets and turnips to determine the magnitude of BAS 510 F residue in or on these Raw Agricultural Commodities (RAC), as representatives of the Leaves of Root and Tuber Vegetables Crop Group following applications of BAS 510 02 F to bare soil. Each trial, or site, consisted of two plots, an untreated control plot (Treatment 1) and one treated plot (Treatment 2). Three sequential applications of BAS 510 02 F were made to bare ground in the treated plot targeting the maximum proposed seasonal application rate of 1.82 pounds active ingredient per acre (lb ai/A). The first application of BAS 510 02 F targeted 0.72 lb ai/A, followed by two applications targeting 0.55 lb ai/A. There was a 7 (±1) day target interval between successive applications, beginning approximately 28 days prior to planting, resulting in a 14 day interval between the last application of BAS 510 02 F an planting of sugar beets, garden beets and turnips.

Mature roots and tops were collected from both treatments at all sites and analyzed according to BASF Analytical Method D9908, which measures residues of BAS 510 F as the parent compound, the only residue of concern from BAS 510 F in plants.
The residue summary is shown in Table 6.6.2-5.

Table 6.6.2-5: Summary of boscalid residues in succeeding crops after application of 2.04 kg/ha on bare soil

Crop RAC	Plantback interval*	Number of RAC samples	Range of BAS 510 F residues (mg/kg)
Sugar beet, roots	14	14	<0.05
Sugar beet, tops	14	14	<0.05 - 0.05
Garden beet, roots	14	4	<0.05
Garden beet, tops	14	4	<0.05
Turnip, roots	13 - 14	10	<0.05
Turnip, tops	13 - 14	10	<0.05 - 0.07

* The interval between the last application to the soil and planting of the root and tuber crop

Report: J. Beck, A. Lehmann, C. Grote, Ch. Mackenroth
Study on the residue behaviour of Boscalid (BAS 510 F) on succeeding crops after application of BAS 510 01 F on bare soil and cultivation of potatoes under field conditions in Denmark, France, Germany and Great Britain, 2002
2003/1001358

Guidelines: US, EPA Residue Chemistry Test Guidelines
OPPTS 860.1500, Crop Field Trials
OPPTS 860.1900, Rotational Crop Field Trials

GLP: yes

Abstract

During the 2002 growing season, a total of 4 trials were conducted as rotational crop study with potatoes. BAS 510 01 F, a WG formulation of boscalid was spray applied once at a rate of 4.2 kg/ha (formulated product) on the bare soil, 28-29 days before planting of the potatoes, resulting in a maximum pre-seasonal target rate of 2.1 kg a.i./ha in order to determine the magnitude of the residues in the succeeding crop potatoes. This rate of 2.1 kg/ha is more than double than the intended GAP.

According to the commercial agricultural procedures, the creation of the potato dams and the planting of the potatoes was – with one exception- performed after a shallow soil preparation.

Soil specimens were collected directly from the dams at application timing (0 DAA), at potato planting (28-29 DAA) and at crop maturity (106-148 DAA). Potato tuber specimens were collected at commercial harvest time (BBCH 91-97 and 106-148 DAA) of the potatoes.

For further investigations, potatoes of some trials were washed and peeled. Then the peeled potatoes and the potato peel were additionally analyzed.

The results are summarized in Table 6.6.2-6.

Table 6.6.2-6: Summary of boscalid residues in succeeding crops after application of 2.1 kg/ha on bare soil

Specimen material	Growth stage range	Timing range (DAA/PHI)	Range of residues mg/kg
Soil core	0	0	0.36 – 2.5
Soil core	N/a and 3	28-29	0.37 – 1.1
Soil core	N/a and 97	28-29	0.051-0.91
Potato Tuber	91-97	106-148	<0.05-0.06
Potato Tuber, peeled	95	106	0.027
Peel	95	106	0.365

From four potato tuber samples analyzed, two did not contain boscalid residues above the limit of quantitation, the other two showed residues of 0.06 mg/kg.

In order to find the exact location of the residues, a re-analysis was done analyzing the peeled potato and peel separately. The peel contained the major part, while the peeled potatoes where almost free of residues.

Additionally also soil samples were analyzed. In the soil significant residues of boscalid could be detected.

Report: Schroth E., Martin T. 2008
Study on the residue behavior of BAS 510 F on the rotational crop: Carrots, after the application to the soil of BAS 510 01 F under field conditions in France (South), Germany, Netherlands and Spain, 2007
2008/1036949
Guidelines: EEC 91/414 Annex II (Part A Section 6), EEC 91/414 Annex III (Part A Section 8), EEC 1607/VI/97 rev. 2 10.06.1999, EEC 7029/VI/95 rev. 5,EEC 7525/VI/95 rev. 7
GLP: yes

Abstract
During the 2007 growing season, 4 trials in rotational crop: carrots were conducted in different representative growing areas in France (South), Germany, Netherlands and Spain to determine the residue level of BAS 510 F (boscalid) in or on Raw Agricultural Commodities (RAC).

BAS 510 01 F (500 g/kg boscalid, WG) was applied once to bare soil at a rate equivalent to 2.1 kg a.i./ha of boscalid. The application was made at early spring and the spray volume used was 300 l/ha. After an interval of 30 ± 1 days, carrots were planted as succeeding crop

Soil specimens were collected at the day of the application and about 30 days thereafter (immediately before seeding) and as well when the crop reached BBCH 41 and BBCH 49. Specimens of whole plants without roots were collected at BBCH 41 and specimens of carrot tops and carrots roots were collected at BBCH 49.

Table 6.6.2-7: Summary of boscalid residues in rotational crop: carrots

Portion analyzed	Sampling occasion	Growth stage (BBCH)	DALA[1]	Range of boscalid residues (mg/kg)
soil	1	-	-	<0.01 – 0.013 (0.010[2])
	2	-	0	0.273 – 0.459 (0.319[2])
	3	-	29	0.467 – 0.904 (0.349[2])
	4	-	86 – 126 (80[2])	0.403 – 0.569 (0.249[2])
	5	-	147 – 165 (112[2])	0.311 – 0.775 (0.273[2])
plant w/o root	4	41	86 – 126 (80[2])	0.02 – 0.12 (1.13[2])
top	5	49	147 – 165 (112[2])	<0.01 – 0.03 (0.84[2])
root	5	49	147 – 165 (112[2])	0.01 – 0.08 (0.37[2])

1 Days after last application
2 Result from the Spanish trial 07ES/001R

The residue levels of boscalid found in soil were relatively constant throughout the study.

Regarding the residue results obtained in carrot, one point is noticeable: three trials show residues of very comparable levels whereas in the Spanish trial values were found which are considerably higher in plants than the values in the other trials. The results of this trial were confirmed by double analysis. However, a comprehensible explanation cannot be given. This trial was not considered for the estimation of potential residues.

To take these findings into account, the summary table shows the range of residues found in the three comparable trials and in brackets the results from the deviating trial.

As mentioned in chapter M-CA 6.6, a new OECD draft guidance document on residues in rotational crops proposed crop grouping for field trials for MRL setting. Accordingly, the data set of field rotational crop studies were completed by an additional study with representative crops from leafy vegetables and fruiting vegetables.

This new rotational crop study is summarized below and will cover zucchini/cucumber and tomato for fruiting vegetables and lettuce for leafy vegetables at a plant back interval of 30 days:

Report:	CA 6.6.2/1 Martin T., 2015 a BAS 510 F on rotational crops: Fruiting (cucumber, zucchini, tomato) and leafy vegetables (lettuce) after one application of BAS 510 01 F to bare soil 30 days before seeding/planting, field conditions in Germany Netherlands Italy Spain 2015 2015/1117845
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), OECD 504, OECD 509 Crop Field Trial (2009)
GLP:	yes (certified by ENAC, Entidad Nacional de Acreditación, Madrid Spain)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	Boscalid (BAS 510 F), BAS 510 01 F, WG
Lot/Batch #:	FRE-001071, 500 g/kg (BAS 510 F, nominal concentration)
Purity:	Not reported
CAS#:	188425-85-6
Development code:	
Spiking levels:	0.01 and 0.1 mg/kg

Stability of test compound: Test compound was stable over investigation time.

2. Test Commodity:

Crop:	(1) Zucchini (2) Cucumber (3) Tomato (4) Lettuce
Type:	(1+2) Fruiting vegetables, cucurbits (3) Fruiting vegetables, other than cucurbits (4) Leafy vegetables
Variety:	(1) Black beauty, Milos; (2) Modan (3) Moneymaker, Rio Grande, Vilma (4) Lorenzo, Lolio Bionda, Paradai RZ
Botanical name:	(1) *Cucurbita pepo subsp. pepo* (2) *Cucumis sativus* (3) *Solanum lycopersicum* (4) *Lactuca sativa*
Crop part/processed commodity:	Zucchini / Cucumber (fruit), tomato (fruit), lettuce (leaves)
Sample size:	Min. 0.5 kg plant material / 12 plants

B. STUDY DESIGN

1. Test procedure

During the 2015 growing season, four field trials were conducted with three rotational crops (cucumber or zucchini, tomato and seeded lettuce) in different representative growing areas in Northern and Southern Europe in order to determine the residue uptake of boscalid after soil application. Each field trial consisted of one untreated plot (plot 1) and one treated plot (plot 2). Each plot consisted of three subplots where the different crops, cucumber / zucchini, tomato or lettuce were sowed or planted, respectively.

The test item BAS 510 01 F (WG, 500 g boscalid/kg) was applied once to bare soil 30-31 days before seeding / planting at a rate of 2.1 kg/ha of BAS 510 F. The application volume of spray solution was 200 L/ha. Specimens of plant were collected at BBCH 75-79 (cucumber or zucchini, fruits), BBCH 80-89 (tomato, fruits) and BBCH 41 and 49 (lettuce, leaves).

All specimens were stored frozen at or below -18°C until analysis for a maximum period of 100 days for plant material.

2. Description of analytical procedures

BASF method No 535/1 (L0076/01) was used for the analysis of specimens. The limit of quantitation (LOQ) was 0.01 mg/kg for each analyte. A summary of procedural recoveries is given in Table 6.6.2-8.

BAS 510 F was extracted with a mixture of methanol, water and hydrochloric acid. An aliquot of the extract was centrifuged and partitioned at alkaline conditions against cyclohexane. The final determination was performed by HPLC-MS/MS.

Table 6.6.2-8: Summary of recoveries for boscalid (BAS 510 F)

Crop	Matrix	Fortification level (mg/kg)	Summary recoveries		
			n	Mean (%)	RSD (%)
BASF method No L0076/01			Boscalid (BAS 510 F)		
Zucchini	Fruit	0.010 and 0.10	6	90.4	4.2
Cucumber	Fruit	0.010 and 0.10	6	87.9	5.5
Tomato	Fruit	0.010 and 0.10	6	87.7	3.8
Lettuce (seeded)	Leaves	0.010 and 0.10	6	79.0	4.5

n Number of recoveries

II. RESULTS AND DISCUSSION

At a replant interval of 30±1 days, no residues of BAS 510 F in zucchini, cucumber and tomato fruits above the LOQ were detected at harvest at BBCH 75-79, 75 and 80-89, respectively. In seeded lettuce (leaves), residues of BAS 510 F ranged from 0.018 to 0.120 mg/kg at BBCH 41 and between 0.014 and 0.055 mg/kg at harvest.

No residues of BAS 510 F above the limit of quantitation (0.01 mg/kg) were detected in any of the analyzed control specimens.

Table 6.6.2-9: Summary of residues in rotational crops - 30 days planting interval

Crop	Portion analyzed	Growth stage (BBCH)	DALA[1]	n	Residues (mg/kg)
					BAS 510 F
30±1 days replant interval					
Zucchini	Fruit	75-79	66-73	3	<0.010
Cucumber	Fruit	75	80	1	<0.010
Tomato	Fruit	80-89	129-140	4	<0.010
Lettuce (Seeded)	Leaves	41	60-104	4	0.018-0.120
Lettuce (Seeded)	Leaves	49	86-119	4	0.014-0.055

1 Days after Last Application
n Number of samples

III. CONCLUSION

The results of the study show that no residues above the limit of quantitation of BAS 510 F are taken up into edible parts of following crops such as zucchini/cucumber and tomato planted 30 days after application of BAS 510 01 F to bare soil. In seeded lettuce, only low residues of up to 0.055 mg/kg were found at harvest.

Table 6.6.2-10: Residues in succeeding crops

Study details		Formulation, Appl. rate (kg a.s./ha)	DALA[1]	Crop	Residues (mg/kg)	
					Matrix	BAS 510 F
Study code:	781793			**30±1 day plant back interval**		
DocID:	2015/1117845	BAS 510 01 F	73	Zucchini	Fruit	<0.01
GLP:	Yes	1 x 2.1	129	Tomato	Fruit	<0.01
Year:	2015	to bare soil	73	Lettuce	Leaves	0.078
Trial:	L150339		86	Lettuce	Leaves	0.055
Country:	Germany					
Study code:	781793			**30±1 day plant back interval**		
DocID:	2015/1117845	BAS 510 01 F	73	Zuchini	Fruit	<0.01
GLP:	Yes	1 x 2.1	129	Tomato	Fruit	<0.01
Year:	2015	to bare soil	73	Lettuce	Leaves	0.018
Trial:	L150340		86	Lettuce	Leaves	0.014
Country:	The Netherlands					
Study code:	781793			**30±1 day plant back interval**		
DocID:	2015/1117845	BAS 510 01 F	66	Zucchini	Fruit	<0.01
GLP:	Yes	1 x 2.1	129	Tomato	Fruit	<0.01
Year:	2015	to bare soil	104	Lettuce	Leaves	0.036
Trial:	L150341		119	Lettuce	Leaves	0.038
Country:	Italy					
Study code:	781793			**30±1 day plant back interval**		
DocID:	2015/1117845	BAS 510 01 F	80	Cucumber	Fruit	<0.01
GLP:	Yes	1 x 2.1	140	Tomato	Fruit	<0.01
Year:	2015	to bare soil	60	Lettuce	Leaves	0.120
Trial:	L150342		87	Lettuce	Leaves	0.022
Country:	Spain					

1 Days after Last Application

The study on the residue behavior of boscalid in rotational crops after growing on aged boscalid-treated soil alluded in chapter M-CA 6.6 is summarized below.

Report:	CA 6.6.2/2 Schweda Z.,Mackenroth C., 2009a Study on the residue behavior of BAS 510 F in the rotational crops: Wheat, spinach and radish after growing on soil with different aged Boscalid 2009/1069175
Guidelines:	EEC 91/414 Annex II (Part A Section 6), EEC 91/414 Annex III (Part A Section 8), EEC 1607/VI/97 rev. 2 10.06.1999, EEC 7029/VI/95 rev. 5, EEC 7525/VI/95 rev. 7, EEC 7524/VI/95 rev. 2 (July 22 1997)
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

This study was designed to further elucidate the bioavailability of boscalid in aged soil with special regard to succeeding crops. The fungicidal active ingredient boscalid is known to degrade very slowly in soil. The uptake of boscalid into succeeding crops on aged and freshly applied soil was analyzed.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	Boscalid
Lot/Batch #:	01893-55
Purity:	100.0%
CAS#:	188425-85-6
Development code:	BAS 510 F
Spiking levels:	0.01-1.0 mg/kg

2. Test Commodity:

Crop:	1) Wheat, 2) radish, 3) spinach
Type:	1) Cereals, 2) root and tuber vegetables, 3) leafy vegetables
Variety:	1) Thassos, 2) April Cross, 3) Monopa
Botanical name:	1) *Triticum aestivum*, 2) *Raphanus sativus*, 3) *Spinacia oleracea*
Crop parts(s) or processed Commodity:	1) Whole plant without roots, grain, straw, 2) root, leaves, 3) leaves

The soils used for this investigation derive from a former study: soil 1 is an untreated control material, soil 2 is a boscalid-containing aged soil which was treated over three years (2005: 1800 g a.s./ha, 2006: 750 g a.s./ha, 2007: 1800 g a.s./ha).

B. STUDY DESIGN AND METHODS

1. Test procedure

It is the intention of this study to investigate if boscalid accumulated in soil over a longer time period is equally available for plant up-take as freshly applied boscalid.
For that purpose a comparison between a soil treated with boscalid over three years (soil 2) and a freshly treated soil (soil 1) containing the same amount of boscalid was performed in a greenhouse trial.
The study comprised three plots: plot 1 was used for the control, plot 2 was freshly treated at a rate equivalent to the concentration found in the aged soil, and plot 3 consisted of a soil treated with boscalid over 3 years. The plots were divided into three subplots which were planted with wheat (subplot 1), radish (subplot 2) and spinach (subplot 3). The maintenance of the crops was performed in accordance with normal agricultural practice. The crops were cultivated in climatic chambers simulating climatic conditions of Southwest Germany.
At the beginning of the study both plot 1 and 3 were analyzed for boscalid. The difference of the mean values found in the aged soil and in the control material was calculated. The result was taken as application rate for plot 2. Wheat and radish were sowed within 24 h following application, spinach was sowed within 15 days after application.
Wheat plant without root specimens were taken at growth stage BBCH 39 to 49. Grain and straw were sampled at BBCH 89. Radish root and leaf specimens were sampled at BBCH 49. Spinach leaves were collected at BBCH 14 to 49.

2. Description of analytical procedures

Soil specimens were analyzed for boscalid with BASF method No L0096/1 which quantifies the analyte with a limit of quantitation of 0.01 mg/kg.
In principle, boscalid is extracted from soil samples by methanol/aqueous acetate buffer. Final determination is performed by HPLC-MS/MS monitoring two parent-daughter ion transitions for quantitation and confirmation.
The results of procedural recovery experiments averaged at about 97% for boscalid at fortification levels between 0.01 and 1.0 mg/kg.

Plant specimens were analyzed for boscalid with BASF method No L0076/03 which quantifies the analyte with a limit of quantitation of 0.01 mg/kg.
In principle, boscalid is extracted from plant material with a mixture of methanol, water and hydrochloric acid. An aliquot of the extract is centrifuged and partitioned at alkaline conditions against cyclohexane. The final determination is performed by HPLC-MS/MS.
The results of procedural recovery experiments averaged at about 73% for boscalid at fortification levels between 0.01 and 0.1 mg/kg.

II. RESULTS AND DISCUSSION

A summary is given in the table below.

Table 6.6.2-11: Boscalid residues in succeeding crops grown on different soils

Succeeding crop	Portion analyzed	Growth stage (BBCH)	Mean boscalid residues (mg/kg		Proportion (%) (residue found in soil 2 expressed as percentage of residue found in soil 1)
			Soil 1 (freshly treated)	Soil 2 (treated over 3 years)	
Wheat	Whole plant	39-49	0.544	0.300	55.1
	Grain	89	0.015	0.007	46.5
	Straw	89	3.875	2.015	52.0
Radish	Root	49	0.029	0.019	66.5
	Leaves	49	0.150	0.087	58.2
Spinach	Leaves	14	0.075	0.041	54.8
		49	0.063	0.023	36.5
Mean (%) (mean residue found in soil 2 expressed as percentage of residue found in soil 1)					**52.8**

III. CONCLUSION

In the succeeding crops grown on soil freshly treated with boscalid, residues were found to range between 0.015 and 3.875 mg/kg depending on the crop. In the aged soil however, residues were between 0.007 and 2.015 mg/kg. The residues found in soil 2, expressed as percentage of residues found in soil 1, were between 36.5% and 66.5%. On average, in plants grown on aged soil only 53% of the residues were found in comparison to those grown on freshly treated soil.
The results of the study show that the availability of boscalid for plant uptake from soil is dependent on the aging time of the soil. In comparison to a soil aged over 3 years, residues in succeeding crops grown on freshly treated soil were about double as high.

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 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 representative uses in grapes, oilseed rape, beans and peas.

Table 6.7.1-1: Residue definition - boscalid

End-Point	**Active substance: Boscalid**	
	EU agreed endpoints (Reasoned opinion on the review of the existing maximum residue levels (MRLs) for boscalid according to Article 12 of Regulation (EC) No 396/2005. EFSA Journal 2014;12(7):3799, 127 pp.	Residue definitions proposed in the context of this dossier
Residue definition in plant matrices for risk assessment	Parent compound (boscalid)	Parent compound (boscalid)
Residue definition in plant matrices for monitoring	Parent compound (boscalid)	Parent compound (boscalid)
Residue definition in animal matrices for risk assessment	Parent boscalid (BAS 510 F); Liver and kidney: parent boscalid (BAS 510 F) and hydroxylation product M510F01 (including its conjugates); Liver (ruminant and pig): parent boscalid (BAS 510 F and hydroxylation product M510F01 (including its conjugates) and the bound residues (measured as M510F52 or M510F53)	Parent boscalid (BAS 510 F); Liver and kidney: parent boscalid (BAS 510 F) and hydroxylation product M510F01 (including its conjugates); Liver (ruminant and pig): parent boscalid (BAS 510 F and hydroxylation product M510F01 (including its conjugates) and the bound residues (measured as M510F52 or M510F53)
Residue definition in animal matrices for monitoring	Parent boscalid (BAS 510 F); Liver and kidney: parent boscalid (BAS 510 F) and hydroxylation product M510F01 (including its conjugates)	Parent boscalid (BAS 510 F); Liver and kidney: parent boscalid (BAS 510 F) and hydroxylation product M510F01 (including its conjugates)
Conversion factors between residue definitions (animal)	-	-

For deriving appropriate residue definitions for monitoring and risk assessment purposes the principles described in the following document were considered:

- OECD GUIDANCE DOCUMENT ON THE DEFINITION OF RESIDUE (as revised in 2009), SERIES ON TESTING AND ASSESSMENT No. 63 and SERIES ON PESTICIDES No. 31 (ENV/JM/MONO(2009)30)
- EFSA Scientific Opinion on Evaluation of the Toxicological Relevance of Pesticide Metabolites for Dietary Risk Assessment, EFSA Journal 2012;10(07): 2799

The first document covers both aspects whereas the purpose of the PPR Scientific Opinion is limited to the residue definition for risk assessment purposes. The corresponding EU guidance document is in preparation and will be available earliest by end of 2015.

Plant Matrices

For proposing a suitable residue definition in plant matrices, multiple investigations were performed. As presented in chapter M-CA 6.2, 6.5 and 6.6, plant studies were performed in which boscalid was applied according to the intended use patterns.

For deriving a suitable **residue definition for food of plant origin**, three peer-reviewed crop metabolism studies in three different crop categories were considered covering the categories of fruits and fruiting vegetables, pulses and oilseeds, and leafy vegetables. Therefore, foliar treatment on grapes, beans and lettuce were conducted using U-^{14}C-diphenyl and 3-^{14}C-pyridine labelled boscalid (Monograph 2002). The effect of processing on the nature of the residue was investigated in the framework of the original inclusion into Annex I according to Directive 91/414/EEC using test conditions simulating pasteurization, baking, brewing, boiling and sterilization. Information on the residue situation in succeeding or rotational crops is available from one peer-reviewed crop rotational study conducted with boscalid (Monograph 2002).

In general, metabolism of boscalid in plants comprises

- Hydroxylation of the parent compound
- Conjugation of the parent compound

In addition, hydroxylation in the diphenyl and the pyridine rings and cleavage reactions between both ring systems had been observed, but were less pronounced. The hydroxylation reaction is followed by glycosylation. A part of the residue was also incorporated into and/or associated with natural products, such as starch, cellulose and lignin (EFSA Journal 2014;12(7):3799).
The plant metabolism studies indicate that parent compound is the predominant residue in edible plant parts after foliar treatment. In rotational crops, boscalid is the main metabolite as well. Metabolic patterns in primary and rotational crops are found to be similar and a specific residue definition for rotational crops is not deemed necessary (EFSA Reasoned Opinion 2014, EFSA Journal 2014;12(7):3799)
Processing conditions are not expected to have a significant impact on the composition of residues in matrices of plant origin. Boscalid was found to be stable to hydrolysis simulated by pasteurization, baking/brewing/boiling and sterilization. Thus, for processed commodities the same residue definition as for raw agricultural commodities (RAC) is applicable.

Residue definition for monitoring purposes

According to the OECD Guidance Document, the residue definition for tolerance/MRL enforcement purposes should focus on those analytes which would indicate a possible misuse of the pesticide and which can be easily detected/measured by a broad base of national laboratories.

In case of boscalid and its metabolites in food of plant origin, there is only one component which meets all criteria listed in the OECD guidance document. Based on the plant studies available, in which parent BAS 510 F was the most prominent residue, the following residue definition is proposed for monitoring purposes in plant commodities (including process fractions thereof):

Residue definition for monitoring / enforcement:	**boscalid (BAS 510 F), parent only**

This is compliant with the current EU residue definition and is in line with the one in force for JMPR (CODEX).

Residue definition for data generation / risk assessment purposes

The derivation of a suitable residue definition for risk assessment purposes is much more complex; according to the OECD guidance document the contribution of each metabolite/degradate to a potential dietary risk needs to be considered.

In general, two factors must be addressed:

- **Potential for exposure** to the metabolite/degradate in the human diet
- **Relative toxicity** of the metabolite/degradate to the parent

Metabolites/degradates with higher potential exposures and toxicities are more likely to be included in the dietary assessment. The OECD guidance document provides a first hint on how an indicative risk assessment can be performed if metabolites are not readily available as reference substances. For such cases, the document recommends to calculate parent/metabolite ratios from the metabolism studies and to apply these ratios in a second step to the residue level being measured during e.g. supervised field trials. The approach is described much more in detail in the EFSA Scientific Opinion 2012 (EFSA Journal 2012;10(7):2799), which also includes the concept of the threshold of toxicological concern (TTC) as screening tool for pesticide metabolites. Main “purpose” of the TTC concept is to check whether there is negligible exposure.

The dietary exposure for each metabolite was assessed separately for identifying the contributions of the plant metabolites to the total dietary risk (for details see chapter M-CA 6.9). The assessments were limited to those crops from which a contribution to the dietary risk could be expected.

The relevant chronic exposure assessment for plant commodities are summarized in more detail in chapter M-CA 6.9. The data show that the contributions of boscalid metabolites in plants to the dietary risk are small even under unrealistic worst case assumptions. None of the metabolites should be included in the residue definition for dietary risk assessment.

Based on the findings summarized above the following residue definition for risk assessment in plant commodities is proposed:

Residue definition for dietary risk assessment:	**boscalid (BAS 510 F), parent only**

This is compliant with the current EU residue definition and is in line with the one in force for JMPR (CODEX).

Animal matrices

For proposing a suitable residue definition in animal matrices, multiple investigations were performed in the framework of Directive 91/414/EEC (Monograph 2002) considering livestock metabolism (for detail see chapter M-CA 6.2).

For deriving a suitable **residue definition for food of animal origin**, metabolism studies were performed in lactating goats for ruminants and in laying hens for poultry, both using U-^{14}C-diphenyl labelled boscalid. (Monograph 2002, EFSA Journal 2014;12(7):3799).

In general, metabolism of boscalid follows a common pathway in different livestock species, which is comparable to the one observed in rats. Following metabolic conversion steps were observed in the relevant studies:

- Hydroxylation of the parent compound
- Formation of glucuronide conjugates of metabolites via hydroxylation of the parent compound

The biotransformation reactions (hydroxylation and conjugation) were observed in rats, goats, laying hens and fish, thus there is a consistent picture of the metabolism of ^{14}C-boscalid in all animal species investigated.

In ruminants and poultry boscalid is rapidly absorbed, distributed and excreted.

In goats dosed with 1.46-1.73 mg/kg bw per day, boscalid is rapidly absorbed, distributed with a relatively low transfer of residues to tissues and extensively excreted. The residues in milk, fat, muscle and kidney mainly consisted of unchanged parent and its hydroxy metabolite M510F01 including its conjugates. Metabolites are formed by hydroxylation of the biphenyl and the pyridine ring and by conjugation with glutathione. M510F02, the glucuronide conjugate of M510F01, is the most abundant compound in kidney and was also detected in muscle and milk. Low extractability could be observed in liver due to a high level of bound residues. After further extraction with either a mixture of acetic acid and acetone or with formic acid, released either M510F52 or M510F53. Therefore, both M510F52 and M510F53 were used as marker for the bound residues in the liver.

In hens dosed with 0.80-1.14 mg/kg bw per day, boscalid is rapidly absorbed, distributed with a relatively low transfer of residues to tissues and eggs and extensively excreted. The residues in eggs, fat, and muscle mainly consisted of unchanged parent. In eggs, beside the parent compound also its hydroxy metabolite M510F01 including conjugates was present. Low extractability could be also observed in hen liver due to a high level of bound residues. By application of the same microwave extraction method used in the metabolism study on goats (only with formic acid), it was possible to characterise bound residues and the same marker molecule M510F52 was found. Therefore, the results are similar to those observed in goats, bound parent (measured as M510F52) being the main compound in the liver.

In the "Reasoned opinion on the review of the existing maximum residue levels (MRLs) for boscalid according to Article 12 of Regulation (EC) No 396/2005 (EFSA Journal 2014;12(7):3799) it is stated: *"M510F53 and M510F52 are deemed to be representative of the bound residues in liver. It was demonstrated that residues mainly included components containing the unchanged diphenyl moiety, but also that a cleavage on the amine bound of boscalid cannot be excluded."* However, the harsh extraction conditions, used to release liver bound residues do not apply under normal conditions in food preparation. Therefore, cleavage of the amide bound and release of liver-bound residues during cooking of liver as well as cleavage of the amine bound of boscalid is very unlikely. Additionally, the amide bound of boscalid was very stable under metabolic conditions in different animal matrices and therefore, the cleavage is negligible. According to BASF opinions the metabolites M510F52 and M510F53 were not formed under biotic conditions. In the liver, parent is bound to the proteins mainly resulting from a substitution of the chlorine of the pyridine system by thiol groups of liver proteins.

The metabolism studies on both ruminant and poultry show that parent compound, its hydroxy metabolite M510F01 and its glucuronide conjugate M510F02 are the main components of the residue in animal tissues and products, except in liver where the bound residues (parent measured as M510F53 and M510F52) were found to be the main components of the residue.

The general metabolic pathways in rodents, ruminants and poultry were found to be comparable.

Thus, the metabolism and the residue situation of BAS 510 F can be extrapolated from ruminants to pigs.

Residue definition for monitoring purposes

For the residue definition in animal commodities the same criteria apply as for plants. The analyte(s) to be selected for monitoring purposes should occur in large quantities, and should be common to all commodities in which residues are expected. Ideally, the monitoring method should be based on one single analyte (‘marker or indicator compound’).

In the livestock studies available, in all tissues hydroxy metabolite M510F01 as well as its glucuronide conjugate M510F02 were the most prominent residue. Thus, the following residue definition is proposed for monitoring purposes in animal commodities:

Residue definition for monitoring / enforcement:	**parent boscalid (BAS 510 F);** **liver and kidney: parent boscalid (BAS 510 F) and hydroxylation product M510F01 (including its conjugates)**

This is compliant with the current EU residue definitions and the current JMPR (CODEX) residue definitions.

Residue definition for data generation / risk assessment purposes

As in plants, the derivation of a suitable residue definition for risk assessment purposes is much more complex; according to the OECD guidance document the contribution of each metabolite/degradate to a potential dietary risk needs to be considered.

In order to propose a suitable residue definition for risk assessment purposes, the boscalid metabolites found in animal metabolism studies were considered.

The dietary exposure to each metabolite was assessed separately for identifying the contributions of the livestock metabolites to the total dietary risk (for details see M-CA 6.9). To derive input values, two routes were considered: livestock metabolism studies were carefully evaluated for the presence of relevant metabolites.

The relevant chronic exposure assessments for livestock metabolites are summarized in more detail in M-CA 6.9. The data show that the contributions of boscalid metabolites to the dietary risk are small even under unrealistic worst case assumptions. No additional metabolite should be included in the residue definition for dietary risk assessment; hydroxy metabolite M510F01 as well as its conjugate M510F02 and the liver-bound residues (with M510F52 and M510F53 as marker) are already part of the current residue definition.

Based on the findings summarized above the following residue definition for risk assessment in animal matrices is proposed:

Residue definition for dietary risk assessment:	**boscalid (BAS 510 F), parent** **liver and kidney: boscalid and hydroxylation product M510F01 (including its conjugates)** **liver (ruminant and pig): boscalid and hydroxylation product M510F01 (including its conjugates) and the bound residues**

These risk assessment residue definitions are in alignment with the latest evaluation of boscalid according to Article 12 Regulation (EC) No 396/2005 published by EFSA (EFSA Reasoned Opinion 2014, EFSA Journal 2014;12(7):3799).
The definition for enforcement derived by the JMPR (CODEX) is the same in muscle, fat, milk and eggs, but differs for liver and kidney, for which the residue definition is limited to boscalid only (FAO 2006, Plant Production and Protection Paper 187). However, EFSA considers that the residue definition derived by JMPR for liver and kidney is not adequate, based on the results of the available feeding studies (EFSA Journal 2014;12(7):3799).

CA 6.7.2 Proposed maximum residue levels (MRLs) and justification of the acceptability of the levels proposed

For boscalid, residue trials and derived MRLs for a wide range of crops comprising major agronomic crops, such as cereals, oilseeds, but also fruits and specialty crops have been reported and submitted during the registration processes, which will not be reported in detail. The following table shows the MRLs for boscalid (mg/kg) in accordance to SANTE/10530/2015 (pending) (source: European Commission website http://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/public/?event=pesticide.residue.selection&language=EN).

Table 6.7.2-1: Boscalid - MRL values in accordance to SANTE/10530/2015 (pending)

Code number	Groups and examples of individual products to which the MRLs apply	Boscalid[1] MRL (mg/kg), SANTE/10530/2015
0100000	. FRUITS, FRESH or FROZEN; TREE NUTS	-
0110000	. Citrus fruits	2[a]
0120000	. Tree nuts	-
0120010	. Almonds	0.05*[a]
0120020	. Brazil nuts	0.05*[a]
0120030	. Cashew nuts	0.05*[a]
0120040	. Chestnuts	0.05*[a]
0120050	. Coconuts	0.05*[a]
0120060	. Hazelnuts/cobnuts	0.05*[a]
0120070	. Macadamias	0.05*[a]
0120080	. Pecans	0.05*[a]
0120090	. Pine nut kernels	0.05*[a]
0120100	. Pistachios	1[a]
0120110	. Walnuts	0.05*[a]
0120990	. Others	0.05*
0130000	. Pome fruits	-
0130010	. Apples	2[a]
0130020	. Pears	1.5[a]
0130030	. Quinces	1.5[a]
0130040	. Medlars	0.01*
0130050	. Loquats/Japanese medlars	0.01*
0130990	. Others	0.01*
0140000	. Stone fruits	-
0140010	. Apricots	5[a]
0140020	. Cherries (sweet)	4[a]
0140030	. Peaches	5[a]
0140040	. Plums	3[a]
0140990	. Others	0.01*
0150000	. Berries and small fruits	-
0151000	. (a) grapes	5[a]
0152000	. (b) strawberries	6[b]
0153000	. (c) cane fruits	10[a]
0154000	. (d) other small fruits and berries	15[a]
0160000	. Miscellaneous fruits with	-
0161000	. (a) edible peel	0.01*

Table 6.7.2-1: Boscalid - MRL values in accordance to SANTE/10530/2015 (pending)

Code number	Groups and examples of individual products to which the MRLs apply	Boscalid[1] MRL (mg/kg), SANTE/10530/2015
0162000	. (b) inedible peel, small	-
0162010	. Kiwi fruits (green, red, yellow)	5[a]
0162020	. Litchis/lychees	0.01*
0162030	. Passionfruits/maracujas	0.01*
0162040	. Prickly pears/cactus fruits	0.01*
0162050	. Star apples/cainitos	0.01*
0162060	. American persimmons/Virginia kaki	0.01*
0162990	. Others	0.01*
0163000	. (c) inedible peel, large	-
0163010	. Avocados	0.01*
0163020	. Bananas	0.6[a]
0163030	. Mangoes	0.01*
0163040	. Papayas	0.01*
0163050	. Granate apples/pomegranates	0.01*
0163060	. Cherimoyas	0.01*
0163070	. Guavas	0.01*
0163080	. Pineapples	0.01*
0163090	. Breadfruits	0.01*
0163100	. Durians	0.01*
0163110	. Soursops/guanabanas	0.01*
0163990	. Others	0.01*
0200000	. VEGETABLES, FRESH or FROZEN	-
0210000	. Root and tuber vegetables	-
0211000	. (a) potatoes	2[b]
0212000	. (b) tropical root and tuber vegetables	2[b]
0213000	. (c) other root and tuber vegetables except sugar beets	-
0213010	. Beetroots	4[b]
0213020	. Carrots	2[b]
0213030	. Celeriacs/turnip rooted celeries	2[b]
0213040	. Horseradishes	2[b]
0213050	. Jerusalem artichokes	2[b]
0213060	. Parsnips	2[b]
0213070	. Parsley roots/Hamburg roots parsley	2[b]
0213080	. Radishes	2[b]
0213090	. Salsifies	2[b]
0213100	. Swedes/rutabagas	2[b]
0213110	. Turnips	2[b]
0213990	. Others	2
0220000	. Bulb vegetables	-
0220010	. Garlic	5[b]
0220020	. Onions	5[b]
0220030	. Shallots	5[b]
0220040	. Spring onions/green onions and Welsh onions	6[b]
0220990	. Others	0.5
0230000	. Fruiting vegetables	-
0231000	. (a) solanacea	3[b]
0232000	. (b) cucurbits with edible peel	4[b]
0233000	. (c) cucurbits with inedible peel	3[b]
0234000	. (d) sweet corn	0.05[b]

Table 6.7.2-1: Boscalid - MRL values in accordance to SANTE/10530/2015 (pending)

Code number	Groups and examples of individual products to which the MRLs apply	Boscalid[1] MRL (mg/kg), SANTE/10530/2015
0239000	. (e) other fruiting vegetables	0.9
0240000	. Brassica vegetables (excluding brassica roots and brassica baby leaf crops)	-
0241000	. (a) flowering brassica	5[b]
0242000	. (b) head brassica	5[b]
0243000	. (c) leafy brassica	9[b]
0244000	. (d) kohlrabies	5[b]
0250000	. Leaf vegetables, herbs and edible flowers	-
0251000	. (a) lettuces and salad plants	50[b]
0252000	. (b) spinaches and similar leaves	-
0252010	. Spinaches	50[b]
0252020	. Purslanes	0.9[b]
0252030	. Chards/beet leaves	30[b]
0252990	. Others	0.9
0253000	. (c) grape leaves and similar species	0.01*[b]
0254000	. (d) watercresses	0.01*[b]
0255000	. (e) witloofs/Belgian endives	7[b]
0256000	. (f) herbs and edible flowers	50[b]
0260000	. Legume vegetables	-
0260010	. Beans (with pods)	5[b] / 6[f]
0260020	. Beans (without pods)	3[b]
0260030	. Peas (with pods)	5[b] / 6[f]
0260040	. Peas (without pods)	3[b]
0260050	. Lentils	3[b]
0260990	. Others	0.06
0270000	. Stem vegetables	-
0270010	. Asparagus	0.9[b]
0270020	. Cardoons	0.9[b]
0270030	. Celeries	9[b]
0270040	. Florence fennels	9[b]
0270050	. Globe artichokes	5[b]
0270060	. Leeks	9[b]
0270070	. Rhubarbs	0.9[b]
0270080	. Bamboo shoots	0.01*[b]
0270090	. Palm hearts	0.01*[b]
0270990	. Others	0.5
0280000	. Fungi, mosses and lichens	0.01*
0290000	. Algae and prokaryotes organisms	0.01*
0300000	. PULSES	3[b]

Table 6.7.2-1: Boscalid - MRL values in accordance to SANTE/10530/2015 (pending)

Code number	Groups and examples of individual products to which the MRLs apply	Boscalid[1] MRL (mg/kg), SANTE/10530/2015
0400000	. OILSEEDS AND OIL FRUITS	-
0401000	. Oilseeds	-
0401010	. Linseeds	1[b]
0401020	. Peanuts/groundnuts	1[b]
0401030	. Poppy seeds	1[b]
0401040	. Sesame seeds	1[b]
0401050	. Sunflower seeds	1[b]
0401060	. Rapeseeds/canola seeds	1[b]
0401070	. Soyabeans	3[b]
0401080	. Mustard seeds	1[b]
0401090	. Cotton seeds	1[b]
0401100	. Pumpkin seeds	1[b]
0401110	. Safflower seeds	1[b]
0401120	. Borage seeds	1[b]
0401130	. Gold of pleasure seeds	1[b]
0401140	. Hemp seeds	1[b]
0401150	. Castor beans	1[b]
0401990	. Others	0.06
0402000	. Oil fruits	0.01*
0500000	. CEREALS	-
0500010	. Barley	4[b]
0500020	. Buckwheat and other pseudo-cereals	0.15[b]
0500030	. Maize/corn	0.15[b]
0500040	. Common millet/proso millet	0.15[b]
0500050	. Oat	4[b]
0500060	. Rice	0.15[b]
0500070	. Rye	0.8[b]
0500080	. Sorghum	0.15[b]
0500090	. Wheat	0.8[b]
0500990	. Others	0.15
0600000	. TEAS, COFFEE, HERBAL INFUSIONS, COCOA AND CAROBS	-
0610000	. Teas	0.01*
0620000	. Coffee beans	0.05*[a]
0630000	. Herbal infusions from	-
0631000	. (a) flowers	0.9[c]
0632000	. (b) leaves and herbs	0.9[c]
0633000	. (c) roots	3[c]
0639000	. (d) any other parts of the plant	0.01*
0640000	. Cocoa beans	0.01*
0650000	. Carobs/Saint John's breads	0.01*
0700000	. HOPS	80[c]
0800000	. SPICES	-
0810000	. Seed spices	0.9[c]
0820000	. Fruit spices	0.9[c]
0830000	. Bark spices	0.9[c]

Table 6.7.2-1: Boscalid - MRL values in accordance to SANTE/10530/2015 (pending)

Code number	Groups and examples of individual products to which the MRLs apply	Boscalid[1] MRL (mg/kg), SANTE/10530/2015
0840000	. Root and rhizome spices	-
0840010	. Liquorice	0.4[c]
0840020	. Ginger	0.4[c]
0840030	. Turmeric/curcuma	0.4[c]
0840040	. Horseradish	2[b]
0840990	. Others	0.4
0850000	. Bud spices	0.9[c]
0860000	. Flower pistil spices	0.9[c]
0870000	. Aril spices	0.9[c]
0900000	. SUGAR PLANTS	-
0900010	. Sugar beet roots	0.4[b]
0900020	. Sugar canes	7[b]
0900030	. Chicory roots	0.4[b]
0900990	. Others	0.5
1000000	. PRODUCTS OF ANIMAL ORIGIN -TERRESTRIAL ANIMALS	-
1010000	. Tissues from	-
1011000	. (a) swine	-
1011010	. Muscle	0.03[e]
1011020	. Fat tissue	0.07
1011030	. Liver	0.05*
1011040	. Kidney	0.05*
1011050	. Edible offals (other than liver and kidney)	0.07
1011990	. Others	0.05*
1012000	. (b) bovine	-
1012010	. Muscle	0.03[e]
1012020	. Fat tissue	0.3
1012030	. Liver	0.2[d]
1012040	. Kidney	0.2
1012050	. Edible offals (other than liver and kidney)	0.3
1012990	. Others	0.05*
1013000	. (c) sheep	-
1013010	. Muscle	0.03[e]
1013020	. Fat tissue	0.3
1013030	. Liver	0.2[d]
1013040	. Kidney	0.2
1013050	. Edible offals (other than liver and kidney)	0.3
1013990	. Others	0.05*
1014000	. d) goat	-
1014010	. Muscle	0.03[e]
1014020	. Fat tissue	0.3
1014030	. Liver	0.2[d]
1014040	. Kidney	0.2
1014050	. Edible offals (other than liver and kidney)	0.3
1014990	. Others	0.05*

Table 6.7.2-1: Boscalid - MRL values in accordance to SANTE/10530/2015 (pending)

Code number	Groups and examples of individual products to which the MRLs apply	Boscalid[1] MRL (mg/kg), SANTE/10530/2015
1015000	. (e) equine	-
1015010	. Muscle	0.03[e]
1015020	. Fat tissue	0.3
1015030	. Liver	0.2
1015040	. Kidney	0.2
1015050	. Edible offals (other than liver and kidney)	0.3
1015990	. Others	0.05*
1016000	. (f) poultry	-
1016010	. Muscle	0.03[e]
1016020	. Fat tissue	0.15[g]
1016030	. Liver	0.3[d, h]
1016040	. Kidney	0.05*
1016050	. Edible offals (other than liver and kidney)	0.3[h]
1016990	. Others	0.05*
1017000	. (g) other farmed terrestrial animals	-
1017010	. Muscle	0.03[e]
1017020	. Fat tissue	0.3
1017030	. Liver	0.2
1017040	. Kidney	0.2
1017050	. Edible offals (other than liver and kidney)	0.3
1017990	. Others	0.05*
1020000	. Milk	0.02
1030000	. Birds eggs	0.05[i]
1040000	. Honey and other apiculture products	0.5[j]
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

1 The residue definition differs for the following combinations pesticide-code number: Boscalid - code 1000000 except 1040000: Sum of boscalid and metabolite 2-chloro-N-(4'-chloro-5-hydroxybiphenyl-2-yl)nicotinamide (M510F01) including its conjugates expressed as boscalid

a The European Food Safety Authority identified some information on residues after repeated application in permanent crops and data to confirm the plateau level in soil as unavailable. When re-viewing the MRLs, the Commission will take into account the information referred to in the first sentence, if submitted by [Office of Publication: please insert date 2 years after publication], or, if that information is not submitted by that date, the lack of it.

b The European Food Safety Authority identified some information on residues for rotational crops and data to confirm the plateau level in soil as unavailable. When re-viewing the MRLs, the Commission will take into account the information referred to in the first sentence, if submitted by [Office of Publication: please insert date 2 years after publication], or, if that information is not submitted by that date, the lack of it.

c The European Food Safety Authority identified some information on analytical methods, residues after repeated application in permanent crops and data to confirm the plateau level in soil as unavailable. When re-viewing the MRLs, the Commission will take into account the information referred to in the first sentence, if submitted by [Office of Publication: please insert date 2 years after publication], or, if that information is not submitted by that date, the lack of it.

d The European Food Safety Authority identified some information on the fate of the pyridine moiety as unavailable. When re-viewing the MRLs, the Commission will take into account the information referred to in the first sentence, if submitted by [Office of Publication: please insert date 2 years after publication], or, if that information is not submitted by that date, the lack of it.

e In SANTE/10530/2015, an MRL of 0.01* mg/kg is set for muscle. However, in the feeding studies, residues were below the LOQ of 0.025 mg/kg. An MRL of 0.03 mg/kg is therefore proposed within this document.

f An MRL of 6 mg/kg is calculated based on the Northern European residue data for treatment of beans with pods, which is not covered by the current, pending EU MRL of 5 mg/kg (SANTE/10530/2015). Therefore, an MRL of 6 mg/kg is proposed within this document. According to "Guidelines on comparability, extrapolation, group tolerances and data requirements for setting MRLs" (SANCO 7525/VI/95 rev. 9, March 2011), this MRL is extrapolated to peas with pods.

g In SANTE/10530/2015, an MRL of 0.08 mg/kg is set for fat. However, based on the feed burden calculation and the derived factor, residues above this value were derived. Therefore, an MRL of 0.15 mg/kg is proposed within this document.
h In SANTE/10530/2015, an MRL of 0.15 mg/kg is set for liver and edible offals. However, based on the feed burden calculation and the derived factor, residues above this value were derived. Therefore, an MRL of 0.3 mg/kg is proposed within this document.
i In SANTE/10530/2015, an MRL of 0.01* mg/kg is set for eggs. However based on the feed burden calculation and the derived factor, residues above this value were derived. An MRL of 0.05 mg/kg according to Reg. (EU) No 441/2012 is therefore proposed to be maintained.
j In SANTE/10530/2015, an MRL of 0.05* mg/kg is set for honey and other apiculture products. However, treatment of oilseed rape is performed during flowering and in residue studies. As residues were found above LOQ, the MRL of 0.5 mg/kg according to Reg. (EU) No 441/2012 and SANTE/10377/2015 (pending) is proposed to be maintained.

Comments to the footnotes of Table 6.7.2-1 are given in the following:

Footnote a to c: A field soil accumulation study is currently in progress. Interim results covering a period of about 5.5 years are presented in chapter M-CA 7.1.2. As a result, the plateau reported in this study is clear below the application rates used in the rotational field studies (for details see chapter M-CA 6.6 and 7.1). Additionally, a study on the residue behavior of boscalid in rotational crops after growing on aged boscalid-treated soil shows that aging of boscalid residues in soil leads to a reduction of the bioavailability for plant of about 50%, starting about 3 months after the application (for details see chapter M-CA 6.6.2). Therefore, after multi-year use of boscalid, the results of the rotational crop study should be corrected. Where a significant residue uptake could not be excluded, for MRL proposal additional residue via soil uptake was taken into account (EFSA Reasoned Opinion 2014, EFSA Journal 2014;12(7):3799) and permuted in the currently pending EU MRLs. This MRL values including the uptake from residues from soil are valid because the tested concentration of boscalid in the rotational crop studies is significantly below the calculated plateau in the soil after multi-year use of boscalid.

Footnote d: In liver, parent boscalid is bound to the proteins, mainly resulting from a substitution of the chlorine of the pyridine system by thiol groups of liver proteins. Only harsh extraction conditions lead to cleavage products M510F52 or M510F53, both used as marker for the bound residues in the liver. EFSA Reasoned Opinion 2014 (EFSA Journal 2014;12(7):3799) discussed the possibility that the bound liver residues may be released during cooking and a cleavage of the amine bond of boscalid cannot be excluded (only a diphenyl label was applied in animal studies). BASF is of the opinion that the M510F52 and M510F53 residues are not formed under biotic conditions: the amide bound of BAS 510 F was very stable under metabolic conditions in hens. Additionally, the harsh extraction conditions used to release liver bound residues do not apply under normal conditions in food processing. Thus, the residue definition for animal matrices is considered valid and MRL values for boscalid according to SANTE/10530/2015 (pending) are applicable.

Plant Matrices

For boscalid MRLs are established in several crops. EU MRLs have been established according to Reg. (EU) No 441/2012. New MRLs are pending based on SANTE/10530/2015, summarizing the outcome of the recent evaluations according to Article 12 of Reg 396/2005.

In order to support the renewal of approval for boscalid, additional residue trials are presented for some of the intended uses (beans, oilseed rape). In the chapters below, the residue data summarised in chapter M-CA 6.3 are evaluated using statistical means and compared with the data being included in the most recent EFSA Reasoned Opinions. The established EU MRLs cover the representative uses (grapes, beans, peas, oilseed rape).

Grapes

The use in grapes was part of the previous active substance inclusion process. Sufficient data supporting the representative GAP were submitted to the designated Rapporteur Member State and were evaluated on EU level (Addendum 2006). In the growing season 2003 and 2004, 17 trials on grapes were conducted in the EU (9 in the EU North, 8 in the EU South) according to the critical GAP.
After application of boscalid formulation BAS 510 01 F at a rate of 0.6 kg boscalid/ha at target BBCH stages 60-81, residue levels at were considered for the MRL calculation. The intended PHI was 21 days. The residue results at PHI 21 days±25%, as listed below, were used for MRL derivation. If higher residues occurred at later sampling times, those values were used.

Northern Europe (n=8), field: 0.24, 0.26, 0.39, 0.41, 0.71, 0.78, 1.03, 1.12 mg/kg
Southern Europe (n=9); field: 0.19, 0.23, 0.24, 0.28, 0.34, 0.50, 0.78, 0.88, 1.47 mg/kg

MRL calculation was performed using the OECD calculator.

Table 6.7.2-2: MRL calculation for grapes in the EU for boscalid based on parent residues (mg/kg)

OECD calculator[#]	BAS 510 F [mg/kg]	
	N-EU	S-EU
Total number of data (n)	8	9
Highest residue	1.12	1.47
Mean + 4 SD	1.989	2.252
CF x 3 Mean	1.853	1.637
Rounded MRL	**2**	**3**
STMR	0.560	0.340

[#] OECD calculator spreadsheet, taken from the OECD page:
http://www.oecd.org/document/34/0,3746,en_2649_37465_48447010_1_1_1_37465,00.html

An MRL of 3 mg/kg is calculated based on the Southern European residue data for treatment of grapes and is covered by the existing EU MRL of **5 mg/kg** for boscalid in grapes.

Beans

The use in beans was part of the previous active substance inclusion process. Data were submitted to the designated Rapporteur Member State and were evaluated on EU level. Since those residue trials are not fully GAP compliant (the number of applications was 3 instead of 2), they are not considered here even though they were considered acceptable during the peer-review process. Further studies have been conducted and will be submitted in this dossier. 15 trials in beans were conducted in the EU (8 in the EU North, 8 in the EU South in the period from 2008 to 2011) according to the critical GAP. Formulation BAS 510 01 F was applied twice at a rate of 0.5 kg boscalid/ha at target BBCH stages 60 and 69 for the first and second application. The intended PHI was 7 days. The residue results at PHI 7 days±25%, as listed below, were used for MRL derivation. If residues where higher at a later than the targeted harvest time these values were used.

Northern Europe (n=8), field: 0.12, 0.61, 0.62, 0.77, 1.40, 1.59, 1.62, 3.62 mg/kg
Southern Europe (n=8), field 0.23, 0.33, 0.37, 0.41, 0.77, 0.83, 1.34, 1.4 mg/kg

MRL calculation was performed using the OECD calculator.

Table 6.7.2-3: MRL calculation for beans in the EU for boscalid based on parent residues (mg/kg)

OECD calculator[#]	BAS 510 F [mg/kg]	
	N-EU	S-EU
Total number of data (n)	8	8
Highest residue	3.620	1.400
Mean + 4 SD	5.621	2.544
CF x 3 Mean	3.881	2.130
Rounded MRL	**6**	**3**
STMR	1.085	0.590

[#] OECD calculator spreadsheet, taken from the OECD page:
http://www.oecd.org/document/34/0,3746,en_2649_37465_48447010_1_1_1_37465,00.html

An MRL of 6 mg/kg is calculated based on the Northern European residue data for treatment of beans with pods, which is not covered by the current EU MRL of **5 mg/kg** for boscalid in beans with pods.

Therefore, it is proposed to raise the EU MRL to

6 mg/kg for boscalid in beans with pods (code number 0260010).

According to "Guidelines on comparability, extrapolation, group tolerances and data requirements for setting MRLs" (SANCO 7525/VI/95 rev. 9, March 2011), it is further suggested to extrapolate this MRL to

peas with pods (code number 0260030).

Oilseed rape

The use in oilseed rape was part of the previous active substance inclusion process. Data supporting the corresponding GAP were submitted to the designated Rapporteur Member State and were evaluated on EU level. Further studies have been conducted and will be submitted in this dossier. In total, 14 trials in oilseed rape were conducted in the EU (10 in the EU North, 4 in the EU South in the period from 2000 to 2008) according to the critical GAP. Formulation BAS 510 01 F was applied twice at a rate of 0.20-0.25 kg boscalid/ha at target BBCH stages between 13 and 75 for the first and second application. There is no intended PHI; sampling is defined by growth stage at latest application timing. The residue results at 35 to 81 DALA as listed below, were used for MRL derivation. If residues where higher at a later than the targeted harvest time these values were used.

Northern Europe (n=8), field 3x<0.01, 0.03, 4x<0.05, 0.06, 0.07 mg/kg
Southern Europe (n=4), field: 2x<0.05, 0.11, 0.25 mg/kg

MRL calculation was performed using the OECD calculator.

Table 6.7.2-4: MRL calculation for oilseed rape in the EU for boscalid based on parent residues (mg/kg)

OECD calculator[#]	BAS 510 F [mg/kg]	
	N-EU	S-EU
Total number of data (n)	10	4
Highest residue	0.070	0.250
Mean + 4 SD	0.128	0.492
CF x 3 Mean	0.062	0.230
Rounded MRL	**0.15**	**0.5**
STMR	0.050	0.080

[#] OECD calculator spreadsheet, taken from the OECD page:
http://www.oecd.org/document/34/0,3746,en_2649_37465_48447010_1_1_1_37465,00.html

An MRL of **0.5 mg/kg** is calculated based on the Southern European residue data for treatment of oilseed rape and is covered by the existing EU MRL of **1 mg/kg** for boscalid in oilseed rape.

Animal Matrices

In 2014, the most recent calculation of the overall feed burden has been performed by EFSA in context of the MRL re-evaluation according to EEC 396/2005, Article 12. The output is shown in Table 6.7.2-5.

Table 6.7.2-5: Results of the dietary burden calculation by EFSA (EFSA Reasoned Opinion, 2014)[1)]

	Median dietary burden (mg/kg bw/d)	Maximum dietary burden (mg/kg bw/d)	Highest contributing commodity	Max dietary burden (mg/kg DM)	Trigger exceeded (Y/N)
Risk assessment residue definition: boscalid					
Dairy ruminants	0.41	1.43	Grass, fresh	39.9	Y
Meat ruminants	0.99	2.04	Wheat straw	47.5	Y
Poultry	0.10	0.23	Kale	3.66	Y
Pigs	0.10	0.37	Grass silage	9.31	Y

1) EFSA Journal 2014;12(7):3799

In this document, the current version of the OECD feed burden calculator (using the OECD methodology) was applied in the first place. All boscalid uses were considered as a worst-case scenario.

The following input values were used for calculation of the Results for Reasonable Worst Case Feeding Levels (RWCFL).

Table 6.7.2-6: Input values for the revised dietary burden calculation (OECD methodology)

Commodity[1]	RWCFL EU	
	Input value (mg/kg)	Comment
Forages		
Alfalfa (fresh) forage	1.46	HR
Alfalfa, hay	5.84	HR
Alfalfa, silage	1.46	HR
Barley, straw	33.7	HR
Beet, mangel, fodder[2]	0.84	
Beet, sugar, tops	0.84	HR
Cabbage heads, leaves	2.82	HR
Clover (fresh), forage	1.46	HR
Clover, hay	5.84	HR
Clover, silage	1.46	HR
Corn, field, forage/silage	6.8	HR
Grass, forage (fresh)	6.8	HR
Grass, hay	27.2	HR
Grass, silage	6.8	HR
Kale, leaves	4.1	HR
Oat, straw	33.7	HR
Rape, forage	1.46	HR

Table 6.7.2-6: Input values for the revised dietary burden calculation (OECD methodology)

Commodity[1]	RWCFL EU	
	Input value (mg/kg)	Comment
Rye, straw	39.5	HR
Wheat, straw	52.7	HR
Roots & Tubers		
Carrot, culls[3]	0.38	HR
Potatoes, culls[4]	0.42	HR
Swede	0.37	HR
Turnip	0.65	HR
Cereal Grain/Crops Seeds		
Barley, grain	1.07	STMR
Bean, seed	0.13	STMR
Corn, field, grain	0.05	STMR
Cotton, undelinted seed	0.05	STMR
Lupin, seed	0.05	STMR
Oat, grain	1.07	STMR
Pea, seed	0.13	STMR
Rye, grain	0.17	STMR
Wheat, grain	0.17	STMR
By-Products		
Almond, hulls[5]	0.05	STMR
Apple, pomace, wet	2.52	STMR (0.42 mg/kg) x PF (6)
Beet, sugar, ensiled pulp[6]	0.37	STMR
Cotton, meal	0.07	STMR (0.05 mg/kg) x default PF (1.3)
Flaxseed/linseed, meal	0.2	STMR (0.1 mg/kg) x default PF (2)
Grape, pomace, wet	3.55	STMR (1.42 mg/kg) x PF (2.5)
Peanut, meal	0.2	STMR (0.1 mg/kg) x default PF (2)
Potato, process waste[4]	0.1	STMR
Rape, meal	0.08	STMR (0.15 mg/kg) x PF (0.56)
Soybean, meal	0.02	STMR (0.1 mg/kg) x PF (0.16)
Sunflower, meal	0.32	STMR (0.16 mg/kg) x default PF (2)
Wheat, milled byproducts[7]	0.73	STMR (0.17 mg/kg) x PF (4.32)

1 See EFSA Journal 2014;12(7):3799 (considering primary crops and uptake of residues from previously treated soil) except indicated otherwise
2 Fodder beet leaves
3 Carrots
4 Potatoes
5 Almonds
6 Sugar beets
7 Wheat bran

The results of the total maximum dietary burdens are presented below (see Table 6.7.2-7).

Table 6.7.2-7: Summary of the results for RWCFL (EU)

	Cattle Beef	Cattle Dairy	Sheep Ram/Ewe	Sheep Lamb	Swine Breeding	Swine Finishing	Poultry Broiler	Poultry Layer	Poultry Turkey
Body weight (kg)	500	650	75	40	260	100	1.7	1.9	7
Daily intake (kg DM)	12	25	2.5	1.7	6	3	0.12	0.13	0.5
Dietary burden (mg/kg bw)	**0.422**	**0.751**	**0.942**	**1.089**	**0.194**	**0.074**	**0.102**	**0.485**	**0.086**
Feed burden (mg/kg DM)	**17.565**	**19.532**	**28.252**	**25.619**	**8.402**	**2.463**	**1.450**	**7.083**	**1.207**

The tables below show the detailed results of the feed burden calculation. It should be noted that the doses assume that the diet completely consists of plant material which had been treated with boscalid.

Table 6.7.2-8: Detailed results for RWCFL (EU): cattle (beef)

Category	Crop	Feedstuff	% of diet	Dietary contribution (mg/kg bw)	Cumulative % diet	Adjusted % Diet	Adjusted dietary contribution (mg/kg bw)	Feed burden (mg/kg DM)
Forages	Grass	hay	50	0.371	50	50	0.371	15.455
Roots/tubers	Swede	roots	40	0.036	90	40	0.036	1.480
By-products	Apple	pomace, wet	20	0.030	100	10	0.015	0.630
Cereal grains	Barley	grain	70	0.020	100	0	0.000	0.000
Total			**180**	**0.457**	**-**	**100**	**0.422**	**17.565**

Table 6.7.2-9: Detailed results for RWCFL (EU): cattle (dairy)

Category	Crop	Feedstuff	% of diet	Dietary contribution (mg/kg bw)	Cumulative % diet	Adjusted % Diet	Adjusted dietary contribution (mg/kg bw)	Feed burden (mg/kg DM)
Forages	Grass	hay	60	0.713	60	60	0.713	18.545
By-products	Beet, sugar	ensiled pulp	40	0.038	100	40	0.038	0.987
Roots/tubers	Turnip	roots	20	0.033	100	0	0.000	0.000
Cereal grains	Barley	grain	40	0.019	100	0	0.000	0.000
Total			**160**	**0.803**	**-**	**100**	**0.751**	**19.532**

Table 6.7.2-10: Detailed results for RWCFL (EU): sheep (ram/ewe)

Category	Crop	Feedstuff	% of diet	Dietary contribution (mg/kg bw)	Cumulative % diet	Adjusted % Diet	Adjusted dietary contribution (mg/kg bw)	Feed burden (mg/kg DM)
Forages	Grass	hay	90	0.927	90	90	0.927	27.818
Roots/tubers	Turnip	roots	30	0.043	100	10	0.014	0.433
By-products	Apple	pomace, wet	10	0.021	100	0	0.000	0.000
Cereal grains	Barley	grain	40	0.016	100	0	0.000	0.000
Total			170	1.008	-	100	**0.942**	**28.252**

Table 6.7.2-11: Detailed results for RWCFL (EU): sheep (lamb)

Category	Crop	Feedstuff	% of diet	Dietary contribution (mg/kg bw)	Cumulative % diet	Adjusted % Diet	Adjusted dietary contribution (mg/kg bw)	Feed burden (mg/kg DM)
Forages	Wheat	straw	40	1.018	40	40	1.018	23.955
Roots/tubers	Turnip	roots	30	0.055	70	30	0.055	1.300
Cereal grains	Barley	grain	60	0.031	100	30	0.016	0.365
By-products	Apple	pomace, wet	10	0.027	100	0	0.000	0.000
Total			140	1.131	-	100	**1.089**	**25.619**

Table 6.7.2-12: Detailed results for RWCFL (EU): swine (breeding)

Category	Crop	Feedstuff	% of diet	Dietary contribution (mg/kg bw)	Cumulative % diet	Adjusted % Diet	Adjusted dietary contribution (mg/kg bw)	Feed burden (mg/kg DM)
Forages	Grass	hay	20	0.143	20	20	0.143	6.182
Roots/tubers	Turnip	roots	40	0.040	60	40	0.040	1.733
Cereal grains	Barley	grain	80	0.022	100	40	0.011	0.486
By-products	Wheat	milled bypdts	50	0.010	100	0	0.000	0.000
Total			190	0.215	-	100	**0.194**	**8.402**

Table 6.7.2-13: Detailed results for RWCFL (EU): swine (finishing)

Category	Crop	Feedstuff	% of diet	Dietary contribution (mg/kg bw)	Cumulative % diet	Adjusted % Diet	Adjusted dietary contribution (mg/kg bw)	Feed burden (mg/kg DM)
Roots/tubers	Turnip	roots	40	0.052	40	40	0.052	1.733
Cereal grains	Barley	grain	80	0.029	100	60	0.022	0.730
By-products	Wheat	milled bypdts	50	0.012	100	0	0.000	0.000
Forages	Wheat	silage	0	0.000	100	0	0.000	0.000
Total			170	0.094	-	100	**0.074**	**2.463**

Table 6.7.2-14: Detailed results for RWCFL (EU): poultry (broiler)

Category	Crop	Feedstuff	% of diet	Dietary contribution (mg/kg bw)	Cumulative % diet	Adjusted % Diet	Adjusted dietary contribution (mg/kg bw)	Feed burden (mg/kg DM)
Cereal grains	Barley	grain	70	0.060	70	70	0.060	0.851
Roots/tubers	Turnip	roots	10	0.031	80	10	0.031	0.433
By-products	Wheat	milled bypdts	20	0.012	100	20	0.012	0.166
Forages	Wheat	silage	0	0.000	100	0	0.000	0.000
Total			100	0.102	-	100	**0.102**	**1.450**

Table 6.7.2-15: Detailed results for RWCFL (EU): poultry (layer)

Category	Crop	Feedstuff	% of diet	Dietary contribution (mg/kg bw)	Cumulative % diet	Adjusted % Diet	Adjusted dietary contribution (mg/kg bw)	Feed burden (mg/kg DM)
Forages	Wheat	straw	10	0.410	10	10	0.410	5.989
Cereal grains	Barley	grain	100	0.083	100	90	0.075	1.094
Roots/tubers	Turnip	roots	10	0.030	100	0	0.000	0.000
By-products	Wheat	milled bypdts	20	0.011	100	0	0.000	0.000
Total			140	0.534	-	100	**0.485**	**7.083**

Table 6.7.2-16: Detailed results for RWCFL (EU): poultry (turkey)

Category	Crop	Feedstuff	% of diet	Dietary contribution (mg/kg bw)	Cumulative % diet	Adjusted % Diet	Adjusted dietary contribution (mg/kg bw)	Feed burden (mg/kg DM)
Cereal grains	Barley	grain	50	0.043	50	50	0.043	0.608
Roots/tubers	Turnip	roots	10	0.031	60	10	0.031	0.433
By-products	Wheat	milled bypdts	20	0.012	80	20	0.012	0.166
Forages	Wheat	silage	0	0.000	80	0	0.000	0.000
Total			80	0.086	-	80	**0.086**	**1.207**

Proposed animal MRLs

Thus, the doses (mg/kg bw/d) calculated for ruminants and pigs with the OECD calculator result in the most critical values and will consequently be used for estimating the maximum residues in products of animal origin:

dairy cattle	0.75 mg/kg bw/d (19.53 mg/kg feed DM)
beef cattle	0.42 mg/kg bw/d (17.56 mg/kg feed DM)
lamb	1.09 mg/kg bw/d (25.62 mg/kg feed DM)
poultry	0.49 mg/kg bw/d (7.08 mg/kg feed DM, layer)
pig	0.19 mg/kg bw/d (8.40 mg/kg feed DM, breeding)

Cattle (sheep, goat) products

A residue transfer study (BASF DocID 2008/7015330) with boscalid was conducted in cows and was evaluated during Article 12 review (EFSA Reasoned Opinion 2014, EFSA Journal 2014;12(7):3799). The animals were dosed with 35.8 and 116.3 mg/kg feed equivalent to 1.22 and 3.36 mg/kg bw/d for a period of 28 days (see chapter M-CA 6.4). In muscle, no residues (<0.025 mg/kg) of boscalid and M510F01 including its conjugates were found in any dose group. Maximum group mean residues of 0.01 and 0.05 mg/kg (expressed as parent equivalents) were found in milk of the low and high dose groups, respectively. In both kidney and liver, maximum residues of 0.11 and 0.24 mg/kg were found, respectively. In fat, maximum residues of 0.22 and 0.25 mg/kg were found, respectively.
The calculated feed burdens are covered by the feeding study. Therefore, no residues are to be expected in ruminant muscle. Estimated residues in milk, kidney, liver and fat are described below.
The feed burden calculation presented above, considering lamb as worst case of the meat ruminant species and dairy ruminants, indicates that the low dose level (1.22 mg/kg bw/d) for tissues and for milk is the most appropriate for covering the uses of boscalid. Thus, a factor was calculated (calculated feed burden / dose level in feeding study, in mg/kg bw/d).
The factor derived for liver, kidney and fat is 0.89 (1.09 mg/kg/d / 1.22 mg/kg/d). Applying this factor to the maximum residue of 0.11 mg/kg found in liver and kidney at the low dose level results in a calculated residue of 0.10 mg/kg. Applying this factor to the maximum residue of 0.22 mg/kg found in fat at the low dose level results in a calculated residue of 0.20 mg/kg.
In case of milk, a factor of 0.61 (0.75 mg/kg/d / 1.22 mg/kg/d) was calculated. The mean residue was 0.01 mg/kg in the low dose group, resulting in a calculated residue of 0.006 mg/kg.

Therefore, no residues above the pending EU MRLs (SANTE/10530/2015) of

0.3 mg/kg for ruminant fat and edible offals,
0.2 mg/kg for ruminant liver and kidney and
0.02 mg/kg for milk

are anticipated. Accordingly, no new MRLs are proposed for these matrices.

For muscle, the pending EU MRL (SANTE/10530/2015) is 0.01* mg/kg (lower limit of analytical determination). Since the LOQ of the feeding study is actually 0.025 mg/kg for boscalid and M510F01 including its conjugates, which is the residue definition for enforcement, it is proposed to set the MRL to 0.03 mg/kg for muscle.

An MRL of 0.03 mg/kg is proposed for ruminant muscle.

Pig products

No separate feeding study with pigs has been performed since common metabolic pathways have been observed in rats and goats and therefore significant differences in the metabolic pathways from pigs as compared to ruminants are very unlikely. The proposals for maximum residue levels for pig products can therefore be derived from the cattle feeding study.

The calculated feed burden is below the low dose level (1.22 mg/kg bw/d) of the cow feeding study. Thus, a factor of 0.16 (0.19 mg/kg/d / 1.22 mg/kg/d) is derived. Applying this factor to the maximum residue of 0.11 mg/kg found in liver and kidney at the low dose level results in a calculated residue of 0.018 mg/kg. Applying this factor to the maximum residue of 0.22 mg/kg found in fat at the low dose level results in a calculated residue of 0.035 mg/kg.

Therefore, no residues above the pending EU MRLs (SANTE/10530/2015) of

0.07 mg/kg for swine fat and edible offals and
0.05* mg/kg for swine liver and kidney

are anticipated. Accordingly, no new MRLs are proposed for these matrices.

For muscle, the pending EU MRL (SANTE/10530/2015) is 0.01* mg/kg (lower limit of analytical determination). Since the LOQ of the feeding study is actually 0.025 mg/kg for boscalid and M510F01 including its conjugates, which is the residue definition for enforcement, it is proposed to set the MRL to 0.03 mg/kg.

An MRL of 0.03 mg/kg is proposed for swine muscle.

Poultry products

The results of the hen feeding study (BASF DocID 2002/5002466) with boscalid was evaluated during Article 12 review (EFSA Reasoned Opinion 2014, EFSA Journal 2014;12(7):3799). The animals were dosed with 1, 5 and 20 mg/kg feed equivalent to 0.06, 0.32 and 1.26 mg/kg bw/d for a period of 29 days. In muscle, no residues (<0.025 mg/kg) of boscalid and M510F01 including its conjugates were found in any dose group. Maximum group residues of <0.01, <0.01 and 0.037 mg/kg were found in eggs of the low, medium and high dose groups, respectively. In liver, maximum residues of 0.05, 0.18 and 0.47 mg/kg were found, respectively. In fat, maximum residues of <0.025, 0.10 and 0.17 mg/kg were found, respectively.
The calculated feed burden is covered by the feeding study. Therefore, no residues are to be expected in poultry muscle. Estimated residues in eggs, liver and fat are described below.

The feed burden calculation presented above indicates that the medium dose level (0.32 mg/kg bw/d) is the most appropriate for covering the uses of boscalid. Thus, a factor was calculated (calculated feed burden / dose level in feeding study, in mg/kg bw/d).
The factor derived is 1.5 (0.49 mg/kg/d / 0.32 mg/kg/d). Applying this factor to the maximum residue of 0.18 mg/kg found in liver at the medium dose level results in a calculated residue of 0.27 mg/kg. Applying this factor to the maximum residue of 0.10 mg/kg found in fat at the medium dose level results in a calculated residue of 0.15 mg/kg. For eggs, applying this factor to the maximum group mean residues of <0.01 mg/kg results in a calculated residue of 0.015 mg/kg.

For liver and edible offals as well as for fat the pending EU MRL (SANTE/10530/2015) is 0.15 mg/kg and 0.08 mg/kg, respectively.
Therefore, it is proposed to set the MRL for poultry matrices to

0.3 mg/kg for liver and edible offals and

0.15 mg/kg for fat.

For muscle, the pending EU MRL (SANTE/10530/2015) is 0.01* mg/kg (lower limit of analytical determination). Since for muscle the LOQ of the feeding study is actually 0.025 mg/kg for boscalid and M510F01 including its conjugates, which is the residue definition for enforcement, it is proposed to set the MRL to

0.03 mg/kg for muscle.

For eggs, residues above the pending EU MRLs (SANTE/10530/2015) of 0.01* mg/kg (lower limit of analytical determination) were determined.
Therefore, the MRL according to EU Regulation No 441/2012 is proposed to be maintained:

0.05 mg/kg for eggs.

Fish

For calculation purposes, the procedure specified in the EU Working Document SANCO/11187/2013 on the nature of pesticide residues in fish (as of 31 January 2013) has been used. According to the working document, fish diet for trout and carp mainly consists of cereals, pulses, oilseeds and processed fractions thereof. The input values for calculation of the fish feed burden are shown in Table 6.7.2-17. Additionally, for vegetable oil an input value of 0.039 mg/kg (based on STRM oilseed rape seed 0.15 mg/kg x PF 1.26 for refined oil taken from EFSA Reasoned Opinion 2014, EFSA Journal 2014;12(7):3799) was used.

The calculation resulted in the following approximate maximum feed burdens:

- Carp: 0.997 mg /kg feed DM
- Trout: 1.270 mg/kg feed DM

Though the calculated feed burden is above the trigger for fish metabolism and fish feeding studies of 0.1 mg/kg DM, there are no agreed guidelines how to conduct these studies. Therefore no proposal of a future EU MRL for fish commodities is provided.

Table 6.7.2-17: Input values fish feed burden calculation (Fraunhofer IME)

Crop	Matrix	IFN Code	Type	Input value (mg/kg)	Source
Barley	Bran fractions	4-00-515	STMR-P	4.62*	STMR (1.07 mg/kg*) x PF wheat bran (4.32)
	Brewers grain	5-00-516	STMR-P	0.51*	STMR (1.07 mg/kg*) x PF (0.48)
Corn field	Grain meal	4-12-208	STMR	0.05*	STMR Maize grain
	Bran	5-28-235	STMR-P	0.22*	STMR Maize grain (0.05* x PF wheat bran (4.32)
	Hominy meal	4-03-010	STMR	0.05*	STMR Maize grain
Cotton seed	Meal	5-01-617	STMR-P	0.07*	STMR (0.05 mg/kg*) x default PF (1.3)
Linseed	Meal (treated)	5-02-048	STMR-P	0.2*	STMR (0.1 mg/kg*) x default PF (2)
Lupin seed white	Meal	5-27-717	STMR-P	0.1*	STMR (0.05 mg/kg*) x default PF (2)
Mustard	Meal		STMR-P	0.2*	STMR (0.1 mg/kg*) x default PF (2)
Peanut	Meal decorticated	5-03-649	STMR-P	0.2*	STMR (0.1 mg/kg*) x default PF (2
Rape seed	Meal (toxic)	5-26-093	STMR-P	0.028*	STMR (0.05 mg/kg*) x PF oilseed rape meal (0.56)
Canola	Meal	5-08-136	STMR-P	0.028*	STMR (0.05 mg/kg*) x PF oilseed rape meal (0.56)
Rice	Bran de-oiled		STMR-P	0.22*	STMR (0.05 mg/kg*) x PF wheat bran (4.32)
Sesame seed	Meal	5-04-220	STMR-P	0.1*	STMR (0.05 mg/kg*) x default PF (2)
Safflower	Meal decorticated	5-26-095	STMR-P	0.1*	STMR (0.05 mg/kg*) x default PF (2)
Soya bean	Meal decorticated	5-20-638	STMR-P	0.02	STMR (0.1 mg/kg) x PF (0.16)
Sunflower	Meal decorticated	5-26-098	STMR-P	0.32*	STMR (0.16 mg/kg*) x default PF (2)
Wheat	Extruded grain	4-12-208	STMR	0.17*	
	Bran	4-05-190	STMR-P	0.73*	STMR (0.17 mg/kg*) x PF (4.32)
	Flour	4-05-199	STMR-P	0.06*	STMR (0.17 mg/kg*) x PF (0.34)
Corn	Grain	4-20-698	STMR	0.05*	
Cow pea	Treated seed	5-01-661	STMR	0.13*	
Faba bean	Treated seed		STMR	0.13*	
Lupin (white)	Treated seed	5-02-707	STMR	0.05*	
Pea	Treated seed	5-03-600	STMR	0.13*	
Rice	Broken grains	4-03-939	STMR	0.05*	

Table 6.7.2-17: Input values fish feed burden calculation (Fraunhofer IME)

Crop	Matrix	IFN Code	Type	Input value (mg/kg)	Source
Sorghum	Grain	4-04-383	STMR	0.05*	
Soya bean	Treated seed	5-64-610	STMR	0.1*	
Sunflower	Seed		STMR	0.16*	
Triticale	Grain	4-20-362	STMR	0.17*	
Vetch	Seed	5-26-351	STMR	0.13*	
Wheat	Grain (extruded)	4-05-211	STMR	0.17*	
Fat	Vegetable oil		STMR-P	0.19*	STMR (0.15 mg/kg*) x PF refined rape oil (1.26)

* Combined contribution of primary crop and rot crop (EFSA Journal 2014;12(7):3799)

Table 6.7.2-18: Detailed results of the fish feed burden calculation (Fraunhofer IME)

Scenario	Common carp	Rainbow trout
Without PC, CC, F	0.997	1.191
Without PC, CC, F (MRBD)	0.972	-
PC	0.997	1.270
PC (MRBD)	0.974	1.060
CC	0.997	1.191
CC (MRBD)	0.972	-
F	0.997	1.191
F (MRBD)	0.972	-
PC and CC	0.997	1.270
PC and CC (MRBD)	0.974	1.060
PC and F	0.997	1.270
PC and F (MRBD)	0.974	1.060
CC and F	0.997	1.191
CC and F (MRBD)	0.972	-
PC, CC and F	0.997	1.270
PC, CC and F (MRBD)	0.974	1.060
Worst case	**0.997**	**1.270**

PC Unloaded protein concentrate is added as feed component
CC Unloaded carbohydrate concentrate is added as feed component
F Unloaded fat is added as feed concentrate
MRBD Maximum reasonable balanced diet

Honey

12 field trials were conducted in representative oilseed rape growing areas in Germany to determine the magnitude of the residues in oilseed rape honey (see also chapter M-CA 6.10). Formulation BAS 510 01 F (WG) was foliar applied to winter oilseed rape once at a target rate of 0.25 kg boscalid/ha. The application was conducted during full flowering at BBCH 65, the most critical time for honey production. The residue results of honey specimens sampled are listed below.

EU North outdoor (n=7): 0.01; 0.02; <0.05 (7x); 0.06; 0.064; 0.08 mg/kg

MRL calculation was performed using the OECD calculator.

Table 6.7.2-19: MRL calculation for honey in the EU for boscalid based on parent residues (mg/kg)

OECD calculator[#]	BAS 510 F [mg/kg]
	N-EU
Total number of data (n)	12
Highest residue	0.080
Mean + 4 SD	0.122
CF x 3 Mean	0.146
Rounded MRL	**0.15**
STMR	0.050

[#] OECD calculator spreadsheet, taken from the OECD page:
http://www.oecd.org/document/34/0,3746,en_2649_37465_48447010_1_1_1_37465,00.html

An MRL of 0.15 mg/kg is calculated based on the Northern European residue data for treatment of oilseed rape, the relevant crop for the production of honey. The calculated MRL is covered by the current **EU MRL** of **0.5 mg/kg**. Nevertheless, the residues are not covered by the pending EU MRL (SANTE/10530/2015) of 0.05 mg/kg. Therefore, the **MRL of 0.5 mg/kg** according to Reg. (EU) No 441/2012 and SANTE/10377/2015 (pending) is proposed to be maintained.

CA 6.7.3 Proposed maximum residue levels (MRLs) and justification of the acceptability of the levels proposed for imported products (import tolerance)

The MRLs listed in chapter M-CA 6.7.2 include domestic uses, but also values for imported crops. Prior to final approval, the import tolerances have been carefully evaluated by EFSA. Parts of them are resulting from the adoption of CODEX MRLs (CXLs). Boscalid and its crops have been assessed by JMPR (2006, 2009).

CA 6.8 Proposed safety intervals

Boscalid is intended for post-emergence use (BBCH 60-81 for grapes, BBCH 60-69 for peas and beans and BBCH 13-75 for oilseed rape). Residue trials have been conducted with applications made at the latest recommended crop growth stage with harvest taking place at the time of crop maturity following good agricultural practice.

Pre-harvest interval

The pre-harvest interval is for grape 21 days, and for peas and beans 7 days. In oilseed rape, applications are intended at growth stages BBCH 13-75. No specific pre-harvest interval is defined; the interval between last application and crop harvest is defined by crop maturity.

Re-entry period (in days) for livestock, to areas to be grazed

Because boscalid 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 crops, buildings or spaces treated

Re-entry assessments are provided for the representative uses in the supplemental product dossier (see M-CP 7.2). It was concluded that there is no unacceptable risk anticipated for workers wearing adequate working clothing when re-entering crops treated with BAS 510 01 F after the spray dilute has dried.

Withholding period (in days) for animal feeding stuffs

The products of treated grapes, beans and peas and oilseed rape (referred to as meal) may be used as fodder for livestock. Boscalid derived residues in those feed items are assessed in this dossier by providing the respective calculations of livestock dietary burdens MRLs (see M-CA 6.7) for animal products covering the intended uses. There is no additional withholding period needed for animal feeds with regard to boscalid derived residues.

Waiting period between last application and sowing or planting

No waiting period is necessary since boscalid containing products are intended for post emergence use.

Waiting periods between application and handling treated products

Not relevant since a post-harvest treatment is not intended.

Waiting period before sowing/planting succeeding crops

No minimum waiting periods are necessary to be considered neither in terms of phytotoxicity nor in terms of residues in succeeding crops (Information on potential residues of boscalid in succeeding crops is provided in M-CA 6.6).

No replant restriction is needed since either the MRL proposals included in M-CA 6.7 are covering potential residues taken up from soil, or any residue above LOQ can be excluded in crops investigated following current guidelines.

CA 6.9 Estimation of the potential and actual exposure through diet and other sources

Assessments of the potential chronic dietary consumer risk resulting from exposure to residues of boscalid (BAS 510 F) were performed using revision 2 (August 2008) of the EFSA Pesticide Residues Intake Model (PRIMo). The EFSA model was used since it considers all the different diets and all consumer groups in the EU.

The ADI and ARfD values for the active substance boscalid are summarized in the table below.

Table 6.9-1: Toxicological endpoints - boscalid

End-Point	Value	Study	Safety Factor	Reference
Acceptable Daily Intake (ADI)	0.04 mg/kg bw	2-year oral feeding study in rats	100	Dir. 2008/44/EC
Acute Reference Dose (ARfD)	Not allocated	Not necessary, based on low acute toxicity and lack of developmental toxicity concerns		Dir. 2008/44/EC

Since the data for parent do not lead to the need for an Acute Reference Dose (ARfD), no ARfD is proposed for the metabolites, either. The ADI values used for metabolites M510F01 and M510F02 (being part of the residue definition for risk assessment in animal matrices) are included in Table 6.9-2. Based on their structural similarity to BAS 510 F, the use of the parent ADI is feasible. However, for all other boscalid metabolites the use of the stricter Cramer Class III TTC endpoints were applied in the dietary risk assessment. The risk assessment for M510F01 and M510F02 according to the TTC concept is provided within the indicative risk assessment (see below).

Table 6.9-2: Toxicological endpoints – boscalid metabolites

Metabolite	Acceptable Daily Intake (ADI) used [mg/kg bw/d]	Study	Safety factor	Reference
M510F01	0.04	None	Not relevant	Residue definition
M510F02	0.04	None	Not relevant	Residue definition
M510F47	0.0015	None	Not relevant	TTC Cramer Class III value
M510F54	0.0015	None	Not relevant	TTC Cramer Class III value
M510F61	0.0015	None	Not relevant	TTC Cramer Class III value

Acceptable Daily Intake (ADI) and Dietary Exposure Calculation

Boscalid

TMDI calculation

An assessment of the potential chronic dietary consumer risk due to exposure to residues of boscalid was performed using the following scenario:

- EFSA calculation model for acute and chronic consumer exposure (Version 2) as of October 2007, source: Internet: http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178620776373.htm

The diets are regarded as sufficiently representative for Europe and should cover the use in other countries as well. Theoretical maximum daily intake (TMDI) calculations use a number of worst-case scenarios with respect to dietary burden. It is assumed that the market penetration is 100% i.e. all crops have been treated with the respective active substance and, furthermore, all residues are at the upper tolerable limit, the MRL. The MRL is always set for the tradable commodity. Effects of processing are not considered. Thus, the actual consumer exposure will be significantly lower in reality.

A revised chronic exposure assessment was performed, for which all crops and maximum residue levels used are summarized in Table 6.9-3. The risk assessment was based on MRLs pending according to the Article 12 evaluation (SANCO/10530/2015). Where a new maximum residue level was proposed in this document the new proposed value was considered. For the assessment, the ADI of 0.04 mg/kg bw/day was used.

The TMDI calculation according to the EFSA model is presented in Table 6.9-4. 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 6.9-3: Boscalid - MRL values used for risk assessment

Code number	Groups and examples of individual products to which the MRLs apply	Boscalid[1] MRL (mg/kg), SANTE/10530/2015
0100000	. FRUITS, FRESH or FROZEN; TREE NUTS	-
0110000	. Citrus fruits	2[a]
0120000	. Tree nuts	-
0120010	. Almonds	0.05*[a]
0120020	. Brazil nuts	0.05*[a]
0120030	. Cashew nuts	0.05*[a]
0120040	. Chestnuts	0.05*[a]
0120050	. Coconuts	0.05*[a]
0120060	. Hazelnuts/cobnuts	0.05*[a]
0120070	. Macadamias	0.05*[a]
0120080	. Pecans	0.05*[a]
0120090	. Pine nut kernels	0.05*[a]
0120100	. Pistachios	1[a]
0120110	. Walnuts	0.05*[a]
0120990	. Others	0.05*

Table 6.9-3: Boscalid - MRL values used for risk assessment

Code number	Groups and examples of individual products to which the MRLs apply	Boscalid[1] MRL (mg/kg), SANTE/10530/2015
0130000	. Pome fruits	-
0130010	. Apples	2[a]
0130020	. Pears	1.5[a]
0130030	. Quinces	1.5[a]
0130040	. Medlars	0.01*
0130050	. Loquats/Japanese medlars	0.01*
0130990	. Others	0.01*
0140000	. Stone fruits	-
0140010	. Apricots	5[a]
0140020	. Cherries (sweet)	4[a]
0140030	. Peaches	5[a]
0140040	. Plums	3[a]
0140990	. Others	0.01*
0150000	. Berries and small fruits	-
0151000	. (a) grapes	5[a]
0152000	. (b) strawberries	6[b]
0153000	. (c) cane fruits	10[a]
0154000	. (d) other small fruits and berries	15[a]
0160000	. Miscellaneous fruits with	-
0161000	. (a) edible peel	0.01*
0162000	. (b) inedible peel, small	-
0162010	. Kiwi fruits (green, red, yellow)	5[a]
0162020	. Litchis/lychees	0.01*
0162030	. Passionfruits/maracujas	0.01*
0162040	. Prickly pears/cactus fruits	0.01*
0162050	. Star apples/cainitos	0.01*
0162060	. American persimmons/Virginia kaki	0.01*
0162990	. Others	0.01*
0163000	. (c) inedible peel, large	-
0163010	. Avocados	0.01*
0163020	. Bananas	0.6[a]
0163030	. Mangoes	0.01*
0163040	. Papayas	0.01*
0163050	. Granate apples/pomegranates	0.01*
0163060	. Cherimoyas	0.01*
0163070	. Guavas	0.01*
0163080	. Pineapples	0.01*
0163090	. Breadfruits	0.01*
0163100	. Durians	0.01*
0163110	. Soursops/guanabanas	0.01*
0163990	. Others	0.01*

Table 6.9-3: Boscalid - MRL values used for risk assessment

Code number	Groups and examples of individual products to which the MRLs apply	Boscalid[1] MRL (mg/kg), SANTE/10530/2015
0200000	. VEGETABLES, FRESH or FROZEN	-
0210000	. Root and tuber vegetables	-
0211000	. (a) potatoes	2[b]
0212000	. (b) tropical root and tuber vegetables	2[b]
0213000	. (c) other root and tuber vegetables except sugar beets	-
0213010	. Beetroots	4[b]
0213020	. Carrots	2[b]
0213030	. Celeriacs/turnip rooted celeries	2[b]
0213040	. Horseradishes	2[b]
0213050	. Jerusalem artichokes	2[b]
0213060	. Parsnips	2[b]
0213070	. Parsley roots/Hamburg roots parsley	2[b]
0213080	. Radishes	2[b]
0213090	. Salsifies	2[b]
0213100	. Swedes/rutabagas	2[b]
0213110	. Turnips	2[b]
0213990	. Others	2
0220000	. Bulb vegetables	-
0220010	. Garlic	5[b]
0220020	. Onions	5[b]
0220030	. Shallots	5[b]
0220040	. Spring onions/green onions and Welsh onions	6[b]
0220990	. Others	0.5
0230000	. Fruiting vegetables	-
0231000	. (a) solanacea	3[b]
0232000	. (b) cucurbits with edible peel	4[b]
0233000	. (c) cucurbits with inedible peel	3[b]
0234000	. (d) sweet corn	0.05[b]
0239000	. (e) other fruiting vegetables	0.9
0240000	. Brassica vegetables (excluding brassica roots and brassica baby leaf crops)	-
0241000	. (a) flowering brassica	5[b]
0242000	. (b) head brassica	5[b]
0243000	. (c) leafy brassica	9[b]
0244000	. (d) kohlrabies	5[b]
0250000	. Leaf vegetables, herbs and edible flowers	-
0251000	. (a) lettuces and salad plants	50[b]
0252000	. (b) spinaches and similar leaves	-
0252010	. Spinaches	50[b]
0252020	. Purslanes	0.9[b]
0252030	. Chards/beet leaves	30[b]
0252990	. Others	0.9
0253000	. (c) grape leaves and similar species	0.01*[b]
0254000	. (d) watercresses	0.01*[b]
0255000	. (e) witloofs/Belgian endives	7[b]
0256000	. (f) herbs and edible flowers	50[b]

Table 6.9-3: Boscalid - MRL values used for risk assessment

Code number	Groups and examples of individual products to which the MRLs apply	Boscalid[1] MRL (mg/kg), SANTE/10530/2015
0260000	. Legume vegetables	-
0260010	. Beans (with pods)	5[b] / 6[f]
0260020	. Beans (without pods)	3[b]
0260030	. Peas (with pods)	5[b] / 6[f]
0260040	. Peas (without pods)	3[b]
0260050	. Lentils	3[b]
0260990	. Others	0.06
0270000	. Stem vegetables	-
0270010	. Asparagus	0.9[b]
0270020	. Cardoons	0.9[b]
0270030	. Celeries	9[b]
0270040	. Florence fennels	9[b]
0270050	. Globe artichokes	5[b]
0270060	. Leeks	9[b]
0270070	. Rhubarbs	0.9[b]
0270080	. Bamboo shoots	0.01*[b]
0270090	. Palm hearts	0.01*[b]
0270990	. Others	0.5
0280000	. Fungi, mosses and lichens	0.01*
0290000	. Algae and prokaryotes organisms	0.01*
0300000	. PULSES	3[b]
0400000	. OILSEEDS AND OIL FRUITS	-
0401000	. Oilseeds	-
0401010	. Linseeds	1[b]
0401020	. Peanuts/groundnuts	1[b]
0401030	. Poppy seeds	1[b]
0401040	. Sesame seeds	1[b]
0401050	. Sunflower seeds	1[b]
0401060	. Rapeseeds/canola seeds	1[b]
0401070	. Soyabeans	3[b]
0401080	. Mustard seeds	1[b]
0401090	. Cotton seeds	1[b]
0401100	. Pumpkin seeds	1[b]
0401110	. Safflower seeds	1[b]
0401120	. Borage seeds	1[b]
0401130	. Gold of pleasure seeds	1[b]
0401140	. Hemp seeds	1[b]
0401150	. Castor beans	1[b]
0401990	. Others	0.06
0402000	. Oil fruits	0.01*

Table 6.9-3: Boscalid - MRL values used for risk assessment

Code number	Groups and examples of individual products to which the MRLs apply	Boscalid[1] MRL (mg/kg), SANTE/10530/2015
0500000	. CEREALS	-
0500010	. Barley	4[b]
0500020	. Buckwheat and other pseudo-cereals	0.15[b]
0500030	. Maize/corn	0.15[b]
0500040	. Common millet/proso millet	0.15[b]
0500050	. Oat	4[b]
0500060	. Rice	0.15[b]
0500070	. Rye	0.8[b]
0500080	. Sorghum	0.15[b]
0500090	. Wheat	0.8[b]
0500990	. Others	0.15
0600000	. TEAS, COFFEE, HERBAL INFUSIONS, COCOA AND CAROBS	-
0610000	. Teas	0.01*
0620000	. Coffee beans	0.05*[a]
0630000	. Herbal infusions from	-
0631000	. (a) flowers	0.9[c]
0632000	. (b) leaves and herbs	0.9[c]
0633000	. (c) roots	3[c]
0639000	. (d) any other parts of the plant	0.01*
0640000	. Cocoa beans	0.01*
0650000	. Carobs/Saint John's breads	0.01*
0700000	. HOPS	80[c]
0800000	. SPICES	-
0810000	. Seed spices	0.9[c]
0820000	. Fruit spices	0.9[c]
0830000	. Bark spices	0.9[c]
0840000	. Root and rhizome spices	-
0840010	. Liquorice	0.4[c]
0840020	. Ginger	0.4[c]
0840030	. Turmeric/curcuma	0.4[c]
0840040	. Horseradish	2[b]
0840990	. Others	0.4
0850000	. Bud spices	0.9[c]
0860000	. Flower pistil spices	0.9[c]
0870000	. Aril spices	0.9[c]
0900000	. SUGAR PLANTS	-
0900010	. Sugar beet roots	0.4[b]
0900020	. Sugar canes	7[b]
0900030	. Chicory roots	0.4[b]
0900990	. Others	0.5

Table 6.9-3: Boscalid - MRL values used for risk assessment

Code number	Groups and examples of individual products to which the MRLs apply	Boscalid[1] MRL (mg/kg), SANTE/10530/2015
1000000	. PRODUCTS OF ANIMAL ORIGIN -TERRESTRIAL ANIMALS	-
1010000	. Tissues from	-
1011000	. (a) swine	-
1011010	. Muscle	0.03[e]
1011020	. Fat tissue	0.07
1011030	. Liver	0.05*
1011040	. Kidney	0.05*
1011050	. Edible offals (other than liver and kidney)	0.07
1011990	. Others	0.05*
1012000	. (b) bovine	-
1012010	. Muscle	0.03[e]
1012020	. Fat tissue	0.3
1012030	. Liver	0.2[d]
1012040	. Kidney	0.2
1012050	. Edible offals (other than liver and kidney)	0.3
1012990	. Others	0.05*
1013000	. (c) sheep	-
1013010	. Muscle	0.03[e]
1013020	. Fat tissue	0.3
1013030	. Liver	0.2[d]
1013040	. Kidney	0.2
1013050	. Edible offals (other than liver and kidney)	0.3
1013990	. Others	0.05*
1014000	. d) goat	-
1014010	. Muscle	0.03[e]
1014020	. Fat tissue	0.3
1014030	. Liver	0.2[d]
1014040	. Kidney	0.2
1014050	. Edible offals (other than liver and kidney)	0.3
1014990	. Others	0.05*
1015000	. (e) equine	-
1015010	. Muscle	0.03[e]
1015020	. Fat tissue	0.3
1015030	. Liver	0.2
1015040	. Kidney	0.2
1015050	. Edible offals (other than liver and kidney)	0.3
1015990	. Others	0.05*
1016000	. (f) poultry	-
1016010	. Muscle	0.03[e]
1016020	. Fat tissue	0.15[g]
1016030	. Liver	0.3[d, h]
1016040	. Kidney	0.05*
1016050	. Edible offals (other than liver and kidney)	0.3[h]
1016990	. Others	0.05*

Table 6.9-3: Boscalid - MRL values used for risk assessment

Code number	Groups and examples of individual products to which the MRLs apply	Boscalid[1] MRL (mg/kg), SANTE/10530/2015
1017000	. (g) other farmed terrestrial animals	-
1017010	. Muscle	0.03[e]
1017020	. Fat tissue	0.3
1017030	. Liver	0.2
1017040	. Kidney	0.2
1017050	. Edible offals (other than liver and kidney)	0.3
1017990	. Others	0.05*
1020000	. Milk	0.02
1030000	. Birds eggs	0.05[i]
1040000	. Honey and other apiculture products	0.5[j]
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

1 The residue definition differs for the following combinations pesticide-code number: Boscalid - code 1000000 except 1040000: Sum of boscalid and metabolite 2-chloro-N-(4'-chloro-5-hydroxybiphenyl-2-yl)nicotinamide (M510F01) including its conjugates expressed as boscalid

a The European Food Safety Authority identified some information on residues after repeated application in permanent crops and data to confirm the plateau level in soil as unavailable (A soil accumulation study is currently ongoing. For detail please refer to chapter 6.6; 6.7 and 7.1). When re-viewing the MRLs, the Commission will take into account the information referred to in the first sentence, if submitted by [Office of Publication: please insert date 2 years after publication], or, if that information is not submitted by that date, the lack of it.

b The European Food Safety Authority identified some information on residues for rotational crops and data to confirm the plateau level in soil as unavailable. When re-viewing the MRLs, the Commission will take into account the information referred to in the first sentence, if submitted by [Office of Publication: please insert date 2 years after publication], or, if that information is not submitted by that date, the lack of it.

c The European Food Safety Authority identified some information on analytical methods, residues after repeated application in permanent crops and data to confirm the plateau level in soil as unavailable. When re-viewing the MRLs, the Commission will take into account the information referred to in the first sentence, if submitted by [Office of Publication: please insert date 2 years after publication], or, if that information is not submitted by that date, the lack of it.

d The European Food Safety Authority identified some information on the fate of the pyridine moiety as unavailable. When re-viewing the MRLs, the Commission will take into account the information referred to in the first sentence, if submitted by [Office of Publication: please insert date 2 years after publication], or, if that information is not submitted by that date, the lack of it.

e In SANTE/10530/2015, an MRL of 0.01* mg/kg is set for muscle. However, in the feeding studies, residues were below the LOQ of 0.025 mg/kg. An MRL of 0.03 mg/kg is therefore proposed within this document.

f An MRL of 6 mg/kg is calculated based on the Northern European residue data for treatment of beans with pods, which is not covered by the current, pending EU MRL of 5 mg/kg (SANTE/10530/2015). Therefore, an MRL of 6 mg/kg is proposed within this document. According to "Guidelines on comparability, extrapolation, group tolerances and data requirements for setting MRLs" (SANCO 7525/VI/95 rev. 9, March 2011), this MRL is extrapolated to peas with pods.

g In SANTE/10530/2015, an MRL of 0.08 mg/kg is set for fat. However, based on the feed burden calculation and the derived factor, residues above this value were derived. Therefore, an MRL of 0.15 mg/kg is proposed within this document.

h In SANTE/10530/2015, an MRL of 0.15 mg/kg is set for liver and edible offals. However, based on the feed burden calculation and the derived factor, residues above this value were derived. Therefore, an MRL of 0.3 mg/kg is proposed within this document.

i In SANTE/10530/2015, an MRL of 0.01* mg/kg is set for eggs. However, based on the feed burden calculation and the derived factor, residues above this value were derived. An MRL of 0.05 mg/kg according to Reg. (EU) No 441/2012 is therefore proposed to be maintained.

j In SANTE/10530/2015, an MRL of 0.05* mg/kg is set for honey and other apiculture products. However, treatment of oilseed rape is performed during flowering and in residue studies. As residues were found above LOQ, the MRL of 0.5 mg/kg according to Reg. (EU) No 441/2012 and SANTE/10377/2015 (pending) is proposed to be maintained.

Comments to the footnotes of Table 6.9-3 are given in the following:

Footnote a to c: A field soil accumulation study is currently in progress. Interim results covering a period of about 5.5 years are presented in chapter M-CA 7.1.2. As a result, the plateau reported in this study is clear below the application rates used in the rotational field studies (for details see chapter M-CA 6.6 and 7.1). Additionally, a study on the residue behavior of boscalid in rotational crops after growing on aged boscalid-treated soil shows that aging of boscalid residues in soil leads to a reduction of the bioavailability for plant of about 50%, starting about 3 months after the application (for details see chapter M-CA 6.6.2). Therefore, after multi-year use of boscalid, the results of the rotational crop study should be corrected. Where a significant residue uptake could not be excluded, for MRL proposal additional residue via soil uptake was taken into account (EFSA Reasoned Opinion 2014, EFSA Journal 2014;12(7):3799) and permuted in the currently pending EU MRLs. This MRL values including the uptake from residues from soil are valid because the tested concentration of boscalid in the rotational crop studies is significantly below the calculated plateau in the soil after multi-year use of boscalid.

Footnote d: In liver, parent boscalid is bound to the proteins, mainly resulting from a substitution of the chlorine of the pyridine system by thiol groups of liver proteins. Only harsh extraction conditions lead to cleavage products M510F52 or M510F53, both used as marker for the bound residues in the liver. EFSA Reasoned Opinion 2014 (EFSA Journal 2014;12(7):3799) discussed the possibility that the bound liver residues may be released during cooking and a cleavage of the amine bond of boscalid cannot be excluded (only a diphenyl label was applied in animal studies). BASF is of the opinion that the M510F52 and M510F53 residues are not formed under biotic conditions: the amide bound of BAS 510 F was very stable under metabolic conditions in hens. Additionally, the harsh extraction conditions used to release liver bound residues do not apply under normal conditions in food processing. Thus, the residue definition for animal matrices is considered valid and MRL values for boscalid according to SANTE/10530/2015 (pending) are applicable.

Using the current EFSA model, the chronic risk ranges from 55 to 279% of the ADI. The diet with the highest TMDI is “WHO Cluster diet B” with 279.0% of the ADI. For this diet, the highest contributor is “lettuce and other salad plants” with 49.6% of the ADI. The diet with the second highest TMDI is “NL child” with 276.1% of the ADI where “spinach” is the major contributor (46.5% of the ADI).

The ADI utilization exceeds the ADI for some diets when using MRL values. Thus, a refinement was performed (see IEDI calculation below).

Table 6.9-4: Boscalid (BAS 510 F): TMDI calculation based on input values listed in Table 6.9-3

Boscalid			
Status of the active substance:	**Approved**	Code no.	**BAS 510 F**
LOQ (mg/kg bw):		proposed LOQ:	
Toxicological end points			
ADI (mg/kg bw/day):	**0,04**	ARfD (mg/kg bw):	**n.a.**
Source of ADI:	**Dir. 2008/44/EC**	Source of ARfD:	**Dir. 2008/44/EC**
Year of evaluation:	**2002-2008**	Year of evaluation:	**2002-2008**

Chronic risk assessment

	TMDI (range) in % of ADI minimum - maximum
	55 279
No of diets exceeding ADI	20

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)
279,0	WHO Cluster diet B	49,6	Lettuce and other salad plants	29,2	Solanacea	26,8	Table and wine grapes	
276,1	NL child	46,5	Spinach	38,6	Lettuce and other salad plants	31,7	Apples	
247,6	FR toddler	88,4	Spinach	25,3	Potatoes	16,6	Beans (with pods)	
240,2	DE child	60,3	Apples	25,5	Spinach	23,0	Citrus fruit	
215,5	E adult	17,7	Tropical root and tuber vegetables	15,6	Spinach	14,6	Table and wine grapes	
170,9	SE general population 90th percentile	50,4	Lettuce and other salad plants	20,8	Potatoes	8,3	Spinach	
168,9	WHO cluster diet E	24,8	Lettuce and other salad plants	22,4	Table and wine grapes	19,2	Potatoes	
167,1	FR infant	55,4	Spinach	20,7	Potatoes	13,2	Carrots	
159,2	WHO regional European diet	51,5	Lettuce and other salad plants	20,1	Potatoes	9,8	Solanacea	
145,2	IT adult	69,8	Lettuce and other salad plants	11,9	Spinach	10,1	Solanacea	
144,3	ES adult	67,0	Lettuce and other salad plants	9,3	Spinach	7,3	Solanacea	
141,4	FR all population	51,4	Table and wine grapes	40,0	Lettuce and other salad plants	6,6	Wheat	
140,5	ES child	52,2	Lettuce and other salad plants	11,7	Citrus fruit	10,1	Spinach	
138,1	NL general	29,9	Lettuce and other salad plants	17,7	Spinach	13,7	Potatoes	
133,2	WHO Cluster diet F	37,5	Lettuce and other salad plants	17,1	Potatoes	9,2	Table and wine grapes	
131,0	IT kids/toddler	51,5	Lettuce and other salad plants	13,3	Wheat	11,9	Solanacea	
126,3	WHO cluster diet D	20,3	Potatoes	13,0	Wheat	9,1	Head brassica	
126,3	PT General population	34,6	Table and wine grapes	26,7	Potatoes	13,1	Lettuce and other salad plants	
121,9	UK Toddler	22,9	Sugar beet (root)	17,5	Potatoes	11,5	Citrus fruit	
117,8	DK child	17,6	Lettuce and other salad plants	16,4	Cucurbits - edible peel	12,2	Potatoes	
95,3	UK Infant	16,3	Potatoes	10,1	Sugar beet (root)	7,8	Apples	
86,2	UK vegetarian	17,8	Lettuce and other salad plants	11,1	Table and wine grapes	6,9	Potatoes	
74,7	DK adult	18,4	Table and wine grapes	16,2	Lettuce and other salad plants	7,3	Potatoes	
72,0	UK Adult	14,6	Lettuce and other salad plants	14,2	Table and wine grapes	7,0	Potatoes	
67,2	PL general population	17,2	Potatoes	10,2	Apples	7,3	Solanacea	
59,7	LT adult	15,9	Potatoes	9,3	Apples	7,9	Lettuce and other salad plants	
54,9	FI adult	9,8	Lettuce and other salad plants	6,5	Other small fruit & berries	6,1	Potatoes	

IEDI calculations

For the EFSA model, a more refined assessment is required since the calculation resulted in an ADI utilization above 100% ADI. In a first step, STMRs are included in the assessment instead of the MRL. The used values are summarized in Table 6.9-5. They were taken from EFSA Reasoned Opinion 2014 (EFSA Journal 2014;12(7):3799), considering the combined assessment of primary uses and uptake of residues from previously treated soil. The refinements performed clearly indicate that there is no chronic risk for any subpopulation group. The results of refinements are summarized in Table 6.9-6.

Table 6.9-5: Supervised trial medium residues (STMR) used for a refined exposure assessment

Code number	Groups and examples of individual products to which the STMRs apply	Boscalid[1] STMR[2] (mg/kg), incl. residues from rotational crops
0120100	. Pistachios	0.27
0130010	. Apples	0.42
0130020	. Pears	0.42
0130030	. Quinces	0.42
0140010	. Apricots	0.77
0140020	. Cherries (sweet)	1.51
0140030	. Peaches	0.77
0140040	. Plums	0.29
0151000	. (a) grapes	1.42
0152000	. (b) strawberries	1.95
0153000	. (c) cane fruits	2.47
0154010	. Blueberries	3.6
0154020	. Cranberries	3.6
0154030	. Currants (black, red and white)	3.6
0154040	. Gooseberries (green, red and yellow)	3.6
0154050	. Rose hips	2.6
0154060	. Mulberries (black and white)	2.6
0154070	. Azaroles/Mediterranean medlars	3.6
0154080	. Elderberries	2.6
0154990	. Others	3.6
0162010	. Kiwi fruits (green, red, yellow)	0.08
0163020	. Bananas	0.05
0211000	. (a) potatoes	0.1
0212020	. Sweet potatoes	0.1
0212030	. Yams	0.1
0212040	. Arrowroots	0.1
0213010	. Beetroots	0.38
0213020	. Carrots	0.24
0213030	. Celeriacs/turnip rooted celeries	0.39
0213040	. Horseradishes	0.24
0213060	. Parsnips	0.14
0213070	. Parsley roots/Hamburg roots parsley	0.14
0213080	. Radishes	0.33
0213090	. Salsifies	0.14
0213110	. Turnips	0.14
0220010	. Garlic	0.2
0220020	. Onions	0.2
0220030	. Shallots	0.2

Table 6.9-5: Supervised trial medium residues (STMR) used for a refined exposure assessment

Code number	Groups and examples of individual products to which the STMRs apply	Boscalid[1] STMR[2] (mg/kg), incl. residues from rotational crops
0220040	. Spring onions/green onions and Welsh onions	2.3
0231010	. Tomatoes	0.40
0231020	. Sweet peppers/bell peppers	0.56
0231030	. Aubergines/eggplants	0.40
0232000	. (b) cucurbits with edible peel	0.73
0233000	. (c) cucurbits with inedible peel	0.40
0241010	. Broccoli	1.55
0241020	. Cauliflowers	1.55
0242010	. Brussels sprouts	0.3
0242020	. Head cabbages	1.1
0243010	. Chinese cabbages/pe-tsai	1.1
0243020	. Kales	1.1
0244000	. (d) kohlrabies	0.08
0251000	. (a) lettuces and salad plants	5.6
0252010	. Spinaches	5.6
0255000	. (e) witloofs/Belgian endives	1.16
0256010	. Chervil	5.60
0256020	. Chives	5.60
0256030	. Celery leaves	5.60
0256040	. Parsley	5.60
0256050	. Sage	5.60
0256060	. Rosemary	5.60
0256070	. Thyme	5.60
0256080	. Basil and edible flowers	14.50
0256090	. Laurel/bay leave	5.60
0256100	. Tarragon	5.60
0256990	. Others	5.60
0260010	. Beans (with pods)	1.28[3]
0260020	. Beans (without pods)	0.11
0260030	. Peas (with pods)	1.28[3]
0260040	. Peas (without pods)	0.11
0270010	. Asparagus	0.1
0270030	. Celeries	2.18
0270040	. Florence fennels	2.18
0270050	. Globe artichokes	1.23
0270060	. Leeks	2.35
0300010	. Beans	0.13
0300020	. Lentils	0.13
0300030	. Peas	0.13
0401010	. Linseeds	0.1
0401020	. Peanuts/groundnuts	0.1
0401030	. Poppy seeds	0.1
0401050	. Sunflower seeds	0.16
0401060	. Rapeseeds/canola seeds	0.15
0401070	. Soyabeans	0.1
0401080	. Mustard seeds	0.1
0401120	. Borage seeds	0.1
0401130	. Gold of pleasure seeds	0.1
0500010	. Barley	1.07

Table 6.9-5: Supervised trial medium residues (STMR) used for a refined exposure assessment

Code number	Groups and examples of individual products to which the STMRs apply	Boscalid[1] STMR[2] (mg/kg), incl. residues from rotational crops
0500050	. Oat	1.07
0500070	. Rye	0.17
0500090	. Wheat	0.17
0633000	. (c) roots	0.95
0700000	. HOPS	24.5

* Indicates lower limit of analytical determination

1 The residue definition differs for the following combinations pesticide-code number: Boscalid - code 1000000 except 1040000: Sum of boscalid and metabolite 2-chloro-N-(4'-chloro-5-hydroxybiphenyl-2-yl)nicotinamide (M510F01) including its conjugates expressed as boscalid

2 EFSA Reasoned Opinion 2014 (EFSA Journal 2014;12(7):3799), considering the combined assessment of primary uses and uptake of residues from previously treated soil

3 STMR related to the MRL proposed within this document (see chapter 6.7).

Using the STMRs and the current EFSA model for refinement, the chronic risk ranges from 10 to 72.2% of the ADI. The diet with the highest IEDI is “WHO Cluster diet B” with 72.2% of the ADI. For this diet, the highest contributor is “sugar cane” with 12.6% of the ADI. The diet with the second highest IEDI is “DE child” with 64.1% of the ADI where “citrus fruit” is the major contributor (23.0% of the ADI).

According to the presented IEDI calculation, a chronic intake of boscalid residues is unlikely to present a public health concern.

Table 6.9-6: Boscalid (BAS 510 F): IEDI calculation based on input values listed in Table 6.9-3 and Table 6.9-5

Boscalid			
Status of the active substance:	**Approved**	Code no.	**BAS 510 F**
LOQ (mg/kg bw):		proposed LOQ:	
Toxicological end points			
ADI (mg/kg bw/day):	**0,04**	ARfD (mg/kg bw):	**n.a.**
Source of ADI:	**Dir. 2008/44/EC**	Source of ARfD:	**Dir. 2008/44/EC**
Year of evaluation:	**2002-2008**	Year of evaluation:	**2002-2008**

Chronic risk assessment

TMDI (range) in % of ADI minimum - maximum	
10	72
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)
72,2	WHO Cluster diet B	12,6	Sugar cane	7,8	Citrus fruit	7,6	Table and wine grapes	
64,1	DE child	23,0	Citrus fruit	12,7	Apples	4,5	Table and wine grapes	
62,3	NL child	20,3	Citrus fruit	6,6	Apples	5,2	Spinach	
54,3	E adult	13,4	Citrus fruit	4,5	Other leafy brassica	4,1	Table and wine grapes	
49,5	FR toddler	11,7	Citrus fruit	9,9	Spinach	4,2	Leek	
48,9	UK Toddler	22,9	Sugar beet (root)	11,5	Citrus fruit	1,8	Apples	
37,0	WHO cluster diet E	6,4	Table and wine grapes	4,2	Citrus fruit	3,9	Other tropical root and tuber	
35,2	ES child	11,7	Citrus fruit	6,0	Beet leaves (chard)	5,8	Lettuce and other salad plants	
32,2	ES adult	7,5	Lettuce and other salad plants	7,2	Citrus fruit	6,0	Beet leaves (chard)	
31,1	UK Infant	10,1	Sugar beet (root)	6,7	Citrus fruit	1,9	Milk and cream,	
31,1	FR all population	14,6	Table and wine grapes	4,5	Lettuce and other salad plants	3,3	Citrus fruit	
31,0	SE general population 90th percentile	6,9	Citrus fruit	5,6	Lettuce and other salad plants	1,7	Head cabbage	
30,8	FR infant	6,2	Spinach	5,3	Citrus fruit	2,7	Beans (with pods)	
30,3	NL general	9,3	Citrus fruit	3,4	Lettuce and other salad plants	3,0	Table and wine grapes	
27,3	IT adult	7,8	Lettuce and other salad plants	4,7	Beet leaves (chard)	2,9	Citrus fruit	
26,8	IT kids/toddler	5,8	Lettuce and other salad plants	4,5	Beet leaves (chard)	3,7	Citrus fruit	
26,7	WHO regional European diet	5,8	Lettuce and other salad plants	4,1	Citrus fruit	1,4	Table and wine grapes	
26,2	WHO Cluster diet F	5,7	Citrus fruit	4,2	Lettuce and other salad plants	2,6	Table and wine grapes	
24,6	WHO cluster diet D	2,8	Wheat	2,5	Beet leaves (chard)	2,3	Citrus fruit	
24,2	PT General population	9,8	Table and wine grapes	3,8	Citrus fruit	1,7	Wheat	
21,7	UK vegetarian	5,2	Citrus fruit	3,8	Sugar beet (root)	3,2	Table and wine grapes	
21,1	DK child	3,0	Cucurbits - edible peel	2,4	Apples	2,3	Wheat	
18,7	UK Adult	4,0	Table and wine grapes	4,0	Sugar beet (root)	3,5	Citrus fruit	
14,5	DK adult	5,2	Table and wine grapes	1,8	Lettuce and other salad plants	1,3	Citrus fruit	
14,1	FI adult	5,7	Citrus fruit	1,2	Table and wine grapes	1,1	Lettuce and other salad plants	
10,3	PL general population	2,1	Apples	1,1	Table and wine grapes	1,0	Head cabbage	
9,6	LT adult	2,0	Apples	1,1	Head cabbage	0,9	Lettuce and other salad plants	

Metabolites

The main purpose of the information presented below is to support the development of a robust residue definition for risk assessment purposes. Therefore, the data being summarized are going far beyond the scope of this dossier. The indicative assessment is based on the available exposure data. This includes extrapolations for feed item metabolites and the use of metabolism studies, rotational crop studies and magnitude of the residue studies for a comprehensive evaluation of residue input values.

For facilitating the evaluation of the performed exposure assessment, the relevant data for the metabolites are derived in a separate report summarized below.

Report: CA 6.9/1
Rabe U.,Bohner A., 2015 a
Supplemental information - Boscalid (BAS 510 F) - Application of the TTC-Concept to BAS 510 F metabolites in plant and animal commodities
2015/1245132
Guidelines: none
GLP: no

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test Material:** BAS 510 F (Boscalid)
Description: not relevant
Lot/Batch #: not relevant
Purity: not relevant
CAS#: 188425-85-6
Development code: not applicable
Spiking levels: not relevant

B. STUDY DESIGN AND METHODS

In order to assess the contribution of boscalid metabolites to the chronic dietary risk, the input data for the exposure assessment are derived differently for the individual metabolites.

For M510F47 input values were derived from the bean metabolism study, while input values for M510F61 were derived from the confined rotational crop study.

For metabolite M510F54 found in animal matrices, input values were taken from the livestock metabolism study. As metabolites M510F01 and M510F02 are being part of the residue definition for risk assessment, parent MRL values were used as base to derive input values.

II. RESULTS AND DISCUSSION

M510F47

With the current EFSA PRIMo model the chronic risk assessment ranges from 0 to 9.9% of ADI. The diet with the highest TMDI is “FR toddler” with 9.9% of ADI. For this diet the highest contributors are beans with pods.

M510F61

With the current EFSA PRIMo model the chronic risk assessment ranges from 0.3 to 5.8% of ADI. The diet with the highest TMDI is “FR toddler” with 5.8% of ADI. For this diet the highest contributors are other root and tuber vegetables.

M510F01 and M510F02

With the current EFSA PRIMo model the chronic risk assessment ranges from 0.2 to 2.4% of ADI. The diet with the highest TMDI is “FR toddler” with 2.4% of ADI. For this diet the highest contributors are milk and cream.

M510F54

With the current EFSA PRIMo model the chronic risk assessment ranges from 0.8 to 4.5% of ADI. The diet with the highest TMDI is “UK infant” with 4.5% of ADI. For this diet the highest contributors are birds’ eggs.

III. CONCLUSION

Boscalid follows a common pathway in crops and livestock. In general, the following key transformation steps were found:

- Glutathione conjugation of the pyridine and the diphenyl ring system
- Hydroxylation of the aromatic ring systems

These steps are common in metabolism of all tested species; the differences observed are more on the quantitative level.

For performing an indicative assessment, the exposure was estimated based on all available data (mainly of metabolism information and data from succeeding crop residue). Subsequently, chronic dietary exposure assessments were performed for identifying the contributions of the metabolites to the total dietary risk.

The exposure estimates applying worst-case assumptions did not indicate any dietary concern. The calculation of the % ADI utilization resulted in values far below 100%. No significant contribution to the chronic dietary risk can be expected from any plant or livestock metabolite.

Acute Reference Dose (ARfD) and Dietary Exposure Calculation

Boscalid

No Acute Reference Dose (ARfD has been set or is considered necessary (Monograph 2002; EFSA Journal 2014;12(7):3799).

The 2006 JMPR also decided that an ARfD is unnecessary: "*The Meeting concluded that it was not necessary to establish an ARfD for boscalid in view of the well-demonstrated lack of toxicity in studies of acute toxicity, the absence of relevant developmental toxicity that could have occurred as a consequence of a single exposure, the absence of any indication of neurotoxicity and the absence of any other adverse effects that would be likely to be induced after a single or a small number of exposures in repeat-dose studies."*

The statements of EFSA and JMPR are still valid - no ARfD is necessary for boscalid. Therefore, acute exposure calculations were not carried out for boscalid (BAS 510 F).

Metabolites

Boscalid technical material has a low acute toxicity and is devoid of genotoxic, carcinogenic, neurotoxic and reprotoxic concerns. Since the data for parent do not lead to the need for an ARfD, no ARfD is proposed for the metabolites, either.

Based on the different calculations made to estimate the risk for consumer through diet and other means it can be concluded that the use of the BAS 510 F does not lead to unacceptable risk for consumer when applied according to the recommendations.

CA 6.10 Other studies

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. Studies determining the residues in oilseed rape honey after application of BAS 510 01 F are summarized in the following.

CA 6.10.1 Effect on the residue level in pollen and bee products

The representative uses in this dossier are grapes, oilseed rape, beans and peas. Of these crops, grapes and oilseed rape could be relevant for bees and honey production. In the EFSA Guidance Document on the risk assessment of plant protection products on bees (*Apis mellifera, Bombus* spp. and solitary bees (see EFSA Journal 2013;11(7):3295) the attractiveness of grapes for honeybees is only mentioned for pollen, while for nectar the attractiveness is low. For oilseed rape however, an attractiveness to honeybees for nectar and the presence of nectar/honeydew, i.e. melliferous capacity is stated. Therefore, new studies have been performed to determine boscalid residues in bee products for human consumption resulting from residues taken up by honey bees from oilseed rape at blossom. According to the proposed GAP for oilseed rape, application was performed at growth stage BBCH 13-75, i.e. leaf unfolding, inflorescence, flowering and development of fruit. Boscalid residues in oilseed rape honey are considered representative for all sorts of honey and therefore sufficient to cover all boscalid uses with application at blossom.

Report:	CA 6.10.1/1 Rabe U.,Mackenroth C., 2004 a Report of the analytical phase - Non-GLP study: Residue in honey from bee colonies (Apis mellifera L.) after the application of Cantus (BAS 510 01 F) at different dates on winter oil-seed rape 2004/1006463
Guidelines:	none
GLP:	no

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description: BAS 510 01 F (WG), Boscalid (BAS 510 F)
Lot/Batch #: 2002-7: 0.5 kg nominal
CAS#: 188425-85-6, Boscalid (BAS 510 F)
Development code: BAS 510 01 F (WG),
Spiking levels: 0.05, 0.5 mg/kg

3. Test Commodity:

Crop: Winter oilseed rape / Honey
Type: Honey bee
Variety: Not reported
Botanical name: *Brassica napus / Apis mellifera*
Crop parts(s) or processed Commodity: Honey
Sample size: Not reported

B. STUDY DESIGN AND METHODS

1. Test procedure

In 2003, one trial was conducted under semi field conditions using tunnel tents in Germany to determine the magnitude of the residues in oilseed rape honey of bee colonies placed in the tents. The fungicidal product BAS 510 01 F (WG, 500 g/kg boscalid), was foliar applied to winter oilseed rape once at a target rate equivalent to 0.25 kg a.s./ha of formulated product in a spray volume of 400 L/ha. The application was conducted before flowering at BBCH growth stage 59 in a first variant and during full flowering (BBCH 65) in the second and third variant. Thereby, the application was done in the evening before exposure of the bees or during foraging activity of the bees, in the second and third variant, respectively. The colonies remained inside the tents up to the end of flowering. Honey samples were taken twice after the start of bee exposure in tents, from all three variants. The first sampling was carried out 21, 8 or 7 days after the respective treatment. After the end of flowering, the second sampling was performed 26, 13 and 12 days after the application.
The honey collected from the bees was analyzed for residues of BAS 510 F.
All specimens were frozen immediately after specimen preparation and remained frozen (≤ 18°C) until analysis including transportation. The maximum storage interval from harvest until extraction for analysis of boscalid was 208 days.

Table 6.10.1-1: Target application rates and timings for oilseed rape (honey production)

Year	No of trials	No of appl.	F P or G	Test item	Active substance	Appl. rate (kg a.s./ha)	Water volume (L/ha)	Target date/BBCH
2003	1	1	P	BAS 510 01 F (WG)	BAS 510 F	0.25	400	BBCH 59 BBCH 65

2. Description of analytical procedures

The honey collected from the bees was homogenized with dry ice prior to analysis using BASF method No. 445/0 (L0076/01). Boscalid (BAS 510 F) was extracted with a mixture of methanol, water and hydrochloric acid. An aliquot of the extract was centrifuged and partitioned against cyclohexane. The final determination of the analytes was performed by HPLC-MS/MS with a limit of quantitation (LOQ) of 0.05 mg/kg. The results of procedural recovery experiments were about 95% for BAS 510 F.

Table 6.10.1-2: Summary of procedural recovery data for boscalid

Matrix	Fortification level (mg/kg)	Summary recoveries		
		n	Mean (%)	RSD (%)
BASF method No 445/0 (L0076/01); LOQ = 0.05 mg/kg		**Boscalid**		
Honey	0.05, 0.5	2	94.9	1.5

II. RESULTS AND DISCUSSION

The residue ranges of boscalid treated with formulation BAS 510 01 F are summarized in the table below. Details are presented in Table 6.10.1-4.
At the first sampling, no boscalid residues were found above LOQ in honey from variant 1 (application before flowering) and variant 2 (application in the evening), 8 to 21 days after treatment. In honey from variant 3, where the application of BAS 510 01 F was performed during foraging activity of the bees, boscalid residues of 0.064 mg/kg were found 7 days after application. No residues of boscalid above the LOQ were observed in honey samples of the second sampling.
In control samples, no residues of BAS 510 F at or above LOQ was observed.

Table 6.10.1-3: Summary of residues in bee honey from oilseed rape treated with BAS 510 01 F

Crop	Year	Sampling	Variant	DAA[1]	Growth stage[2] (BBCH)	Boscalid (mg/kg)	
						Matrix	BAS 510 F
Oilseed rape	2003	1	1	21	59	Honey	<0.05
			2	8	65	Honey	<0.05
			3	7	65	Honey	0.064
Oilseed rape	2003	2	1	26	59	Honey	<0.05
			2	13	65	Honey	<0.05
			3	12	65	Honey	<0.05

1 Days after last application
2 At application

III. CONCLUSION

After application of BAS 510 01 F, before flowering and during full flowering at 7-26 days after application, the residues of boscalid in oilseed rape honey ranged between <0.05 mg/kg and 0.064 mg/kg.

Table 6.10.1-4: Residues of boscalid in oilseed rape honey after application of BAS 510 01 F in Northern Europe

Trial details		Crop	Country	Formulation, Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	Sampling	DALA[1]	Residues found (mg/kg)	
								Matrix	BAS 510 F
Study code:	20031165/01-BZEU	Oilseed rape	Germany	BAS 510 01 F 1 x 0.25	59	1	7	Honey	0.064
Doc ID:	2004/1006463				65		8		<0.05
Trial No:	1				65		21		<0.05
GLP:	No				59	2	12		<0.05
Year:	2003				65		13		<0.05
					65		26		<0.05

0 Actual application rates varied by 10% at most
1 Days after last application
2 At application
_ underlined values used for MRL calculation

Report: CA 6.10.1/2
Rabe U.,Mackenroth C., 2004 b
Report on the analytical phase - Non-GLP study: Residue in honey from bee colonies (Apis mellifera L.) after the application of Cantus (BAS 510 01 F) at different dates on oil-seed rape
2004/1006464
Guidelines: none
GLP: no

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:
Description: BAS 510 01 F (WG)
Lot/Batch #: 2002-7: 0.5 kg nominal
Purity:
CAS#: 188425-85-6, Boscalid
Development code:
Spiking levels: 0.05, 0.5 mg/kg

3. Test Commodity:
Crop: Winter oilseed rape / Honey
Type: Honey bee
Variety: **N**ot reported
Botanical name: *Brassica napus / Apis mellifera*
Crop parts(s) or processed
Commodity: Honey
Sample size: **N**ot reported

B. STUDY DESIGN AND METHODS

1. Test procedure

In 2003, six trials were conducted in Germany to determine the magnitude of the residues in oilseed rape honey. Bee colonies were placed in or nearby oilseed rape fields treated with the fungicidal product BAS 510 01 F (WG, 500 g/kg boscalid). During exposure of the colonies for 4-6 weeks in May/June, BAS 510 01 F was applied once at a target rate equivalent of 0.4 to 0.5 kg/ha of the formulated product. The application was performed between 2 and 14 days after placing the colonies to oilseed rape fields. After the exposure time, honey samples were taken.
The samples collected from the bees was analyzed for residues of BAS 510 F.
All specimens were frozen immediately after specimen preparation and remained frozen (≤18°C) until analysis including transportation. The maximum storage interval from harvest until extraction for analysis of boscalid was 132 days.

Table 6.10.1-5: Target application rates and timings for oilseed rape (honey production)

Year	No of trials	No of appl.	F P or G	Test item	Active substance	Appl. rate (kg a.s./ha)	Water volume (L/ha)	Target date/ BBCH
2003	6	1	F	BAS 510 01 F (WG)	BAS 510 F	0.25	n.r.	n.r.

n.r. Not reported

1. Description of analytical procedures

The honey collected from the bees was homogenized with dry ice prior to analysis using BASF method No. 445/0 (L0076/01). Boscalid (BAS 510 F) was extracted with a mixture of methanol, water and hydrochloric acid. An aliquot of the extract was centrifuged and partitioned against cyclohexane. The final determination of the analytes was performed by HPLC-MS/MS with a limit of quantitation (LOQ) of 0.05 mg/kg. The results of procedural recovery experiments were about 95% for BAS 510 F.

Table 6.10.1-6: Summary of procedural recovery data for boscalid

Matrix	Fortification level (mg/kg)	Summary recoveries		
		n	Mean (%)	RSD (%)
BASF method No 445/0 (L0076/01); LOQ = 0.05 mg/kg		**Boscalid**		
Honey	0.05, 0.5	2	95	N/A

N/A Not applicable

II. RESULTS AND DISCUSSION

The residue ranges of boscalid treated with formulation BAS 510 01 F are shown in the table below. Details are presented in Table 6.10.1-8.
Boscalid residues above LOQ were detected in none of the honey samples analyzed. In control samples, no residues of BAS 510 F at or above LOQ were observed.

Table 6.10.1-7: Summary of residues in bee honey from oilseed rape treated with BAS 510 01 F

Crop	Year	DALA[1]	Growth stage[2] (BBCH)	Boscalid (mg/kg)	
				Matrix	BAS 510 F
Oilseed rape	2003	15-42	n r	Honey	<0.05

1 Days after last application
2 At application

III. CONCLUSION

After application of BAS 510 01 F on oilseed rape, the residues of boscalid in oilseed rape honey were below the LOQ of 0.05 mg/kg.

Table 6.10.1-8: Residues of boscalid in oilseed rape honey after application of BAS 510 01 F in Northern Europe

Trial details	Crop	Country	Formulation, Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1]	Residues found (mg/kg)	
						Matrix	BAS 510 F
Study code: n.r. **Doc ID:** 2004/1006464 **Trial No:** 1 (24245 Kleinbarkau) **GLP:** No **Year:** 2003	Oilseed rape	Germany	BAS 510 01 F 1 x 0.25	n.r.	28	Honey	<u>≤0.05</u>
Study code: n.r. **Doc ID:** 2004/1006464 **Trial No:** 2 (78199 Braeunlingen) **GLP:** No **Year:** 2003	Oilseed rape	Germany	BAS 510 01 F n r.	n.r.	42	Honey	<u>≤0.05</u>
Study code: n.r. **Doc ID:** 2004/1006464 **Trial No:** 3 (73072 Donzdorf) **GLP:** No **Year:** 2003	Oilseed rape	Germany	BAS 510 01 F 1 x 0.400	n.r.	17	Honey	<u>≤0.05</u>
Study code: n.r. **Doc ID:** 2004/1006464 **Trial No:** 4 (73072 Donzdorf) **GLP:** No **Year:** 2003	Oilseed rape	Germany	BAS 510 01 F 1 x 0.20	n.r.	15	Honey	<u>≤0.05</u>

Table 6.10.1-8: Residues of boscalid in oilseed rape honey after application of BAS 510 01 F in Northern Europe

Trial details		Crop	Country	Formulation, Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1]	Residues found (mg/kg)	
							Matrix	BAS 510 F
Study code:	n.r.	Oilseed rape	Germany	BAS 510 01 F 1 x 0.25	n.r.	22	Honey	<u>≤0.05</u>
Doc ID:	2004/1006464							
Trial No:	5 (25361 Elskop)							
GLP:	No							
Year:	2003							
Study code:	n.r.	Oilseed rape	Germany	BAS 510 01 F n r.	n.r.	29	Honey	<u>≤0.05</u>
Doc ID:	2004/1006464							
Trial No:	6 (27318 Wienbergen)							
GLP:	No							
Year:	2003							

0 Actual application rates varied by 10% at most
1 Days after last application
2 At application
n.r. Not reported
_ underlined values used for MRL calculation

Report:	CA 6.10.1/3 Renner G., 2006 a Determination of residues of Boscalid in rape honey after application of BAS 510 01 F (Cantus) in oilseed rape during full flowering (BBCH 65) in Germany 2006/1002522
Guidelines:	EEC 7029/VI/95 rev. 5, BBA IV 3-3, IVA Guideline I-III (1992), EPPO PP 1/170 (3) (2000), BBA VI 23-1
GLP:	yes (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	BAS 510 01 F (WG)
Lot/Batch #:	1302, 50% nominal
Purity:	
CAS#:	188425-85-6, Boscalid
Development code:	
Spiking levels:	0.01, 5.0 mg/kg

3. Test Commodity:

Crop:	Winter oilseed rape / Honey
Type:	Honey bee
Variety:	Express, Maja, Aviso, Maplus
Botanical name:	*Brassica napus / Apis mellifera*
Crop parts(s) or processed Commodity:	Honey
Sample size:	1.2-2.5 kg (shoot); 0.88-1.02 kg (honey)

B. STUDY DESIGN AND METHODS

1. Test procedure

During the 2008 growing season, four field trials were conducted in different representative oilseed rape growing areas in Germany to determine the residual behavior of BAS 510 F (boscalid) in oilseed rape shoots and rape honey. The fungicidal product BAS 510 01 F (WD, boscalid, 50% nominal) was foliar applied to winter oilseed rape once at a target rate corresponding to 0.5 kg/ha of product formulation (nominal 250 g a.s./ha) in a spray volume of 200 L/ha. The application was conducted during full flowering at BBCH 65. Three hives with one brood body each were placed at the border of each field 2-3 days before application. Immediately before application (day 0), one honey body with empty honey combs was set up on each brood body. Whole plant without roots specimens were sampled directly before and after application and the honey specimens were sampled 23±3 days after application. All specimens were frozen immediately after specimen preparation and remained frozen (≤18°C) until analysis including transportation. The maximum storage interval from harvest until extraction for analysis of boscalid was 420 days.

Table 6.10.1-9: Target application rates and timings for oilseed rape (honey production)

Year	No of trials	No of appl.	F P or G	Test item	Active substance	Appl. rate (kg a.s./ha)	Water volume (L/ha)	Target date/BBCH
2004	4	1	F	BAS 510 01 F (WG)	BAS 510 F	0.25	200-300	BBCH 65

2. Description of analytical procedures

Samples were analyzed for boscalid residues using BASF method No 445/0 (L0076/01). Boscalid (BAS 510 F) was extracted with a mixture of methanol, water and hydrochloric acid. An aliquot of the extract was centrifuged and partitioned at alkaline conditions against cyclohexane. The final determination was performed by LC-MS/MS with a limit of quantitation (LOQ) of 0.01 mg/kg for honey and 0.05 mg/kg for the shoots.

Table 6.10.1-10: Summary of procedural recovery data for boscalid

Matrix	Fortification level (mg/kg)	Summary recoveries		
		n	Mean (%)	RSD (%)
BASF method No 445/0 (L0076/01); LOQ = 0.01 mg/kg (honey); 0.05 mg/kg (shoots)		**Boscalid**		
Whole plant w/o root	0.05, 0.5, 5.0	3	94	4.9
Honey	0.01, 0.1	12	79	11

II. RESULTS AND DISCUSSION

The residue ranges of boscalid treated with formulation BAS 510 01 F are summarized in Table 6.10.1-11. Details are presented in Table 6.10.1-12.
Boscalid residues in whole plant without root specimens at 0 DALA (BBCH 65) ranged between 0.53-2.37 mg/kg. Residues in honey samples taken 20-26 DALA ranged between 0.01-0.08 mg/kg. No residues of boscalid were found in the control samples above the LOQ.

Table 6.10.1-11: Summary of residues in oilseed rape treated with BAS 510 01 F

Crop	Year	DALA[1]	Growth stage[2] (BBCH)	Boscalid (mg/kg)	
				Matrix	BAS 510 F
Oilseed rape	2004	0	65	Whole plant without roots	0.53-2.37
		20-26	69	Honey	0.01-0.08

1 Days after last application
2 At sampling

III. CONCLUSION

After application of BAS 510 01 F on oilseed rape, the residues of boscalid in oilseed rape honey were between 0.01 to 0.08 mg/kg.

Table 6.10.1-12: Residues of boscalid in oilseed rape honey after application of BAS 510 01 F in Northern Europe

Trial details		Crop	Country	Formulation, Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1]	Residues found (mg/kg)	
							Matrix	BAS 510 F
Study code: **Doc ID:** **Trial No:** **GLP:** **Year:**	200248 2006/1002522 FR 07/04/37 Yes 2004	Oilseed rape	Germany	BAS 510 01 F 1 x 0.250	65	0 26	Whole plant without root Honey	0.53 0.08
Study code: **Doc ID:** **Trial No:** **GLP:** **Year:**	200248 2006/1002522 FR 07/04/51 Yes 2004	Oilseed rape	Germany	BAS 510 01 F 1 x 0.250	65	0 20	Whole plant without root Honey	1.89 0.06
Study code: **Doc ID:** **Trial No:** **GLP:** **Year:**	200248 2006/1002522 FR 07/04/52 Yes 2004	Oilseed rape	Germany	BAS 510 01 F 1 x 0.250	65	0 24	Whole plant without root Honey	1.18 0.01
Study code: **Doc ID:** **Trial No:** **GLP:** **Year:**	200248 2006/1002522 FR 07/04/61 Yes 2004	Oilseed rape	Germany	BAS 510 01 F 1 x 0.250	65	0 21	Whole plant without root Honey	2.37 0.02

0 Actual application rates varied by 10% at most
1 Days after last application
2 At application
_ underlined values used for MRL calculation

Appendix: Tier 1 Summaries of the Supervised Field Residue Trials

Bean

Green Bean

Northern Europe

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	BAS 510 F (Boscalid)	**Commercial Product (name)**	-
Crop/crop group:	Green Bean/Beans	**Producer of commercial product**	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	**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)	50%		
Formulation (e.g. WP)	WG (BAS 510 01 F)	**Residues calculated as:**	BAS 510 F

1	2	3		4	5			6	7	8	9	10	11
Report-No Location (Trial No)	**Commodity/ Variety**	**1 2 3**	**Date of Sowing / Planting Flowering Harvest**	**Method of treatment**	**Application rate[0] per treatment**			**No of treatm. and last date**	**Growth stage (BBCH)[2]**	**Portion analyzed**	**Residues (mg/kg)**	**DALA[1]**	**Remarks**
					kg a.s./hL	**Water (L/ha)**	**kg a.s./ha**				**BAS 510 F**		
309368 2008/1028266 8255 RE Swifterbant Netherlands (L070848)	VP 0526 Cantara	1 2 3	24.04.2007 10.6.07-08.07.07 19.07.2007	Plotsprayer (Agrartest)	0.167	300	0.500	2 23.07.2007	88	Beans with pods Rest of plant* Beans with pods Rest of plant* Beans with pods Rest of plant* Beans with pods Rest of plant*	0.98 19.73 0.29 6.40 0.12 1.90 0.10 1.52	0 0 3 3 7 7 15 15	BASF method SOP-PA.0243 (based on BASF Method No. 445/0 and No. 535/1) LOQ = 0.01 mg/kg
309368 2008/1028266 80400 Esmery Hallon France (L070849)	VP 0526 Sopra	1 2 3	15.06.2007 20.07.07-05.08.07 04.09.2007	Agrotop - Sprayer with booms	0.167	300	0.500	2 21.08.2007	81	Beans with pods Rest of plant* Beans with pods Rest of plant* Beans with pods Rest of plant* Beans with pods Rest of plant*	0.76 12.86 0.50 6.94 0.42 7.78 0.77 6.60	0 0 3 3 7 7 14 14	

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	BAS 510 F (Boscalid)	**Commercial Product (name)**	-
Crop/crop group:	Green Bean/Beans	**Producer of commercial product**	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	**Indoor/Glasshouse/Outdoor**	Outdoor
Country	Germany	**Other active substance in the formulation**	None
Content of active substance (g/kg or g/L)	50%	**(common name and content)**	
Formulation (e.g. WP)	WG (BAS 510 01 F)	**Residues calculated as:**	BAS 510 F

1	2	3		4	5			6	7	8	9	10	11
Report-No Location (Trial No)	**Commodity/ Variety**	**1 2 3**	**Date of Sowing / Planting Flowering Harvest**	**Method of treatment**	**Application rate[0] per treatment**			**No of treatm. and last date**	**Growth stage (BBCH)[2]**	**Portion analyzed**	**Residues (mg/kg)**	**DALA[1]**	**Remarks**
					kg a.s./hL	**Water (L/ha)**	**kg a.s./ha**				**BAS 510 F**		
309368 2008/1028266 69121 Heidelberg Germany (L070850)	VP 0526 Phantheon	1 2 3	19.06.2007 10.07.07-18.08.07 24.08.2007	Plotsprayer (Agrartest)	0.167	300	0.500	2 23.08.2007	83	Beans with pods Rest of plant* Beans with pods Rest of plant* Beans with pods Rest of plant* Beans with pods Rest of plant*	1.14 12.74 0.49 11.73 0.61 7.05 0.32 7.28	0 0 3 3 7 7 15 15	BASF method SOP-PA.0243 (based on BASF Method No. 445/0 and No. 535/1) LOQ = 0.01 mg/kg
309368 2008/1028266 CV37 9SJ Warwickshire United Kingdom (L070851)	VP 0526 White Apollo	1 2 3	25.03.2007 01.06.07-27.07.07 02.07.2007	Stihl Mistblower SR420	0.167	300	0.500	2 08.08.2007	79	Beans with pods Rest of plant* Beans with pods Rest of plant* Beans with pods Rest of plant* Beans with pods Rest of plant*	2.80 20.19 1.92 14.49 1.59 15.11 0.76 11.49	0 0 3 3 7 7 14 14	

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
* Without roots

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	BAS 510 F (Boscalid)	**Commercial Product (name)**	-
Crop/crop group:	Green Beans/Beans	**Producer of commercial product**	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	**Indoor/Glasshouse/Outdoor**	Outdoor
Country	Germany	**Other active substance in the formulation**	None
Content of active substance (g/kg or g/L)	500 g/kg	**(common name and content)**	
Formulation (e.g. WP)	WG (BAS 510 01 F)	**Residues calculated as:**	BAS 510 F

1	2	3		4	5			6	7	8	9	10	11
Report-No Location (Trial No)	**Commodity/ Variety**	**1. 2. 3.**	**Date of Sowing / Planting Flowering Harvest**	**Method of treatment**	**Application rate[0] per treatment**			**No of treatm. and last date**	**Growth stage (BBCH)[2]**	**Portion analyzed**	**Residues (mg/kg)**	**DALA[1]**	**Remarks**
					kg a.s./hL	**Water (L/ha)**	**kg a.s./ha**				**BAS 510 F**		
309349 2010/1165744 69121 Heidelberg Germany (L090151)	VP 0526 Tamara	1. 2. 3.	10.07.2009 15.08.-18.08.09 03.09.-16.09.09	BPS Agrotop	0.17	300	0.50	2 22.09.2009	78	Pods with seeds Rest of plant* Pods with seeds Rest of plant* Pods with seeds Rest of plant* Pods without seeds Seeds Pods with seeds Rest of plant* Pods without seeds Seeds	2.03 32.40 1.18 31.40 1.44 37.10 1.72 0.25 1.62 34.60 2.41 0.09	0 0 2 2 7 7 7 7 14 14 14 14	BASF method Method No. L0076/01 LOQ = 0.01 mg/kg
309349 2010/1165744 8255 RE Swifterbant The Netherlands (L090152)	VP 0526 Nagano	1. 2. 3.	11.05.2009 05.06.-14.07.09 04.08.2009	Plot sprayer (Agrartest)	0.17	300	0.50	2 11.08.2009	83	Pods with seeds Rest of plant* Pods with seeds Rest of plant* Pods with seeds Rest of plant* Pods without seeds Seeds Pods with seeds Rest of plant* Pods without seeds Seeds	0.76 30.70 0.58 20.00 0.97 18.30 1.18 0.09 1.40 22.30 1.57 0.12	0 0 3 3 6 6 6 6 14 14 14 14	BASF method Method No. L0076/01 LOQ = 0.01 mg/kg

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	BAS 510 F (Boscalid)	**Commercial Product (name)**	-
Crop/crop group:	Green Beans/Beans	**Producer of commercial product**	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	**Indoor/Glasshouse/Outdoor**	Outdoor
Country	Germany	**Other active substance in the formulation**	None
Content of active substance (g/kg or g/L)	500 g/kg	**(common name and content)**	
Formulation (e.g. WP)	WG (BAS 510 01 F)	**Residues calculated as:**	BAS 510 F

1	2	3		4	5			6	7	8	9	10	11
Report-No Location (Trial No)	**Commodity/ Variety**	**1. 2. 3.**	**Date of Sowing / Planting Flowering Harvest**	**Method of treatment**	**Application rate[0] per treatment**			**No of treatm. and last date**	**Growth stage (BBCH)[2]**	**Portion analyzed**	**Residues (mg/kg)**	**DALA[1]**	**Remarks**
					kg a.s./hL	**Water (L/ha)**	**kg a.s./ha**				**BAS 510 F**		
309349 2010/1165744 72250 Parigné l'Evèque Northern France (L090153)	VP 0526 Flagrano	1. 2. 3.	29.05.2009 18.07.-29.07.09 23.09.2009	Sprayer with booms (Agrotop)	0.17	300	0.50	2 08.09.2009	79	Pods with seeds Rest of plant* Pods with seeds Rest of plant* Pods with seeds Rest of plant* Pods without seeds Seeds Pods with seeds Rest of plant* Pods without seeds Seeds	3.81 32.20 2.42 26.20 3.62 23.00 5.00 1.46 1.86 10.70 8.15 0.59	0 0 2 2 6 6 6 6 13 13 13 13	BASF method Method No. L0076/01 LOQ = 0.01 mg/kg
309349 2010/1165744 6280 Gerpinnes Belgium (L090154*)	VP 0526 Flagrano	1. 2. 3.	10.06.2009 20.07.-04.08.09 15.09.2009	Sprayer with booms (Agrotop)	0.17	300	0.50	2 02.09.2009	79	Pods with seeds Rest of plant* Pods with seeds Rest of plant* Pods with seeds Rest of plant* Pods without seeds Seeds Pods with seeds Rest of plant* Pods without seeds Seeds	1.90 47.00 1.20 45.50 1.28 44.90 2.01 0.07 1.12 28.70 2.37 0.03	0 0 2 2 6 6 6 6 13 13 13 13	BASF method Method No. L0076/01 LOQ = 0.01 mg/kg

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
* Without roots

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	BAS 510 F (Boscalid)	**Commercial Product (name)**	-
Crop/crop group:	Green Beans/Beans	**Producer of commercial product**	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	**Indoor/Glasshouse/Outdoor**	Outdoor
Country	Germany	**Other active substance in the formulation**	None
Content of active substance (g/kg or g/L)	500 g/kg	**(common name and content)**	
Formulation (e.g. WP)	WG (BAS 510 01 F)	**Residues calculated as:**	BAS 510 F

1	2	3		4	5			6	7	8	9	10	11
309371 2011/1251203 6280 Gerpinnes Belgium (L100613)	VP 0526 Flagrano	1. 2. 3.	05.07.2010 28.07.-13.08.10 16.09.2010	Agrotop Plot Sprayer	0.17	300	0.50	2 09.09.2010	78	Pods with seeds Rest of plant* Pods with seeds Rest of plant* Pods with seeds Rest of plant* Pods with seeds Rest of plant* Pods without seeds Seeds	0.46 20 0.39 11 0.41 8.7 0.62 11 0.59 0.032	0 0 4 4 7 7 13 13 13 13	BASF method Method No. L0076/01 LOQ = 0.01 mg/kg

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
* Without roots

Southern Europe

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	BAS 510 F (Boscalid)	**Commercial Product (name)**	-
Crop/crop group:	Green Beans/Beans	**Producer of commercial product**	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	**Indoor/Glasshouse/Outdoor**	Outdoor
Country	Germany	**Other active substance in the formulation**	None
Content of active substance (g/kg or g/L)	500 g/kg	**(common name and content)**	
Formulation (e.g. WP)	WG (BAS 510 01 F)	**Residues calculated as:**	BAS 510 F

1	2	3		4	5			6	7	8	9	10	11
309349 2010/1165744 47120 Duras Southern France (L090155)	VP 0526 Flagrano	1. 2. 3.	18.06.2009 05.08.-25.08.09 20.09.2009	Agrotop plot sprayer	0.17	300	0 50	2 08.09.2009	79	Pods with seeds Rest of plant* Pods with seeds Rest of plant* Pods with seeds Rest of plant* Pods without seeds Seeds Pods with seeds Rest of plant* Pods without seeds Seeds	1.01 35.00 0.56 12.20 0.33 4.54 0.56 <0.01 0.15 1.84 0.23 <0.01	0 0 2 2 8 8 8 8 14 14 14 14	BASF method Method No. L0076/01 LOQ = 0.01 mg/kg
309349 2010/1165744 GR 57500 Greece (L090156)	VP 0526 Etna	1. 2. 3.	23.06.2009 01.08.-28.08.09 05.09.-30.09.09	Pressurized gas sprayer AZO with 8-nozzle boom	0.17	300	0 50	2 08.09.2009	77	Pods with seeds Rest of plant* Pods with seeds Rest of plant* Pods with seeds Rest of plant* Pods without seeds Seeds Pods with seeds Rest of plant* Pods without seeds Seeds	0.45 25.30 0.62 19.40 0.23 7.55 0.25 0.02 0.15 4.77 0.18 0.03	0 0 3 3 7 7 7 7 14 14 14 14	BASF method Method No. L0076/01 LOQ = 0.01 mg/kg
309349 2010/1165744 48010 Barbianto di Cotignola Italy (L090157)	VP 0526 Flavert	1. 2. 3.	08.05.2009 21.06.-02.07.09 17.07.2009	Echo SHR 150-SI	0.17	300	0 50	2 10.07.2009	75-77	Pods with seeds Rest of plant* Pods with seeds Rest of plant* Pods with seeds Rest of plant* Pods without seeds Seeds Pods with seeds Rest of plant* Pods without seeds Seeds	1.15 30.20 0.37 6.10 0.28 4.55 0.39 0.07 0.37 2.31 0.36 0.05	0 0 3 3 7 7 7 7 14 14 14 14	BASF method Method No. L0076/01 LOQ = 0.01 mg/kg

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	BAS 510 F (Boscalid)	**Commercial Product (name)**	-
Crop/crop group:	Green Beans/Beans	**Producer of commercial product**	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	**Indoor/Glasshouse/Outdoor**	Outdoor
Country	Germany	**Other active substance in the formulation**	None
Content of active substance (g/kg or g/L)	500 g/kg	**(common name and content)**	
Formulation (e.g. WP)	WG (BAS 510 01 F)	**Residues calculated as:**	BAS 510 F

1	2	3		4	5			6	7	8	9	10	11
309349 2010/1165744 29001 Málaga Spain (L090158	VP 0526 Marconi	1. 2. 3.	25.05.2009 01.07.-15.07.09 15.07.2009	Atomizer Stihl SR 420	0.17	300	0 50	2 06.07.2009	79	Pods with seeds Rest of plant* Pods with seeds Rest of plant* Pods with seeds Rest of plant* Pods without seeds Seeds Pods with seeds Rest of plant* Pods without seeds Seeds	4.61 34.90 3.36 48.80 1.34 46.50 31.20 0.02 0.15 14.50 35.00 0.03	0 0 3 3 7 7 7 7 14 14 14 14	BASF method Method No. L0076/01 LOQ = 0.01 mg/kg

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
* Without roots

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	BAS 510 F (Boscalid)	**Commercial Product (name)**	-
Crop/crop group:	Green Beans/Beans	**Producer of commercial product**	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	**Indoor/Glasshouse/Outdoor**	Outdoor
Country	Germany	**Other active substance in the formulation**	None
Content of active substance (g/kg or g/L)	500 g/kg	**(common name and content)**	
Formulation (e.g. WP)	WG (BAS 510 01 F)	**Residues calculated as:**	BAS 510 F

1	2	3		4	5			6	7	8	9	10	11
Report-No Location (Trial No)	**Commodity/ Variety**	**1. 2. 3.**	**Date of Sowing / Planting Flowering Harvest**	**Method of treatment**	**Application rate[0] per treatment**			**No of treatm. and last date**	**Growth stage (BBCH)[2]**	**Portion analyzed**	**Residues (mg/kg)**	**DALA[1]**	**Remarks**
					kg a.s./hL	**Water (L/ha)**	**kg a.s./ha**				**BAS 510 F**		
309371 2011/1251203 47250 Bougnon France (L100342)	VP 0526 Pedra	1. 2. 3.	19.06 2010 25.07.-10.08.10 29.09 2010	Pulvexper boom sprayer	0.17	300	0.50	2 17.09.2010	81	Pods with seeds Rest of plant* Pods with seeds Rest of plant* Pods without seeds Seeds	0.84 15 0.83 15 0.82 0.010	0 0 3 3 7 7	BASF method Method No. 535/1 LOQ = 0.01 mg/kg
309371 2011/1251203 57500 Epanomi Greece (L100343)	VP 0526 Etna	1. 2. 3.	11.05 2010 18.06.-15.07.10 15.07.-10.08.10	AZO boom sprayer	0.17	300	0.50	2 12.07.2010	77	Pods with seeds Rest of plant* Pods with seeds Rest of plant* Pods with seeds Rest of plant* Pods without seeds Seeds Pods with seeds Rest of plant* Pods without seeds Seeds	0 59 20 0 55 13 0 29 8.5 0.44 0.024 0.41 7.3 0.84 0.033	0 0 3 3 7 7 7 7 15 15 15 15	
309371 2011/1251203 47030 San Mauro Pascoli Italy (L100344)	VP 0526 Flavert	1. 2. 3.	27.06 2010 10.08.-20.08.10 31.08 2010	ECHO SHR 150-SI	0.17	300	0.50	2 24.08.2010	71-75	Pods with seeds Rest of plant* Pods with seeds Rest of plant* Pods with seeds Rest of plant* Pods without seeds Seeds Pods with seeds Rest of plant* Pods without seeds Seeds	1.7 38 1.6 33 1.1 21 1.3 0 12 1.4 40 2.2 0.027	0 0 3 3 7 7 7 7 14 14 14 14	

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	BAS 510 F (Boscalid)	**Commercial Product (name)**	-
Crop/crop group:	Green Beans/Beans	**Producer of commercial product**	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	**Indoor/Glasshouse/Outdoor**	Outdoor
Country	Germany	**Other active substance in the formulation**	None
Content of active substance (g/kg or g/L)	500 g/kg	**(common name and content)**	
Formulation (e.g. WP)	WG (BAS 510 01 F)	**Residues calculated as:**	BAS 510 F

1	2	3		4	5			6	7	8	9	10	11
Report-No Location (Trial No)	**Commodity/ Variety**	**1. 2. 3.**	**Date of Sowing / Planting Flowering Harvest**	**Method of treatment**	**Application rate[0] per treatment**			**No of treatm. and last date**	**Growth stage (BBCH)[2]**	**Portion analyzed**	**Residues (mg/kg)**	**DALA[1]**	**Remarks**
					kg a.s./hL	**Water (L/ha)**	**kg a.s./ha**				**BAS 510 F**		
309371	VP 0526	1.	23.03 2010	Stihl	0.17	300	0.50	2	75	Pods with seeds	0.81	0	
2011/1251203	Marconi	2.	21.06.-05.07.10	Atomizer				09.07.2010		Rest of plant*	8.0	0	
29001 Málaga		3.	12.07 2010							Pods with seeds	0.67	3	
Spain										Rest of plant*	12	3	
(L100345)										Pods with seeds	0.77	7	
										Rest of plant*	13	7	
										Pods with seeds	0.43	14	
										Rest of plant*	6.3	14	

1 Days after last application
2 At last application
* Without roots

Oilseed rape

Northern Europe

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	BAS 510 F (Boscalid)	**Commercial Product (name)**	-
Crop/crop group:	Spring Rape/Oilseeds	**Producer of commercial product**	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	**Indoor/Glasshouse/Outdoor**	Outdoor
Country	Germany	**Other active substance in the formulation**	None
Content of active substance (g/kg or g/L)	500 g/kg	**(common name and content)**	
Formulation (e.g. WP)	WG (BAS 510 01 F), 0.5 kg/ha	**Residues calculated as:**	BAS 510 F

1	2	3		4	5			6	7	8	9	10	11
Report-No Location (Trial No)	**Commodity/ Variety**	**1. 2. 3.**	**Date of Sowing / Planting Flowering Harvest**	**Method of treatment**	**Application rate[0] per treatment**			**No of treatm. and last date**	**Growth stage (BBCH)[2]**	**Portion analyzed**	**Residues (mg/kg)**	**DALA[1]**	**Remarks**
					kg a.s./hL	**Water (L/ha)**	**kg a.s./ha**				**BAS 510 F**		
150307 2005/1004971 16833 Lentzke Germany ACK/24/04	SO 0495 Helios	1. 2. 3.	12.03.2004 25.05.-19.06.04 21.08.-22.08.04	Bicyde monted boom sprayer	0.083	300	0 250	2 30.06.2004	75	Whole plant* Pods with seed Whole plant*[3] Pods with seed Whole plant*[3] Pods with seed Whole plant* Seeds	3.18 0.24 0.35 0.11 0.18 0.09 0.13 0.07	0 20 20 27 27 35 35 43	BASF method Method No 445/0 LOQ = 0.05 mg/kg
150307 2005/1004971 267 32 Bjuv Sweden HUS/12/04	SO 0495 Heros	1. 2. 3.	13.04.2004 10.06,-30.06.04 19.08.-24.08.04	Sprayer with a 3 m boom to one side	0.083	300	0 250	2 07.07.2004	75	Whole plant* Pods with seed Whole plant*[3] Pods with seed Whole plant*[3] Pods with seed Whole plant* Seeds	3.30 0.08 0.14 <0.05 0.06 <0.05 0.09 <0.05	0 21 21 28 28 35 35 43	
150307 2005/1004971 CV37 8UJ Stratford United Kingdom OAT/26/04	SO 0495 Heros	1. 2. 3.	20.02.2004 27.05.-10.06.04 15.08.-16.08.04	T.E.UK battery powered compressed air sprayer	0.083	300	0 250	2 30.06.2004	75	Whole plant* Pods with seed Whole plant*[3] Pods with seed Whole plant*[3] Seeds Seeds	4.39 0.84 0.79 1.19 0.91 0.06 0.06	0 21 21 28 28 35 42	

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	BAS 510 F (Boscalid)	**Commercial Product (name)**	-
Crop/crop group:	Spring Rape/Oilseeds	**Producer of commercial product**	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	**Indoor/Glasshouse/Outdoor**	Outdoor
Country	Germany	**Other active substance in the formulation**	None
Content of active substance (g/kg or g/L)	500 g/kg	**(common name and content)**	
Formulation (e.g. WP)	WG (BAS 510 01 F), 0.5 kg/ha	**Residues calculated as:**	BAS 510 F

1	2	3		4	5			6	7	8	9	10	11
Report-No Location (Trial No)	**Commodity/ Variety**		**Date of Sowing / Planting Flowering Harvest**	**Method of treatment**	**Application rate[0] per treatment**			**No of treatm. and last date**	**Growth stage (BBCH)[2]**	**Portion analyzed**	**Residues (mg/kg)**	**DALA[1]**	**Remarks**
					kg a.s./hL	**Water (L/ha)**	**kg a.s./ha**				**BAS 510 F**		
		1. 2. 3.											

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
3 Without pods
* Without roots

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	BAS 510 F (Boscalid)	**Commercial Product (name)**	-
Crop/crop group:	Spring Rape/Oilseeds	**Producer of commercial product**	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	**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)	500 g/kg		
Formulation (e.g. WP)	WG (BAS 510 01 F), 0.5 kg/ha	**Residues calculated as:**	BAS 510 F

1	2	3		4	5			6	7	8	9	10	11
Report-No Location (Trial No)	**Commodity/ Variety**	**1. 2. 3.**	**Date of Sowing / Planting Flowering Harvest**	**Method of treatment**	**Application rate[0] per treatment**			**No of treatm. and last date**	**Growth stage (BBCH)[2]**	**Portion analyzed**	**Residues (mg/kg)**	**DALA[1]**	**Remarks**
					kg a.s./hL	**Water (L/ha)**	**kg a.s /ha**				**BAS 510 F**		
334204 2008/1074165 47574 Goch-Pfalzdorf Germany L080238	SO 0495 PR45001 D	1. 2. 3.	12.08.2007 01.05.-14.05.08 14.07.2008	Boom sprayer	0.067	200	0.133	2 20.05.2008	73	Whole plant* Rest of plant*[3] Pods with seed Rest of plant*[3] Pods with seed Seed	1 30 0 10 0.08 0 17 0 34 <0.01	0 36 36 43 43 55	BASF method Method No 535/1 (L0076/01) LOQ = 0.05 mg/kg
					0.125	200	0.250	2 20.05.2008	73	Whole plant* Rest of plant*[3] Pods with seed Rest of plant*[3] Pods with seed Seed	1 36 0 11 0 14 0 14 0 17 <0.01	0 36 36 43 43 55	
334204 2008/1074165 47652 Weeze Germany L080239	SO 0495 Billy	1. 2. 3.	01.09.2007 02.05.-16.05.08 14.07.2008	Boom sprayer	0.067	200	0.133	2 20.05.2008	73	Whole plant* Rest of plant*[3] Pods with seed Rest of plant*[3] Pods with seed Seed	0 90 0 15 0 20 0.07 0.06 <0.01	0 36 36 43 43 55	
					0.125	200	0.250	2 20.05.2008	73	Whole plant* Rest of plant*[3] Pods with seed Rest of plant*[3] Pods with seed Seed	1.60 0 24 0 27 0 15 0 18 <0.01	0 36 36 43 43 55	

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	BAS 510 F (Boscalid)	**Commercial Product (name)**	-
Crop/crop group:	Spring Rape/Oilseeds	**Producer of commercial product**	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	**Indoor/Glasshouse/Outdoor**	Outdoor
Country	Germany	**Other active substance in the formulation**	None
Content of active substance (g/kg or g/L)	500 g/kg	**(common name and content)**	
Formulation (e.g. WP)	WG (BAS 510 01 F), 0.5 kg/ha	**Residues calculated as:**	BAS 510 F

1	2	3		4	5			6	7	8	9	10	11
Report-No Location (Trial No)	**Commodity/ Variety**	**1. 2. 3.**	**Date of Sowing / Planting Flowering Harvest**	**Method of treatment**	**Application rate[0] per treatment**			**No of treatm. and last date**	**Growth stage (BBCH)[2]**	**Portion analyzed**	**Residues (mg/kg)**	**DALA[1]**	**Remarks**
					kg a.s./hL	**Water (L/ha)**	**kg a.s /ha**				**BAS 510 F**		
334204 2008/1074165 41500 Suevres North France L080240	SO 0495 Fidness	1. 2. 3.	03.07.2007 18.04.-24.04.08 12.07.08	Boom sprayer	0.067	200	0.133	2 30.05.2007	77	Whole plant* Rest of plant*[3] Pods with seed Rest of plant*[4] Seed	2.00 0.43 0 99 0.73 0.02	0 35 35 43 43	BASF method Method No 535/1 (L0076/01) LOQ = 0.05 mg/kg
					0.125	200	0.250	2 30.05.2008	77	Whole plant* Rest of plant*[3] Pods with seed Rest of plant*[4] Seed	2.49 0.40 0.65 0 59 0.03	0 35 35 43 43	
334204 2008/1074165 51220 Brimont Northern France L080241	SO 0495 Exagone	1. 2. 3.	21.08.2007 21.04.-30.04.08 21.07.2008	Boom sprayer	0.067	200	0.133	2 21.05.2008	73	Whole plant* Rest of plant*[3] Pods with seed Rest of plant*[3] Pods with seed Seed	0.67 0.09 0 10 0.05 0.07 0.02	0 35 35 43 43 61	
					0.125	200	0.250	2 21.05.2008	73	Whole plant* Rest of plant*[3] Pods with seed Rest of plant*[3] Pods with seed Seed	1 12 0 13 0.05 0.07 0.03 <0.01	0 35 35 43 43 61	

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
3 Without pods
4 With pods
* Without roots

Southern Europe

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	BAS 510 F (Boscalid)	**Commercial Product (name)**	-
Crop/crop group:	Oilseed rape/Oilseeds	**Producer of commercial product**	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	**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)	500 g/kg		
Formulation (e.g. WP)	WG (BAS 510 01 F), 0.5 kg/ha	**Residues calculated as:**	BAS 510 F

1	2	3		4	5			6	7	8	9	10	11
Report-No Location (Trial No)	**Commodity/ Variety**	**1. 2. 3.**	**Date of Sowing / Planting Flowering Harvest**	**Method of treatment**	**Application rate[0] per treatment**			**No of treatm. and last date**	**Growth stage (BBCH)[2]**	**Portion analyzed**	**Residues (mg/kg)**	**DALA[1]**	**Remarks**
					kg a.s./hL	**Water (L/ha)**	**kg a.s./ha**				**BAS 510 F**		
241888 2007/1007952 82290 Meauzac South France AF/10506/BA/1	SO 0495 Fantasio	1. 2. 3.	07.04.2006 n.a. 21.06-02.08.06	Foliar application with boom	0.08	300	0.25	2 21.06.2006	75	Whole plant* Pods with seed Whole plant*[3] Seed Seed Seed	3.16 1.1 0.45 0.61 0.25 0.21	0 21 21 28 35 42	BASF method Method No 535/0 LOQ = 0.01 mg/kg
241888 2007/1007952 82290 Meauzac South France AF/10506/BA/2	SO 0495 Fantasio	1. 2. 3.	17.04.2006 n.a. 05.07-16.08.06	Foliar application with boom	0.08	300	0.25	2 05.07.2006	73	Whole plant* Pods with seed Whole plant*[3] Seed Seed Seed	3.69 3.04 0.35 0.83 0.11 0.06	0 21 21 28 35 42	

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
3 Without pods
* Without roots

We create chemistry

Boscalid

Document M-CA, Section 7

FATE AND BEHAVIOUR IN THE ENVIRONMENT

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Version history[1]

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

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

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CA 7 FATE AND BEHAVIOUR IN THE ENVIRONMENT

All relevant information on the first Annex I review of BAS 510 F – boscalid and the endpoints used in environmental risk assessments can be found in the monograph of boscalid (Monograph 12945/ECCO/BBA/01, August 2001) and in the review report (SANCO/3919/2007 – rev.5). In this Supplemental Dossier for renewal of approval, only those environmental fate studies are summarized which are submitted for the first time. However, relevant results of the studies already evaluated are also summarized briefly in order to provide an overall picture of the fate and behaviour of boscalid in the environment.

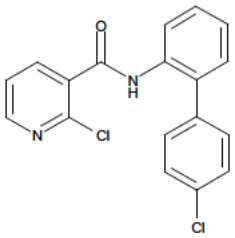
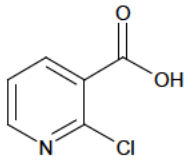
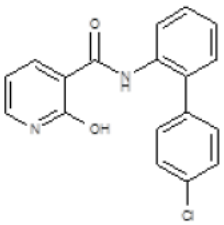
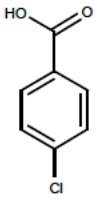
For the current renewal of approval under Regulation 1107/2009, a data gap analysis according to new guidelines, new guidance documents and new procedures in kinetic evaluations and exposure assessments were performed and new studies or kinetic evaluations were initiated where considered necessary. All new data are provided in this section.

The new data include four kinds of studies:
1) Studies that are triggered by results from previously peer-reviewed studies due to current trigger values.
2) Supplemental studies that were not required for the first Annex I listing and are also not required for the current process, but elucidate the environmental fate and behavior. Results from these supplemental studies are considered with regard to current trigger values.
3) Studies that are triggered by results from the supplemental studies.
4) Re-evaluations of data from previously peer-reviewed studies to derive updated kinetic endpoints according to current guidance.

Furthermore, a literature search was performed and scientific publications were evaluated for their endpoint relevance and quality. No publications were found that provided a reliable endpoint for use in the risk assessment. Two relevant articles were however found describing monitoring in EU countries for multiple substances including boscalid. These articles are presented in M-CA 7.5. Further information on the literature assessment and respective justifications can be found in M-CA 9.

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

An overview of metabolites discussed in this section is given below.

Substance/ Metabolite Code	Reference code (Reg. No.)	Synonyms	Structure
BAS 510 F	300355	Boscalid M510F00	
M510F47	107371	M47	
M510F49	391572	M49	
M510F64	309572	M64	

CA 7.1 Fate and behaviour in soil

CA 7.1.1 Route of degradation in soil

Information on the route and rate of degradation in soil was derived from the peer-reviewed laboratory studies with [pyridine-3-^{14}C]- and [diphenyl-U-^{14}C]-labeled boscalid [*BASF DocID 1999/11807; BASF DocID 2000/1013279; BASF DocID 1998/10607; BASF DocID 2000/1014986; BASF DocID 2000/1014990; BASF DocID 2000/1014989*]. Supplemental already peer-reviewed degradation studies were performed with the two subunits of the active substance M510F62 and M510F47 [*BASF DocID 1999/11102; BASF DocID 2000/1013280*] to further elucidate the degradation pathway of boscalid to CO_2 and bound residues. The very fast binding and mineralization of M510F62 support the finding that the metabolite was not observed in the environmental fate studies with the parent applied. Therefore, M510F62 is not considered to be a metabolite that is formed in environmental matrices. No further information on degradation was needed for the previous Annex I and current renewal procedures.

Two additional studies on degradation of boscalid are included in the current dossier:

Metabolism and degradation of boscalid in soil was investigated in four US soils in the laboratory [*BASF DocID 2002/5002772, submitted under CA 7.1.1.1/1*].
For the current registration renewal, the study is included in the dossier for completeness and considered for the current data requirements.

In a further study [*BASF DocID 2008/1013108, submitted under CA 7.1.1.1/2*] degradation of freshly applied boscalid was compared to degradation of aged residues of boscalid. The study elucidates degradation and long-term sorption behaviour of boscalid.

CA 7.1.1.1 Aerobic degradation

For the previous Annex I listing, information on the route of degradation in aerobic soil were available from two studies with [pyridine-3-^{14}C]-labeled and [diphenyl-U-^{14}C]-labeled boscalid.

Table 7.1.1.1-1: Studies on aerobic soil degradation of boscalid and metabolites

Reference	BASF DocID	Soil type (USDA)	Application rate [mg kg^{-1}]	MWHC [%]	Incubation temperature [°C]	Incubation period [days]	Remark
Boscalid							
Stephan A., 1999a	1999/11807	sandy loam	1.0	40	20±1	364	
Ebert D., Harder U., 2000a	2000/1013279	loamy sand	1.0	40	20	120	
		loamy sand		40 20 40 40 40	20 20 5 30 20 (sterile)		
		sandy loam		40	20		
		loam		40	20		

MWHC = Maximum water holding capacity

In the first study, degradation was investigated in one soil and boscalid showed substantial mineralisation with both radio-labels, but was still detectable in amounts of about 17% TAR after 360 days. No major metabolites were observed during the study. Two transformation products could be identified by MS-analysis (M510F49 and M510F50), although they never reached more than 0.2% TAR. The majority of the degradates were bound residues.

In the second study, degradation was investigated in four soils and boscalid was degraded slowly in all cases (down to 53.6% to 80.9% TAR after about 120 days), but no metabolites were detected. The majority of the degradates were bound residues.

For the renewal of approval, two additional studies are considered in the supplementary dossier:

Degradation of boscalid in soil was also investigated in an US study in the laboratory [*see CA 7.1.1.1/1 2002/5002772*]. For the current registration renewal, the study is included in the dossier for completeness. Results from the study are considered regarding the current data requirements. Aerobic degradation of [pyridine-3-^{14}C]-labeled boscalid was studied in soil from four different US locations. Boscalid degraded slowly with DT_{50} values for the soils between 159 and 573 days. A small percentage of M510F49 was observed at 0 DAT in two soils increasing to a maximum of 5.4% TAR and 14.5% TAR at the end of the study. M510F47 was found in three the soils but was always less than 5% TAR (in two soils less than 1.8% TAR).

A more recent study compared degradation of freshly applied boscalid to degradation of aged residues [*see CA 7.1.1.1/2 2008/1013108*]. The study was performed to further elucidate the degradation behavior of boscalid. The DT_{50} of aged residues was 746 days, whereas the DT_{50} of freshly applied boscalid was distinctly shorter with a value of 336 days. The difference was attributed to the known effect of time-dependent sorption (non-equilibrium sorption). This effect reduces the amount of dissolved residues that is available e.g. for microbial degradation. As a result, the apparent degradation of aged residues is slower than that of fresh residues.

Report:	CA 7.1.1.1/1 Paulick R.C., 2002a Aerobic soil metabolism of 14C-BAS 510 F 2002/5002772
Guidelines:	EPA 162-1, DACO 8.2.3.4.2
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary

The aerobic soil metabolism of ^{14}C-BAS 510 F - boscalid was determined in soil from four different US locations: California (CA), Idaho (ID), Illinois (IL) and North Dakota (ND). The study utilized [pyridine-3-^{14}C]-boscalid.

Aliquots of 40 g soil adjusted to approximately 75% of the maximum water holding capacity at 1/3 bar were weighed into crystallization dishes. The nominal application rate was 0.825 µg g^{-1} which is approximately equal to a field rate of 0.55 lb a.s. $acre^{-1}$ in the top 30 cm of soil (corresponding to 0.616 kg ha^{-1}). Soil samples were individually treated with ^{14}C-boscalid resulting in concentrations of 0.88 µg g^{-1} ^{14}C-boscalid for CA and ID and 0.95 µg g^{-1} for IL and ND. After treatment, the time 0 samples were analyzed immediately, and the other samples were placed into glass metabolism towers and incubated in darkness at 27 °C until the appropriate sampling time.

Samples were taken at 0, 7, 14, 29, 63, 91, 127, 179/181, 273 and 371 days after treatment (DAT). At each sampling time, the volatiles were purged from the towers by drawing the gasses through two 1N NaOH traps. The NaOH solutions were analyzed for radioactive residues by LSC. The soil from each container was extracted 2-3 times with methanol and then 1-3 times with methanol/water (1:1, v/v). The volume of the extracts was determined and the radioactivity was measured by LSC. The extraction solutions were combined, concentrated, and analyzed by HPLC. The radioactivity remaining in the soil was determined by combustion. Boscalid, M510F49 and M510F47 (chloronicotinic acid, CNA) in the extracts were confirmed by HPLC co-injection with analytical standards and by LC-MS analysis.

Average recoveries of radioactivity ranged generally from 90.2% to 102.5% TAR throughout the incubation period, with one exception (CA, 371 DAT) with an average of 79.4% TAR, probably due to an error in the measurement.

The amount of extractable radioactive residues decreased continuously throughout the study period to 35-64% TAR at 371 DAT. The non-extractable (bound) residues increased from 1.3-2.2% TAR at day 0 to 23.8-44.9% TAR at 371 DAT. Therein, the major part of radioactivity was associated to the humin fraction (up to 23.4% TAR). The formation of volatiles was exclusively related to the mineralization to $^{14}CO_2$ and reached a total amount of 2.2-8.3% TAR after 371 days of incubation.

In the soil extracts, the unchanged test item boscalid represented the major radioactive component, decreasing from 93.7-97.2% TAR at day 0 to 33.1-58.7% TAR at 371 DAT. A small percentage of the 2-hydroxy metabolite M510F49 was observed at 0 DAT, increasing to a maximum of 14% TAR at 371 DAT in the Illinois soil and a maximum of 5.4% TAR at 371 DAT in the ldaho soil. M510F047 (CNA) was found in three of the soils but was always less than 5% TAR.
The degradation of boscalid in all soils was reasonably slow, although mineralization occurred in all. The soils from CA and ND produced the greatest degradation, while those from IL and ID showed a slower process. The DT_{50} values for the soils were calculated using linear regression and ranged from 159 days (CA) to 573 days (ID).

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

Internal code:	BAS 510 F
Common name:	Boscalid
Reg. No.:	300355
Chemical name (IUPAC):	2-chloro-N-(4'-chloro-biphenyl-2-yl)nicotinamide
Molar mass:	343.2 g mol^{-1}
Chemical Purity:	> 97%
Position of radiolabel:	[pyridin-3-^{14}C]
Batch No.:	640-2037
Specific Activity:	310,000 dpm μg^{-1}
Radiochemical purity:	> 99%

2. Soil

The soils were obtained from four different US locations. Soil was partially dried, if necessary, and sieved through a 2 mm screen to remove debris. The soil characteristics are summarized in Table 7.1.1.1-2.

Table 7.1.1.1-2: Characterization of Soils

Soil designation	**Santa Maria, California (CA)**	**Payette, Idaho (ID)**	**Carlyle, Illinois (IL)**	**Gardner, North Dakota (ND)**
USDA Particle size distribution [%]				
Sand 0.050 – 2 mm	44	32	28	46
Silt 0.002 – 0.050 mm	28	38	54	34
Clay < 0.002 mm	28	30	18	20
Textural class	Clay loam	Clay loam	Silt loam	Loam
German BBA texture [%]				
Sand 0.063 – 2 mm	42	30	26	44
Silt 0.002 – 0.063 mm	30	40	56	36
Clay < 0.002 mm	28	30	18	20
Textural class	Sandy clay loam	Sandy clay loam	Silty loam	Sandy loam
Organic matter [%]	4.6	3.4	2.3	3.6
pH [in saturated paste] [a]	7.8	6.8	6.5	7.7
Cation exchange capacity [$cmol^+ kg^{-1}$]	23.1	22.0	12.9	22.7
Max. water holding capacity [%]	39.8	43.5	40.4	44.2
% Moisture Capacity at 1/3 bar	24.1	35.7	30.2	26.1
Microbial biomass [$\mu g\ g^{-1}$ dry weight soil]	218.9	301.5	24.3	80.1

[a] in the US soil pH is commonly measured in H_2O; this is however not explicitly stated in the report

B. STUDY DESIGN

1. Experimental conditions

The soil moisture was then adjusted to approximately 75% of the maximum water holding capacity at 1/3 bar. The soil was allowed to equilibrate in the dark for at least 3 days.

Soil samples (40 g) were weighed into individual glass crystallizing dishes (test vessels). The concentration of ^{14}C-boscalid in each dosing solution was approximately 0.20 µg of test substance per µL of solution. ^{14}C-boscalid was added to each test system as a 170 µL aliquot for CA and ID soils (or 180 µL for IL and ND soils), containing approximately 33 µg of test substance. The nominal application rate was 0.825 µg g^{-1} which is approximately equal to a field rate of 0.55 lb a.s. $acre^{-1}$ in the top 30 cm of soil (corresponding to 0.616 kg ha^{-1}). The soil samples were placed into glass metabolism towers which were then incubated at 27°C in the dark. Aliquots of the application solutions were taken as dose verification samples before the applications to each soil test system. These values were used to establish the total applied radioactivity (TAR) and the total applied µg of ^{14}C-boscalid.

2. Sampling

Sampling dates were 0, 7, 14, 29, 63, 91, 127, 179/181, 273 and 371 days after treatment (DAT). At the 0, 29,127, and 371 DAT sampling times, duplicate samples were analyzed to demonstrate the reproducibility of the analysis procedures.

3. Description of analytical procedures

The soil samples were extracted sequentially 2-3 times with 100 mL of methanol and 1-3 times with 100 mL of methanol/water (1:1 v/v) depending on the sampling period (later time periods were extracted more extensively). The samples were shaken for 45 min at 300 strokes per minute and then centrifuged. The extracts were assayed by LSC and then combined for HPLC analysis.

The soil samples from the 127, 179/181, 273 and 371 DAT time periods were air-dried sufficiently for combustion analysis. The remaining samples were subjected to bound residue analysis. Thereto, the soil residue was extracted twice with 0.5 N NaOH. The mixtures were centrifuged after each extraction. The solid residue (humins, RRRII) was dried before assay by combustion and LSC. The supernatants were acidified to precipitate the humic acids. The mixture was centrifuged to remove the humic acids. The humic acid precipitate was resuspended in 1 N NaOH and assayed by LSC. The acidified supernatant was extracted with ethyl acetate and the extracts combined and assayed by LSC and HPLC.

At each sampling interval the $^{14}CO_2$ was measured by evacuating the gases from the tower through a pair of tubes containing NaOH and then continuing to draw air through the system for at least 60 min. Aliquots of the NaOH trapping solutions were then analyzed by LSC to determine the volatile residues.

4. Calculation of the degradation rate

The DT_{50} values for boscalid were calculated using linear regression. For the determination of half-lives, % TAR was converted to ppm by using the nominal concentrations of 0.88 ppm (CA and ID soils) and 0.95 ppm (IL and ND soils) x (% TAR x 0.01) = sample ppm.
The residue concentration per sampling period was transformed to a natural log expression before analysis by linear regression.

The linear regression was solved using Origin 6.0 Scientific Graphics Software. The Hamaker equation is one of the most commonly used methods for describing dissipation kinetics using a linear fit. The basic computational form of the equation is described below.

$$\frac{dC}{dt} = Co * e^{(-k*t)}$$

The natural log of 2 (0.693) is then divided by the slope of the line for determination of the rate constant.
The kinetic analysis was carried out according to previous requirements in the summarized study. A new kinetic analysis following the recommendations of the FOCUS work group on degradation kinetics [*FOCUS (2006) "Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration" Report of the FOCUS Work Group on Degradation Kinetics, EC Document Reference Sanco/10058/2005 version 2.0, 434 pp., June 2006*] is provided in section CA 7.1.2.1.1/1 [*BASF DocID 2014/1261100*].

II. RESULTS AND DISCUSSION

A. MASS BALANCE

The overall mass balance was excellent for all of the soils at all of the time periods. Average recoveries of radioactivity ranged from 90.2-102.5% TAR with the exception of one sample (CA; 371 DAT), in which the average was 79.4% TAR, but this may have been due to an error in the measurement.

B. EXTRACTABLE AND BOUND RESIDUES

In the CA and ND soils the extractable residues decreased with time to 35-50% TAR at 371 DAT. In the ID and IL soils the extractable residues decreased more slowly and by 371 DAT comprised 62-64% TAR. The bound residues correspondingly increased over time reaching a maximum of 24-45% TAR at 371 DAT for the CA and ND soils and 24-26% TAR for the ID and IL soils (Table 7.1.1.1-3 to Table 7.1.1.1-6).

Table 7.1.1.1-3: Distribution of radioactivity after application of ^{14}C-boscalid to soil sample CA [% TAR]

Sampling Time	Replicate Number	Volatiles	Extractable Residues			Bound Residues	Total
			MeOH	MeOH/water	Total		
0	1	-	96.8	1.8	98.6	2.1	100.7
	2	-	95.6	1.7	97.3	2.3	99.6
	Mean	-	96.2	1.8	98.0	2.2	100.2
7	1	0.0	83.9	2.7	86.6	12.5	99.1
14	1	0.2	75.6	3.0	78.6	17.1	95.9
29	1	0.2	70.7	3.5	74.2	22.1	96.5
	2	0.2	72.0	3.3	75.3	23.3	98.8
	Mean	0.2	71.4	3.4	74.8	22.7	97.7
63	1	1.1	61.2	5.7	66.9	29.9	97.9
91	1	1.7	54.5	6.2	60.7	34.0	96.4
127	1	2.4	50.3	5.6	55.9	35.2	93.5
	2	2.1	50.8	5.6	56.4	35.9	94.4
	Mean	2.3	50.6	5.6	56.2	35.6	94.1
179		4.3	42.7	5.5	48.2	39.2	91.7
273		5.8	37.0	5.4	42.4	42.0	90.2
371	1	9.1	30.8	6.1	37.0	35.3	81.4
	2	7.5	27.5	4.5	32.0	37.9	77.4
	Mean	8.3	29.2	5.3	34.5	36.6	79.4

MeOH = methanol

Table 7.1.1.1-4: Distribution of radioactivity after application of ^{14}C-boscalid to soil sample ID [% TAR]

Sampling Time	Replicate Number	Volatiles	Extractable Residues			Bound Residues	Total
			MeOH	MeOH/water	Total		
0	1	-	96.7	1.6	98.4	1.3	99.7
	2	-	97.4	1.6	99.0	1.3	100.3
	Mean	-	97.1	1.6	98.7	1.3	100.0
7	1	0.3	90.4	2.1	92.5	6.7	99.5
14	1	0.7	88.3	2.3	90.6	8.0	99.3
29	1	1.1	84.5	2.3	86.8	10.8	98.7
	2	0.6	85.8	2.3	88.2	10.5	99.3
	Mean	0.9	85.2	2.3	87.5	10.7	99.1
63	1	1.4	78.2	3.6	81.8	14.4	97.6
91	1	1.5	77.2	4.0	81.3	17.7	100.5
127	1	1.7	76.8	3.8	80.6	17.7	100.0
	2	1.8	74.8	3.8	78.6	17.3	97.7
	Mean	1.8	75.8	3.8	79.6	17.5	98.9
179		2.0	65.9	3.9	69.8	20.5	92.3
273		2.2	67.4	4.0	71.4	22.2	95.8
371	1	2.5	60.9	4.2	65.1	24.1	91.7
	2	2.8	59.8	3.7	63.5	23.4	89.7
	Mean	2.7	60.4	4.0	64.3	23.8	90.8

MeOH = methanol

Table 7.1.1.1-5: Distribution of radioactivity after application of ^{14}C-boscalid to soil sample IL [% TAR]

Sampling Time	Replicate Number	Volatiles	Extractable Residues			Bound Residues	Total
			MeOH	MeOH/water	Total		
0	1	-	97.6	0.0 [a]	97.6	2.1	99.7
	2	-	91.8	1.4	93.2	1.0	94.2
	Mean	-	94.7	0.7	95.4	1.6	97.0
7	1	0.4	90.6	1.8	92.4	6.5	99.3
14	1	0.5	89.8	2.7	92.5	8.2	101.2
29	1	0.6	81.9	7.9	89.7	12.6	102.9
	2	0.4	82.6	7.3	89.9	11.7	102.0
	Mean	0.5	82.3	7.8	89.8	12.2	102.5
63	1	0.7	76.8	4.3	81.2	13.6	95.5
91	1	0.8	76.6	4.0	80.6	18.9	100.3
127	1	0.8	73.9	3.6	77.5	21.6	99.9
	2	0.7	74.4	3.8	78.1	21.8	100.6
	Mean	0.8	74.2	3.7	77.8	21.7	100.3
181	2	1.2	65.0	4.0	69.0	23.4	93.6
273	1	1.7	70.3	3.4	73.7	24.4	99.8
371	1	2.5	62.7	4.0	66.7	26.9	96.1
	2	1.9	53.7	4.1	57.8	24.5	84.2
	Mean	2.2	58.2	4.1	62.3	25.7	90.2

MeOH = methanol
[a] = sample lost during extraction

Table 7.1.1.1-6: Distribution of radioactivity after application of ^{14}C-boscalid to soil sample ND [% TAR]

Sampling Time	Replicate Number	Volatiles	Extractable Residues			Bound Residues	Total
			MeOH	MeOH/water	Total		
0	1	-	98.0	1.8	99.8	1.9	101.7
	2	-	96.4	1.8	98.2	2.3	100.5
	Mean	-	97.2	1.8	99.0	2.1	101.1
7	1	0.1	74.9	4.6	79.4	22.8	102.3
14	1	0.5	68.5	5.6	74.1	24.4	99.0
29	1	0.8	59.1	11.1	70.2	26.3	97.3
	2	0.5	62.3	12.1	74.4	30.0	104.9
	Mean	0.7	60.7	11.6	72.3	28.2	101.2
63	1	1.0	55.8	10.0	65.8	32.8	99.6
91	1	1.1	50.8	10.3	61.1	35.8	98.0
127	1	1.3	48.3	8.6	56.9	41.4	99.6
	2	1.2	48.1	9.0	57.1	39.3	97.6
	Mean	1.3	48.2	8.8	57.0	40.4	98.7
181	1	1.7	44.4	11.3	55.7	40.9	98.3
273	1	2.2	44.3	8.6	52.9	44.9	100.0
371	1	2.8	38.2	9.2	47.4	44.7	94.9
	2	2.7	42.8	8.8	51.6	45.0	99.3
	Mean	2.8	40.5	9.0	49.5	44.9	97.2

MeOH = methanol

The bound residues from the 127 through 371 DAT time periods were further analyzed (Table 7.1.1.1-7).

Table 7.1.1.1-7: Analysis of Bound Residues in four soils treated with ^{14}C-boscalid under aerobic conditions [%TAR]

DAT	Replicate Number	RRRI [a]	Humic Acid	Fulvic Acid	Ethyl Acetate Extract	Aqueous Fraction	Humins RRRII
California (CA)							
127	1	32.9	6.1	7.2	5.8	1.0	17.9
	2	33.2	6.4	7.3	6.1	1.1	20.6
	Mean	33.1	6.3	7.3	6.0	1.1	19.3
179 [b]	1	38.3	6.4	6.4	5.1	1.1	20.2
273	1	41.1	5.2	6.5	5.1	1.3	28.9
371	1	33.6	6.9	4.1	3.1	1.0	29.2
371	2	36.9	7.2	4.1	2.9	1.0	30.3
	Mean	35.3	7.1	4.1	3.0	1.0	29.8
Idaho (ID)							
127	1	17.3	6.0	3.7	3.2	0.2	5.6
	2	16.9	6.0	3.7	3.0	0.6	5.5
	Mean	17.1	6.0	3.7	3.1	0.4	5.6
179 [b]	1	19.9	7.1	4.3	3.4	0.6	6.8
273	1	21.6	7.4	4.2	3.7	0.6	8.2
371	1	22.9	6.3	4.9	4.2	0.7	10.2
371	2	22.8	6.4	4.4	3.9	0.8	10.7
	Mean	22.9	6.4	4.7	4.1	0.8	10.5
Illinois (IL)							
127	1	21.0	8.6	5.9	5.0	0.7	5.6
	2	21.4	8.4	5.8	4.7	0.8	5.5
	Mean	21.2	8.5	5.9	4.9	0.8	5.6
181	1	22.7	9.1	5.6	4.7	0.8	6.9
273	1	23.7	9.5	6.0	5.1	1.0	7.0
371	1	25.7	9.6	5.0	4.3	1.0	6.7
371	2	24.1	9.3	5.0	4.0	1.0	7.4
	Mean	24.9	9.5	5.0	4.2	1.0	7.1
North Dakota (ND)							
127	1	40.7	8.1	10.6	9.7	0.5	22.0
	2	38.4	8.0	11.8	10.9	0.6	23.4
	Mean	39.6	8.1	11.2	10.3	0.6	22.7
181	1	39.6	6.8	10.0	9.2	0.5	21.7
273	1	43.3	7.6	10.9	10.1	0.5	24.8
371	1	43.3	8.2	4.7	4.2	0.4	26.2
371	2	43.7	8.2	5.5	4.7	0.4	25.9
	Mean	43.5	8.2	5.1	4.5	0.4	26.1

[a] %TAR available; this is less than reported in Table 7.1.1.1-3 to Table 7.1.1.1-6 because of aliquots removed for analysis (e.g. combustion, LSC)
[b] DAT 181 corrected to 179 for CA and ID soil, as DAT 181 is presumably wrong reported in the corresponding report table

C. VOLATILIZATION

The level of radiolabeled volatile metabolites increased during the course of the study. By the end of the study, the CO_2 production reached 2.2 to 8.3% TAR (Table 7.1.1.1-3 to Table 7.1.1.1-6).

D. TRANSFORMATION OF PARENT COMPOUND

HPLC analysis of the soil extracts showed predominantly boscalid (Table 7.1.1.1-8). A small percentage of the 2-hydroxy metabolite M510F49 was observed at 0 DAT and it increased over time, primarily in the Illinois soil with a maximum of 14% TAR at 371 DAT and in the ldaho soil where the maximum was 5.4% TAR also at 371 DAT. M510F47 (chloronicotinic acid, CNA) was found in three of the soils but was always less than 5% TAR (in two soils less than 1.8% TAR).

Boscalid residues of parent, M510F49 and M510F47 (CNA) in the extracts were confirmed by HPLC co-injection with analytical standards and by LC-MS analysis.

Table 7.1.1.1-8: Radio HPLC analysis of extracts in four soils treated with ^{14}C-boscalid under aerobic conditions [%TAR]

Days of treatment [DAT]	California (CA)			Idaho (ID)		
	Boscalid	M510F49	M510F47	Boscalid	M510F49	M510F47
0	96.6	1.4		97.1	1.6	
7	85.1	1.5		91.2	1.3	
14	77.7	0.9		88.3	1.5	0.8
29	73.9	0.9		86.1	1.4	
63	65.5	1.4		80.0	1.8	
91	59.7	1.0		78.7	2.6	
127	54.7	0.7		76.5	2.8	
179	46.3	0.5		66.3	3.3	
273	41.7	0.7		67.9	3.5	
371	33.1	0.6		58.7	5.4	
Days of treatment [DAT]	**Illinois (IL)**			**North Dakota (ND)**		
	Boscalid	**M510F49**	**M510F47**	**Boscalid**	**M510F49**	**M510F47**
0	93.7	1.7	0.0	97.2	1.8	0.0
7	90.5	1.6	0.0	77.0	1.4	1.0
14	90.1	1.6	0.8	73.0	1.1	0.0
29	85.6	2.8	1.4	70.7	1.2	0.8
63	74.8	5.7	0.6	64.0	1.1	0.5
91	71.4	8.4	0.9	59.1	1.1	0.9
127	66.9	9.0	1.3	53.2	0.9	2.2
181	57.8	9.0	1.2	51.3	0.5	3.2
273	61.1	12.6	0.0	47.9	0.7	4.3
371	47.6	14.5	0.0	44.8	1.4	3.1

E. CHARACTERIZATION OF FULVIC ACID EXTRACT

Analysis of the ethyl acetate extracts of the fulvic acid fractions indicated the same three components as found in the soil extracts, although the relative levels varied. The levels of boscalid were highest in the CA (2.6-5.3% TAR) and ND (4.0-9.5% TAR) soils, in which the extractable residues decreased the fastest over time. In the ID and IL soils residues of boscalid were lower at 2.0-3.0% TAR. The levels of the metabolites were low and ranged from < 0.1 to 1.0% TAR for M510F49 and from 0.1-0.2% TAR for M510F47 (see Table 7.1.1.1-9).

Table 7.1.1.1-9: Characterization of ethyl acetate extracts of fulvic acid fractions in four soils treated with ^{14}C-boscalid under aerobic conditions [% TAR]

Days of treatment [DAT]	California (CA)			Idaho (ID)		
	boscalid	M510F49	M510F47	boscalid	M510F49	M510F47
127	5.3	0.1	0.1	2.3	0.1	0.2
179	4.5	0.1	0.2	2.5	0.2	0.2
273	4.5	0.1	0.1	2.9	0.2	0.2
371	2.6	0.0	0.1	2.8	0.4	0.2
Days of treatment [DAT]	**Illinois (IL)**			**North Dakota (ND)**		
	boscalid	**M510F49**	**M510F47**	**boscalid**	**M510F49**	**M510F47**
127	3.1	0.7	0.2	9.5	0.1	0.2
181	2.7	0.8	0.2	8.6	0.2	0.2
273	3.0	0.9	0.2	9.4	0.1	0.1
371	2.0	1.0	0.1	4.0	0.1	0.2

F. DEGRADATION RATES

The degradation rates were calculated by linear regression based on the Hamaker equation. The DT_{50} values for the four soils were 159 days in the California soil, 277 days in the North Dakota soil, 378 days in the Illinois soil and 573 days (degradation rate constant) in the Idaho soil.

III. CONCLUSION

The results of this study indicate that boscalid undergoes aerobic metabolism in a number of different soils. The rates of degradation, as evidenced by CO_2 production, vary with the soil, and the metabolite profile shows similar differences. The greatest rate of CO_2 production occurred in the CA soil and reached 8.3% TAR by the end of the study. The other soils gave somewhat lower CO_2 production.

In all soils the major component of the residue was boscalid. In the IL soil significant levels of the metabolite M510F49 were found and reached 14.5% TAR by the end of the study. The metabolite M510F47 was found at a maximum level of 4.3% TAR in the ND soil.
Bound residues were found in maximum of 24-45% TAR at 371 DAT for the CA and ND soils and 24-26% TAR for the ID and IL soils. Residues in the extractable portion of the bound residues were similar to those in the soil extracts, but at much lower levels.

DT_{50} values were determined by transformation of concentrations to a natural log expression before analysis by linear regression using the Hamaker equation. For the four soils DT_{50} values were found in a range from 159 to 573 days.

A new kinetic analysis following the recommendations of the FOCUS work group on degradation kinetics [*FOCUS (2006)*] is provided in section CA 7.1.2.1.1/1 [*BASF DocID 2014/1261100*].

Report:	CA 7.1.1.1/2 Beck I.-C., 2008a Boscalid (BAS 510 F): Study on soil degradation and long-term sorption in soil 2008/1013108
Guidelines:	SETAC Procedures for assessing the environmental fate and ecotoxicity for pesticides (March 1995), BBA IV 4-1, OECD 106 (2000), OECD 307 (2002)
GLP:	yes (certified by Umweltministerium Baden-Wuerttemberg, Stuttgart)

Executive Summary

The objective of this study was to examine the degradation and long-time sorption of boscalid in soil. Two field-fresh charges of one soil (“Studernheim”) were used in this study. One charge of the soil was from a field plot that had received repeated applications of boscalid over several years and thus contained residues that had been in contact with the soil for an extended time period (aged residues). The other charge of the soil was from a nearby area of the same field that had not yet received any boscalid treatments.

The soils were acclimatized, and soil moisture was adjusted to about 40 % of the maximum water holding capacity. The soil concentration of aged boscalid from the treated plot was determined by residue analysis, and the untreated soil was spiked to a similar concentration with non-radiolabeled boscalid. 100 g aliquots of the soils containing aged residues or freshly applied boscalid, respectively, were incubated at 20°C in the dark. Samples were taken after 0, 7, 14/15, 29, 58/62, 87/91, 119/120, 149/152 and 179/182 days of incubation.

Extractable residues of the test item were determined after extraction with methanol/water (1/1, v/v). Desorption of boscalid residues was determined by extraction with 0.01 M $CaCl_2$ solution. Analysis was performed using LC-MS/MS.

Aged residues of boscalid decreased only by about 10 %, whereas for freshly applied boscalid, a degradation of about 30 % during the incubation of about 180 days was observed. The aerobic degradation behavior was evaluated using single first-order kinetics (SFO). The estimated degradation time (DT_{50}) of aged residues was 746 days, whereas the DT_{50} of freshly applied boscalid was distinctly shorter with a value of 336 days. The difference can be attributed to the known effect of time-dependent sorption (non-equilibrium sorption), expressed by increasing apparent distribution coefficients K_d and carbon normalized adsorption coefficients K_{OC} in freshly treated soil samples and almost constant high K_d and K_{OC} values in the soil containing the aged boscalid residues. This effect reduces the amount of dissolved residues that is available e.g. for microbial degradation. As a result, the apparent degradation of aged residues is slower than that of fresh residues.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

Internal code:	BAS 510 F
Common name:	boscalid
Reg. No.:	300355
Chemical name (IUPAC):	2-chloro-N-(4'-chloro-biphenyl-2-yl)nicotinamide
Molecular formula:	$C_{18}H_{12}Cl_2N_2O$
Molar mass:	343.2 g mol^{-1}
Batch No.:	01893-55
Purity:	100.0%

2. Soil

Two field-fresh charges of one soil ("Studernheim") were used in this study. One charge of the soil was from a field plot that had received repeated applications of boscalid over several years and thus contained residues that had been in contact with the soil for an extended time period (aged residues). The other charge of the soil was from a nearby area of the same field that had not yet received any boscalid treatments. A summary of the soil characteristics is given in Table 7.1.1.1-10. The soils were acclimatized and soil moisture was adjusted to about 40 % of the maximum water holding capacity.

Table 7.1.1.1-10: Soil Characteristics

Soil designation	**Studernheim 21/03/07**
Origin	Studernheim, Germany
DIN Particle size distribution [%] sand 0.063 – 2 mm silt 0.002 – 0.063 mm clay < 0.002 mm textural class	 37.9 41.2 21.0 sandy loam
USDA Particle size distribution [%] sand 0.050 – 2 mm silt 0.002 – 0.050 mm clay < 0.002 mm textural class	 40.6 38.4 21.0 loam
Organic C [%]	2.00
Organic matter [%] [b]	3.45
pH [H_2O]	8.3
pH [$CaCl_2$]	7.5
Cation exchange capacity [$cmol^+ kg^{-1}$]	15.5
Maximum water holding capacity [g/100 g dry soil] [a]	45.2
Microbial biomass (start of study) [mg C/100 g dry soil] [a]	11.2 [c] / 9.0 [d]
Microbial biomass (end of study) [mg C/100 g dry soil] [a]	4.7 [c] / 9.7 [d]

[a] determined at the test facility PTRL Europe
[b] organic matter = organic carbon x 1.724
[c] "aged soil"
[d] "virgin soil"

B. STUDY DESIGN

1. Experimental conditions

The concentration of aged boscalid in the soil from the treated plot was determined to be about 0.3 mg kg^{-1}. The soil from the untreated plot was spiked to a similar concentration with non-radiolabeled boscalid (about 0.21 mg kg^{-1}).

100 g (dry weight) aliquots of both soils containing aged residues or freshly applied boscalid, respectively, were weight into 1 L incubation flasks, septum-sealed, connected by tubes to allow aeration with a continuous flow of humidified air, placed in thermostat-controlled cabinets, and incubated at 20°C in the dark.

2. Sampling

Samples were taken 0, 7, 14/15, 29, 58/62, 87/91, 119/120, 149/152, and 179/182 days after treatment (DAT).
At each sampling time, two replicate vessels were sampled. One replicate sample was worked up, whereas the other replicate sample was immediately frozen, except for samplings 0, 87/91 and 179/182 DAT, where both replicates were worked up.

3. Description of analytical procedures

Determination of boscalid in soil
A 10 g aliquot of the soil specimen (dry mass) was weighed into a centrifuge bottle, 10 mL of methanol were added, and the bottle was shaken at 300 rpm for 30 min. Thereafter, the specimens were centrifuged at 3000 rpm for 5 min, the supernatant decanted and filtered through a funnel plugged with glass wool into a 25 mL volumetric flask.
Subsequently, a 15 mL aliquot of solvent mixture (methanol/water, 1/1, v/v) was added to the soil pellet and the soil was loosened, followed by another 30 min period of shaking and 5 min of centrifugation. The supernatant was again decanted, filtered, and combined with the first extract. The volume was adjusted with methanol to the mark, and an aliquot was further diluted and finally used for LC-MS/MS determination.
External calibration was used for quantification of the analyte (quantitation ion 307 m/z) by LC/MS/MS using ESI. Calibrations were established with standard solutions prepared in solvent injected interspersed with the soil extracts. Calibrations usually ranged from 0.1 ng mL^{-1} to 250 ng mL^{-1}.

Additionally, appropriate samples were set up and analyzed for concurrent recovery control and individual recoveries.

The concentration of boscalid extracted with organic solvent after given incubation times from aliquots of soil containing aged residues or spiked soil was used to evaluate the aerobic degradation of boscalid.

Aqueous extraction (desorption) of boscalid from soil
A 20 g aliquot of the soil specimen (dry mass) were transferred to centrifuge flasks for desorption by shaking for 24 h with 36 mL aqueous 0.01 M $CaCl_2$ solution on a horizontal shaker at room temperature. Subsequently, the soil/water mixtures were centrifuged for 5 min at 2000 rpm. An aliquot was further centrifuged, diluted with injection solvent, and analyzed by LC-MS/MS.

For method validation, untreated and incubated soil samples were fortified with the test item solved in 0.01 M $CaCl_2$ solution. Aliquots of the solution were diluted and analyzed by LC-MS/MS to determine the recovery.
The extractability of boscalid extracted by aqueous $CaCl_2$ solution is defined as the amount of the substance which is desorbed, related to the quantity of substance initially present (desorption in %).

Apparent distribution coefficients K_d and K_{OC} were calculated.

4. Calculation of the degradation rate

The residue behavior was evaluated by separately fitting kinetic models to the observed residues of boscalid (aged or freshly spiked, expressed as µg kg^{-1}) using the software package KinGUI version 1.1.
The kinetic analyses followed the "Recommended procedures to derive endpoints for parent compounds" as outlined by FOCUS [*FOCUS (2006*] in Chapter 7.

II. RESULTS AND DISCUSSION

A. AEROBIC SOIL DEGRADATION

The amount of boscalid extracted with organic solvent was expressed as a percentage of the total residue determined at the beginning of the incubation (aged: 0.30 mg kg^{-1}, freshly applied: 0.21 mg kg^{-1}). Table 7.1.1.1-11 shows the aerobic soil degradation for aged and freshly applied residues of boscalid.

Table 7.1.1.1-11: Degradation of aged and freshly applied residues of boscalid in soil

Degradation of aged residues		Degradation of fresh residues	
Time [Days]	**%TR [a]**	**Time [Days]**	**%TAR [b]**
0	103	0	106
0	102	0	109
7	100	7	81
14	108	15	91
29	90	29	88
62	105	58	76
91	88	87	77
91	86	87	81
120	90	119	75
152	87	149	64
182	90	179	73
182	87	179	71

[a] % of total initial residues
[b] % of total applied residues

Aged residues of boscalid decreased only for about 10 %, whereas for freshly applied boscalid a degradation of about 30 % during the incubation of about 180 days was observed.

B. LONG-TERM SORPTION BEHAVIOR

The amount of boscalid extracted from soil with $CaCl_2$ solution is expressed as a percentage of the total residue determined at the beginning of the incubation. Results are shown in Table 7.1.1.1-12.

Table 7.1.1.1-12: Desorption of aged and freshly applied residues of boscalid in soil

Desorption of aged residues		Desorption of fresh residues	
Time [Days]	**%TR [a]**	**Time [Days]**	**%TAR [b]**
0	11	0	21
0	10	0	21
7	14	7	17
14	11	15	14
29	11	29	15
62	11	58	9
91	9	87	8
91	8	87	8
120	8	119	7
152	7	149	7
182	9	179	7
182	9	179	7

[a] % of total initial residues
[b] % of total applied residues

The desorption of aged residues of boscalid ("aged" soil) shows a slight decline from 11 % to about 9 % after 182 days of incubation. For freshly applied boscalid ("virgin" soil), the aqueous extractability decreased more significant: from 21 % to 7 % at the end of incubation.

Apparent distribution coefficients K_d and carbon normalized adsorption coefficient K_{OC} of freshly applied residues increased with incubation time. In contrast, K_d and K_{OC} values of aged residues remained almost constant (see Table 7.1.1.1-13).

Table 7.1.1.1-13: Apparent distribution coefficients K_d and K_{OC} of soil containing aged and freshly applied residues of boscalid

Aged residues			Fresh residues		
Time [Days]	**K_d [mL g^{-1}]**	**K_{OC} [mL g^{-1}]**	**Time [Days]**	**K_d [mL g^{-1}]**	**K_{OC} [mL g^{-1}]**
0	17.3	864	0	7.9	393
0	17.7	883	0	8.4	420
7	11.9	595	7	7.6	378
14	16.8	841	15	11.0	548
29	15.0	749	29	9.9	495
62	17.9	895	58	14.2	711
91	18.3	914	87	16.2	812
91	19.0	948	87	19.3	964
120	21.1	1053	119	18.2	910
152	22.6	1130	149	15.6	778
182	18.0	901	179	20.2	1008
182	17.9	896	179	19.7	986

C. KINETIC MODELING RESULTS

The observed residues of boscalid (aged or freshly spiked) were described best by the fitted curves based on single first-order (SFO) kinetics. DT_{50} and DT_{90} values based on SFO kinetics are shown in Table 7.1.1.1-14.

Table 7.1.1.1-14: DT_{50}/DT_{90} values of aged boscalid residues and freshly applied boscalid

Soil	Kinetic	χ^2 error [%]	DT_{50} [d]	DT_{90} [d]
Aged boscalid residues	SFO	4.4	745.7	>1000
Freshly applied boscalid	SFO	7.0	336.2	>1000

III. CONCLUSION

The evaluation of the aerobic degradation applying single first-order kinetics resulted in a degradation time (DT_{50}) for aged residues of boscalid in soil of 746 days, whereas for boscalid freshly applied to soil a significantly shorter DT_{50} of 336 days could be calculated.

The observed increase of the adsorption of boscalid to the soil over time was attributed to the known effect of time-dependent sorption (non-equilibrium sorption). This effect reduces the amount of dissolved residues that is available e.g. for microbial degradation. As a result, the apparent degradation of aged residues is slower than that of fresh residues.

CA 7.1.1.2 Anaerobic degradation

Although anaerobic conditions over extended time periods are not expected to occur following application of boscalid, two anaerobic soil metabolism studies with [pyridine-3-^{14}C]-labeled and [diphenyl-U-^{14}C]-labeled boscalid were considered for previous Annex I listing (Table 7.1.1.2-1).

Table 7.1.1.2-1: Studies on anaerobic soil degradation of boscalid

Reference	BASF DocID	Soil type (USDA)	Application rate [mg kg^{-1}]	Moisture [% MWHC]	Incubation temperature [°C]	Incubation period [days]	Remark
Staudenmaier H., Schaefer C., 2000a	2000/ 1014986	sandy loam	1.0	flooded	20±2	120	
Staudenmaier H., 2000a	2000/ 1014990	sandy loam	1.0	flooded	20±2	120	

MWHC = Maximum water holding capacity

Boscalid showed slow degradation and very low levels of mineralization (<1% TAR). Bound residues were formed in moderate amounts. No major metabolites were detected in the study with the diphenyl label [*BASF DocID 2000/1014986*]. In the study with the pyridine label, metabolite M510F47 was detected and reached a maximum of 6.7% TAR [*BASF DocID 2000/1014990*].

CA 7.1.1.3 Soil photolysis

A photolysis study with pyridine-^{14}C-labeled boscalid was submitted for the previous Annex I listing (Table 7.1.1.3-1).

Table 7.1.1.3-1:Studies on soil photolysis of boscalid

Reference	BASF DocID	Soil type (USDA)	Application rate [mg kg^{-1}]	Moisture [% MWHC]	Incubation temperature [°C]	Irradiation period [days]	Remark
von Goetz N., 2000b	2000/1014989	sandy loam	4.6	40	22±1	15	

MWHC = Maximum water holding capacity

Boscalid degraded very slowly under irradiated conditions whereas in the dark control practically no degradation could be observed within 15 days. Mineralisation was negligible under both photolysis and dark control conditions. In total, a slight difference was observed between photolysis and dark control, indicating that light might enhance the degradation of BAS 510 F on soil.

Report: CA 7.1.1.3/1
Pape L., 2014a
Kinetic evaluation of a laboratory soil photolysis study with Boscalid according to FOCUS Degradation Kinetics
2014/1261102
Guidelines: FOCUS Kinetics (2006) SANCO/10058/2005 version 1.0 of Nov. 2011
GLP: no

Executive Summary

The photolytic degradation behavior of BAS 510 F - boscalid in soil was investigated with one soil using pyridine-^{14}C-labeled boscalid. The purpose of this evaluation was to analyze the degradation kinetics observed in the study, taking into account the current guidance of the FOCUS workgroup on degradation kinetics.

Kinetic evaluation was performed in order to derive degradation parameters for environmental fate models (modeling endpoints).

The calculated kinetic endpoints for modeling are summarized in the following table.

Table 7.1.1.3-2: Modeling endpoints for boscalid

Compound	Test system	Kinetic model	χ^2 error	DT_{50} [d]	k [d^{-1}]
Boscalid	Irradiated soil	SFO	0.9	126.8	0.0055

Since the estimated DT_{50} of 126.8 days is considerably longer than the 15 days study period, the value needs to be treated with caution.

I. MATERIAL AND METHODS

The photolytic degradation of pyridine-^{14}C-labeled boscalid was investigated in one soil taking into account the current guidance of the FOCUS workgroup on degradation kinetics [*FOCUS (2006): "Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration" Report of the FOCUS Work Group on Degradation Kinetics, EC Document Reference Sanco/10058/2005 version 1.0, 436 pp*].

1. Kinetic modeling strategy

The kinetic evaluation was performed in order to derive modeling endpoints. The appropriate kinetic model was identified based on visual and statistical assessment considering the procedures and kinetic models proposed by the FOCUS workgroup on degradation kinetics [*FOCUS (2006)*]. The appropriate modelling endpoints for use in environmental fate models were derived depending on the kinetic model.

2. Kinetic models included in the evaluations

The kinetic evaluation started with testing the single first order (SFO) model proposed by FOCUS Kinetics [*FOCUS (2006)*]. The goodness-of-fit of the SFO kinetic model was assessed by visual inspection and statistical measures, as recommended by the FOCUS Kinetics guidance [*FOCUS (2006)*].

Since the resulting model fit was already good, testing of bi-phasic models was not required.

3. Data handling and software for kinetic evaluation

The experimental data were derived from the study reports and adjusted according to FOCUS [*FOCUS (2006)*].

The software package KinGUI version 2.2012.320.1629 was used for parameter fitting. The error tolerance and the number of iterations of the optimization tool (IRLS) were set to $1x10^{-6}$ and 100, respectively.

4. Experimental data

The photolytic degradation of boscalid (pyridine-^{14}C-labeled) was investigated in one soil [*already peer-reviewed study BASF DocID 2000/1014989*]. The soil characteristics are summarized in Table 7.1.1.3-3.

Table 7.1.1.3-3: Soil characteristics of study 2000/1014989

Parameter	**Limburgerhof, Bruch West**
Soil type (USDA)	Sandy loam
Particle size distribution [%]	
Sand (50 - 2000 µm)	63
Silt (2 - 50 µm)	27
Clay (< 2 µm)	10
Organic carbon [%]	1.9
CEC [mVal 100g^{-1} dry weight]	9.8
pH ($CaCl_2$)	7.3
Max. water holding capacity [g 100g^{-1} soil]	35.9

Two test systems were used: irradiated test system and dark control test system. The test substance was applied at a rate of 4.6 µg a.s. g^{-1} dry soil, corresponding to a field application rate of 700 g a.s. ha^{-1}. Both test systems were incubated for 15 days at 22 ± 1 °C and soil moisture of 40 % of the maximum water holding capacity (MWHC). The irradiated test system was irradiated continuously at a light intensity of 3 mW cm^{-2}. For both test systems samples were taken at 0, 2, 6, 9, 12, and 15 days after treatment (DAT).

The measured data as well as the resulting dataset submitted to kinetic analysis are given in Table 7.1.1.3-4.

Table 7.1.1.3-4: Data for kinetic evaluation of photolytic degradation of boscalid

day	Experimental data [a] [%TAR]	Input data according to FOCUS [%TAR]
0	99.40	100.10 [b]
2	98.70	98.70
6	96.70	96.70
9	97.10	97.10
12	95.20	95.20
15	90.60	90.60

TAR = total applied radioactivity
[a] Mean of two measurements
[b] Set to material balance

II. RESULTS AND DISCUSSION

The kinetic evaluation of the irradiated test system showed that the SFO kinetic model is appropriate to describe the observed degradation behavior of boscalid and can be used to derive modeling endpoints from this study. Calculated DT_{50} and DT_{90} values for the irradiated system were 126.8 days and 421.3 days, respectively. No degradation was observed in the dark during the 15 d study period. Since the estimated modeling endpoints are considerably longer than the study duration (15 d) the values need to be treated with caution.

III. CONCLUSION

According to the guidance of the FOCUS workgroup on degradation kinetics, modeling endpoints for photolytic degradation of boscalid in soil were derived with the SFO kinetic model. Boscalid degraded in the irradiated test system with a DT_{50} of 126.8 days, while no degradation was observed in the dark during the 15 d study period.

CA 7.1.2 Rate of degradation in soil

The peer-reviewed and new studies on degradation of boscalid under laboratory conditions are presented in CA 7.1.1 together with the already peer-reviewed metabolite studies.

Two new metabolite studies are included in the dossier that were triggered by results from parent studies presented in CA 7.1.1: A new soil degradation study was initiated for M510F47 that was formed in the already peer-reviewed anaerobic degradation study. For the previous Annex I listing degradation was not investigated further due to the relevant trigger value of >10% TAR valid at that time. Another new soil study was initiated for M510F49. The metabolite was observed in the US study that is now included in the dossier. The new metabolite study is reported to comply with current trigger values.

Information about the dissipation and accumulation behavior of boscalid in the field was already derived from the peer-reviewed studies. The final report of the accumulation study that was still ongoing at the time of the previous Annex I listing is included as a new study in the current dossier. Additional studies were conducted to enhance understanding of the dissipation and accumulation behavior and are now included as supplemental studies in the dossier.

New field soil dissipation studies are included in the dossier to elucidate the occurrence and behavior of M510F49 in the field to comply with current data requirements.

Summary tables of all obtained soil degradation values for boscalid and major metabolites can be found at the end of chapter “Rate of degradation in soil” CA 7.1.2.

CA 7.1.2.1 Laboratory studies

CA 7.1.2.1.1 Aerobic degradation of the active substance

For the previous Annex I listing, information on the rate of degradation in aerobic soil were available from three studies with [pyridine-3-^{14}C]-labeled or [diphenyl-U-^{14}C]-labeled boscalid [see Table 7.1.2.1.1-1].

Table 7.1.2.1.1-1 Studies on aerobic soil degradation rates of boscalid

Reference	BASF DocID	Soil type USDA)	Application rate [mg kg^{-1}]	Moisture [% MWHC]	Incubation temperature [°C]	Incu-bation period [days]	Remark
Hein W., 1998a	1998/10607	loamy sand	4 x 10.0 [a] + 7.0 [b] 3 x 5.0 [c] + 7.0 [b] 4 x 7.0 [d] + 7.0 [b]	50	20±1	120	
			3 x 5.0 [c] + 7.0 [b] 3 x 5.0 [d] + 7.0 [b]				
			4 x 7.0 [d] + 7.0 [b]				
			0.0 + 7.0 [b]				
		loam	5.25 + 2 x 7.0 [c] + 7.0 [b]				
			2.25 + 2 x 3.0 [c] + 7.0 [b]				
			0.0 + 7.0 [b]				
Stephan A., 1999a	1999/11807	sandy loam	1.0	40	20±1	364	
Ebert D., Harder U., 2000a	2000/1013279	loamy sand	1.0	40	20	120	
		loamy sand		40 20 40 40 40	20 20 5 30 20 (sterile)		
		sandy loam		40	20		
		Loam		40	20		

MWHC = Maximum water holding capacity
[a] Pre-applications made in 1st year
[b] Application in laboratory degradation study
[c] Pre-applications made in 2nd year
[d] Pre-applications made in 3rd year

New soil studies with boscalid that provided degradation rates are summarized in chapter M-CA 7.1.1.1 [*see CA 7.1.1.1/1, BASF DocID 2002/5002772, CA 7.1.1.1/2, BASF DocID 2008/1013108*].

Both peer-reviewed and new studies with boscalid were evaluated to calculate degradation parameters for modelling according to the current FOCUS guidance. Where possible also degradation parameters for modelling were calculated for the metabolites M510F47 and M510F49. The evaluation also includes an already peer-reviewed soil metabolism study with M510F47 applied that is included in chapter M-CA 7.1.2.1.2 [*already peer-reviewed study BASF DocID 2000/1013280*].

Report: CA 7.1.2.1.1/1
Pape L., 2014b
Kinetic evaluation of laboratory soil degradation studies with Boscalid and Chloronicotinic acid according to FOCUS Degradation Kinetics 2014/1261100
Guidelines: FOCUS Kinetics (2006) SANCO/10058/2005 version 1.0 of Nov. 2011
GLP: no

Executive Summary

The degradation of the fungicide BAS 510 F - boscalid in soil has been investigated under aerobic laboratory conditions in four studies using different soils from Europe and the USA. In addition, the degradation of chloronicotinic acid (M510F47), an intermediate degradation product during soil degradation of boscalid, has been investigated in one study with one soil. The purpose of this evaluation was to analyze the degradation kinetics of boscalid and its metabolites M510F49 and M510F47 observed in these studies according to the current guidance of the FOCUS workgroup on degradation kinetics, in order to derive degradation parameters for environmental fate models (modeling endpoints).

For the experiments under standard incubation conditions (EU: 20°C, 40% of MWHC; US: 27°C, 75% of MWHC at 0.33 bar) the resulting DT_{50} values were further normalized to reference conditions (20°C, pF 2) according to the recommendations of the FOCUS groundwater workgroup. The calculated DT_{50} at study conditions as well as the corresponding DT_{50} values normalized to reference conditions for boscalid and the metabolites M510F49 and M510F47 are summarized in the following tables.

Table 7.1.2.1.1-2: Modeling endpoints for boscalid derived from standard degradation studies (EU or US)

Study DocID (label)	Soil	Study duration [d]	Temp. [°C]	Moisture [% MWHC]	Kinetic model	χ^2	DT_{50act} [d]	DT_{50ref} [d]
Aerobic soil degradation of boscalid								
1999/11807 (diphenyl + pyridine)	Bruch West	364	20	40	DFOP	5.3	300.2	280.0
2000/1013279 (diphenyl)	Li35b	120	20	40	SFO	3.9	430.7	413.3
	Lufa 2.2	120	20	40	SFO	4.1	418.0	418.0
	US-soil	120	20	40	SFO	3.6	526.4	372.7
	Canadian soil	119	20	40	DFOP	4.1	212.2 [a]	163.3
2002/5002772 (pyridine)	California	371	27	75 (at 0.33 bar)	DFOP	2.3	305.8 [a]	437.0
	Idaho	371	27	75 (at 0.33 bar)	DFOP	2.3	645.4 [a]	1214.4
	Illinois	371	27	75 (at 0.33 bar)	DFOP	3.1	613.4 [a]	1081.3
	North Dakota	371	27	75 (at 0.33 bar)	DFOP	3.6	531.2 [a]	869.0

DT_{50act} = DT_{50} at study conditions
DT_{50ref} = DT_{50} at reference conditions (20°C, pF 2)
[a] Calculated from slow phase of DFOP model (DT_{50} = ln(2)/k2)

Table 7.1.2.1.1-3: Modeling endpoints for boscalid derived from non-standard degradation studies

Study DocID (label)	Soil	Study duration [d]	Temp. [°C]	Moisture [% MWHC]	Kinetic model	χ^2	DT_{50act} [d]
Aerobic soil degradation of boscalid, influence of pretreatment							
1998/10607 (diphenyl)	Limburgerhof soil I	120	20	50	DFOP	1.9	*No reliable endpoint found.*
	Limburgerhof soil II	120	20	50	DFOP	1.1	889.8 [a]
	Limburgerhof soil III	120	20	50	DFOP	1.2	605.9 [a]
	Limburgerhof soil IV (no pretreatment)	120	20	50	DFOP	1.8	418.6 [a]
	Edesheim soil V	120	20	50	SFO	2.0	139.8
	Edesheim soil VI	120	20	50	SFO	3.0	152.4
	Edesheim soil VII (no pretreatment)	120	20	50	SFO	1.6	194.4
Aerobic soil degradation of boscalid							
2000/1013279 (diphenyl)	*Lufa 2.2*	120	30	40	*SFO*	1.1	*358.4*
	Lufa 2.2	119	5	40	*Not evaluated, because degradation was not observed.*		
	Lufa 2.2	120	20	20			
	*Lufa 2.2 (*sterile)	120	20	40			

DT_{50act} = DT_{50} at study conditions
[a] Calculated from slow phase of DFOP model (DT_{50} = ln(2)/k2)

Table 7.1.2.1.1-4: Modeling endpoints for the metabolites M510F49 and M510F47 derived from standard degradation studies (EU or US)

Study DocID (label)	Soil	Study duration [d]	Temp. [°C]	Moisture [% MWHC]	Kinetic model	χ^2	DT_{50act} [d]	DT_{50ref} [d]	Formation fraction
Aerobic soil degradation of M510F49									
2002/5002772 (pyridine labeled boscalid)	California	371	27	75 (at 0.33 bar)	SFO [a]	18.9	248.9	355.7	n/a [a]
	Idaho	371	27	75 (at 0.33 bar)	SFO [b]	15.8	No reliable endpoint found.		0.100
	Illinois	371	27	75 (at 0.33 bar)	SFO [b]	8.9	No reliable endpoint found.		0.291
	North Dakota	371	27	75 (at 0.33 bar)	No acceptable fit due to scatter of data.				
Aerobic soil degradation of M510F47									
2000/1013280 (pyridine labeled M510F47)	Bruch West	14	20	40	SFO [c]	12.1	3.3	2.9	n/a [c]
2002/5002772 (pyridine labeled boscalid)	California	371	27	75 (at 0.33 bar)	Metabolite not detected.				
	Idaho	371	27	75 (at 0.33 bar)	Metabolite detected only in one sample				
	Illinois	371	27	75 (at 0.33 bar)	Metabolite data too scattered for kinetic evaluation				
	North Dakota	371	27	75 (at 0.33 bar)					

n/a = Not applicable
[a] Metabolite decline fit
[b] DFOP kinetics for parent
[c] Metabolite applied as parent

I. MATERIAL AND METHODS

The degradation of boscalid and its soil metabolites M510F49 and M510F47 was analyzed in four different studies under laboratory conditions using different soils from Europe and the USA [*already peer-reviewed studies BASF DocID 1998/10606, BASF DocID 1999/11807, BASF DocID 2000/1013279; new study BASF DocID 2002/5002772 submitted under CA 7.1.1.1/1*]. In addition, the degradation of chloronicotinic acid (M510F47), an intermediate degradation product during soil degradation of boscalid, has been investigated in one study with one soil [*already peer-reviewed study BASF DocID 2000/1013280*].
For the kinetic evaluation of these studies, the current guidance of the FOCUS workgroup on degradation kinetics [*FOCUS (2006)*] was taken into account.

1. Kinetic modeling strategy

Kinetic evaluation was performed in order to derive modeling endpoints. The appropriate kinetic model was identified considering the procedures and kinetic models proposed by the FOCUS workgroup on degradation kinetics [*FOCUS (2006)*]. The appropriate model was selected based on visual and statistical assessment. Appropriate DT_{50} values for use in environmental fate models were derived depending on the kinetic model.

2. Kinetic models included in the evaluations

For each data set, the kinetic models proposed by FOCUS Kinetics [*FOCUS (2006)*] were tested in order to identify the appropriate model, i.e. single first order (SFO) kinetics, and the bi-exponential (DFOP) kinetics. The respective model descriptions and corresponding equations for calculating endpoints (DT_{50}, DT_{90}) are shown in the FOCUS Kinetics guidance [*FOCUS (2006)*].

The goodness-of-fit of the kinetic models was assessed by visual inspection and statistical measures, as recommended by the FOCUS Kinetics guidance [*FOCUS (2006)*].

3. Data handling and software for kinetic evaluation

Where available, replicate measurements were used in the parameter estimation. The experimental data were derived from the study reports and adjusted according to FOCUS [*FOCUS (2006)*].

The software package KinGUI version 2.2012.320.1629 was used for parameter fitting. The error tolerance and the number of iterations of the optimization tool (IRLS) were set to $1x10^{-6}$ and 100, respectively.

4. Normalization of degradation rates to reference conditions

In accordance with FOCUS guidance [*FOCUS (2006)*] the DT_{50} values obtained from laboratory studies were normalized for modeling purposes to reference conditions at a temperature of 20°C and soil moisture at pF2 to account for the different soil temperature and moisture conditions during incubation.

5. Experimental data

The kinetic evaluation was conducted for different soils from Europe and the USA deriving from five aerobic laboratory soil degradation studies, thereof four for the parent compound [*BASF DocID 1998/10607; BASF DocID 1999/11807; BASF DocID 2000/1013279; BASF DocID 2002/5002772*] and one for metabolite M510F47 [*BASF DocID 2000/1013280*]. The test substance was applied at a nominal rate of 0.7 mg kg^{-1} dry soil [*BASF DocID 1998/10607*], 1.0 mg kg^{-1} dry soil [*BASF DocID 1999/11807; BASF DocID 2000/1013279*], or 0.88 – 0.95 mg kg^{-1} dry soil [*BASF DocID 2002/5002772*]. Metabolite M510F47 was applied at a nominal rate of 0.25 mg kg^{-1} soil [*BASF DocID 2000/1013280*]. The soil characteristics are summarized in Table 7.1.2.1.1-5 to Table 7.1.2.1.1-9.

Table 7.1.2.1.1-5: Soil characteristics of study 1998/10607

Parameter	**Limburgerhof soil IV** [a]	**Edesheim soil VII** [a]
Soil type (USDA)	Loamy sand	Loam
Particle size distribution [%]		
Sand (50 - 2000 µm)	84	48
Silt (2 - 50 µm)	12	35
Clay (< 2 µm)	4	17
Organic carbon [%]	1.4	1.6
Microbial biomass [mg C kg^{-1} dry soil] at start of study	Soil I: 1005.59 Soil II: 1036.79 Soil III: 1007.64 Soil IV: 1028.13	Soil V: 1126.14 Soil VI: 1036.80 Soil VII: 1102.24
pH ($CaCl_2$)	5.9	6.9
MWHC [g 100g^{-1} dry soil]	not reported	

[a] Soils were considered representative for the respective sampling sites

Table 7.1.2.1.1-6: Soil characteristics of study 1999/11807

Parameter	**Limburgerhof, Bruch West**
Soil type (USDA)	Sandy loam
Particle size distribution [%]	
Sand (50 - 2000 µm)	77
Silt (2 - 50 µm)	17
Clay (<2 µm)	6
Organic carbon [%]	1.3
Microbial biomass [mg C kg^{-1} dry soil] **at start of study** **at end of study**	 35.1 21.9
pH ($CaCl_2$)	7.4
MWHC [g 100g^{-1} dry soil]	43

Table 7.1.2.1.1-7: Soil characteristics of study 2000/1013279

Parameter	Li35b	Lufa 2.2 [a]	US soil	Canadian soil	Lufa 2.2 [b]
Soil type (USDA)	Loamy sand	Loamy sand	Sandy loam	Loam	Loamy sand
Particle size distribution [%]					
Sand (50 - 2000 µm)	80	84	72	49	81
Silt (2 - 50 µm)	11	10	14	36	16
Clay (< 2 µm)	9	6	14	15	3
Organic carbon [%]	1.0	2.5	0.6	5.2	2.2
Microbial biomass [mg C kg^{-1} dry soil] at start of study	30.4	58.1	70.3	49.0	49.4
pH ($CaCl_2$)	6.6	5.6	7.0	7.7 [c]	5.7
MWHC [g $100g^{-1}$ dry soil]	33	39	29	43	38

[a] Incubated at 20 °C, 40 % MWHC
[b] Incubated at 30 °C, 40 % MWHC
[c] Solution was not reported

Table 7.1.2.1.1-8: Soil characteristics of study 2002/5002772

Parameter	California	Idaho	Illinois	North Dakota
Soil type (USDA)	Clay loam	Clay loam	Silt loam	Loam
Particle size distribution [%]				
Sand (50 - 2000 µm)	44	32	28	46
Silt (2 - 50 µm)	28	38	54	34
Clay (< 2 µm)	28	30	18	20
Organic carbon [%] [a]	2.7	2.0	1.3	2.1
Microbial biomass [µg g^{-1} dry soil] at start of study	218.9	301.5	24.3	80.1
pH (saturated paste)	7.8	6.8	6.5	7.7
MWHC [g $100g^{-1}$ dry soil]	39.8	43.5	40.4	44.2
MWHC at 1/3 bar [g $100g^{-1}$ dry soil]	24.1	35.7	30.2	26.1

[a] Calculated from organic matter content by division by 1.724

Table 7.1.2.1.1-9: Soil characteristics of study 2000/1013280

Parameter	Bruch West
Soil type (USDA)	Sandy loam
Particle size distribution [%]	
Sand (50 - 2000 µm)	59.3
Silt (2 - 50 µm)	29.8
Clay (<2 µm)	10.8
Organic carbon [%]	1.90
Microbial biomass [mg C kg^{-1} dry soil] at start of study	31.9
pH($CaCl_2$)	7.6
MWHC [g $100g^{-1}$ dry soil]	40.0

An overview of the experimental conditions of the studies is given in Table 7.1.2.1.1-10.

Table 7.1.2.1.1-10: Overview on experimental conditions

Study (DocID)	Analyte	Soil	Soil type (USDA)	Moisture [% MWHC]	Temp. [°C]	Duration [d]	Residues at end of study [%TAR]
1998/10607	Boscalid (d)	Limburgerhof soil I – soil IV	Loamy sand	50	20	120	72.83 – 89.41
		Edesheim soil V – soil VII	Loam	50	20		52.95 – 62.97
1999/11807	Boscalid (d+p)	Bruch West	Sandy loam	40	20	364	16.7 / 17.3
2000/1013279	Boscalid (d)	Li35b	Loamy sand	40	20	120	77.7
		Lufa 2.2	Loamy sand	40	20	120	78.8
		US soil	Sandy loam	40	20	120	80.9
		Canadian soil	Loam	40	20	119	53.6
		Lufa 2.2	Loamy sand	20	20	119	98.8
		Lufa 2.2	Loamy sand	40	5	120	103.8
		Lufa 2.2	Loamy sand	40	30	120	84.4
		Lufa 2.2 (sterile)	Loamy sand	40	20	120	100.5
2002/5002772	Boscalid (p), M510F47, M510F49	California	Clay loam	75 [a]	27	371	33.1
		Idaho	Clay loam	75 [a]	27		58.7
		Illinois	Silt loam	75 [a]	27		47.6
		North Dakota	Loam	75 [a]	27		44.8
2000/1013280	M510F47	Bruch West	Sandy loam	40	20	14	0.4

(d) = Diphenyl-^{14}C-labeled boscalid
(p) = Pyridine-^{14}C-labeled boscalid
[a] Referring to MWHC at 0.33 bar

The measured data as well as resulting datasets submitted to kinetic analysis are given in Table 7.1.2.1.1-11 to Table 7.1.2.1.1-15. In the US study [*BASF DocID 2002/5002772*], M510F47 was not formed in the California soil and was detected only once in the Idaho soil (0.8% TAR). The metabolite occurred in subsequent samples in the Illinois and North Dakota soils (max. 4.3% TAR), but a kinetic evaluation was not possible due to the scatter of data from the beginning of the incubation phase. Therefore, Table 7.1.2.1.1-14 reports experimental data for boscalid and M510F49 only.

Table 7.1.2.1.1-11: Experimental data from study 1998/10607 used for kinetic evaluation (diphenyl-^{14}C-labeled boscalid)

DAT	Experimental data [%TAR]	Input data according to FOCUS [%TAR]
Limburgerhof soil I		
0	98.82	103.09 [a]
7	93.43	93.43
20	90.29	90.29
40	89.26	89.26
60	86.76	86.76
90	83.73	83.73
120	89.41	89.41
Limburgerhof soil II		
0	97.86	101.60 [a]
7	91.90	91.90
20	87.19	87.19
40	88.53	88.53
60	85.36	85.36
90	81.75	81.75
120	82.34	82.34
Limburgerhof soil III		
0	94.47	99.23 [a]
7	89.27	89.27
20	88.67	88.67
40	86.90	86.90
60	85.10	85.10
90	79.08	79.08
120	80.09	80.09
Limburgerhof soil IV		
0	96.31	100.53 [a]
7	92.34	92.34
20	84.71	84.71
40	86.12	86.12
60	84.25	84.25
90	77.99	77.99
120	72.83	72.83
Edesheim soil V		
0	94.74	98.06 [a]
7	90.44	90.44
20	87.58	87.58
40	81.30	81.30
60	74.48	74.48
90	59.83	59.83
120	52.95	52.95

Table 7.1.2.1.1-11: Experimental data from study 1998/10607 used for kinetic evaluation (diphenyl-^{14}C-labeled boscalid)

DAT	Experimental data [%TAR]	Input data according to FOCUS [%TAR]
Edesheim soil VI		
0	93.47	97.33 [a]
7	88.51	88.51
20	84.71	84.71
40	81.71	81.71
60	76.77	76.77
90	59.83	59.83
120	54.65	54.65
Edesheim soil VII		
0	94.63	98.63 [a]
7	91.93	91.93
20	89.81	89.81
40	85.72	85.72
60	80.37	80.37
90	68.93	68.93
120	62.97	62.97

DAT = Days after treatment
TAR = Total Applied Radioactivity
[a] Set to material balance

Table 7.1.2.1.1-12: Experimental data from study 1998/10807 used for kinetic evaluation (diphenyl-^{14}C- and pyridine-^{14}C-labeled boscalid)

DAT	Experimental data [a] [%TAR]	Input data according to FOCUS [%TAR]
Bruch West soil		
0	99.70	100.0 [b]
0	99.60	100.0 [b]
7	95.5	95.5
7	95.5	95.5
14	93.0	93.0
14	85.6	85.6
29	81.6	81.6
29	77.9	77.9
57	61.4	61.4
57	62.7	62.7
93	51.7	51.7
93	48.0	48.0
119	44.2	44.2
119	42.0	42.0
182	32.7	32.7
182	28.8	28.8
266	26.3	26.3
266	20.5	20.5
364	16.7	16.7
364	17.3	17.3

DAT = Days after treatment
TAR = Total Applied Radioactivity
[a] Two labels were considered as replicates
[b] Set to material balance

Table 7.1.2.1.1-13: Experimental data from study 2000/1013279 used for kinetic evaluation (diphenyl-^{14}C-labeled boscalid)

DAT	Experimental data [%TAR]	Input data according to FOCUS [%TAR]
Li35b soil (20 °C, 40% MWHC)		
0	96.50	97.20 [a]
3	96.80	96.80
7	98.70	98.70
14	82.60	82.60
30	90.60	90.60
60	84.50	84.50
91	85.20	85.20
120	77.70	77.70
Lufa 2.2 soil (20 °C, 40% MWHC)		
0	97.60	98.30 [a]
3	102.60	102.60
7	100.20	100.20
14	84.80	84.80
30	92.60	92.60
60	93.00	93.00
91	85.20	85.20
120	78.80	78.80
US-soil (20 °C, 40% MWHC)		
0	96.30	97.80 [a]
3	95.60	95.60
7	84.70	84.70
14	99.00	99.00
30	93.80	93.80
60	89.30	89.30
91	83.90	83.90
120	80.90	80.90
Canadian soil (20 °C, 40% MWHC)		
0	108.70	114.00 [a]
3	99.10	99.10
7	95.50	95.50
14	78.40	78.40
30	78.20	78.20
60	74.40	74.40
90	68.40	68.40
119	53.60	53.60
Lufa 2.2 soil (30 °C, 40% MWHC)		
0	105.10	106.20 [a]
3	103.80	103.80
7	101.60	101.60
14	100.50	100.50
30	97.10	97.10
60	91.40	91.40
90	86.20	86.20
120	84.40	84.40

DAT = Days after treatment
TAR = Total Applied Radioactivity
[a] Set to material balance

Table 7.1.2.1.1-14: Experimental data from study 2002/5002772 used for kinetic evaluation (pyridine-^{14}C-labeled boscalid and metabolite M510F49)

DAT	Experimental data [%TAR]		Input data according to FOCUS [%TAR]	
	Boscalid	M510F49	Boscalid	M510F49
California soil				
0	97.2	1.4	99.3 [a]	1.4 [b]
0	95.9	1.4	98.2 [a]	1.4 [b]
7	85.1	1.5	85.1	1.5
14	77.7	0.9	77.7	0.9
29	73.2	1.0	73.2	1.0
29	74.4	0.9	74.4	0.9
63	65.5	1.4	65.5	1.4
91	59.7	1.0	59.7	1.0
127	54.3	0.6	54.3	0.6
127	55.0	0.7	55.0	0.7
179	46.3	0.5	46.3	0.5
273	41.7	0.7	41.7	0.7
371	35.3	0.9	35.3	0.9
371	30.9	0.3	30.9	0.3
Idaho soil				
0	96.9	1.5	98.2 [a]	1.5 [b]
0	97.3	1.7	98.6 [a]	1.7 [b]
7	91.2	1.3	91.2	1.3
14	88.3	1.5	88.3	1.5
29	85.4	1.4	85.4	1.4
29	86.8	1.4	86.8	1.4
63	80	1.8	80.0	1.8
91	78.7	2.6	78.7	2.6
127	77.4	3.0	77.4	3.0
127	75.7	2.6	75.7	2.6
179	66.3	3.3	66.3	3.3
273	67.9	3.5	67.9	3.5
371	58.6	6.3	58.6	6.3
371	58.8	4.5	58.8	4.5
Illinois soil				
0	96.1	1.5	98.2 [a]	1.5 [b]
0	91.4	1.8	92.4 [a]	1.8 [b]
7	90.5	1.6	90.5	1.6
14	90.1	1.6	90.1	1.6
29	85.5	2.8	85.5	2.8
29	85.7	2.8	85.7	2.8
63	74.8	5.7	74.8	5.7
91	71.4	8.4	71.4	8.4
127	66.8	8.6	66.8	8.6
127	66.9	9.4	66.9	9.4
179	57.8	9.0	57.8	9.0
273	61.1	12.6	61.1	12.6
371	50.6	15.8	50.6	15.8
371	44.5	13.1	44.5	13.1

Table 7.1.2.1.1-14: Experimental data from study 2002/5002772 used for kinetic evaluation (pyridine-^{14}C-labeled boscalid and metabolite M510F49)

DAT	Experimental data [%TAR]		Input data according to FOCUS [%TAR]	
	Boscalid	M510F49	Boscalid	M510F49
North Dakota soil				
0	97.9	1.9	99.8 [a]	1.9 [b]
0	96.5	1.7	98.8 [a]	1.7 [b]
7	77.0	1.4	77.0	1.4
14	73.0	1.1	73.0	1.1
29	69.0	1.2	69.0	1.2
29	72.4	1.2	72.4	1.2
63	64.0	1.1	64.0	1.1
91	59.1	1.1	59.1	1.1
127	53.3	0.8	53.3	0.8
127	53.1	0.9	53.1	0.9
179	51.3	0.5	51.3	0.5
273	47.9	0.7	47.9	0.7
371	42.4	1.4	42.4	1.4
371	47.2	1.3	47.2	1.3

DAT = Days after treatment
TAR = Total Applied Radioactivity
[a] Set to difference between material balance and the measured values for M510F49
[b] Set to measured value as metabolite was present in application solution
[c] Set to 0.5 LOD

Table 7.1.2.1.1-15: Experimental data from study 2000/1013280 used for kinetic evaluation (metabolite M510F47)

DAT	Experimental data [%TAR]	Input data according to FOCUS [%TAR]
Bruch West soil		
0	98.00	100.00 [a]
1	88.60	88.60
3	68.10	68.10
7	13.00	13.00
14	0.40	0.40

DAT = Days after treatment
TAR = Total Applied Radioactivity
[a] Set to material balance

II. RESULTS AND DISCUSSION

Degradation kinetics of boscalid and its metabolites M510F49 and M510F47 were evaluated according to FOCUS guidance [*FOCUS (2006)*] from the results of laboratory studies on aerobic soil degradation of boscalid and M510F47 in order to derive modeling endpoints.

The non-normalized DT_{50} values at study conditions as well as parameters from the kinetic modelling evaluations for boscalid and the metabolites M510F49 and M510F47 are summarized in Table 7.1.2.1.1-16 to Table 7.1.2.1.1-21. For the US study [*see CA 7.1.1.1/1, BASF DocID 2002/5002772*], endpoints are reported for boscalid and M510F49 only. M510F47 was not detected, occurred only in one sample or detections were too scattered for a kinetic evaluation.

The DT_{50} values that were estimated from data obtained under standard incubation conditions (EU: 20°C, 40% of MWHC; US: 27°C, 75% of MWHC at 0.33 bar) were normalized to reference conditions according to the recommendations of the FOCUS groundwater workgroup [*FOCUS (2002) Generic guidance for FOCUS groundwater scenarios, v 2.1, December 2012*]. Parameters included in the normalization procedure of the standard degradation studies and the resulting DT_{50} values for modeling are summarized in Table 7.1.2.1.1-22.

Table 7.1.2.1.1-16: Modeling endpoints for boscalid derived from study 1998/10607

Soil	Kinetic model	χ^2	Estimated parameters	DT_{50} [d] [a]
Limburgerhof soil I	DFOP	1.9	k1: not sig. k2: not sig. g: 0.1407	no reliable endpoint derived [b]
Limburgerhof soil II	DFOP	1.1	k1: 0.2137 d^{-1} k2: 0.0008 d^{-1} g: 0.1195	889.8 [c]
Limburgerhof soil III	DFOP	1.2	k1: 1.9990 d^{-1} k2: 0.0011 d^{-1} g: 0.0890	605.9 [c]
Limburgerhof soil IV	DFOP	1.8	k1: 0.2085 d^{-1} k2: 0.0017 d^{-1} g: 0.1008	418.6 [c]
Edesheim soil V	SFO	2.0	k: 0.0050 d^{-1}	139.8
Edesheim soil VI	SFO	3.0	k: 0.0045 d^{-1}	152.4
Edesheim soil VII	SFO	1.6	k: 0.0036 d^{-1}	194.4

[a] Calculated from unrounded values for the degradation parameters provided in the KinGUI report
[b] Degradation rates were not considered for endpoint calculation as they were not significantly different from zero
[c] Calculated as $DT_{50} = \ln(2)/k2$

Table 7.1.2.1.1-17: Modeling endpoints for boscalid derived from study 1999/11807

Soil	Kinetic model	χ^2	Estimated parameters	DT_{50} [d] [a]
Bruch West soil	DFOP	1.1	k1: 0.0131 d^{-1} k2: 0.0023 d^{-1} g: 0.615	300.2

[a] Calculated from unrounded values for the degradation parameters provided in the KinGUI report

Table 7.1.2.1.1-18: Modeling endpoints for boscalid derived from study 2000/1013279

Soil	Kinetic model	χ^2	Estimated parameters	DT_{50} [d] [a]
Li35b (20 °C, 40% MWHC)	SFO	3.9	k: 0.0016 d^{-1}	430.7
Lufa 2.2 (20 °C, 40% MWHC)	SFO	4.1	k: 0.0017 d^{-1}	418.0
US (20 °C, 40% MWHC)	SFO	3.6	k: 0.0013 d^{-1}	526.4
Canadian (20 °C, 40% MWHC)	DFOP	4.1	k1: 0.1991 d^{-1} k2: 0.0033 d^{-1} g: 0.2420	212.2 [b]
Lufa 2.2 (30 °C, 40% MWHC)	SFO	1.1	k: 0.0019 d^{-1}	358.4
Lufa 2.2 (5°C, 40% MWHC)	*Not evaluated, because degradation was not observed.*			
Lufa 2.2 (20°C, 20% MWHC)				
Lufa 2.2 (20°C, 40% MWHC, sterile)				

[a] Calculated from unrounded values for the degradation parameters provided in the KinGUI report
[b] Calculated as $DT_{50} = \ln(2)/k2$

Table 7.1.2.1.1-19: Modeling endpoints for boscalid derived from study 2002/5002772

Soil	Kinetic model	χ^2	Estimated parameters	DT_{50} [d] [a]
California	DFOP (parent only)	2.3	k1: 0.0880 d^{-1} k2: 0.0023 d^{-1} g: 0.2432	305.8 [b]
Idaho	DFOP (pathway fit)	2.3	k1: 0.0793 d^{-1} k2: 0.0011 d^{-1} g: 0.1077	645.4 [b]
Illinois	DFOP (pathway fit)	3.1	k1: 0.0146 d^{-1} k2: 0.0011 d^{-1} g: 0.2198	613.4 [b]
North Dakota	DFOP (parent only)	3.6	k1: 0.1331 d^{-1} k2: 0.0013 d^{-1} g: 0.3090	531.2 [b]

[a] Calculated from unrounded values for the degradation parameters provided in the KinGUI report
[b] Calculated as $DT_{50} = \ln(2)/k2$

Table 7.1.2.1.1-20: Modeling endpoints for M510F49 derived from study 2002/5002772

Soil	Kinetic model	χ^2	Estimated parameters	DT_{50} [d] [a]	Formation fraction [-]	Std. deviation of formation fraction
California	SFO (decline fit)	18.9	k: 0.0028 d^{-1}	248.9	not applicable [b]	
Idaho	SFO [c] (combined fit)	15.8	k: not sig.	no reliable endpoint derived	0.100	0.047
Illinois	SFO [c] (combined fit)	8.9	k: not sig.	no reliable endpoint derived	0.291	0.054
North Dakota	SFO fit was visually poor and, therefore, no reliable endpoints could be derived.					

[a] Calculated from unrounded values for the degradation parameters provided in the KinGUI report
[b] Endpoints were derived from metabolite decline fit
[c] DFOP kinetics for parent

Table 7.1.2.1.1-21: Modeling endpoints for M510F47 derived from study 2000/1013280

Soil	Kinetic model	χ^2	Estimated parameters	DT_{50} [d]
Bruch West	SFO	12.1	k: 0.2115	3.3

Table 7.1.2.1.1-22: Normalization of modeling endpoints from standard degradation studies (EU or US) to reference conditions

Study DocID	Soil	Kinetic model	T_{act}	T_{ref}	θ_{act}	θ_{ref}	f_{temp}	f_{moist}	DT_{50act} [d]	DT_{50ref} [d]
Aerobic soil degradation of boscalid										
1999/11807	Bruch West	DFOP	20	20	17.2	19	1.00	0.93	300.2	280.0
2000/1013279	Li35b	SFO	20	20	13.2	14	1.00	0.96	430.7	413.3
	Lufa 2.2	SFO	20	20	15.6	14	1.00	1.00	418.0	418.0
	US-soil	SFO	20	20	11.6	19	1.00	0.71	526.4	372.7
	Canadian soil	DFOP	20	20	17.2	25	1.00	0.77	212.2	163.3
2002/5002772	California	DFOP	27	20	18.1	28	1.94	0.74	305.8	437.0
	Idaho	DFOP	27	20	26.8	28	1.94	0.97	645.4	1214.4
	Illinois	DFOP	27	20	22.7	26	1.94	0.91	613.4	1081.3
	North Dakota	DFOP	27	20	19.6	25	1.94	0.84	531.2	869.0
Aerobic soil degradation of M510F49										
2002/5002772	California	SFO	27	20	18.1	28	1.94	0.74	248.9	355.7
Aerobic soil degradation of M510F47										
2000/1013280	Bruch West	SFO	20	20	16.0	19	1.00	0.89	3.3	2.9

T_{act} = Actual temperature during incubation [°C]
T_{ref} = Reference temperature (20°C)
θ_{act} =Actual soil moisture [g / 100 g dry soil]
θ_{ref} = Reference soil moisture at field capacity (pF 2, 10 kPa) according to FOCUS [g / 100 g dry soil]
f_{temp} = Temperature correction factor [-]
f_{moist} = Moisture correction factor [-]
DT_{50act} = DT_{50} at study conditions [d]
DT_{50ref} = DT_{50} at reference conditions [d]

III. CONCLUSION

Modeling endpoints were derived for boscalid and its metabolites M510F47 and M510F49 in four laboratory degradation studies with the parent applied and one study with M510F47 applied. The DT_{50} values that were estimated from data obtained under standard incubation conditions (EU: 20°C, 40% of MWHC; US: 27°C, 75% of MWHC at 0.33 bar) were normalized to reference conditions (20°C, pF2).

Normalized DT_{50} values for boscalid ranged from 163.3 to 1214.4 days.

For M510F49, a normalized DT_{50} value of 355.7 days and formation fractions of 0.100 and 0.291 were estimated.

For M510F47, a normalized DT_{50} value of 2.9 days was estimated from the metabolite study. Formation fractions could not be estimated, because the metabolite was not detected in parent studies, occurred only in one sample or the experimental data were too scattered for a kinetic evaluation.

CA 7.1.2.1.2 Aerobic degradation of metabolites, breakdown and reaction products

For the previous Annex I listing, aerobic soil degradation of the two fragments of boscalid, 1-(4-chlorophenyl)-2'-aminobenzene (M510F62) and 2-chloronicotinic acid (M510F47), which were considered at the time as likely candidates for potential major soil metabolites, was investigated [Table 7.1.2.1.2-1].
The studies were not triggered due to data requirements at the time of the first Annex I listing, but were conducted to further elucidate the pathway and intermediary metabolites leading to CO_2 and bound residues as final degradates of boscalid.

Both studies showed fast degradation in soil. M510F62 was preferably bound to the soil matrix (65% after 7 days) and mineralized (11% within 14 days). M510F47 was quickly mineralized (28% within 14 days), but also showed a rapid formation of bound residues (56% after 14 days). Degradates of the metabolites were only observed in low percentages and were of transient nature.

Table 7.1.2.1.2-1: Studies on aerobic soil degradation of boscalid metabolites M510F62 and M510F47

Reference	BASF DocID	Soil type (USDA)	Application rate [mg kg^{-1}]	Moisture [% MWHC]	Incubation temperature [°C]	Incubation period [days]	Remark
M510F62							
Kellner O., 1999a	1999/11102	loamy sand	0.25	40	20±1	52	
M510F47							
Ebert D., Harder U., 2000b	2000/1013280	sandy loam	0.25	40	20±1	14	

MWHC = Maximum water holding capacity

The very fast binding and mineralization of M510F62 support the finding that the metabolite was not observed in the environmental fate studies with the parent applied. Therefore, M510F62 is not considered to be a metabolite that is formed in environmental matrices. No further information on degradation was needed for the previous and current Annex I listing procedures.

The already peer-reviewed study on the degradation of M510F47 was evaluated to obtain an additional modelling endpoint according to the latest FOCUS guidance [*see CA 7.1.2.1.1/1 2014/1261100*].

M510F47 was also detected in an already peer-reviewed soil degradation study with boscalid under anaerobic conditions [*BASF DocID 2000/1014990*]. This anaerobic study was submitted in the course of the previous Annex I listing, but did not trigger additional studies at that time. For the current renewal procedure, a new aerobic soil degradation study with M510F47 was performed followings current triggers. The study showed that the metabolite degraded fast in aerobic soil with DT_{50} values ranging from 9.7 to 15.8 days.

Based on the results of the additional study with boscalid [*see CA 7.1.1.1/1 2002/5002772*], a laboratory soil degradation study was also triggered for M510F49 [*see CA 7.1.2.1.2/2 2014/1049139*]. The study showed that M510F49 degraded slowly with estimated DT_{50} values clearly exceeding the study duration (DT_{50} > 240 d).

Report:	CA 7.1.2.1.2/1 Class T., Heinz N., 2013a Aerobic soil degradation of Reg.No. 107371 (M510F47, a soil metabolite of BAS 510 F, Boscalid) in three soils (OECD Guideline 307) 2013/1341957
Guidelines:	OECD 307 (2002), BBA IV 4-1, EPA 835.4100, Guidance Document on Residue Analytical Methods (SANCO/3029/99 rev.4)
GLP:	yes (certified by Landesamt fuer Umwelt, Messungen und Naturschutz Baden Wuerttemberg, Karlsruhe, Germany)

Executive Summary

The objective of the study was to examine aerobic degradation of M510F47 (Reg. No. 107371, a soil metabolite of BAS 510 F, boscalid) in three soils.

Three field-fresh soils were investigated: Loamy sand Li10, loamy sand LUFA 5M and loamy sand LUFA 2.2 (soil class according to DIN). The soils were acclimatized with the soil moisture adjusted to 40% of their maximum water holding capacities.

The application rate of M510F47 dosed to bulk soil was 0.5 mg kg^{-1}. Assuming a soil depth of 2.5 cm and a soil density of 1.5 g cm^{-3} this corresponds to a theoretical field application rate of about 200 g ha^{-1}.
The dosed soil samples were incubated for various intervals (0, 3, 11, 12, 20, 28 and 39 days) prior to extraction.

The evaluation of the aerobic degradation of M510F47 (Reg. No. 107371) applying FOCUS kinetics analysis and single first-order (SFO) kinetics resulted in degradation times DT_{50} of 9.7 to 15.8 days and DT_{90} of 32.2 to 52.3 days.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test material

Metabolite code:	M510F47
Reg. No.:	107371
CAS-No.:	2942-59-8
Chemical name (IUPAC):	2-chloronicotinic acid
Molecular formula:	$C_6H_4ClNO_2$
Molar mass:	157.6 g mol^{-1}
Batch No.:	01174-232
Purity:	99.8%

2. Soil

The study used three different field-fresh soils. The soil characteristics are summarized in Table 7.1.2.1.2-2.

Table 7.1.2.1.2-2: Soil Characteristics

Soil designation	**Li10 13/1680/02** (Germany, Limburgerhof)	**LUFA 2.2 13/736/02** (Germany, Hanhofen)	**LUFA 5 M 13/1651/02** (Germany, Mechtersheim)
DIN Particle size distribution [%]			
Clay < 0.002 mm	5.6	12.0	6.1
Silt 0.002 – 0.063 mm	13.2	32.1	13.9
Sand 0.063 – 2 mm	81.2	55.9	80.0
Textural class	loamy sand (SI2)	loamy sand (SI4)	loamy sand (SI2)
USDA Particle size distribution %]			
Clay < 0.002 mm	5.6	12.0	6.1
Silt 0.002 – 0.050 mm	11.6	27.7	11.1
Sand 0.050 – 2 mm	82.8	60.3	82.8
Textural class	loamy sand	sandy loam	loamy sand
TOC (total organic carbon [%]	0.84	1.47	2.03
TC (total carbon) [%]	0.84	1.47	2.03
pH [H_2O]	6.9	5.9	7.9
pH [$CaCl_2$]	6.4	5.4	7.2
Cation exchange capacity [$cmol^+ kg^{-1}$]	5.3	7.6	11.4
Max. water holding capacity [g/100g dry weight]	25.1	29.6	25.2
pF 2.0 [$g_{soil\ moisture}\ g_{dry\ soil}^{-1}$]	0.103	0.168	0.214
pF 2.5 [$g_{soil\ moisture}\ g_{dry\ soil}^{-1}$]	0.089	0.125	0.140
Microbial biomass (start of study [17-Jul-13]) [mg C/100g dry soil]	21.7	35.1	28.0
Microbial biomass (after application [31-Jul-13]) [mg C/100g dry soil]	38.1	52.6	37.0
Microbial biomass (end of incubation [29-Aug-13]) [mg C/100g dry soil]	20.2	28.3	26.3

B. STUDY DESIGN

1. Experimental conditions

Three field-fresh soils were provided by the sponsor: Li10, LUFA 5M and LUFA 2.2. Soils were kept under aerobic conditions at about 5°C for about 13 days then soil moisture was adjusted to 40% of their maximum water holding capacities (MWHC). The soil samples were acclimatized 21 days at room temperature in the dark.

The application rate of M510F47 dosed to bulk soil was 0.5 mg kg^{-1}. Assuming a soil depth of 2.5 cm and a soil density of 1.5 g cm^{-3} this corresponds to a theoretical field application rate of about 200 g ha^{-1}.

Incubation flasks with 50 g of dosed dry soil equivalents were loosely covered with plugs of paper tissue to allow air exchange. The flasks were placed in tempered cabinets set to 20°C and kept in the dark. Loss of water was controlled once per week by weighing and was re-adjusted with distilled water if necessary.

2. Sampling

The dosed soil samples were incubated for various intervals (0, 3, 11, 12, 20, 28 and 39 days) prior to extraction.

3. Description of analytical procedures

For analysis, an analytical soil extraction method [*D0004/1, already peer-reviewed study BASF DocID 2001/5000881*] was adapted for LC-MS/MS determination of M510F47 and pre- and concurrently validated for soil extraction and determination of the analyte.
Soil aliquots of 10 g (dry weight) were extracted first with 25 mL methanol, then twice with methanol/water (50/50, v/v). The extracts were combined and the final extract volume was adjusted to 100 mL. The combined extracts were diluted by a factor of 2 or 5 with methanol/water (1/1 v/v, 0.1% formic acid) for subsequent LC-MS/MS determination of the analyte.

4. Calculation of the degradation rate

The observed residues of the analyte in the incubated soil samples (expressed as µg kg^{-1}) were fitted using the software package KinGUI version 1.1.
The kinetics analyses followed the “Recommended procedures to derive endpoints for parent compounds” as outlined by FOCUS [*FOCUS (2006)*] in Chapter 7, page 107ff.

II. RESULTS AND DISCUSSION

A. AEROBIC SOIL DEGRADATION RESULTS

After various incubation periods (0, 3, 11, 12, 20, 28 and 39 days), replicate soil incubations were collected and soil was extracted for subsequent LC-MS/MS. The nominal concentration (NC) of the test item was 0.5 mg kg^{-1} (dry soil weight). A summary of the results are presented in Table 7.1.2.1.2-3.
Soil results in µg kg^{-1} were used for subsequent KinGUI kinetic modeling calculations.

Table 7.1.2.1.2-3: Degradation of M510F47 in three different soils

Days after treatment	Li10		LUFA 2.2		LUFA 5M	
	µg kg^{-1}	% of NC	µg kg^{-1}	% of NC	µg kg^{-1}	% of NC
0	452	90.4	510	102	530	106
0	449	89.7	480	95.9	515	103
3	443	88.5	440	87.9	444	88.7
3	463	92.5	450	90.0	462	92.3
11	339	67.7	251	50.2	310	61.9
11	346	69.1	264	52.8	306	61.1
12	341	68.1	245	48.9	282	56.4
12	345	68.9	248	49.6	289	57.8
20	228	45.6	91	18.1	135	26.9
20	225	44.9	109	21.7	146	29.2
28	107	21.4	40.4	8.08	33.2	6.64
28	111	22.2	43.2	8.64	43.2	8.64
39	18.2	3.65	8.02	1.60	2.46	0.49
39	23.2	4.64	9.18	1.84	2.14	0.43

% of NC = per cent of nominal concentration

B. KINETIC MODELING RESULTS

Degradation kinetics of M510F47 were evaluated according to FOCUS guidance [*FOCUS (2006)*] in order to derive persistence and modelling endpoints. A comparison of SFO and FOMC model fits showed that the SFO model is appropriate to describe the observed degradation behavior of M510F47. The estimated DT_{50} and DT_{90} values are shorter than 60 days. The DT_{50} values to be used as modelling endpoints range between 9.69 and 15.8 days.
The estimated DT_{50} and DT_{90} values are summarized in Table 7.1.2.1.2-4.

Table 7.1.2.1.2-4: Best-fit degradation endpoints and modeling endpoints obtained for M510F47 in laboratory soil studies

Soil	Kinetic model	χ^2 error	Best-fit $DegT_{50}$ / $DegT_{50}$ at study conditions[d]	Best-fit $DegT_{90}$ [d]	$DegT_{50}$ normalized to 20°C, pF2 [d]
Li10	SFO	12.2	15.8	52.3	Not calc.
LUFA 2.2	SFO	8.7	9.69	32.2	Not calc.
LUFA 5M	SFO	10.6	10.7	35.6	Not calc.

III. CONCLUSION

The objective of the study was to examine the aerobic degradation of M510F47 (metabolite of boscalid) in three different soils.
The evaluation of the aerobic degradation of M510F47 applying FOCUS kinetic analyses and single first-order (SFO) kinetics resulted in degradation times DT_{50} in a range from 9.7 to 15.8 days and DT_{90} in a range from 32.2 to 52.3 days.

Normalization of degradation rates
Since for environmental fate modeling $DegT_{50}$ values at reference conditions (temperature of 20°C and soil moisture at field capacity, i.e. pF2) are required, the reported $DegT_{50}$ values for modeling were normalized following the recommendations of *FOCUS (2012):* [*Generic Guidance for Tier 1 FOCUS Ground Water Assessments. Version: 2.1*].

Since the study was performed at 20°C no temperature correction was necessary. The moisture normalization was performed using the moisture dependency equations by Walker as described in Equation 7.1.2.1.2-1.

Equation 7.1.2.1.2-1: Calculation of the moisture correction factor according to Walker

$$f_{moist} = \begin{cases} \left(\frac{\theta_{act}}{\theta_{ref}}\right)^{0.7} & if \quad \theta_{act} < \theta_{ref} \\ 1 & if \quad \theta_{act} \geq \theta_{ref} \end{cases}$$

with:			
	f_{moist}	moisture correction factor	[-]
	θ_{ref}	reference soil moisture at field capacity (pF2, 10 kPa)	[g / 100 g dry soil]
	θ_{act}	actual soil moisture during incubation	[g / 100 g dry soil]

The actual soil moisture was taken from the study report and the corresponding reference moisture at pF 2 was derived from FOCUS (2012). The normalized DT_{50} values were calculated by multiplying the DT_{50} values at study conditions by the correction factor f_{moist} as described in Equation 7.1.2.1.2-2.

Equation 7.1.2.1.2-2: Calculation of the DT_{50} at reference conditions (20°C, pF2)

$$DegT_{50,ref} = DegT_{50,act} \cdot f_{moist}$$

with:	$DegT_{50,ref}$	normalized $DegT_{50}$	[d]
	$DegT_{50,act}$	$DegT_{50}$ at study conditions	[d]
	f_{moist}	moisture correction factor	[-]

A summary of the normalized half-lives of M510F47 is given below:

Table 7.1.2.1.2-5: Normalization of $DegT_{50}$ values for M510F47 to reference conditions

Soil	pH ($CaCl_2$)	Kinetic model	θ_{act}	θ_{ref}	f_{moist}	$DegT_{50,act}$	$DegT_{50,ref}$
Li10 Loamy sand	6.4	SFO	9.6	14	0.77	15.8	12.1
Lufa 2.2 Sandy loam	5.4	SFO	9.6	14	0.77	9.7	7.4
Lufa 5M Loamy sand	7.2	SFO	9.6	14	0.77	10.7	8.2

θ_{act}	actual soil moisture	[g / 100 g dry soil]
θ_{ref}	reference soil moisture at field capacity (pF 2) according to FOCUS (2012)	[g / 100 g dry soil]
f_{moist}	moisture correction factor	[-]
$DegT_{50,act}$	$DegT_{50}$ at study conditions	[d]
$DegT_{50,ref}$	$DegT_{50}$ at reference conditions	[d]

Report:	CA 7.1.2.1.2/2 Heinz N.,Class T., 2014a Aerobic soil degradation of Reg No. 391572 (M510F49, a soil metabolite of BAS 510 F, Boscalid) in three soils (OECD Guideline 307) 2014/1049139
Guidelines:	OECD 307 (2002), BBA VI 4-1 (December 1986), EPA 835.4100, SANCO/3029/99 rev. 4 (11 July 2000)
GLP:	yes (certified by Landesanstalt fuer Umwelt, Messungen und Naturschutz Baden-Wuerttemberg, Karlsruhe, Germany)

Executive Summary

The objective of the study was to examine aerobic degradation of M510F49 (Reg. No. 391572), a soil metabolite of BAS 510 F, boscalid) in three soils.

Three field-fresh soils were investigated: Loamy sand Li10, loamy sand LUFA 5M and loamy sand LUFA 2.2 (soil class according to DIN). The soils were acclimatized with soil moisture adjusted to 40% of their maximum water holding capacities.

The degradation behavior of M510F49 was analyzed to derive persistence and modeling endpoints.
The application rate of M510F49 dosed to bulk soil was 0.5 mg kg^{-1}. Assuming a soil depth of 2.5 cm and a soil density of 1.5 g cm^{-3} this corresponds to a theoretical field application rate of about 200 g ha^{-1}.
The dosed soil samples were incubated for various intervals (0, 3, 7, 14, 30, 58, 91 and 120 days) prior to extraction.

The evaluation of the aerobic degradation of M510F47 applying FOCUS kinetics analyses showed that persistence and modeling endpoints (DT_{50} values) are > 240 days.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

Metabolite code:	M510F49
Reg. No.:	391572
Chemical name (IUPAC):	2-hydroxy-N-(4'-chlorobiphenyl-2-yl)nicotinamide
Molecular formula:	$C_{18}H_{13}ClN_2O_2$
Molar mass:	324.8 g mol^{-1}
Batch No.:	L71-12
Purity:	99.7%

2. Soil

The study used three different soils. The soil characteristics are summarized in Table 7.1.2.1.2-6.

Table 7.1.2.1.2-6: Soil Characteristics

Soil designation	**Li10** **13/1680/02** (Germany, Limburgerhof)	**LUFA 2.2** **13/736/02** (Germany, Hanhofen)	**LUFA 5 M** **13/1651/02** (Germany, Mechtersheim)
DIN Particle size distribution [%]			
Clay < 0.002 mm	5.6	12.0	6.1
Silt 0.002 – 0.063 mm	13.2	32.1	13.9
Sand 0.063 – 2 mm	81.2	55.9	80.0
Textural class	loamy sand (SI2)	loamy sand (SI4)	loamy sand (SI2)
USDA Particle size distribution [%]			
Clay < 0.002 mm	5.6	12.0	6.1
Silt 0.002 – 0.050 mm	11.6	27.7	11.1
Sand 0.050 – 2 mm	82.8	60.3	82.8
Textural class	loamy sand	sandy loam	loamy sand
TOC (total organic carbon [%]	0.84	1.47	2.03
TC (total carbon) [%]	0.84	1.47	2.03
pH [H_2O]	6.9	5.9	7.9
pH [$CaCl_2$]	6.4	5.4	7.2
Cation exchange capacity [$cmol^+ kg^{-1}$]	5.3	7.6	11.4
Max. water holding capacity [g/100g dry weight]	25.1	29.6	25.2
pF 2.0 [$g_{soil\ moisture}\ g_{dry\ soil}^{-1}$]	0.103	0.168	0.214
pF 2.5 [$g_{soil\ moisture}\ g_{dry\ soil}^{-1}$]	0.089	0.125	0.140
Microbial biomass (start of study [02-Jul-13]) [mg C/100g dry soil]	29.1	38.6	31.6
Microbial biomass (after application [24-Sep-13]) [mg C/100g dry soil]	28.0	28.3	28.3
Microbial biomass (end of incubation [22-Nov-13]) [mg C/100g dry soil]	32.5	50.7	35.0

B. STUDY DESIGN

1. Experimental conditions

The three field-fresh soils were provided with characterizations by the sponsor: Li10, LUFA 5M and LUFA 2.2. Soils were first kept under aerobic conditions at about 5°C for about 12 days, then water was adjusted to 40% of their maximum water holding capacities (MWHC) and the soils were acclimatized 21 days at room temperature in the dark with soil moistures adjusted to 40% of their maximum water holding capacities.
The application rate of M510F49 dosed to bulk soil was 0.5 mg kg^{-1}. Assuming a soil depth of 2.5 cm and a soil density of 1.5 g cm^{-3} this corresponds to a theoretical field application rate of about 200 g ha^{-1}.

Incubation flasks with 50 g of dosed dry soil equivalents were loosely covered with plugs of paper tissue to allow air exchange. The flasks were placed in temperated cabinets set to 20°C and kept in the dark. Loss of water was controlled once per week by weighing and was re-adjusted with distilled water if necessary.

The dosed soil samples were incubated for various intervals up to 120 days prior to extraction.

2. Sampling

The dosed soil samples were incubated for various intervals (0, 3, 7, 14, 30, 58, 91 and 120 days) prior to extraction.

3. Description of analytical procedures

For analysis, an analytical soil extraction method [*D0004/1, already peer-reviewed study BASF DocID 2001/5000881*] was adapted for LC-MS/MS determination of the analyte and pre- and concurrently validated for soil extraction and determination of M510F49.
Soil aliquots of 10 g (dry weight) were extracted first with 25 mL methanol, then twice with methanol/water (50/50, v/v). The extracts were combined and the final extract volume was adjusted to 100 mL. The combined extracts were diluted by a factor of 10 with methanol/water (1/1, v/v, 0.1% formic acid) for subsequent LC-MS/MS determination of the analyte.

4. Calculation of the degradation rate

The observed residues of the analyte in the incubated soil samples (expressed as µg kg^{-1}) were fitted using the software package KinGUI version 2.
The kinetics analyses followed the “Recommended procedures to derive endpoints for parent compounds” as outlined by FOCUS [*FOCUS (2006)*] in Chapter 7. Kinetic analyses were performed to derive persistence as well as modeling endpoints.

II. RESULTS AND DISCUSSION

A. AEROBIC SOIL DEGRADATION RESULTS

After various incubation periods (0, 3, 7, 14, 30, 58, 91 and 120 days), replicate soil incubations were collected and soil was extracted for subsequent LC-MS/MS. The nominal concentration (NC) of the test item was 0.5 mg kg^{-1} (dry soil weight). A summary of the results are presented in Table 7.1.2.1.2-7.

Soil results in µg kg^{-1} were used for subsequent KinGUI kinetic modeling calculations.

Table 7.1.2.1.2-7: Degradation of M510F49 in three different soils

Days	Li10		LUFA 2.2		LUFA 5M	
	µg kg^{-1}	% of NC	µg kg^{-1}	% of NC	µg kg^{-1}	% of NC
0	467	93.4	458	91.6	454	90.8
0	441	88.2	451	90.2	439	87.8
3	471	94.2	457	91.4	460	92.0
3	494	98.8	486	97.2	469	93.8
7	413	82.6	395	79.0	385	77.0
7	461	92.2	408	81.6	384	76.8
14	370	74.0	380	76.0	362	72.4
14	398	79.6	373	74.6	377	75.4
30	352	70.4	323	64.6	365	73.0
30	368	73.6	354	70.8	336	67.2
58	317	63.4	315	63.0	311	62.2
58	313	62.6	333	66.6	338	67.6
91	355	71.0	305	61.0	306	61.2
91	350	70.0	311	62.2	289	57.8
120	339	67.8	334	66.8	324	64.8
120	323	64.6	353	70.6	333	66.6

% of NC = per cent of nominal concentration

B. KINETIC MODELING RESULTS

Degradation kinetics of M510F49 were evaluated according to FOCUS guidance [*FOCUS (2006)*] in order to derive persistence and modelling endpoints. The kinetic evaluation showed that the bi-phasic models (FOMC, DFOP, HS) provide visually acceptable fits, but (some of) the corresponding model parameters are estimated with a certain degree of uncertainty. Based on the fitting results obtained with FOMC and DFOP models, it was concluded that the persistence endpoints are longer than two times the study duration (i.e. DT_{50} and DT_{90} > 240 d). DFOP and HS model fits were compared to derive modelling endpoints, but due to the uncertainty associated with the estimated model parameters it could only be concluded that the DT_{50} appropriate for modelling is longer than two times the study duration (DT_{50} > 240 d). The best-fit and modelling endpoints are summarized in Table 7.1.2.1.2-8.

Table 7.1.2.1.2-8: Best-fit degradation endpoints and modeling endpoints obtained for M510F49 in laboratory soil studies

Soil	Kinetic model	χ^2 error	Best-fit $DegT_{50}$ / $DegT_{50}$ at study conditions[d]	Best-fit $DegT_{90}$ [d]	$DegT_{50}$ normalized to 20°C, pF2 [d]
Li10	FOMC DFOP HS	< 5	> 240 [a]	> 240 [b]	Not calc.
LUFA 2.2	FOMC DFOP HS	< 5	> 240 [a]	> 240 [b]	Not calc.
LUFA 5M	FOMC DFOP HS	< 5	> 240 [a]	> 240 [b]	Not calc.

[a] FOMC and DFOP model were considered.

[b] DFOP and HS model were considered.

III. CONCLUSION

The objective of the study was to examine aerobic degradation of M510F49 (metabolite of boscalid) in three different soils.
The evaluation of the aerobic degradation of M510F49 applying FOCUS kinetics analyses showed that persistence and modeling endpoints (DT_{50} values) are > 240 days.

CA 7.1.2.1.3 Anaerobic degradation of the active substance

Anaerobic conditions over extended time periods are not expected to occur following application of boscalid. Nevertheless, for the previous Annex I listing, information on the rate of degradation in anaerobic soil were available from two studies with [pyridine-3-^{14}C]-labeled and [diphenyl-U-^{14}C]-labeled boscalid [Table 7.1.2.1.3-1].

Table 7.1.2.1.3-1: Studies on anaerobic soil degradation rates of boscalid

Reference	BASF DocID	Soil type (USDA)	Application rate [mg kg^{-1}]	Moisture [% MWHC]	Incubation temperature [°C]	Incubation period [days]	Remark
Staudenmaier H., Schaefer C., 2000a	2000/1014986	sandy loam	1.0	flooded	20±2	120	
Staudenmaier H., 2000a	2000/1014990	sandy loam	1.0	flooded	20±2	120	

MWHC = Maximum water holding capacity

Degradation rates were calculated from these already peer-reviewed studies for boscalid and M510F47 according to the current FOCUS guidance. These calculations are reported in CA 7.1.2.1.3/1.

Report: CA 7.1.2.1.3/1
Pape L., 2014c
Kinetic evaluation of anaerobic laboratory soil metabolism studies with Boscalid according to FOCUS Degradation Kinetics
2014/1261101
Guidelines: FOCUS Kinetics (2006) SANCO/10058/2005 version 1.0 of Nov. 2011
GLP: no

Executive Summary

The degradation behavior of BAS 510 F - boscalid in soil under anaerobic conditions was investigated in two laboratory metabolism studies with two soils using diphenyl-^{14}C-labeled and pyridine-^{14}C-labeled boscalid. The purpose of this evaluation was to analyze the degradation kinetics observed in the studies, taking into account the current guidance of the FOCUS workgroup on degradation kinetics. Kinetic evaluation was performed in order to derive degradation parameters for environmental fate models (modeling endpoints).

The calculated modeling endpoints of boscalid and its metabolite M510F47 are summarized in the following table.

Table 7.1.2.1.3-2: Modeling endpoints for boscalid and the metabolite M510F47

Study (DocID)	Compound	Kinetic model	χ^2 error	Model parameters	DT_{50} [d]	Formation fraction [-]
2000/1014986	BAS 510 F (diphenyl-^{14}C-label)	DFOP	0.7	k1: 0.0533 d^{-1} k2: 0.0015 d^{-1} g: 0.1340	477.4 [a]	-
2000/1014990	BAS 510 F (pyridine-^{14}C-label)	DFOP	0.9	k1: 0.0842 d^{-1} k2: 0.0012 d^{-1} g: 0.1152	594.5 [a]	-
	M510F47	SFO	13.4	k: not sig.	no reliable endpoint derived	0.373

Not sig. = Not significant
[a] Calculated as $DT_{50} = \ln(2)/k2$

The estimated DT_{50} values for the parent exceeded the 120 d study period, but can be considered reliable based on the good fit of the DFOP kinetic model. No reliable DT_{50} value could be estimated for M510F47, because the maximum of formation was not reached towards the end of the study. As a worst case no degradation of M510F47 under anaerobic conditions can be assumed.

I. MATERIAL AND METHODS

The degradation of boscalid (diphenyl-^{14}C-labeled and pyridine-^{14}C-labeled) in soil under anaerobic laboratory conditions from two studies [*already peer-reviewed studies BASF DocID 2000/1014986, BASF DocID 2000/1014990*] was investigated taking into account the current guidance of the FOCUS workgroup on degradation kinetics [*FOCUS (2006)*]. In the study using pyridine-^{14}C-labeled boscalid the metabolite M510F47 was detected at a maximum of 6.7 % TAR [*BASF DocID 2000/1014990*]. Therefore, M510F47 was included in the kinetic evaluation.

1. Kinetic modeling strategy

The kinetic evaluation was performed in order to derive modeling endpoints. The appropriate kinetic model was identified based on visual and statistical assessment considering the procedures and kinetic models proposed by the FOCUS workgroup on degradation kinetics [*FOCUS (2006)*]. Appropriate modelling endpoints for use in environmental fate models were derived depending on the kinetic model.

2. Kinetic models included in the evaluations

The kinetic evaluation of the parent datasets started with testing the single first order (SFO) model proposed by FOCUS Kinetics [*FOCUS (2006)*]. Since both datasets indicated bi-phasic degradation behavior, the bi-exponential model (DFOP) was tested in addition.

The kinetic evaluation of the M510F47 data was done using the SFO kinetic model and following the step-wise approach recommended by the FOCUS Kinetics guidance [*FOCUS (2006)*].

The goodness-of-fit of the kinetic models was assessed by visual inspection and statistical measures, as recommended by the FOCUS Kinetics guidance [*FOCUS (2006)*].

3. Data handling and software for kinetic evaluation

The experimental data were derived from the study reports and adjusted according to FOCUS [*FOCUS (2006)*].

The software package KinGUI (version 2.2012.320.1629) was used for parameter fitting. The error tolerance and the number of iterations of the optimization tool (IRLS) were set to $1x10^{-6}$ and 100, respectively.

4. Experimental data

Anaerobic degradation of boscalid (diphenyl-^{14}C-labeled and pyridine-^{14}C-labeled) was investigated in two soils [*BASF DocID 2000/1014986; BASF DocID 2000/1014990*]. The soil characteristics are summarized in Table 7.1.2.1.3-3 and Table 7.1.2.1.3-4.

Table 7.1.2.1.3-3: Soil characteristics of study 2000/1014986

Parameter	Bruch West
Soil type (USDA)	Sandy loam
Particle size distribution [%]	
Sand (50 - 2000 µm)	65.14
Silt (2 - 50 µm)	25.05
Clay (< 2 µm)	9.80
Organic carbon [%]	1.63
Microbial biomass [mg C kg^{-1} dry soil weight]	27.90
CEC [mVal $100g^{-1}$ dry weight]	12.70
pH ($CaCl_2$)	7.2
Max. water holding capacity [g $100g^{-1}$ soil]	40.70

Table 7.1.2.1.3-4: Soil characteristics of study 2000/1014990

Parameter	Bruch West
Soil type (USDA)	Sandy loam
Particle size distribution [%]	
Sand (50 - 2000 µm)	68
Silt (2 - 50 µm)	17
Clay (< 2 µm)	15
Organic carbon [%]	1.7
Microbial biomass [mg C kg^{-1} dry soil weight]	63.9
CEC [mVal $100g^{-1}$ dry weight]	16
pH ($CaCl_2$)	7.5
Max. water holding capacity [g $100g^{-1}$ soil]	39

The soils were treated with diphenyl-^{14}C-labeled boscalid [*BASF DocID 2000/1014986*] or pyridine-^{14}C-labelled boscalid [*BASF DocID 2000/1014990*] at a nominal application rate of 1.0 mg kg^{-1} dry soil (corresponding to 750 g a.s. ha^{-1}) and incubated anaerobically at 20°C in the dark for 120 days. Soil samples were taken at 0, 3, 7, 14, 30, 58, 90 and 120 days after treatment (DAT) [*BASF DocID 2000/1014986*] or 0, 3, 7, 14, 30, 62, 90 and 120 DAT [*BASF DocID 2000/1014990*].

In the study using diphenyl-^{14}C-labeled boscalid [*BASF DocID 2000/1014986*], the amounts of metabolites formed were very low (< 1% TAR). In the study using pyridine-^{14}C-labelled boscalid [*BASF DocID 2000/1014990*], M510F47 was detected at a maximum of 6.7 % TAR and was, therefore, included in the kinetic evaluation.

The measured data as well as resulting datasets submitted to kinetic analysis are given in Table 7.1.2.1.3-5 and Table 7.1.2.1.3-6.

Table 7.1.2.1.3-5: Data for kinetic evaluation of anaerobic degradation of ^{14}C-diphenyl labeled boscalid in Bruch West soil [BASF DocID 2000/1014986]

Day	Experimental data [%TAR]	Input data according to FOCUS [%TAR]
0	100.90	101.40 [a]
3	97.40	97.40
7	94.70	94.70
14	92.80	92.80
30	85.90	85.90
58	81.10	81.10
90	75.80	75.80
120	73.60	73.60

TAR = Total Applied Radioactivity
[a] Set to mass balance

Table 7.1.2.1.3-6: Data for kinetic evaluation of anaerobic degradation of ^{14}C-pyridine labeled boscalid in Bruch West soil [BASF DocID 2000/1014990]

Day	Experimental data [%TAR]		Input data according to FOCUS [%TAR]	
	BAS 510 F	M510F47	BAS 510 F	M510F47
0	96.4	0.0	98.8 [a]	0.0
3	96.2	2.6	96.2	2.6
7	95.1	1.7	95.1	1.7
14	88.6	4.0	88.6	4.0
30	86.2	3.9	86.2	3.9
62	81.9	6.0	81.9	6.0
90	78.0	5.9	78.0	5.9
120	77.0	6.7	77.0	6.7

TAR = Total Applied Radioactivity
[a] Set to mass balance

II. RESULTS AND DISCUSSION

The kinetic evaluation of the parent data showed that the DFOP kinetic model is appropriate to describe the observed degradation behavior of boscalid in both studies. The combined evaluation of parent and metabolite data from the study using pyridine-^{14}C-labelled boscalid [*BASF DocID 2000/1014990*] resulted in good fits to the metabolite residue data. The derived modeling endpoints are summarized in Table 7.1.2.1.3-7.

Table 7.1.2.1.3-7: Modeling endpoints for boscalid and the metabolite M510F47

Study (DocID)	Compound	Kinetic model	χ^2 error	Estimated parameters	DT_{50} [d]	Formation fraction [-]
2000/1014986	BAS 510 F (diphenyl-^{14}C-label)	DFOP	0.7	k1: 0.0533 d^{-1} k2: 0.0015 d^{-1} g: 0.1340	477.4 [a]	-
2000/1014990	BAS 510 F (pyridine-^{14}C-label)	DFOP	0.9	k1: 0.0842 d^{-1} k2: 0.0012 d^{-1} g: 0.1152	594.5 [a]	-
	M510F47	SFO	13.4	k: not sig.	no reliable endpoint derived	0.373

Not sig. = Not significant
[a] Calculated as $DT_{50} = \ln(2)/k2$

III. CONCLUSION

According to the guidance of the FOCUS workgroup on degradation kinetics, modeling endpoints for anaerobic degradation of boscalid and its metabolite M510F47 were derived with the DFOP kinetic model for the parent and the SFO kinetic model for the metabolite. The calculated DT_{50} values of 477.4 days and 594.5 days for diphenyl-^{14}C-labeled and pyridine-^{14}C-labeled boscalid, respectively, exceeded the 120 d study period, but can be considered reliable based on the good fit of the DFOP kinetic model. No reliable DT_{50} value could be estimated for M510F47, because the maximum of formation was not reached towards the end of the study. As a worst case no degradation of M510F47 under anaerobic conditions can be assumed.

CA 7.1.2.1.4 Anaerobic degradation of metabolites, breakdown and reaction products

Information on boscalid metabolites occurring in soil under anaerobic conditions are available from two already peer-reviewed studies with boscalid [*BASF DocID 2000/1014986; BASF DocID 2000/1014990*].

M510F47 is the only metabolite of boscalid formed under anaerobic conditions in amounts >5% TAR and information on its degradation behavior under anaerobic conditions are available from the respective parent study [*BASF DocID 2000/1014990*]. However, no reliable D_{T50} could be derived and no degradation of M510F47 under anaerobic conditions is assumed as a worst case.

New anaerobic studies on metabolites were not conducted.

CA 7.1.2.2 Field studies

CA 7.1.2.2.1 Soil dissipation studies

Based on the results of the peer-reviewed laboratory soil degradation studies, terrestrial field dissipation studies were triggered [see Table 7.1.2.2.1-1]. The corresponding kinetic evaluations were reported separately [see Table 7.1.2.2.1-2].

Table 7.1.2.2.1-1: Studies on terrestrial field dissipation of boscalid

Reference	BASF DocID	Sites	Application rate [kg ha^{-1}]	Crop/ bare soil	Incubation period [days]	Remark
Kellner O., Keller W., 2000a	2000/1000123	Stetten/Germany Schifferstadt/Germany	0.3 0.6 1.2	bare soil	544 545	
Bayer H., Grote C., 2001a	2000/1013295	Manzanilla/Spain Alcala del Rio/Spain Grossharrie/Germany Bjärred/Sweden	0.75	bare soil	349 356 357 352	

Table 7.1.2.2.1-2: Kinetic evaluation of terrestrial field dissipation studies with boscalid

Reference	BASF DocID	Sites	Evaluation	Remark
Platz K., 2001a	2000/1017044	Stetten/Germany Schifferstadt/Germany Manzanilla/Spain Alcala del Rio/Spain Grossharrie/Germany Bjärred/Sweden	Experimental data from 2000/1000123 Experimental data from 2000/1013295	Amended by 2012/1288168

The peer-reviewed studies showed that boscalid degrades under field conditions with best-fit DT_{50} values ranging from 27 to 208 days. The results of these studies triggered soil accumulation studies that were included in the previous Annex I listing [see Table 7.1.2.2.2-1].

The previously submitted kinetic evaluation of the peer-reviewed dissipation studies [already peer-reviewed report *BASF DocID 2000/1017044*] was amended [*BASF DocID 2012/1288168, submitted under CA 7.1.2.2.1/1*]. The amendment was necessary to correct transcription errors in results tables. Further, calculations of the degradation rate constant at 20°C using a Q_{10} value of 2.58 and geometric mean values of the field half-lives were included in the amendment. This study is however superseded by a new kinetic evaluation of the peer-reviewed dissipation studies, included in the current dossier [*CA 7.1.2.2.1/2, BASF DocID 2015/1018173*] to provide updated persistence endpoints according to the latest guidance documents.

Three new terrestrial field dissipation studies conducted at sites across Europe are included in the current dossier to further elucidate the environmental behaviour of boscalid [*CA 7.1.2.2.1/3, BASF DocID 2002/1004283; CA 7.1.2.2.1/5, BASF DocID 2010/1126049; CA 7.1.2.2.1/6, BASF DocID 2010/1140925*]. In two of the studies persistence endpoints were already derived according to latest guidance documents. However, for one study [*CA 7.1.2.2.1/3, BASF DocID 2002/1004283*], persistence endpoints had to be updated and are reported in a separate kinetic study [*CA 7.1.2.2.1/4, BASF DocID 2014/1086103*]. Degradation behaviour of boscalid in the new and already peer-reviewed studies was comparable, since estimated DT_{50} values were in a similar range.

A summary table of the persistence endpoints derived from the already peer-reviewed and newly submitted terrestrial field dissipation studies is provided at end of chapter CA 7.1.2.

Additional field soil dissipation studies were included in the current dossier to provide information on the degradation of M510F49 under field conditions [*CA 7.1.2.2.1/8, BASF DocID 2001/5000833; CA 7.1.2.2.1/9, BASF DocID 2001/5000936; CA 7.1.2.2.1/10, BASF DocID 2001/5000937; CA 7.1.2.2.1/11, BASF DocID 2001/5000938; CA 7.1.2.2.1/12, BASF DocID 2002/5004651*]. The information is required due to the results of the new study on degradation of M510F49 under aerobic conditions in the laboratory [*see CA 7.1.2.1.2/2, BASF DocID 2014/1049139*]. Since in laboratory studies, M510F49 was only observed in US soils, also field soil dissipation studies from the US are cited to address this request. For completeness, an additional US field soil dissipation study is reported where only M510F47 but not M510F49 was analysed [*CA 7.1.2.2.1/7, BASF DocID 2000/5277*]. While M510F49 showed slow degradation under laboratory conditions (DT_{50}> 240 d), continuous residue levels or even accumulation of the metabolite was not observed in the field dissipation studies conducted in the US. M510F49 was only observed in low amounts (max. 0.03 mg/kg, corresponding to 1.1% of the maximum concentration of boscalid after the last application). Since M510F49 was occurring only sporadically and at low levels, it was not possible to derive degradation endpoints for exposure assessment from the results of the US field studies. Therefore, the results from the soil metabolism laboratory studies with M510F49 are used to derive modelling endpoints for exposure assessment. The resulting DT_{50} value of > 240 days is a very conservative estimate in the light of the US field studies.
Also, M510F47 was only observed in low amounts (max. 0.04 mg/kg, corresponding to 2.2% of the maximum concentration of boscalid after the last application). That means that under real outdoor conditions both metabolites, M510F47 and M510F49 occur only in very low concentrations that are of no concern.

The already peer-reviewed and new field studies that are included in the current dossier were conducted at European and US locations. To allow for a direct comparison of the degradation behaviour of boscalid in the field, modelling endpoints normalised to reference conditions (temperature of 20°C and soil moisture at field capacity, i.e. pF2) were derived from all data sets [*see CA 7.1.2.2.1/13, BASF DocID 2013/1285541; CA 7.1.2.2.1/14, BASF DocID 2012/1189904*]. For the European field studies, normalised DT_{50} values ranged between 82.1 and 225.2 days with a geometric mean value of 158.3 days. The DT_{50} values obtained from the US studies ranged between 62.1 and 300.4 days with a geometric mean value of 166.8 days. The comparison of the DT_{50} values shows that the degradation rate of boscalid in the US field studies was similar to the field studies conducted in Europe. Since the US field studies provide no new information compared to the European studies, exposure assessment for boscalid is performed using the modelling endpoints derived from the European studies.

Summary tables of the modelling endpoints derived from the studies at European and US sites are provided at the end of chapter Rate of degradation in soil M-CA 7.1.2.

In addition to the already peer-reviewed storage stability study [see Table 7.1.2.2.1-3], two additional storage stability studies relating to the US field dissipation studies are included in the current dossier [*see CA 7.1.2.2.1/15, BASF DocID 2001/5000904; CA 7.1.2.2.1/16, BASF DocID 2003/5000055*].

Table 7.1.2.2.1-3: Studies on storage stability of boscalid

Reference	BASF DocID	Soil	Fortification rate [mg kg^{-1}]	MWHC [%]	Incubation temperature [°C]	Storage period [days]	Remark
Stephan A., 2000a	2000/1000136	sandy loam	0.93	40	-18 to -22	730	

MWHC = Maximum water holding capacity

The new storage stability studies demonstrate that boscalid and its metabolite M510F47 are stable in soil when stored at ≤ -5°C for 24 months. The recovery of metabolite M510F49 declined slightly, but remained constant at approximately 70-80% up to 24 months.

Report: CA 7.1.2.2.1/1
Platz K., 2012a
Amendment no. 1 to final report: Assessment whether field dissipation studies with BAS 510 F can be used to estimate transformation rates in soil and standardisation of half-lives to reference conditions
2012/1288168
Guidelines: <none>
GLP: no

Executive Summary

Nine field dissipation studies with boscalid (BAS 510 F) conducted at four different trial sites of Central and South Europe were evaluated to determine the usability of their field degradation rates as input parameters for groundwater models as recommended by the FOCUS groundwater workgroup [*FOCUS (2000) "FOCUS groundwater scenarios in the EU review of active substances" Report of the FOCUS Groundwater Scenarios Workgroup, EC Document Reference Sanco/321/2000 rev.2, 202pp.*]. The field studies with BAS 510 F were assessed according to the Dutch CTB Guideline, following a given checklist [*CTB (1999) CTB Guideline: Handleiding Toelating Bestrijdingsmiddelen, HTB. Annex 2: Checklist for assessing whether a field study on pesticide persistence in soil can be used to estimate transformation rates in soil*].

Half-life values for boscalid were estimated and standardized to a reference temperature of 20°C as recommended by the FOCUS groundwater workgroup [*FOCUS (2000)*].

Field half-life values of boscalid (standardized to a reference temperature of 20°C) could be derived for seven out of the nine field trials. Two field dissipation experiments in Spain were rejected in the evaluation process because an acceptable model fit to describe the data and derive endpoints could not be achieved.

Results for calculations under consideration of a Q_{10} value of 2.2:
The field half-lives of boscalid standardized to a reference temperature of 20°C with a Q_{10} value of 2.2 range from 98 to 212 days with an arithmetic mean half-life value of 139 days and a geometric mean of 130 days.

Results for calculations under consideration of a Q_{10} value of 2.58:
After the standardization to a reference temperature of 20°C considering a Q_{10} value of 2.58, half-life values of boscalid ranged from 90 to 201 days with an arithmetic mean value of 130 days and a geometric mean of 122 days.

I. MATERIAL AND METHODS

In total, nine trial experiments at five sites in Europe were assessed: three sites were located in Germany (two of these sites with three trials each with different application rates, the third site had a single trial) and two sites (each with one trial) were located in Spain [*already peer-reviewed studies BASF DocID 2000/1000123, BASF DocID 2000/1013295*]. Boscalid was applied as a formulated product onto bare soil, with application rates ranging from 0.3 to 1.2 kg a.s. ha^{-1}, depending on the individual trial.

The identification, the application rates, the geographical distribution of the trial locations, and the soil parameters of the trial sites are given in Table 7.1.2.2.1-4.

Table 7.1.2.2.1-4: Characterization of the field soil dissipation studies with boscalid

BASF DocID	trial sites	Number of experiments / application rates	location (postal code)	soil type	soil properties	
					% organic C	pH
2000/1000123	DU2/15/97	3 experiments with application rates of 0.3, 0.7 and 1.2 kg ha^{-1}	Germany Stetten (74193)	Silty Loam	0.83	7.5
	DU3/06/97	3 experiments with application rates of 0.3, 0.7 and 1.2 kg ha^{-1}	Germany Schifferstadt (67105)	Silty Sand	0.69	5.4
2000/1013295	ALO/05/98	1 experiment with an application rate of 0.75 kg ha^{-1}	Spain Manzanilla (21890)	Sandy Loam	0.6	7.4
	ALO/06/98	1 experiment with an application rate of 0.75 kg ha^{-1}	Spain Alcala del Rio (41200)	Sandy Loam	0.9	7.7
	D05/03/98	1 experiment with an application rate of 0.75 kg ha^{-1}	Germany Grossharrie (24625)	Loamy Sand	1.2	6.1

The main weather characteristics of the field studies are described in Table 7.1.2.2.1-5 and Table 7.1.2.2.1-6.

Table 7.1.2.2.1-5: Soil moisture and soil temperature at day of application in field soil dissipation studies with boscalid

Trial Site	Location/Code	Application Date	Conditions of Soil at the Day of Application Rel. Humidity, Soil Temperature
1	Germany Stetten (74193) DU2/15/97	April 23, 1997	54%, 5°C
2	Germany Schifferstadt (67105) DU3/06/97	April 18, 1997	46%, 11°C
3	Spain Manzanilla (21890) ALO/05/98	May 07, 1998	19%, 23°C
4	Spain Alcala del Rio (41200) ALO/06/98	May 26, 1998	38%, 21°C
5	Germany Grossharrie (24625) D05/03/98	May 05, 1998	69%, 11°C

Table 7.1.2.2.1-6: Average temperature and accumulated rainfall in the field soil dissipation studies with boscalid

Trial Site	Location/Code	Accumulated Rainfall after Application		Average Temperature after Application	
		3 month [mm]	study period [mm]	3 month [°C]	study period [°C]
1	Germany Stetten (74193) DU2/15/97	282 (0-90 DAT)	1177 (0-544 DAT)	16 (0-90 DAT)	12 (0-544 DAT)
2	Germany Schifferstadt (67105) DU3/06/97	194 (0-90 DAT)	712 (0-545 DAT)	16 (0-90 DAT)	12 (0-545 DAT)
3	Spain Manzanilla (21890) ALO/05/98	33 (0-90 DAT)	455 (0-349 DAT)	25 (0-90 DAT)	18 (0-349 DAT)
4	Spain Alcala del Rio (41200) ALO/06/98	14 (0-90 DAT)	198 (0-356 DAT)	26 (0-90 DAT)	18 (0-356 DAT)
5	Germany Grossharrie (24625) D05/03/98	203 (0-90 DAT)	870 (0-357 DAT)	15 (0-90 DAT)	9 (0-357 DAT)

DAT = Days after treatment

The studies cover a wide range of experimental weather conditions over Europe.

In the South European studies (Spain) very dry periods occurred in the first 3 months after the application (precipitation 33 mm and 14 mm/ 3 months), with high temperatures (about 26°C).

In the studies at the other sites the weather conditions were moderate, with rainfall between 200 and 300 mm and average air temperatures of 13.6 – 16.4°C up to 90 days after application.

The daily temperatures for the trial sites are given in the study report.

Evaluation criteria

Evaluation of the suitability of field dissipation data for kinetic analysis was performed according to the evaluation criteria for standardization compiled by the Dutch regulatory authority (CTB criteria).

II. RESULTS AND DISCUSSION

Intrinsic physical chemical properties

Volatility
A volatilization of about 0.5% from soil and about 1% from plant surfaces [*already peer-reviewed study BASF DocID 2000/1014979*] showed that boscalid has a very low volatilization potential. Volatilization will not be a major dissipation pathway of boscalid in terrestrial field dissipation studies.

Photodegradation on soil
A photolysis study [*already peer-reviewed study BASF DocID 2000/1014989*] determined the half-live of boscalid with about 135 d under continuous irradiation, which equals a half-life of about 270 d, assuming a 12h/12h day/night cycle. The degradation study revealed that light might only slightly increase the degradation rate of boscalid on soil.

Rain fall events up to day 30 after application at the different trial sites
In all studies rainfall events during the first days after application of boscalid were noted. These events will have leached the compound into the top few millimeter layer of the soil. For that reason, boscalid was assumed no more available for photodegradation or volatilization. Because of the high adsorption of boscalid, a leaching of significant residues to deeper soil layers is highly unlikely.

Initial residues of boscalid in soil after application
Acceptable initial soil concentrations in a range from 76% to 103% of the actual applied amounts calculated from spray broth were observed in glass dishes containing soil. The observed residues in the glass dishes were used as input model data (initial concentrations at the day of application) for the parameter estimation process.

Estimation of degradation parameters and standardization to reference conditions

Degradation rates were estimated by adjusting this parameter in a first-order kinetic model to optimize the agreement between calculated and measured soil residues. The effect of temperature variations on the degradation rates during the studies was taken into account in the model by applying temperature corrections to the degradation rate each day according to the measured daily temperature with an Arrhenius function. The rates were corrected to a reference temperature of 20°C. The procedure is described in the EU Guidance Document on Persistence in Soil [9188/VI/97 rev. 8; 12.07.2000]. Parameter estimations were carried out using two different Q_{10} values for the temperature corrections, namely 2.2 and 2.58.

For seven out of the nine field dissipation studies the measured soil residues could be well fitted by the model and degradation rates reliably estimated according to the applied criteria. The specified seven studies were located at the three sites in Central Europe. Adequate rainfall after application led to an infiltration of the compound, minimizing the effect of surface loss processes in these studies.

For the two field dissipation studies in Spain the degradation rates could not be reliably estimated to derive standardized degradation parameters. In these studies a long dry period of >3 months occurred after the second soil sampling. As both soil moisture and temperature are known to influence degradation rates in soil, a temperature correction alone was found to be not suitable to estimate standardized degradation parameters.

At two of the remaining three sites in Germany different application rates (three rates: 0.3, 0.6 and 1.2 kg ha^{-1}) were tested. The three application rates were considered as replicates for each test site in the further evaluation. Therefore, the results of the three replicates were averaged per trial site and a single degradation rate reported.

The best fit DT_{50}-values calculated for the residue curves of boscalid in the field dissipation studies together with the standardized half-lives are listed in Table 7.1.2.2.1-7.

Table 7.1.2.2.1-7: DT_{50} (best fit) values of boscalid in the field dissipation studies and half-life values standardized to a reference temperature of 20°C

Trial code/location	DT_{50} (best-fit) [d]	half-life standardized to 20°C [d]
Germany Stetten (3 replicates) DU2/15/97	55.7	Arithmetic mean Q_{10} 2.2: 106 Geometric mean Q_{10} 2.2: 106 Arithmetic mean Q_{10} 2.58: 101 Geometric mean Q_{10} 2.58: 100
Germany Schifferstadt (3 replicates) DU3/06/97	176.7	Arithmetic mean Q_{10} 2.2: 212 Geometric mean Q_{10} 2.2: 213 Arithmetic mean Q_{10} 2.58: 200 Geometric mean Q_{10} 2.58: 200
Spain Manzanilla ALO/05/98	27	-[a]
Spain Alcala del Rio ALO/06/98	78	-[a]
Germany Grossharrie D05/03/98	144	Q_{10} 2.2: 98 Q_{10} 2.5.8: 90
Average half-life for all trial sites	96.3	Arithmetic mean Q_{10} 2.2: 139 Geometric mean Q_{10} 2.2: 130 Arithmetic mean Q_{10} 2.58: 130 Geometric mean Q_{10} 2.58: 122

[a] Because of the high standard deviations of the degradation rate, a reasonable calculation of the half-life was not possible.

The best fit DT_{50}-values of boscalid for the five field trial sites range from 27 to 176.7 days, with an average of 96.3 days.

The half-lives of boscalid standardized to 20°C at the three suitable field study sites considering a Q Q_{10} of 2.2 ranged from 98 - 212 days with an arithmetic mean of 139 days and a geometric mean of 130 days.

The half-lives of boscalid standardized to 20°C at the three suitable field study sites considering a Q Q_{10} of 2.58 ranged from 90 - 201 days with an arithmetic mean of 130 days and a geometric mean of 122 days.

The half-lives standardized to 20°C are higher than the best-fit DT_{50} values obtained by curve fitting from the field studies without standardization. This can be attributed to the conservative evaluation and to standardization process.

III. CONCLUSION

With the chosen evaluation method, reliable estimations of the transformation rates of boscalid in the seven out of nine field dissipation trials were possible

A mean value of the half-lives of 130 days at 20°C, when considering a Q_{10} of 2.58 (Q_{10} 2.2: 139 days), is a reasonable conservative estimate for the prediction of the degradation of boscalid in the field.

Therefore, the calculated half-lives can be used in simulation models to assess the degradation in soil and potential leaching to groundwater of boscalid under different climatic conditions.

Report:	CA 7.1.2.2.1/2 Sachers S., 2015a Kinetic evaluation of two field dissipation studies with BAS 510 F - Boscalid conducted between 1997 and 1998 in Europe: Determination of trigger endpoints according to FOCUS 2015/1018173
Guidelines:	FOCUS Kinetics (2006) SANCO/10058/2005 version 1.0 of Nov. 2011
GLP:	no

Note: This study was not listed in the application submitted for renewal of approval. The reason for submission is to provide updated persistence endpoints according to the latest guidance documents.

Executive Summary

The dissipation behavior of BAS 510 F – boscalid in soil has been investigated in two field dissipation studies including 10 field trials. The purpose of this evaluation was to analyze the degradation kinetics of boscalid observed in the field according to the current guidance of the FOCUS workgroup on degradation kinetics in order to derive reliable best-fit $DisT_{50}$ and $DisT_{90}$ values.

For each trial, the best-fit model was selected based on a visual and statistical assessment. Kinetic evaluation showed that the dissipation behavior of boscalid was best described using the bi-phasic models double first-order in parallel (DFOP) and first-order multi-compartment (FOMC).

Kinetic evaluation resulted in field dissipation half-lives ($DisT_{50}$) for boscalid for eight of the field trials between 26.3 days and 228.3 days. The corresponding $DisT_{90}$ values for boscalid were between 519.2 days and >1000 days. For two of the field trials no reliable endpoints could be derived.

I. MATERIAL AND METHODS

The degradation behavior of boscalid was investigated in two field dissipation studies with 10 field trials in total [*already peer-reviewed studies BASF DocID 2000/1000123, BASF DocID 2000/1013295*]. The trials were situated in typical agricultural regions in Germany (seven trials), Spain (two trials) and Sweden (one trial), considering a range of different soils and climatic conditions.

Kinetic modeling

The software package KinGUI version 2.2012.320.1629 was used for parameter fitting [*SCHÄFER et al. (2007)*]. The error tolerance and the number of iterations of the optimization tool (IRLS) were set to the default values of 1×10^{-6} and 100, respectively.

Datasets were prepared for kinetic evaluation as follows.

- Values below the quantification or detection limit were treated as recommended by the FOCUS workgroup [*FOCUS (2006)*]. The limit of quantification (LOQ) for boscalid reported in the five studies was 0.01 mg kg^{-1}. A limit of detection (LOD) was not provided in the study reports and was therefore set equal to LOQ. According to FOCUS, values below LOD were set to 0.5 × LOD=0.005 mg kg^{-1}.

- For each sampling point, the residues of the single core segments given in mg kg^{-1} were transformed to residues given in g ha^{-1} according to Equation 7.1.2.2.1-1 considering the thickness of the respective segment and undisturbed soil bulk densities for each soil layer. In case soil bulk densities were not provided, a bulk density of 1.5 g cm^{-3} was used. The total residues in the sampled soil core were calculated as the sum of residues of the single soil core segments.

Equation 7.1.2.2.1-1: Transformation of residues from mg kg^{-1} to kg ha^{-1}

$$residues\left[\frac{kg}{ha}\right] = residues\left[\frac{mg}{kg}\right] \cdot thickness[m] \cdot density\left[\frac{g}{dm^3}\right] / 100$$

with:	residues	residues in soil segment	[mg kg^{-1} or kg ha^{-1}]
	thickness	thickness of soil segment	[m]
	density	undisturbed soil bulk density in soil layer	[g dm^{-3}]

The measured data as well as resulting datasets submitted to kinetic analysis are provided in the original evaluation report.

Kinetic models included in the evaluations

For each data set, the kinetic models proposed by FOCUS Kinetics [*FOCUS (2006)*] were tested. The recommended kinetic models, i.e. single-first order (SFO), double first-order in parallel (DFOP) and first-order multi-compartment (FOMC), were applied to the boscalid data. The respective model descriptions and corresponding equations for calculating trigger endpoints (DT_{50}, DT_{90}) are shown in the FOCUS Kinetics guidance [*FOCUS (2006)*].

The goodness-of-fit of the kinetic models was assessed by visual inspection and statistical measures, as recommended by the FOCUS Kinetics guidance [*FOCUS (2006)*]. A kinetic model is considered appropriate if the residuals are randomly distributed around zero, the χ^2 error value is ideally <15% and the estimated degradation parameters differ significantly from zero.

II. RESULTS AND DISCUSSION

Kinetic evaluation showed that the dissipation behavior of boscalid was best described using the bi-phasic models double first-order in parallel (DFOP) and first-order multi-compartment (FOMC). Due to the scatter of the measured residue data none of the models proposed by FOCUS was appropriate to derive reliable endpoints from field trials ALO/06/98 (Spain) and HUS/10/98 (Sweden). A summary of the results of the kinetic evaluation are presented in Table 7.1.2.2.1-8.

Table 7.1.2.2.1-8: Summary of trigger endpoints of boscalid

Field trial	Soil type (DIN)	Best-fit kinetic model	Trigger endpoints	
			$DisT_{50}$ [d]	**$DisT_{90}$ [d]**
DU2/15/97 (300 g ha^{-1})	Silty loam	FOMC	102.0	794.7
DU2/15/97 (600 g ha^{-1})	Silty loam	FOMC	46.1	519.2
DU2/15/97 (1200 g ha^{-1})	Silty loam	DFOP	26.3	527.0
DU2/15/97		**Geometric mean**	**49.8**	**601.3**
DU3/06/97 (300 g ha^{-1})	Silty sand	FOMC	228.3	>1000
DU3/06/97 (600 g ha^{-1})	Silty sand	FOMC	194.5	>1000
DU3/06/97 (1200 g ha^{-1})	Silty sand	FOMC	175.3	>1000
DU3/06/97		**Geometric mean**	**198.2**	**>1000**
ALO/05/98	Sandy loam	DFOP	73.4	Not calculated
ALO/06/98	Sandy loam	Due to the scatter of measured residue data SFO, FOMC and DFOP fit are visually not acceptable. Reliable endpoints cannot be derived.		
D05/03/98	Loamy sand	FOMC	140.5	>1000
HUS/10/98	Loamy sand	Due to the scatter of measured residue data SFO, FOMC and DFOP model are not appropriate to derive reliable endpoints		

III. CONCLUSION

Kinetic evaluation of 10 field trials with boscalid, originating from two field dissipation studies, was conducted in order to derive reliable trigger endpoints according to the current guidance of the FOCUS workgroup on degradation kinetics.

The best-fit model to derive trigger endpoints was selected based on a visual and statistical assessment. Kinetic evaluation showed that the dissipation behavior of boscalid was best described using the bi-phasic models double first-order in parallel (DFOP) and first-order multi-compartment (FOMC). Kinetic evaluation resulted in field dissipation half-lives ($DisT_{50}$) for boscalid for eight of the field trials between 26.3 days and 228.3 days. The corresponding $DisT_{90}$ values for boscalid were between 519.2 days and >1000 days. For two of the field trials no reliable endpoints could be derived.

Report:	CA 7.1.2.2.1/3 Schulz H., 2002a Field soil dissipation of BAS 510 F following application of BAS 510 01 F at sites in northern and southern France 2000 2002/1004283
Guidelines:	BBA IV 3-3, SETAC, EU Guideline 8064/VI/97 rev. 4 15.12.1998
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Landwirtschaft und Forsten, Wiesbaden)

Executive Summary

Dissipation of BAS 510 F (as formulation BAS 510 01 F), applied onto bare soil, was investigated in two field trials in Northern and Southern France.

The product, formulated as a water dispersible granule (WG), was broadcast applied to bare soil in a single application at a nominal rate of 750 g a.s. ha^{-1} (corresponding to about 1.5 kg ha^{-1} of the product).

The amounts determined by calculation of spray liquid applied ranged from 787.5 to 808.6 g ha^{-1} representing 83.4 to 122.4% of the target rate. Dose verification conducted via application monitors (glass fiber filter pads evenly distributed over the field before application) yielded an average recovery of 106% of the target rate over all sites.

Soil sampling was carried out up to one year after application.

The soil samples were analyzed for BAS 510 F (Reg. No. 300355) using the analytical method No. 408/1 of BASF.

The sampled top horizon (0 - 10 cm) of trial BKA/666/00/RES test 1 showed a steady decline in the residues of BAS 510 F from 0.454 mg kg^{-1} (day after treatment (DAT 7) to 0.145 mg kg^{-1} (DAT 364). In the second horizon (10 – 20 cm), no residues of BAS 510 F above the limit of quantitation (LOQ) were found in the samples of DAT 7, DAT 14 and DAT 28, whereas in the following samples (DAT 59 – DAT 364), residues in the range of 0.012 - 0.038 mg kg^{-1} were detected. Samples from horizons of 20 - 30 cm and 30 to 50 cm were taken at DAT 189 and DAT 364 and analyzed for BAS 510 F. No residues above the LOQ were found in any of these samples.

The sampled top horizon (0 - 10 cm) of trial BKA/666/00/RES test 2 showed a steady decline in the residues of BAS 510 F from 0.499 mg kg^{-1} (DAT 7) to 0.150 mg kg^{-1} (DAT 351). In the second horizon (10 – 20 cm), no residues of BAS 510 F above the LOQ were found in the samples of DAT 7, DAT 34, DAT 184 and DAT 351, whereas in the samples of DAT 14, DAT 62 and DAT 100, residues in the range of 0.017 - 0.043 mg kg^{-1} were detected. Samples from horizons of 20 - 30 cm and 30 to 50 cm were taken at DAT 184 and DAT 351 and analyzed for BAS 510 F. No residues above the LOQ were found in any of these samples.

The LOQ was 0.01 mg kg^{-1}. No peak of BAS 510 F was observed in the control soil samples.

In order to validate the analytical method used in this study, control soil samples were fortified with BAS 510 F at the levels 0.01 and 0.1 mg kg^{-1}. The mean recovery rate was 90.6% (standard deviation (SD): 4.45, coefficient of variation (CV): 4.9%).

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

Test item (formulation):	BAS 510 01 F
Active substance (a.s.):	Boscalid (BAS 510 F, Reg. No. 300355)
Type of formulation:	WG
Batch No.:	99-10
Content of a.s.:	50.0% (nominal)

2. Test sites

The selected sites in France are located in crop growing areas where the climate, soil type, and other pertinent characteristics are typical for areas in which BAS 510 F may be used to control fungi in vegetable fields and vineyards. The sites were in recent agricultural production. The site characteristics including the basic soil parameters of the corresponding soil horizons are presented in Table 7.1.2.2.1-9.

Table 7.1.2.2.1-9: Characteristics of the trial sites used to investigate the field dissipation of BAS 510 F

Trial	BKA/666/00/RES test 1	BKA/666/00/RES test 2
Location	**Loire Valley Northern France**	**Languedoc-Roussillon Southern France**
Soil properties		
Soil class (DIN 4220)	Medium loamy sand (Sl3)	Silty loamy sand (Slu)
sand [%]	70.1	39.3
silt [%]	18.5	47.7
clay [%]	11.3	13.4
Total organic C [%]	0.6	0.7
Organic matter [%] [a]	1.03	1.21
pH	7.2	5.4
CEC [meq/100g dry weight]	12.0	15.9
MWHC [g/100g dry weight]	36.4	39.8
Dry bulk density [g cm^{-3}]	2.56	2.61

[a] organic matter = organic carbon x 1.724
CEC = cation exchange capacity

The field was flat and leveled. The soil allowed coring down to 50 cm; it was not stony.

Although the soil parameters were determined in a non-GLP area of Institut Fresenius (Taunusstein, Germany), the staff were instructed and inspected by the Quality Assurance according to GLP requirements and determinations were carried out according to the relevant SOPs.

B. STUDY DESIGN

1. Experimental treatments

The trial area at each site was divided into two plots, one untreated control plot (one row: XX; size: 29.4 m^2, consisting of 5 subplots) and one treated plot (size: 74.2 m^2). The treated plot consisted of three rows, AA, BA, CA and 13 subplots per treated row.

The product, formulated as a water dispersible granule (WG), was broadcast applied to bare soil in a single application at a nominal rate of 750 g a.s. ha^{-1} using a target water volume of approx. 200 L ha^{-1}. The application was conducted on May 12, 2000 (Northern France; NF), and May 10, 2000 (Southern France; SF), using a calibrated boom sprayer.
The actual application rates determined by quantifying the amount of spray discharged, ranged from 787.5 to 797.5 g a.s. ha^{-1} (NF) and 793.5 to 808.6 g a.s. ha^{-1} (SF). In addition, the dose was verified by means of sampling Petri dishes with glass fiber filters. Four petri dishes with an inner diameter of 9 cm were placed in each row before application. Immediately after application, the glass filters were retrieved and each of them transferred into a glass bottle. The glass filter samples were put onto dry-ice or into a freezer within 2 hours after application. Further details of application are presented in Table 7.1.2.2.1-10.

Table 7.1.2.2.1-10: Application parameters of field trial sites treated with BAS 510 01 F (WG)

Trial	Application method	No. of applications	Subplot (m^2)	Application rate per treatment				No. of treated replicates (rows)	Application date
				nominal	actual [a]	dose verification [b]			
				[g a.s ha^{-1}]	[g a.s. ha^{-1}]	[g a.s. ha^{-1}]	% of nominal		
BKA/666/00/RES test 1	broadcast spray to bare soil	1	BA (74.2)	750	797.5	683.3	91.1	2 [c]	12-May-2000
BKA/666/00/RES test 1	broadcast spray to bare soil	1	CA (74.2)	750	787.5	625.3	83.4		12-May-2000
BKA/666/00/RES test 2	broadcast spray to bare soil	1	AA (74.2)	750	798.5	865.8	115.4	3	10-May-2000
BKA/666/00/RES test 2	broadcast spray to bare soil	1	BA (74.2)	750	793.5	917.7	122.4		10-May-2000
BKA/666/00/RES test 2	broadcast spray to bare soil	1	CA (74.2)	750	808.6	881.0	117.5		10-May-2000

[a] Determined by calculation of spray liquid applied; mean of three (BKA/666/00/RES test 1 two) replicates
[b] Determined by means of Petri dishes containing glass fiber filters
[c] Due to nozzle malfunction no samples of the first row were taken

No irrigation was conducted during the trial period. The sites were not cultivated by any agricultural techniques such as ploughing, harrowing, grubbing, etc. from the beginning of the field part of the study.

The objective of this study required that the site should remain free of weed. Glyphosate or quizalofop-ethyl and diquat were used to remove the weed. The control plots were maintained in the same way as the treated plots. Glyphosate was applied on June 07, Sept 07, 2000 and Mar 16, 2001 on trial site NF. Diquat (June 13, July 07, Aug 11, 2000) and quizalofop-ethyl (June 13, July 07, 2000) were applied to the soil at site SF. The plots of both Northern France and Southern France were not covered by weed by more than 5%.

Precipitation values were obtained from a weather station situated about 2 km, temperature values from a station situated about 16 km from the trial site in Northern France. Weather data for the trial site in Southern France were recorded about 10 km from the trial site. Results on temperature and precipitation are presented in Table 7.1.2.2.1-11.

Table 7.1.2.2.1-11: Summary of climatic conditions at field trial site used to investigate the dissipation of BAS 510 F

Loire Valley Northern France				Languedoc-Roussillon Southern France			
Climatic conditions	T_{mean} Air [°C]		Prec. [mm]	**Climatic conditions**	T_{mean} Air [°C]		Prec. [mm]
Month	Min	Max		**Month**	Min	Max	
May 01-11, 2000	11.6	21.2	31.9	May 01-09, 2000	13.0	24.8	21.2
May 12-31, 2000			18.7	May 09-31, 2000			58.4
June 2000	13.7	24.9	25.9	June 2000	16.3	27.6	90.4
July 2000	13.3	23.7	114.3	July 2000	16.3	28.6	42.2
August 2000	14.6	26.8	50.8	August 2000	18.6	31.0	56.0
September 2000	12.7	23.2	73.0	September 2000	14.9	26.4	87.2
October 2000	9.2	16.2	109.2	October 2000	11.0	20.0	80.6
November 2000	6.6	12.8	105.0	November 2000	6.2	14.6	99.6
December 2000	6.2	11.8	100.2	December 2000	6.5	13.3	102.0
January 2001	3.9	8.8	97.9	January 2001	4.8	11.8	131.8
February 2001	3.4	10.6	46.7	February 2001	4.3	14.0	36.0
March 2001	7.1	13.5	120.2	March 2001	8.0	17.9	92.4
April 2001	6.1	14.6	68.5	April 01-26, 2001	7.8	18.5	22.8
May 01-11, 2001	10.9	22.2	28.0				

2. Sampling

Replicate soil specimens of the treated plot in NF (32 replicates [7 to 59 DAT], 28 replicates (101 DAT), and 64 replicates [189 and 364 DAT]) were taken at intervals up to about 864 days and down to a maximum soil depth of 50 cm. From day 7 until day 101 the treated plots were sampled down to 20 cm only. The control plot was sampled at day -1 down to a soil depth of 50 cm (40 replicates) and 364 days after treatment down to 20 cm (20 replicates). On the trial site located in Southern France, soil specimen of the treated plot (30 replicates [7 to 100 DAT], 60 replicates [184 DAT] and 66 replicates [351 DAT]) were taken at intervals up to 351 days down to a maximum soil depth of 50 cm. Until 100 days after treatment, the soil was sampled until 20 cm depth only. Control plots were sampled one day prior application down to a soil depth of 50 cm (40 replicates) and 351 days after application to a soil depth of 20 cm (20 replicates). The detailed sampling intervals are presented in Table 7.1.2.2.1-12.

Table 7.1.2.2.1-12: Summary of sampling intervals

Trial	Country	Sampling intervals [days after treatment]	
BKA/666/00/RES test 1	Northern France	Untreated plot	-1, 364
		Treated plot	7, 14, 28, 59, 101, 189, 364
BKA/666/00/RES test 2	Southern France	Untreated plot	-1, 351
		Treated plot	7, 14, 34, 62, 100, 184, 351

The corer (Humax corer type SH) with a tube of 500 mm length, and 50 mm diameter was assembled with a new plastic liner (length: 250 mm, diameter: 50 mm) and driven down by hand to the mark of 10 cm. The handle was unscrewed and the inner casing with the soil sample was taken out while the outer tube of the corer remained in the soil. A suitable plastic hammer was used to drive the corer into the ground to the mark of 20 cm. The corer was removed and the liner containing the sample was disassembled. The same procedure was used for deeper horizons.

When removed from the corer the liners were capped and labeled immediately.

Immediately after sampling the samples were carried to the corresponding testing facility of Biotek for freezing. In all cases, the maximum interval recorded between sampling and storage in frozen conditions was 5h 25 min.

In December 2000, the samples stored in the two testing facilities of Biotek in Northern and Southern France were transferred deep frozen to a freezer in the head office of Biotek at Saint Pouange and later by freezer truck to Institute Fresenius, where they arrived deep frozen.

The corresponding horizons of the soil samples were combined to ensure homogeneous blending; the samples were lyophilized and later homogenized together with dry ice. Between lyophilization and homogenization, samples were stored deep frozen. A pre-experiment proved that the use of lyophilization had no influence on the residue concentrations of BAS 510 F in soil.

3. Analytical procedure

The BASF method No. 408/l was used in this study. This method was validated for the determination of BAS 510 F in soil by Institut Fresenius under the study number IF-100/11800-00.

Principle of the BASF method 408/1:
BAS 510 F was extracted from the soil sample with methanol. After centrifugation of the methanol extract and a silica gel column clean-up, the quantitative determination was performed by GC-MS. The glass fibre filters of the petri dish specimen were extracted with methanol as well. The analytical method has a limit of quantification (LOQ) of 0.01 mg kg^{-1} in soil.

The validity of the results was confirmed by fortification experiments which were carried out together with the analysis of the samples. Soil samples were fortified at nominal concentrations of 0.01 mg kg^{-1} and 0.1 mg kg^{-1}.
Furthermore, one untreated glass fibre filter and three glass fibre filters fortified with about 0.5 mg of BAS 510 F were analyzed.

II. RESULTS AND DISCUSSION

1. Spray broth concentration and application verification

Glass fibre filter pads were used on the plots to control the application. The obtained application rates for the individual trials ranged from 787.5 to 808.6 g ha^{-1} representing 83.4 to 122.4% of the target rate.

Recovery rates were determined using blank glass fibre filters fortified with BAS 510 F. The mean recovery rate was 89.3% with a standard deviation of 6.0 and a coefficient of variation of 6.7% (n = 3).

2. Residues in field soil samples

In order to validate the analytical method used in this study, control soil samples were fortified with BAS 510 F. The mean recovery rate was 90.6% (SD: 4.45; CV: 4.9%; n = 10). Fortification levels were at 0.01 and 0.1 mg kg^{-1}.

The sampled top horizon (0 - 10 cm) of trial NF (BKA/666/00/RES test 1) showed a steady decline in the residues of BAS 510 F from 0.454 mg kg^{-1} (DAT 7) to 0.145 mg kg^{-1} (DAT 364). In the second horizon (10 - 20 cm), no residues of BAS 510 F above the LOQ were found in the samples of DAT 7, DAT 14, and DAT 28, whereas in the following samples (DAT 59 – DAT 364) residues in the range of 0.012 - 0.038 mg kg^{-1} were detected. Samples from horizons of 20 – 30 cm and 30 to 50 cm were taken at DAT 189 and DAT 364 and analyzed for BAS 510 F. No residues above the LOQ were found in any of these samples.

The sampled top horizon (0 - 10 cm) of trial SF (BKA666/00/RES test 2) showed a steady decline in the residues of BAS 510 F from 0.499 mg kg^{-1} (DAT 7) to 0.150 mg kg^{-1} (DAT 351). In the second horizon (10 - 20 cm), no residues of BAS 510 F above the LOQ were found in the samples of DAT 7, DAT 34, DAT 184, and DAT 351, whereas in the samples of DAT 14, DAT 62, and DAT 100, residues in the range of 0.017 - 0.043 mg kg^{-1} were detected. Samples from horizons of 20 - 30 cm and 30 to 50 cm were taken at DAT 184 and DAT 351 and analyzed for BAS 510 F. No residues above the LOQ were found in any of these samples.

No peak of BAS 510 F was observed in the control soil samples.

A summary of analytical results are reported in Table 7.1.2.2.1-13 and Table 7.1.2.2.1-14.

Table 7.1.2.2.1-13: Summary of BAS 510 F residues in treated soil samples of trial Northern France (BK N666/00/RES test 1) measured in mg kg-1

Soil depth [cm]	Days after application (DAT)						
	7	14	28	59	101	189	364
0-10	0.454	0.441	0.397	0.243	0.343	0.180	0.145
10-20	<0.01	<0.01	<0.01	0.029	0.034	0.012	0.038
20-30	-	-	-	-	-	<0.01	<0.01
30-50	-	-	-	-	-	<0.01	<0.01

- = No samples taken

Table 7.1.2.2.1-14: Summary of BAS 510 F residues in treated soil samples of trial Southern France (BK N666/00/RES test 2) measured in mg kg^{-1}

Soil depth [cm]	Days after treatment (DAT)						
	7	14	34	62	100	184	351
0-10	0.499	0.514	0.342	0.297	0.185	0.194	0.150
10-20	<0.01	0.032	<0.01	0.043	0.017	<0.01	<0.01
20-30	-	-	-	-	-	<0.01	<0.01
30-50	-	-	-	-	-	<0.01	<0.01

- = No samples taken

No degradation half-lives were calculated in this study. Kinetic evaluations were performed under separate studies which are reported under [*BASF DocID 2014/1086103, CA 7.1.2.2.1/4*] for trigger endpoints and [*BASF DocID 2013/1285541, CA 7.1.2.2.1/13*] for modeling endpoints.

III. CONCLUSION

Boscalid degraded steadily in two field trials conducted in Northern and Southern France. Within the study period of one year, the amount of boscalid in soil decreased to about one third of the initial amount.
Residues were found in the upper soil layer (0-10 cm) and to a minor extent in the layer 10-20 cm. No residues above the LOQ were found in lower soil layers.

Report:	CA 7.1.2.2.1/4 Budde E.,Bisharat R., 2014a Kinetic evaluation of one field dissipation study with BAS 510 F - Boscalid conducted in 2002 in France: Determination of trigger endpoints according to FOCUS 2014/1086103
Guidelines:	FOCUS Kinetics (2006) SANCO/10058/2005 version 1.0 of Nov. 2011
GLP:	no

Executive Summary

The dissipation behavior of BAS 510 F – boscalid in soil has been investigated in a field dissipation study with two trials situated in France. The purpose of this evaluation was to analyze the dissipation kinetics of boscalid observed in the field according to the current guidance of the FOCUS workgroup on degradation kinetics to obtain DT_{50} and DT_{90} values in soil.

Kinetic evaluation was performed in order to derive degradation parameters that are valid as trigger endpoints.

The best-fit model to derive trigger endpoints was selected based on a visual and statistical assessment. Kinetic evaluation showed that the dissipation behavior of boscalid was best described using single first-order (SFO) kinetics (trial BKA/666/00/RES1) and first-order multi compartment (FOMC) kinetics (trial BKA/666/00/RES2). The best-fit field half-lives (DT_{50}) of boscalid adequate for use as trigger endpoints were 227.4 days (trial BKA/666/00/RES1) and 83.2 days (trial BKA/666/00/RES2), respectively. The corresponding DT_{90} values for boscalid were 755.2, respectively 647.3 days.

I. MATERIAL AND METHODS

The degradation behavior of boscalid was investigated in one field dissipation study with two field trials [*BASF DocID 2002/1004283, CA 7.1.2.2.1/3*]. The degradation of boscalid, applied as WG formulation, was investigated in medium loamy sand and in silty loamy sand at two test sites in Northern and Southern France. The sites represent typical regions of agricultural practice. Soil characteristics (German classification) of the field trials were determined from soil samples before application from a depth of 0 to 25 cm. Detailed soil characteristics in each trial are reported in the cited study.

Boscalid was applied using a knapsack sprayer to bare soil at an intended application rate of 750 g a.s. ha^{-1}. The starting application dates were 12.05.2000 and 10.05.2000 for trials BKA/666/000/RES1 and BKA/666/000/RES2, respectively. The average applied amounts determined by spray broth calculation were 792.5 and 800.2 g a.s. ha^{-1}, and Petri dish verification yielded mean values of 654.3 and 888.2 g a.s. ha^{-1} for trials BKA/666/000/RES1 and BKA/666/000/RES2, respectively.

The study duration was up to one year after application. Soil samples were taken prior to application and at 7, 14, 28, 59, 101, 189 and 364 days after treatment (DAT) for trial BKA/666/000/RES1, and at 7, 14, 34, 62, 100, 184 and 351 DAT for trial BKA/666/000/RES2. The soil cores were divided into 0-10, 10-20, 20-30 and 30-50 cm segments, ground up under frozen conditions and stored deep-frozen until analysis.

Samples were worked up and extracts were analyzed for boscalid by GC/MS. The limit of quantification (LOQ) was 0.01 mg kg^{-1}.

Boscalid residues were mainly found in the upper soil layer (0-10 cm) throughout the study. For both trials, residues above the limit of detection were found at few sampling dates in the layer 10-20 cm. No residues above the LOQ were found in lower soil layers.

Kinetic modeling

The software package KinGUI version 2.2012.320.1629 was used for parameter fitting [*SCHÄFER, et al. (2007)*]. The error tolerance and the number of iterations of the optimization tool (IRLS) were set to the default values of 1×10^{-6} and 100, respectively.

Datasets were prepared for kinetic evaluation as follows:

- Values below the quantification or detection limit were treated as recommended by the FOCUS workgroup [*FOCUS (2006), chapter 6.1.4*]. The LOQ for boscalid reported in the study was 0.01 mg kg^{-1} [*BASF DocID 2002/1004283, CA 7.1.2.2.1/3*]. A limit of detection (LOD) was not provided in the study report and was therefore set equal to LOQ. According to FOCUS, values below LOD were set to 0.5 x LOD = 0.005 mg kg^{-1}.
- For each sampling point, the concentration of a compound in the single soil layer given in mg kg^{-1} was transformed to its residue given in g ha^{-1} considering the thickness of the respective segment and undisturbed soil bulk densities for each soil layer. In case soil bulk densities were not provided, a bulk density of 1.5 g cm^{-3} was used. The total residues in the sampled soil core were calculated as the sum of residues of the single soil core segments.

The measured data as well as resulting datasets submitted to kinetic analysis are provided in the original evaluation report.

Kinetic models included in the evaluations

For each data set, the kinetic models proposed by FOCUS Kinetics [*FOCUS (2006)*] were tested. The recommended kinetic models, i.e. single-first order (SFO), double first-order in parallel (DFOP) and first-order multi-compartment (FOMC), were applied to the boscalid data. The respective model descriptions and corresponding equations for calculating trigger endpoints (DT_{50}, DT_{90}) are shown in the FOCUS Kinetics guidance [*FOCUS (2006), Box 5-1, Box 5-4 and Box 5-2*].

The goodness-of-fit of the kinetic models was assessed by visual inspection and statistical measures, as recommended by the FOCUS Kinetics guidance [*FOCUS (2006)*]. A kinetic model is considered appropriate if the residuals are randomly distributed around zero, the χ^2 error value is ideally <15% and the estimated degradation parameters differ significantly from zero.

II. RESULTS AND DISCUSSION

The kinetic evaluation showed that the SFO model is appropriate to derive trigger endpoints for boscalid from the experimental data obtained in field trial BKA/666/00/RES1. For the experimental data obtained in field trial BKA/666/00/RES2, the kinetic evaluation showed that the FOMC model is appropriate to derive trigger endpoints for boscalid. A summary of the derived DT_{50} and DT_{90} values is given in Table 7.1.2.2.1-15.

Table 7.1.2.2.1-15: Summary of best-fit DT_{50} and DT_{90} values of boscalid

Field trial (Trial no)	Soil type (DIN)	Best-fit kinetic model	χ^2 error	DT_{50} [d]	DT_{90} [d]
Northern France (BKA/666/00/RES 1)	Medium loamy sand	SFO	10.8	227.4	755.2
Southern France (BKA/666/00/RES 2)	Silty loamy sand	FOMC	10.7	83.2	647.3

III. CONCLUSION

Kinetic evaluation of two field trials with boscalid was conducted in order to derive reliable best-fit DT_{50} and DT_{90} values according to the current guidance of the FOCUS workgroup on degradation kinetics.

The best-fit field half-lives (DT_{50}) of boscalid adequate for use as trigger endpoints were 227.4 days (trial BKA/666/00/RES1) and 83.2 days (trial BKA/666/00/RES2), respectively. The corresponding DT_{90} values for boscalid were 755.2, respectively 647.3 days.

Report:	CA 7.1.2.2.1/5 Richter T.,Kuhnke G., 2013a Field soil dissipation study of BAS 510 F in the formulation BAS 510 01 F on bare soil in Denmark, 2007-2009 2010/1126049
Guidelines:	EEC 95/36 of 14 July 1995 amending 91/414/EEC, SETAC Procedures for assessing the environmental fate and ecotoxicity of pesticides - Part 1 Fate and behaviour in the environment - 10 - Aqueous photolysis, BBA VI 4-1 (December 1986), ECPA Guidance Document on Field Soil Dissipation Studies Aug. 1997, 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)

Executive Summary

Dissipation of boscalid (BAS 510 F, as formulation BAS 510 01 F), applied onto bare soil, was investigated at a typical agricultural area in Denmark, representing Northern EU conditions.

The product, formulated as a water dispersible granule (WG), was broadcast applied to bare soil in a single application at a nominal rate of rate of 750 g a.s. ha^{-1} (corresponding to about 1.5 kg ha^{-1} of the product) using a target water volume of 300 L ha^{-1}. Each of the four subplots was sprayed separately. The application was conducted in May 2007 using a calibrated boom sprayer. The actual application rates determined by quantifying the amount of spray discharged, ranged from 735.8 g ha^{-1} a.s. to 764.3 g a.s. ha^{-1} for the 4 replicate plots. Dose verification conducted via application monitors yielded an average recovery of 93% of the target rate over all sites. The application monitor consisted of soil filled petri dish samples, which were evenly distributed over the field before application. After application, they were collected and analyzed together with field samples for test item.

No irrigation was conducted during the trial period. No tillage or fertilization was performed during the course of the study and no crops were grown throughout any of the trials. The plots were kept free of vegetation via the application of glyphosate and MCPA.

Soil specimens were taken at intervals up to nominal 820 days and down to a soil depth of 50 cm. Soil cores were cut into 10 cm sections. Soil segments of the same depth were pooled and homogenized and a representative sub-sample of each depth was taken for residue analysis. All soil specimens were stored at about -18°C within a maximum of about 4. 5 hours of being taken and remained frozen until analysis.

Soil specimens and application monitors were analyzed for BAS 510 F according to analytical method L0096/01, which was validated previously. The analytical method involved extraction of the soil with methanol + acetate buffer (80 : 20, v/v). The final determination of the analyte was performed by LC-MS/MS with a limit of quantitation (LOQ) of 0.01 mg kg^{-1}. Analysis of soil specimens originating from the treated plots was conducted down to a depth ensuring that at least two soil segments were free of boscalid residues (< LOQ of 0.01 mg kg^{-1}). Soil specimens were analyzed to a maximum of about 820 days.

No residues of boscalid above 30% of the LOQ were detected in any of the untreated control samples proving that there were no interferences of the untreated soil material with the analytical procedure used. Procedural recovery experiments performed with field soils spiked with boscalid at concentrations of 0.01, 0.1 and 1.0 mg kg^{-1} yielded overall mean recovery rates of 96.1 ± 9.7%, confirming the validity of the analytical method used in this study.

Residue values of boscalid in mg kg^{-1} dry soil were converted to residue rates in g ha^{-1} taking into account the actual dry soil density of the soil, and were summed up for all depths, where the analyte was found above LOQ. Residue values were not corrected for procedural recoveries.

Boscalid degraded in soil under field conditions at the Danish trial site from 0.733 mg kg^{-1} at day 0 down to a residue level of 0.115 mg kg^{-1} at 824 DAT in the upper 10 cm and to 0.01 mg kg^{-1} in the 10-20 cm segment after about 820 days. The estimated best-fit DT_{50} value was 196 days.

Residues of the test substance boscalid were found only in the upper 20 cm of the soil, indicating a low potential of boscalid residues to displace to the groundwater.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

Test item (formulation):	BAS 510 01 F
Active substance (a.s.):	Boscalid (BAS 510 F, Reg. No. 300355)
Type of formulation:	WG
Batch No.:	453 (study code: 56464_4)
Content of a.s.:	51.2% (nominal 50.0%)

2. Test sites

The dissipation of boscalid under field conditions was investigated at the trial site in Denmark representative of Northern EU conditions. The site characteristics including the basic soil parameters of the corresponding soil horizons are presented in Table 7.1.2.2.1-16. Soil parameters were determined from soil samples taken before application following segmentation according to the soil horizons.

Table 7.1.2.2.1-16: Characteristics of the trial sites used to investigate the field dissipation of boscalid

Trial	**L070707**		
Location	**Middelfart, Denmark**		
Soil properties	0 - 40 cm	40 – 70 cm	70 – 90 cm
Soil class (DIN 4220)	Silty sand (Su)	Sand (S)	Sand (S)
sand [%]	82.5	89.9	92.0
silt [%]	14.0	7.6	5.3
clay [%]	3.6	2.5	2.9
Soil class (USDA)	Loamy sand	Sand	Sand
sand [%]	83.7	92.1	96.6
silt [%]	11.5	5.8	0.9
clay [%]	4.8	2.1	0.4
Total organic C [%]	0.92	0.13	0.08
Organic matter [%] [a]	1.59	0.22	0.14
pH ($CaCl_2$)	5.6	6.2	6.4
pH (H_2O)	6.3	7.2	7.2
CEC [meq/100g dry weight]	7.6	2.6	1.5
MWHC [g/100g dry weight]	34.2	28.6	24.0

[a] organic matter = organic carbon x 1.724
CEC = cation exchange capacity

The trial site represented a typical region of agricultural practice and had been under cultivation for many years. The site was flat without any significant slope. Before commencement of the first sampling, the soil of the trial site was prepared as for sowing and then was left fallow. Field maintenance measurement, crop and pesticide history information were not subject to GLP. No product containing the test item had been used on the test plots in the last three years.

B. STUDY DESIGN

1. Experimental treatments

The trial area at each site was divided into two plots, one untreated control plot (size: 90 m^2) and one treated plot (size: 216 m^2) which consisted of four equal sized subplots A, B, C, and D that were assigned for replicates.

The product, formulated as a water dispersible granule (WG), was broadcast applied to bare soil in a single application at a nominal rate of 750 g a.s. ha^{-1} using a target water volume of 300 L ha^{-1}. The application was conducted on May 02, 2007 using a calibrated boom sprayer. All four subplots (each 54 m^2) were treated. For each replicate, a separate spray mixture was prepared and the test item was applied to each subplot separately.

The actual application rates determined by quantifying the amount of spray discharged, ranged from 735.8 to 764.3 g a.s. ha^{-1}. In addition, the dose was verified by means of sampling Petri dishes filled with standard soil LUFA 2.2 (approximately 50 g per dish filled from batch 07/1701). The petri dishes with an inner diameter of 10.8 cm were placed on the treated plot (5 in each subplot) before application. On completion of the application, the petri dishes were sealed with a lid and taped up and frozen within less than 6 hours. Further details of application are presented in Table 7.1.2.2.1-17.

Table 7.1.2.2.1-17: Application parameters of the field trial site treated with BAS 510 01 F (WG)

Trial Country	Application method	No. of applications	Application rate per treatment			No. of treated replicates	Application Date
			nominal	actual [a]	dose verification [b]		
			[g a.s ha^{-1}]	[g a.s. ha^{-1}]	% of nominal		
L070707 Denmark	broadcast spray to bare soil	1	750	746.5 (mean)	93	4 (54 m^2 each)	02-May-2007

[a] determined by calculation of spray liquid applied; mean of four replicates
[b] determined by means of Petri dishes filled with soil

No irrigation was conducted during the trial period. Tillage was performed on April 07, 2007 (ploughing to 40 cm depth) and on April 30, 2007 (rotor-tilling to about 10 cm depth). No fertilizer was used during the trial period and no crops were grown throughout the trial. The plots were kept free of vegetation via the application of glyphosate or MCPA.

Climatic conditions were based on records of appropriate weather stations located at a distance of maximal 500 m from site. Monthly summary results on temperature and precipitation are presented in Table 7.1.2.2.1-18.

Table 7.1.2.2.1-18: Summary of climatic conditions at field trial site used to investigate the dissipation of boscalid

Location	Middelfart				
	Denmark				
Climatic conditions / Month	T_{mean} Air [°C]	Prec. [mm]	Climatic conditions / Month	T_{mean} Air [°C]	Prec. [mm]
May 07	12.7	72.0	Jul 08	17.3	40.2
Jun 07	16.6	167.9	Aug 08	16.7	130.6
Jul 07	16.0	118.9	Sep 08	13.3	43.4
Aug 07	17.1	70.2	Oct 08	9.6	90.2
Sep 07	13.3	104.7	Nov 08	6.2	56.8
Oct 07	8.7	33.9	Dec 08	2.9	18.0
Nov 07	4.5	36.5	Jan 09	0.9	35.6
Dec 07	3.1	58.6	Feb 09	1.0	27.0
Jan 08	3.6	69.2	Mar 09	4.1	53.4
Feb 08	4.2	49.2	Apr 09	9.5	12.2
Mar 08	3.3	85.9	May 09	11.4	67.2
Apr 08	7.8	38.8	Jun 09	13.6	36.2
May 08	13.0	13.4	Jul 09	17.1	105.2
Jun 08	14.8	22.3	Aug 09	16.6	4.8

Weather data refer to time period from start of trial (day of application) until end of trial (day of last sampling).

2. Sampling

Replicate soil specimens of the treated plot (20 replicates) were taken at intervals up to 824 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. The control plot was sampled at day -1 down to a soil depth of 50 cm and 369 days after treatment down to 10 cm (20 replicates each time). The detailed sampling intervals are presented in Table 7.1.2.2.1-19.

Table 7.1.2.2.1-19: Summary of sampling intervals

Trial	Country	Sampling intervals [days after treatment]	
L070707	Denmark	Untreated plot	-1, 369
		Treated plot	0, 14, 28, 57, 103, 154, 232, 369, 824

The 20 specimens were taken from half of the untreated plot each time. At the sampling event before application specimens were taken using a common soil probe (Humax soil coring system) equipped with acetate tubes of 5 cm diameter. At the event 369 days after application the 0-10 cm layer was taken by pressing a metal core of stainless steel (7.5 cm diameter) into the soil and collecting this soil with a spoon.

Treated soil specimens were taken randomly from 5 points of each of the four treated subplots A - D. All soil specimens from 0-10 cm depth collected from the treated plots were taken separately using a stainless steel tube of 7.5 cm diameter, which left a hole by pressing a metal tube into the ground and collecting the soil with a spoon. Soil specimens deeper than 10 cm were collected through the center of the excavation hole contained by the guard collar, using a Humax soil coring system fitted with acetate tubes of diameter 5 cm.

The soil in the acetate tubes from the main samples was cut into 10 cm segments. Segments from the same depth were pooled, double-bagged, and stored at -18°C in the freezer of the test site.
Additionally to the main sampling described above, a second complete sampling (double sampling) was generally carried out. These reserve samples were not sectioned but directly put into the freezers at the field test sites

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 4 hours and 25 minutes of being taken. The specimens remained frozen at about -18°C until shipment
Specimens were shipped under deep frozen conditions from the field test site to BASF SE, Limburgerhof, Germany, and stored at -18°C upon arrival.

Soil characterization specimens were stored at ambient temperature prior to shipment. The shipment was conducted by courier at ambient temperature as well.

3. Analytical procedure

The soil and Petri dish specimens were analyzed for boscalid according to BASF residue method 0096/01, which was previously validated [*BASF Doc ID 2008/1084832, CA 4.1.2/1*]. The analytical method involved extraction of the soil with methanol : acetate buffer pH 4.65 (80 : 20, v/v) and final determination of the analytes by LC-MS/MS. The analytical method has a limit of quantification (LOQ) of 0.01 mg kg^{-1}.

Analysis of soil specimens originating from the treated plots was conducted down to a depth ensuring that at least two soil segments were free of boscalid (< LOQ of 0.01 mg kg^{-1}). Residue analyses were performed until the last soil sampling after 824 days after treatment (DAT).

The validity of the analytical methods for soil samples was proven within the present study by analysis of untreated control as well as the same soil fortified with boscalid.

4. Storage stability experiments

Storage stability of boscalid in soil at a temperature of -18°C for 730 days was investigated and confirmed in a separate study [*peer-reviewed study BASF DocID 2000/1000136*].

5. Calculation of dissipation times

The kinetic analysis was carried out following the recommendations of the FOCUS work group on degradation kinetics [*FOCUS (2006)*]. The software package KinGUI (version 1.1) was used for parameter fitting.

For each data set, the kinetic models proposed by the FOCUS Kinetic guidance document were tested in order to identify the best-fit model, i.e. single first order kinetics (SFO), the Gustafson-Holden model (FOMC) and bi-exponential kinetics (DFOP).

The appropriateness of the distinct kinetic model to describe soil degradation was tested by visual assessment, estimation of the error percentage at which the χ^2 test was passed, and the t-test to evaluate whether estimated degradation parameters differ from zero.
A kinetic model is deemed appropriate if the residuals are randomly distributed and the χ^2-error value is < 15% and the estimated degradation parameters differ from zero as outlined by FOCUS.

II. RESULTS AND DISCUSSION

1. Spray broth concentration and application verification

Petri dishes filled with 50 g soil were used as application control. Residue levels of boscalid achieved on extraction and analysis of the application monitors were corrected for procedural recovery (see below) and then converted into residue rates (in g ha^{-1}) taking into account the area of the Petri dishes (91.6 cm^2). The mean application rate determined was 694 ± 141 g ha^{-1} (mean ± SD) and corresponds to 93% of the target application rate.

Within each analytical series of the application monitor analysis, procedural recovery experiments were performed to prove the reliability of the analytical method. The recovery experiments were carried out with untreated standard soil LUFA 2.2, which was of the same type of soil as was used in the Petri dishes placed in the field.
The fortification level was 14 mg/kg. The fortification experiments (n = 4) had a recovery of 110.2 ± 7.6% (mean ± RSD) for boscalid, residues in blank samples were not detectable above 30% of the LOQ.

2. Residues in field soil samples

Procedural recovery experiments were conducted with untreated field soil obtained from the control plot. Untreated and fortified samples of field soil were analyzed along with samples from the field. Fortification levels were at 0.01, 0.1, and 1.0 mg kg^{-1}. The fortification experiments yielded average recoveries for boscalid of 96.1 ± 9.7% (mean ± RSD; n = 19).

These data prove that the analytical method applied is able to accurately determine boscalid residues in soil down to a concentration of 0.01 mg kg^{-1}. In all cases the concentration of boscalid in the unfortified control samples was below 30% of the LOQ indicating the absence of interferences.

Field soil samples taken from different depths were analyzed up to 824 days after treatment. Depth increments were analyzed at each sampling interval until, as a minimum, two consecutive residue-free layers were reached. Above the LOQ, the analyte boscalid was only found at maximum depth of 20 cm.

A summary of the analytical results are shown in Table 7.1.2.2.1-20. All residue values presented in this table are related to the dry weight of the soil and were not corrected for procedural recoveries. Residue levels of boscalid in mg kg^{-1} dry soil were converted to residue rates in g ha^{-1} taking into account the actual dry soil density of the field sample. The residue rates of boscalid were summed up for all depths, where the analyte was found above LOQ.

Table 7.1.2.2.1-20: Summary of boscalid residues in treated soil samples of trial L070707 measured in mg kg^{-1} and converted in g ha^{-1}

Sampling No.	3	4	5	6	7	8	9	11	12	13
DAT	0	14	28	57	103	154	232	369	736	824
Sample type	Tre	Tre	Tre	Tre	Tre	Tre	Tre	Tre	Tre	Tre
Depth [cm]	Residues of BAS 510 F [mg kg^{-1}] - measured									
0-10	0.733	0.634	0.600	0.549	0.369	0.355	0.309	0.238	0.159	0.115
10-20	-	<0.01	<0.01	0.027	0.021	0.012	0.023	0.011	0.016	0.010
20-30	-	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
30-40	-	n.a.	n.a.	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
40-50	-	n.a.	n.a.	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Cumulated value	0.733	0.634	0.600	0.576	0.390	0.367	0.332	0.249	0.175	0.125
Depth [cm]	Residues of BAS 510 F [g ha^{-1}] – calculated [a]									
0-10	804	610	534	513	384	379	330	298	196	136
10-20	-	0	0	33	27	15	33	14	21	11
20-30	-	0	0	0	0	0	0	0	0	0
30-40	-	n.a.	n.a.	0	0	0	0	0	0	0
40-50	-	n.a.	n.a.	0	0	0	0	0	0	0
Cumulated value	804	610	534	547	411	394	363	312	218	147
Depth [cm]	Actual dry soil density [g cm^{-3}]									
0-10	1.1	1.0	0.9	0.9	1.0	1.1	1.1	1.3	1.2	1.2
10-20		1.2	1.1	1.2	1.3	1.3	1.4	1.2	1.3	1.1
20-30		1.6	1.5	1.6	1.6	1.5	1.7	1.3	1.5	1.4
30-40		n.a.	n.a.	1.6	1.5	1.6	1.5	1.6	1.5	1.3
40-50		n.a.	n.a.	1.5	1.6	1.6	1.6	1.5	1.5	1.3

- = no samples taken
n.a. = sample not analyzed
DAT = days after treatment
Tre = treated sample
[a] calculations are based on actual dry soil density for individual soil layers; residue values < 10 µg kg^{-1} (< LOQ) were treated as zero
Remarks: sampling no. 1 and 10 refer to untreated plot, sampling 2 to application monitor (petri dishes).

The analytical data demonstrate that boscalid is degraded slowly at the field trial site in Denmark. At day 0, residues measured in the top soil (0-10 cm) amounted to 0.733 mg kg^{-1}. By end of the study after 824 days, residues of 0.125 mg kg^{-1} were found.

Considering the distribution of the boscalid residues in the soil profiles, the compound was measured only in the top 0-20 cm soil layer. From these results it appears that boscalid has no potential to displace to the groundwater.

3. Calculation of the degradation rates

The first step was to fit SFO and FOMC models to the residue data. A comparison of both models showed that the FOMC kinetic model was visually and statistically more appropriate than the SFO model.

The second step was to run modified fitting of the SFO and FOMC models with the initial concentration fixed to the measured residue level on day 0. Since the FOMC kinetic model was still more appropriate than the SFO model, a DFOP model was tested.

The third step was to fit a DFOP model to the data without modifications and also with the initial concentration fixed to the measured residue level on day 0. Both unmodified and modified fit were visually and statistically acceptable and provided better descriptions of the residue data than the FOMC model. Therefore, the unmodified DFOP model is considered as best-fit model to describe the observed degradation behavior of boscalid.

The estimated parameters of the best-fit DFOP model are shown in Table 7.1.2.2.1-21.

Table 7.1.2.2.1-21: Boscalid: Parameters of best fit model (trial Denmark)

Model	Parameter	Estimate	Standard deviation	p (t-test)	DT_{50}	DT_{90}
DFOP	k1	0.0641	0.0239	< 0.05	196	> 1000
	k2	0.0013	2.0e-004	< 0.001		
	g	0.3491	0.0465	< 0.001		
	M0	799.9745	35.1157	-		

III. CONCLUSION

Boscalid degraded slowly in soil under field conditions at the Danish trial site from 0.733 mg kg^{-1} at day 0 to a residue level of 0.125 mg kg^{-1} after 824 days, resulting in a DT_{50} degradation rate of 196 days following DFOP kinetic.

Residues of the test item boscalid were found only in the upper 20 cm of the soil. No residues above the LOQ were found below this layer, indicating no potential of boscalid residues to displace to the groundwater.

Report:	CA 7.1.2.2.1/6 Richter T.,Kuhnke G., 2013b Field soil dissipation study of BAS 510 F in the formulation BAS 510 01 F on bare soil in Italy, 2007-2009 2010/1140925
Guidelines:	EEC 95/36 of 14 July 1995 amending 91/414/EEC, SETAC Procedures for assessing the environmental fate and ecotoxicity for pesticides (March 1995), BBA VI 4-1 (December 1986), ECPA Guidance Document on Field Soil Dissipation Studies Aug. 1997, 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)

Executive Summary

Dissipation of BAS 510 F (as formulation BAS 510 01 F), applied onto bare soil, was investigated at a typical agricultural area in Italy, representing Southern EU conditions.

The product, formulated as a WG, was broadcast applied to bare soil in a single application at a nominal rate of 750 g a.s. ha^{-1} (corresponding to about 1.5 kg ha^{-1} of the product) using a target water volume of 300 L ha^{-1}. Each of the four subplots was sprayed separately. The application was conducted in May 2007 using a calibrated boom sprayer. The amounts determined by calculation of spray liquid applied ranged from 700.0 g a.s. ha^{-1} to 766.7 g a.s. ha^{-1} for the 4 replicate plots. Dose verification conducted via application monitors yielded an average recovery of 90% of the target rate over all sites. The application monitor consisted of soil filled petri dish samples, which were evenly distributed over the field before application.
The plots were irrigated with sprinklers from May 2007 to September 2007 with an average amount of about 14 mm per month.
No tillage or fertilization was performed during the course of the study until sampling at 363 DAT and no crops were grown until this date. The plots were kept free of vegetation via the application of glyphosate. After 363 DAT sampling at end of May 2008, an efficacy trial started in June 2009 with the sowing of maize. In September 2009, maize was harvested. In October 2009, last soil sampling was conducted after 875 DAT.

Soil specimens were taken immediately after application (from 0-10 cm depth) and after 15, 28, 56, 98, 161, 244, 363 and 875 days (from 0-50 cm depth). Soil cores were cut into 10 cm sections. Soil segments of the same depth were pooled and homogenized and a representative sub-sample of each depth was taken for residue analysis. Samples from the control plot were taken 1 day before application (from 0-50 cm depth), 363 days after application (from 0-10 cm depth) and 875 days after application (from 0-50 cm depth).

Soil specimens and application monitors were analyzed for BAS 510 F. The analytical method involved extraction of the soil with methanol + acetate buffer (80+20). The final determination of the analyte was performed by LC-MS/MS with a limit of quantitation (LOQ) of 0.01 mg kg^{-1}. Analysis of soil specimens originating from the treated plots was conducted down to a depth ensuring that at least two soil segments were free of BAS 510 F residues (< LOQ of 0.01 mg kg^{-1}). Soil specimens were analyzed to a maximum of 875 days.
No residues of BAS 510 F above 30% of the LOQ were detected in any of the untreated control samples proving that there were no interferences of the untreated soil material with the analytical procedure used. Procedural recovery experiments performed with field soils spiked with BAS 510 F at concentrations of 0.01, 0.1 and 1.0 mg kg^{-1} yielded overall mean recovery rates of 88 ± 9 %, 96 ± 7 and 96 ± 3 %, respectively, confirming the validity of the analytical method used in this study.
Residue values of BAS 510 F in mg kg^{-1} dry soil were converted to residue rates in g ha^{-1} taking into account the actual dry soil density of the soil, and were summed up for all depths between 0 and 50 cm analyzed. Residue values were not corrected for procedural recoveries.
BAS 510 F degraded in soil under field conditions at the field trial site in Italy from 0.965 mg kg^{-1} at day 0 to a residue level of 0.072 mg kg^{-1} after 875 days.

A kinetic evaluation of the soil residues was conducted out of GLP regulations. The estimated best-fit DT_{50} value was 43.6 days. A meaningful estimation of the DT_{90} value could not be achieved by the best-fit (DFOP) kinetic fit approach. However, the comparison of the measured values at the day of application (916 g ha^{-1}) and at the end of the study (83 g ha^{-1}) gives reason that the DT_{90} endpoint is reached within the study period of 875 d.

Residues of the test substance BAS 510 F were found only in the upper 20 cm of the soil, indicating a low potential of BAS 510 F residues to displace to the groundwater.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

Test item (formulation):	BAS 510 01 F
Active substance (a.s.):	Boscalid (BAS 510 F, Reg. No. 300355)
Type of formulation:	WG
Batch No.:	1453 (study code: 56464_4)
Content of a.s.:	51.2% (nominal 50.0%)

2. Test sites

The dissipation of BAS 510 F under field conditions was investigated at a trial site in Italy representative of Southern EU conditions. The site characteristics including the basic soil parameters of the corresponding soil horizons are presented in Table 7.1.2.2.1-22. Soil parameters were determined from soil samples taken before application following segmentation according to the soil horizons.

Table 7.1.2.2.1-22: Characteristics of the trial sites used to investigate the field dissipation of BAS 510 F

Trial	**L070706**	
Location	**Budrio, Italy**	
Soil properties	0 - 60 cm	60 – 90 cm
Soil class (DIN 4220)	Clay loam (Lt)	Sandy loam (Ls)
sand [%]	17.8	45.0
silt [%]	46.6	32.0
clay [%]	35.5	23.1
Soil class (USDA)	Silty clay loam	Loam
sand [%]	18.4	49.1
silt [%]	47.9	30.7
clay [%]	33.7	20.3
Total organic C [%]	0.85	0.40
Organic matter [%] [a]	1.47	0.69
pH ($CaCl_2$)	7.5	7.6
pH (H_2O)	8.4	8.6
CEC [meq/100g dry weight]	29.5	20.3
MWHC [g/100g dry weight]	43.5	36.5

[a] organic matter = organic carbon x 1.724
CEC = cation exchange capacity

The selected field represented a typical region 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 the trial site was prepared as for sowing (harrowing 20 cm depth). Field maintenance measurement, crop and pesticide history information were not subjected to GLP.

No product containing the active substance of the test item had been used on the test plots in the last three years.

3. Experimental treatments

The trial area at each site was divided into two plots, one untreated control plot (size: 30 m^2) and one treated plot (size: 180 m^2) which consisted of four equal sized subplots A, B, C and D that were assigned for replicates.

The product, formulated as a water dispersible granule (WG), was applied to bare soil in a single application at a nominal rate of 750 g a.s. ha^{-1} using a target water volume of 300 L ha^{-1}. The applications were conducted on May 30, 2007 using a calibrated boom sprayer. Treated plots were four-fold replicated with plot size of 45 m^2. For each replicate, a separate spray mixture was prepared and the test item was applied to each subplot separately.

Homogeneity of spray solution was obtained by thorough stirring or mixing immediately before application. Stability in spray solution was not specified, but it was taken to be sufficient for practical use conditions. The interval between mixing and application was 5 to 15 min.

The actual application rates determined by depletion of spray liquid before and after application ranged from 700.0 to 766.7 g a.s. ha^{-1}. In addition, the dose was verified by means of sampling Petri dishes filled with standard soil LUFA 2.2 (approximately 50 g per dish). The Petri dishes with an inner diameter of 10.8 cm were placed at the borderlines between subplots (5 in each subplot) before application. On completion of the application, the Petri dishes were sealed with a lid and taped up and frozen within 15 min after sampling of the last replicate. Further details of application are presented in Table 7.1.2.2.1-23 below.

Table 7.1.2.2.1-23: Application parameters of the field trial site treated with BAS 510 01 F (WG)

Trial Country	Application method	No. of applications	Application rate per treatment			No. of treated replicates	Application date
			nominal	actual [a]	dose verification [b]		
			[g a.s ha^{-1}]	[g a.s. ha^{-1}]	% of nominal		
L070706 Italy	broadcast spray to bare soil	1	750	720.8	90	4 (45 m² each)	30-May-2007

[a] determined by calculation of spray liquid applied; mean of four replicates
[b] determined by means of Petri dishes filled with soil

Irrigation was performed with sprinklers. Irrigation was verified by placing 3 pluviometers in each replicate of the treated plot and 2 pluviometers in the control plot and calculating the average of the catches. Average irrigation between May and September 2007 was 13.75 mm per month. Between September 2007 and October 2009 (last soil sampling), no irrigation was conducted.

Necessary pesticide applications for e.g. weed control were performed chemically by using glyphosate. No tillage or other soil preparation, fertilization, additional pesticide application or crop cultivation was performed until June 05, 2009.

On June 05, 2009, an efficacy trial was started on the trial area with sowing of Imidacloprid-treated maize. During seeding, glyphosate was applied additionally. Maize was harvested on September 29, 2009 at growth stage 89. During this trial, no fertilizers, other pesticides or irrigations were performed.

Between maize harvest and last soil sampling on October 21, 2009 no soil preparation, fertilization or pesticide applications were conducted.

Climatic conditions were based on records of an appropriate weather station located on-site. Monthly summary results on temperature and precipitation are presented in Table 7.1.2.2.1-24.

Table 7.1.2.2.1-24: Summary of climatic conditions at field trial site used to investigate the dissipation of BAS 510 F

Location	**Budrio**				
	Italy				
Climatic conditions	T_{mean} Air [°C]	Prec. [mm]	**Climatic conditions**	T_{mean} Air [°C]	Prec. [mm]
Month			**Month**		
May 07	17.0	0.5	Aug 08	25.6	12.6
Jun 07	22.7	72.9	Sep 08	19.5	36.2
Jul 07	25.6	11.0	Oct 08	16.2	32.8
Aug 07	23.3	46.9	Nov 08	9.7	103.7
Sep 07	18.5	41.8	Dec 08	4.3	9.5
Oct 07	13.8	102.6	Jan 09	3.0	45.0
Nov 07	7.6	17.8	Feb 09	5.2	35.2
Dec 07	3.5	41.6	Mar 09	9.8	91.3
Jan 08	5.4	45.9	Apr 09	14.2	66.7
Feb 08	5.4	13.1	May 09	20.3	46.9
Mar 08	9.4	48.9	Jun 09	22.3	25.1
Apr 08	13.0	43.7	Jul 09	25.3	16.5
May 08	17.6	45.3	Aug 09	26.3	7.7
Jun 08	21.9	94.9	Sep 09	21.3	33.0
Jul 08	25.1	16.3	Oct 09	15.5	19.2

Weather data refer to time period from start of trial (day of application) until end of trial (day of last sampling).

4. Sampling

Replicate soil specimens (20 per treated plot and 20 per control plot) were taken at intervals up to 875 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. The detailed sampling intervals are presented in Table 7.1.2.2.1-25.

Table 7.1.2.2.1-25: Summary of sampling intervals

Trial	Country	Sampling intervals [days after treatment]	
L070706	Italy	Untreated plot	-1, 363, 875
		Treated plot	0, 15, 28, 56, 98, 161, 244, 363, 875

Untreated specimens were collected from the control plot one day before the application, on day 363 after application and on the final day of sampling (20 samples each time). One day before application the soil was sampled down to 50 cm using a manual non-contamination corer fitted with a tube of 5 x 50 cm (diameter x length) containing an acetate liner. At day 363 after application soil was sampled from 0-10 cm using a stainless steel pipe of 8 x 10 cm (diameter x length).
In addition, samples were taken from the control plot at 875 DAT from 0-50 cm using the described corer.
The 20 specimens were taken randomly from half of the untreated plot each time and were later (after deep-freezing and cutting) pooled according to soil depth.

Treated soil specimens were taken randomly from 5 points of each of the four treated subplots A - D and were later (after deep-freezing and cutting) pooled together according to depth. All soil specimens from 0-10 cm depth collected from the treated plots were taken separately using a stainless steel tube of 8 cm diameter, which left a hole by pressing a metal tube into the ground and collecting the soil with a spoon or shovel. Soil specimens deeper than 10 cm were collected through the center of the excavation hole contained by the guard collar, using a manual non-contamination corer fitted with a tube of 5 x 50 cm (diameter x length) containing an acetate liner. The excess 10 cm were disposed after cutting.

Additionally to the main sampling described above, a second complete sampling (double sampling) was generally carried out. These reserve samples were not sectioned but directly put into the freezers at the field test site.

The treated soil samples were deep frozen within 5 h, the control samples within 8 h. Soil cores of the main sampling (except of 875 DAT sampling) were divided into 10 cm increments (except 0-10 cm samples) in frozen state using an electric circular saw. The segments of the same layer were pooled.

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 8 hours of being taken. The specimens remained frozen at about -18°C until analysis.

Soil characterization samples were taken on November 19, 2007. Four cores of a diameter of 5 cm were taken from 0-90 cm with a soil corer at the boundary of the plots and stored at ambient conditions before shipment to BASF SE, Limburgerhof, Germany. Soil characterization specimens were shipped at ambient conditions.

5. Analytical procedure

The soil and Petri dish specimens were analyzed for BAS 510 F according to BASF residue method 0096/01, which was previously validated [*BASF DocID 2008/1084832, CA 4.1.2/1*]. The analytical method involved extraction of the soil with methanol : acetate buffer pH 4.65 (80 : 20, v/v) and final determination of the analytes by LC-MS/MS. The analytical method has a limit of quantification (LOQ) of 0.01 mg kg^{-1}.

Analysis of soil specimens originating from the treated plots was conducted down to a depth ensuring that at least two soil segments were free of BAS 510 F (< LOQ of 0.01 mg kg^{-1}). Residue analyses were performed until the last soil sampling after nominal 870 days after treatment (DAT).

The validity of the analytical methods for soil samples was proven within the present study by analysis of untreated control and fortified samples within each analytical sample set.

6. Storage stability experiments

Storage stability of BAS 510 F in soil at a temperature of -18°C for 730 days was investigated and confirmed in a separate study [*peer-reviewed study BASF DocID 2000/1000136*].

7. Calculation of dissipation times

The kinetic analysis was carried out following the recommendations of the FOCUS work group on degradation kinetics [*FOCUS (2006)*] in order to derive endpoints that can be used as “Triggers for additional work“ as proposed by the FOCUS kinetics guidance. The software package KinGUI (version 2.2012.320.1629) was used for parameter fitting.

For each data set, the kinetic models proposed by the FOCUS Kinetic guidance document were tested in order to identify the best-fit model, i.e. single first order kinetics (SFO), the Gustafson-Holden model (FOMC) and bi-exponential kinetics (DFOP).

The appropriateness of the distinct kinetic model to describe soil degradation was tested by visual assessment, estimation of the error percentage at which the χ^2 test was passed, and the t-test to evaluate whether estimated degradation parameters differ from zero.

A kinetic model is deemed appropriate if the residuals are randomly distributed and the χ^2-error value is < 15% and the estimated degradation parameters differ from zero as outlined by FOCUS.

II. RESULTS AND DISCUSSION

1. Spray broth concentration and application verification

Petri dishes filled with 50 g soil were used as application control. Residue levels of BAS 510 F achieved on extraction and analysis of the application monitors were corrected for analytical recovery and then converted into residue rates (in g ha^{-1}) taking into account the area of the Petri dishes (91.6 cm^2). The application rate determined from mean residues in Petri dishes was 673 g ha^{-1} ± 256 g ha^{-1} representing 90% of the target application rate.

Within the analytical series of the application monitor analysis, procedural recovery experiments were performed to prove the reliability of the analytical method. The recovery experiments were carried out with untreated and fortified standard soil LUFA 2.2, which was of the same type of soil as was used in the Petri dishes placed in the field.
The fortification levels were 0.01 and 14 mg kg^{-1}. The fortification experiments (n = 2) had a recovery of 100.1% (mean) for BAS 510 F, residues in blank samples were not detectable above 30% of the LOQ.

2. Residues in field soil samples

Procedural recovery experiments were conducted with untreated field soil obtained from the control plot. Untreated and fortified samples of field soil were analyzed along with samples from the field. Fortification levels were at 0.01, 0.1, and 1.0 mg kg^{-1}. The fortification experiments yielded average recoveries for BAS 510 F of 92.2 ± 8.1% (mean ± RSD; n = 20).

These data prove that the analytical method applied is able to accurately determine BAS 510 F residues in soil down to a concentration of 0.01 mg kg^{-1}. Residues in blank samples were below 30 % of the LOQ indicating the absence of interferences.

Field soil samples taken from different depths were analyzed to a maximum of 875 days after treatment. Depth increments were analyzed at each sampling interval until, as a minimum, two consecutive residue-free layers were reached. Above the LOQ, the analyte BAS 510 F was only found at maximum depth of 20 cm.

The analytical results are summarized in Table 7.1.2.2.1-26. All residue values presented in this table are related to the dry weight of the soil and were not corrected for procedural recoveries. Residue levels of BAS 510 F in mg kg^{-1} dry soil were converted to residue rates in g ha^{-1} taking into account the actual dry soil density of the field sample. The residue rates of BAS 510 F were summed up for all depths, where the analyte was found above LOQ.

Table 7.1.2.2.1-26: Summary of BAS 510 F residues in treated soil samples of trial L070706 measured in mg kg^{-1} and converted in g ha^{-1}

Sampling No.	3	4	5	6	7	8	9	11	13
DAT (scheduled)	0	14 (±2)	30 (±2)	60 (±7)	100 (±7)	150 (±7)	240 (±10)	360 (±10)	870 (±20)
DAT (actual)	0	15	28	56	98	161	244	363	875
Sample type	Tre	Tre	Tre	Tre	Tre	Tre	Tre	Tre	Tre
Depth [cm]	**Residues of BAS 510 F [mg kg^{-1}] - measured**								
0-10	0.965	0.747	0.224	0.324	0.284	0.229	0.232	0.209	0.048
10-20	-	0.029	0.288	<0.01	<0.01	<0.01	<0.01	0.039	0.024
20-30	-	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
30-40	-	<0.01	<0.01	<0.01	<0.01	n.a.	n.a.	<0.01	<0.01
40-50	-	<0.01	<0.01	<0.01	n.a.	n.a.	n.a.	<0.01	<0.01
Cumulated value	0.965	0.776	0.512	0.324	0.284	0.229	0.232	0.248	0.072
Depth [cm]	**Residues of BAS 510 F [g ha^{-1}] – calculated [a]**								
0-10	916	656	283	360	203	254	233	242	55
10-20		28	385	0	0	0	0	51	28
20-30		0	0	0	0	0	0	0	0
30-40		0	0	0	0			0	0
40-50		0	0	0				0	0
Cumulated value	916	684	668	360	203	254	233	293	83
Depth [cm]	**Actual dry soil density [g cm^{-3}]**								
0-10	0.9	0.9	1.3	1.1	0.7	1.1	1.0	1.2	1.2
10-20		1.0	1.3	1.1	1.3	1.3	1.3	1.3	1.2
20-30		1.3	1.4	1.4	1.4	1.4	1.3	1.5	1.2
30-40		1.2	1.4	1.4	1.3			1.4	1.3
40-50		0.7	1.4	1.5				1.5	0.8

- no samples taken
n.a. sample not analyzed
DAT days after treatment
[a] calculations are based on actual dry soil density for individual soil layers; residue values < 10 µg kg^{-1} (< LOQ) were treated as zero

The analytical data demonstrate that BAS 510 F is degraded slowly at the field trial site in Italy. At day 0 boscalid residues amounted to 0.965 mg kg^{-1}. By the end of the study after about 875 days, residues of 0.072 mg kg^{-1} were found.

Considering the distribution of the BAS 510 F residues in the soil profiles, the compound was measured only in the top 0-20 cm soil layer. From these results it appears that BAS 510 F has no potential to displace to the groundwater.

3. Calculation of the degradation rates

The first step was to fit SFO and FOMC models to the residue data. A comparison of both models showed that the FOMC kinetic model was visually and statistically more appropriate than the SFO model. The second step was to fit a DFOP model to the data. The DFOP kinetic fit was visually and statistically acceptable and provided better descriptions of the residue data than the FOMC model. Therefore, the DFOP model is considered as best-fit model to describe the observed degradation behavior of boscalid.

The DFOP kinetic model does not include the influence of soil temperature and moisture that periodically vary over the course of a year on the degradation behavior of the compound. Hence, the typical degradation behavior of a compound in a field study with faster degradation in summer and lower degradation during winter cannot be reflected by the best-fit model. For that reason the relatively high type 1 error degradation rate k2 of 17 % of the simple best fit DFOP model is not meaningful. Furthermore, an extrapolation of the DT_{90} value on the basis of the fitted curve would lead to an artificial value and is not suitable. A comparison of the measured values at the day of application (916 g ha^{-1}) and at the end of the study (83 g ha^{-1}) gives reason that the DT_{90} is reached within the study period of 875 d.

The estimated parameters of the best-fit DFOP model are shown in Table 7.1.2.2.1-27.

Table 7.1.2.2.1-27: BAS 510 F – boscalid: Parameters of best fit model (trial Italy)

Model	Parameter	Estimate	Standard deviation	p (t-test)	DT_{50}	DT_{90}
DFOP	k1	0.02727	0.00791	0.0091	43.6	- [a]
	k2	7.9e-004	7.5e-004	0.1694		
	g	0.7050	0.08525	0.0002		
	M0	933.9	71.55	2.3e-05		

[a] The DT_{90} value was not listed in the KinGUI report file as the predicted value is too far beyond the study duration of 875 d

III. CONCLUSION

BAS 510 F degraded slowly in soil under field conditions at the Italian trial site from 0.965 mg kg-1 at day 0 to a residue level of 0.072 mg kg^{-1} after 875 days. Kinetic follows DFOP model resulting in DT_{50} value of 45 days and DT_{90} value of > 1000 days.

Residues of BAS 510 F were found only in the upper 20 cm of the soil. No residues above the LOQ were found below this layer, indicating no potential of BAS 510 F residues to displace to the groundwater.

Report:	CA 7.1.2.2.1/7 Oliver G.et al., 2001a 1999 Field dissipation of BAS 510 .. F in orchard/vineyard use patterns 2000/5277
Guidelines:	EPA 164-1
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary

This study was designed to support an orchard/vineyard use pattern according to EPA Guideline 164-1. The test substance, BAS 510 F, is a broad spectrum fungicide which is used to control many diseases in field and row crops, orchard/vineyards, turfgrass and other uses. The test substance formulation was a 69.6% wettable granule (WG). Three field trials were conducted which represent major use areas for the product in the United States (Georgia, California and New York).

A single untreated control plot (bare soil) and one treated bare soil plot were established at each test site. In addition, a treated cropped plot was established at the Georgia and California sites. The plots were established on soils typical for the agricultural areas in which they were located. At each site, six applications were applied at weekly intervals beginning in May 1999. Applications were made to the bare soil plots using a 374.2 liter per ha (40 gal per acre) spray volume and a spray volume of 1870.8 L ha^{-1} (200 gal ac^{-1}) was used in the cropped plots. The first three applications were made targeting a 260 g ha^{-1} (0.231 lb a.s. ac^{-1}) application rate. The following three applications were made targeting a 403 g ha^{-1} (0.36 lb a.s. ac^{-1}) application rate. Although the crop canopy is the spray target, applications were also made directly to bare soil in an effort to answer guideline questions.

Soil samples were collected to a 122 cm depth using zero contamination sampling equipment. The treated plots were scheduled to be sampled on the following thirty-one sampling events: -T1, T1, T1+1, T1+2, T1+3, T1+5, -T2, T2, -T3, T3, -T4, T4, -T5, T5, -T6, T6 and 1, 2, 3, 5, 7, 10, 14, 21, 30, 60, 90, 120, 180, 270 and 360 Days After Last Application (DALA). The untreated plot was scheduled to be sampled on the following seven sampling events: -T1 and 1, 5, 30, 90, 180, and 360 DALA intervals. A sampling variance of ± 2 days was acceptable after thirty days. After collection samples were analyzed using HPLC MS/MS for parent and one degradate M510F47 (2- chloronicotinic acid).

Calculated soil-water recharge events by depth over time and TDR (time domain reflectometry) measurements were used to determine if recharge events had any effect on movement of BAS 510 F or degradate residues from the top 15 cm (6 inch) of soil. In general, no BAS 510 F residues were found below the 15 cm (6 inch) depth in the cropped plots (except one replicate detect at California site (270 DALA, 15-30 cm [6-12 inch]) at the limit of quantitation). Residues for the bare soil plots were confined to depths of 0-15 cm (New York site), 0-30 cm (0-12 inch, Georgia site) and 0-46 cm (0-18 inch, California site). The deepest residues in the CA bare soil plot (30-46 cm [12-18 inch] depth) were found at three intervals (60, 90, and 360 DALA). No parent residues were found below 46 cm (18 inch) at any site. Recharge occurred in all plots at the 122 cm (4 feet) depth. Soil water recharge was correlated to the limited movement observed based on TDR measurements in bare soil plots. However, little movement was observed in the cropped plots which reflect actual use conditions. Therefore, it can be concluded that there is little probability that residues are mobile at a level of significance.

Based on soil core residue concentrations, half-life values (DT_{50}) were calculated for boscalid and its metabolite M510F47 when possible. A summary of the calculated boscalid and M510F47 dissipation times (DT_{50}/DT_{75}) in soil are presented below:

Table 7.1.2.2.1-28: Estimated first-order DT_{50} and DT_{75} values of boscalid and its metabolite M510F47 (2-chloronicotinic acid)

Compound	Trial site	DT_{50} [d]	DT_{75} [d]	α	β	r^2
BAS 510 F	Georgia – bare	264	>360	599.03124	$4.38962 * 10^{-6}$	0.68
	Georgia – cropped	282	>360	40.14995	$6.18152 * 10^{-5}$	0.94
	California – bare	150	>360	0.81875	0.00888	0.96
	California – cropped	>360	>360	0.11507	0.44607	0.86
	New York - bare	356	>360	0.27562	0.03195	0.74
M510F47	Georgia – bare	no detect	-	-	-	-
	Georgia – cropped	no detect	-	-	-	-
	California – bare	no detect	-	-	-	-
	California – cropped	no detect	-	-	-	-
	New York - bare	no detect	-	-	-	-

BAS 510 F dissipates steadily and does not accumulate when applied under worst case, bare soil conditions.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

Test item (formulation): BAS 510 00 F
Active substance (a.s.): Boscalid (BAS 510 F, Reg. No. 300355)
Chemical name (IUPAC): 2-chloro-N-(4'-chloro-biphenyl-2-yl)nicotinamide
Molar mass: 343.2 g mol^{-1}
Batch No.: AF543-17 (containing 69.6% boscalid)
Type of formulation: WG (wettable granule)

2. Test sites

The dissipation of boscalid under field conditions was investigated at three trial sites representative of major crop producing regions of the U.S. One trial each was performed in the regions Georgia, California, and New York. The site characteristics including the basic soil parameters of the corresponding soil horizons are presented in Table 7.1.2.2.1-29 and Table 7.1.2.2.1-30.

Table 7.1.2.2.1-29: Characteristics of the trial sites used in the field dissipation study (Georgia and California site)

Trial	**RCN 99509 (GA)**							
Location	**Georgia (GA), USA**							
Soil properties	**0-6 in (0-15 cm)**	**6-12 in (15-30 cm)**	**12-18 in (30-46 cm)**	**18-24 in (46-61 cm)**	**24-30 in (61-76 cm)**	**30-36 in (76-91 cm)**	**36-42 in (91-107 cm)**	**42-48 in (107-122 cm)**
Soil class (USDA)	Loamy sand	Sandy loam	Sandy clay loam	Sandy clay loam	Sandy clay loam	Sandy clay	Clay loam	Sandy clay
sand [%]	80	70	54	52	54	48	44	46
silt [%]	14	14	16	14	12	14	18	16
clay [%]	6	16	30	34	34	38	38	38
Organic matter [%]	1.4	0.6	0.6	0.3	0.1	0.1	0.1	0.1
pH [c]	6.6	6.6	6.8	6.1	5.6	5.4	5.3	5.1
CEC [meq $100g^{-1}$]	4.7	4.7	5.5	6.1	6.2	6.4	5.8	5.6
Moisture (gravimetric) at 1/3 bar [%] [a]	10.3	13.2	21.8	26.4	24.3	28.6	30.4	29.8
Bulk density [g cm^{-3}] [b]	0.97-1.34	1.11-1.56	0.70-1.38	0.95-1.34	1.15-1.40	0.93-1.25	1.09-1.34	0.98-1.44
Trial	**RCN 99510 (CA)**							
Location	**California, USA**							
Soil properties	**0-6 in (0-15 cm)**	**6-12 in (15-30 cm)**	**12-18 in (30-46 cm)**	**18-24 in (46-61 cm)**	**24-30 in (61-76 cm)**	**30-36 in (76-91 cm)**	**36-42 in (91-101 cm)**	**42-48 in (101-122 cm)**
Soil class (USDA)	Sandy loam	Sandy loam	Sandy loam	Sandy loam	Sandy loam	Sandy loam	Sandy loam	Sandy loam
sand [%]	75	65	57	53	61	59	61	59
silt [%]	18	28	34	40	32	32	28	28
clay [%]	7	7	9	7	7	9	11	13
Organic matter [%]	1.7	0.7	0.7	0.5	0.4	0.4	0.3	0.3
pH	7.0	7.9	7.9	8.0	8.0	8.1	8.2	8.4
CEC [meq $100g^{-1}$]	11.5	10.8	12	12.6	11.9	13.2	14.0	15.0
Moisture (gravimetric) at 1/3 bar [%] [a]	12.1	16.3	22.3	20.7	18.5	17.3	16.2	16.3
Bulk density [g cm^{-3}] [b]	1.44-1.55	1.37-1.68	1.29-1.69	1.27-1.45	1.32-1.80	1.46-1.56	1.46-1.51	1.07-1.60

CEC = cation exchange capacity
[a] Measured at six composited cores
[b] Measured at four separate, undisturbed cores
[c] in the US soil pH is commonly measured in H_2O; this is however not explicitly stated in the report

Table 7.1.2.2.1-30: Characteristics of the trial sites used in the field dissipation study (New York site)

Trial	**RCN 99511 (NY)**							
	New York, USA							
Soil properties	**0-6 in (0-15 cm)**	**6-12 in (15-30 cm)**	**12-18 in (30-46 cm)**	**18-24 in (46-61 cm)**	**24-30 in (61-76 cm)**	**30-36 in (76-91 cm)**	**36-42 in (91-101 cm)**	**42-48 in (101-122 cm)**
Soil class (USDA)	Loamy sand	Loamy sand	Loamy sand	Sand	Sand	Sand	Sandy loam	Sandy loam
sand [%] silt [%] clay [%]	83 10 7	83 10 7	85 10 5	87 10 3	87 10 3	87 10 3	77 14 9	77 14 9
Organic matter [%]	4.6	2.6	1.2	0.7	0.4	0.2	0.2	0.1
pH [c]	6.1	5.4	5.2	5.3	5.3	5.2	5.2	5.3
CEC [meq $100g^{-1}$]	11.0	8.4	6.6	4.5	3.6	3.2	4.5	3.8
Moisture (gravimetric) at 1/3 bar [%] [a]	13.6	11.8	8.7	6.9	5.5	5.4	8.1	6.9
Bulk density [g cm^{-3}] [b]	0.82-1.19	1.07-1.39	1.28-1.47	1.33-1.43	1.33-1.40	1.37-1.48	1.15-1.57	1.21-1.48

CEC = cation exchange capacity
[a] Measured at six composited cores
[b] Measured at four separate, undisturbed cores
[c] in the US soil pH is commonly measured in H_2O; this is however not explicitly stated in the report

The field trials were conducted in areas of major orchard/vineyard producing regions of the US. Applications of product were made to bare soil and cropped plots under actual field conditions. No product containing boscalid had been used on the test plots in the last three years.

B. STUDY DESIGN

1. Experimental conditions

Each trial area was divided into two to three plots. One plot was used as control plot (untreated), the second plot (bare soil) was treated with the test item. At trial sites Georgia and California, a third, cropped plot (orchard/vineyard) was treated with the test item as well. The treated plot consisted of three sampling sections, each divided into 34-40 equal subplots. For trial site New York, the treated plot consisted of six sections with 20 subplots each. The control plot consisted of three sampling sections, each comprising 8-10 subplots, and was separated from the treated plot by a buffer zone of at least 36 m (118 ft) width. The size of each subplot was 2.32 m^2 (25 sq ft). At each sampling time, three subplots of the control plot and three subplots of the treated plot (one of each sampling section) were randomly selected, sampled, and assigned for replicates A, B, and C.

The product, formulated as a WG (wettable granule), was applied by an air-blast sprayer to cropped soil and by flat-boom broadcast sprayers to bare soil. Six applications at a nominal rate of 260 (0.231 lb a.s. ac^{-1}, applications 1-3) and 403 g a.s. ha^{-1} (0.36 lb a.s. ac^{-1}, applications 4-6) were conducted. Depending on the trial site, the applications were performed from mid-May until mid-June 1999 (California), from mid-June until mid-July 1999 (New York), and from the beginning of July 1999 until the beginning of August 1999 (Georgia). The actual application rates determined by quantifying the amount of spray discharged ranged from 255 to 263 g a.s. ha^{-1} (0.228 – 0.235 lb a.s. ac^{-1}) for applications 1-3 and from 400 to 404 g a.s. ha^{-1} (0.357 – 0.361 lb a.s. ac^{-1}) for applications 4-6. Details of the application are presented in Table 7.1.2.2.1-31.

Table 7.1.2.2.1-31: Application parameters of field trial sites treated with boscalid

Trial Location	Test item/ Actual content [a]/ Formulation type	Application method	No. of applications	Application rate per treatment			No. of treated replicates	Application date
				Nominal [g a.s. ha^{-1}]	Actual [g a.s. ha^{-1}]	Dose verification [b]		
Georgia	BAS 510 F 197 - 203 mg a.s. L^{-1} WG	Air-blast spray to cropped soil	3	259	258 259 260	99.6-100.3% of nominal rate	3	03-Jul-99 10-Jul-99 17-Jul-99
	BAS 510 F 304 - 308 mg a.s. L^{-1} WG		3	403	403 404 402	99.9-100.4% of nominal rate	3	24-Jul-99 31-Jul-99 07-Aug-99
	BAS 510 F 956 – 985 mg a.s. L^{-1} WG	Broadcast spray to bare soil	3	259	255 259 260	98.9-100.0% of nominal rate	3	03-Jul-99 10-Jul-99 17-Jul-99
	BAS 510 F 1530 - 1552 mg a.s. L^{-1} WG		3	403	404 403 403	99.9-100.3% of nominal rate	3	24-Jul-99 31-Jul-99 07-Aug-99
California	BAS 510 F 189 - 195 mg a.s. L^{-1} WG	Air-blast spray to cropped soil	3	259	256 256 256	98.9-99.3% of nominal rate	3	11-May-99 18-May-99 25-May-99
	BAS 510 F 301 – 307 mg a.s. L^{-1} WG		3	403	400 400 400	99.2-99.3% of nominal rate	3	01-Jun-99 08-Jun-99 15-Jun-99
	BAS 510 F 972 – 991 mg a.s. L^{-1} WG	Broadcast spray to bare soil	3	259	260 263 258	99.5-101.9% of nominal rate	3	11-May-99 18-May-99 25-May-99
	BAS 510 F 1450 – 1532 mg a.s. L^{-1} WG		3	403	404 400 401	99.2-100.3% of nominal rate	3	01-Jun-99 08-Jun-99 15-Jun-99
New York	BAS 510 F 993 mg a.s. L^{-1} WG	Broadcast spray to bare soil	3	259	264 261 263	100.9-102.0% of nominal rate	3	16-Jun-99 23-Jun-99 30-Jun-99
	BAS 510 F 1548 mg a.s. L^{-1} WG		3	403	404 404 404	100.3-100.4% of nominal rate	3	07-Jul-99 14-Jul-99 21-Jul-99

[a] Values represent the range of test substance concentration in the spray solution (n = 3)
[b] Determined by sprayer calibration/pass time method.

A sprayer calibration/pass time method was used to confirm applications in this study. In addition to the sprayer calibration/pass time method, a petri dish application verification (AV) technique and a zero-time core recovery method were used to validate application rates.

Verification of application with the petri dish AV technique is simply the amount of test substance found per surface area of the petri dishes adjusted up to represent a value per acre.
The zero-time sample interval is defined as the first sample collected post application to the plot. Normally zero-time calculations are based on the last application in a series, if multiple applications have been made. Zero-time core concentrations (bare soil only) were calculated by subtracting parent residue in the 0-15 cm (0-6 inch) core before the last application was made (-T6) from the parent residue measured immediately after the last application (T6). For calculation purposes, soil weight was based on a furrow acre slice (907184 kg/15 cm; 2,000,000 lb/ 6 inch).

No tillage was performed during the course of the study from first to last sampling. Trials Georgia and California consisted of a treated bare soil plot and a cropped soil plot. The plots were kept free of weeds via the application of glyphosate, simazine, acifluorfen, and paraquat.

Seasonal weather data was collected at each trial site for the entire trial period. Precipitation data was collected onsite while daily minimum and maximum air temperatures, wind speed, solar radiation, percent humidity and evapotranspiration (if available) were collected from an on-site or nearby weather station. Irrigation was applied to supplement normal precipitation so that the plots would receive 110% of the historical average rainfall or crop evapotranspiration (if applicable) for the study period. Historical weather data (average monthly minimum and maximum air temperatures and monthly precipitation totals) were submitted for at least a ten-year period from a reliable source located no more than 56.3 km (35 miles) from the test site.

A summary of monthly weather data (maximum and minimum temperatures, precipitation, as well as volumes of the supplementary irrigation) is presented in Table 7.1.2.2.1-32.

Table 7.1.2.2.1-32: Summary of monthly air temperature (max/min), precipitation, and irrigation at each field trial site

Trial	RCN 99509 (GA)					RCN 99510 (CA)				
Location	Georgia, USA					California, USA				
Month/ Year	T_{min} Air [°C]	T_{max} Air [°C]	Precipi-tation [mm]	Irriga-tion [mm]	Total [mm]	T_{min} Air [°C]	T_{max} Air [°C]	Precipi-tation [cmm]	Irri-gation [cmm]	Total [mm]
May-99	-	-	-	-	-	9.5	26.7	0.0	192.0	192.0
Jun-99	-	-	-	-	-	13.4	30.8	0.0	493.8	493.8
Jul- 99	19.2	32.8	69.3 [a]	96.5 [a]	165.9 [a]	16.4	33.2	0.0	246.9	246.9
Aug- 99	19.3	33.9	116.8	78.7	195.6	15.1	31.7	0.0	219.5	219.5
Sep- 99	13.4	29.0	122.2	58.4	180.6	14.2	31.6	4.8	192.0	196.9
Oct- 99	9.5	22.6	79.5	17.8	97.3	9.0	28.1	0.0	137.2	137.2
Nov- 99	4.6	20.1	87.9	43.2	131.1	4.3	19.9	13.2	123.4	136.7
Dec- 99	0.4	14.6	72.1	61.0	133.1	-1.2	15.4	1.5	54.9	56.4
Jan-00	0.3	10.8	102.1	0.0	102.1	2.4	15.1	41.4	0.0	41.4
Feb-00	1.9	16.8	58.7	88.9	147.6	5.9	16.9	99.8	27.4	127.3
Mar-00	6.5	20.8	92.7	88.9	181.6	5.8	19.2	44.2	0.0	44.2
Apr-00	7.5	21.8	49.8	73.7	123.4	7.9	24.2	34.5	123.4	158.0
May-00	15.5	29.6	35.8	106.7	142.5	11.8	28.3	6.6	205.7	212.3
Jun-00	18.2	32.2	51.3	129.5	180.8	16.0	33.4	6.6	41.1	47.8
Jul-00	20.0	33.4	36.8 [a]	114.3 [a]	151.1 [a]	-	-	-	-	-
Aug-00	19.5	31.8	-	-	-	-	-	-	-	-
Total	-	-	**975.4**	**957.6**	**1932.9**	-	-	**254.0**	**2057.4**	**2311.4**

Trial	RCN 99511 (NY)				
Location	New York, USA				
Month/ Year	T_{min} Air [°C]	T_{max} Air [°C]	Precipi-tation [mm]	Irriga-tion [mm]	Total [mm]
Jun-99	13.5	25.9	38.6 [a]	72.6 [a]	111.3 [a]
Jul- 99	17.2	28.7	83.8	151.1	235.0
Aug- 99	14.4	24.7	85.6	94.0	179.6
Sep- 99	12.3	22.9	131.8	0.0	131.8
Oct- 99	4.8	15.2	111.5	31.8	143.3
Nov- 99	2.7	11.2	85.9	31.8	117.6
Dec- 99	-3.8	3.2	49.5	0.0	49.5
Jan-00	-10.2	-0.3	46.7	0.0	46.7
Feb-00	-5.2	2.3	30.7	0.0	30.7
Mar-00	-0.9	9.1	48.5	0.0	48.5
Apr-00	1.7	10.4	130.6	0.0	130.6
May-00	9.3	19.8	144.8	0.0	144.8
Jun-00	12.6	23.2	125.0	0.0	125.0
Jul-00	13.4	23.9	61.7 [a]	28.7 [a]	90.4 [a]
Total	-	-	**1176.0**	**408.9**	**1585.0**

[a] Precipitation totals for the first and last months of each trial reflect active periods only, not totals for the whole month.

2. Sampling

Five replicate cores (0-122 cm [0-48 inch]) were collected each from three predetermined, randomly selected subplots at each designated sampling interval using a soil corer. A soil depth of up to 122 cm (48 inch) was taken for each soil.

Samples from treated soils were collected from the treated plot on 31 occasions one day prior each of the six applications (-T), on the day of each application (T), between the first and second application (T1+1, T1+2, T1+3, T1+5), and 1, 2, 3, 5, 7, 10, 14, 21, 30, 60, 90, 120, 180, 270, 360 days after the last application (DALA). Samples from untreated soils were collected from the control plot on seven occasions one day before the first application (-T1) and 1, 5, 30, 90, 180 and 360 DALA. The specified dates refer to planned sampling dates. Actual sampling dates are given in brackets in the results tables (Table 7.1.2.2.1-34 to Table 7.1.2.2.1-38).

Soil cores from the treated plots were taken to a depth of 122 cm (48 inch) in two stages at each sampling interval. For each sampling event, five 0-15 cm (0-6 inch) and five 15-122 cm (6-48 inch) soil cores were collected from each of three randomly selected subplots, and were designated as replicates A, B, and C. This resulted in a total of fifteen each of the 0-15 cm (0-6 inch) and 15-122 cm (6-48 inch) cores collected at each sampling interval for each treated plot. The 0-15 cm cores were either sampled in two runs or sectioned after sampling to obtain a 0-8 cm (0-3 inch) and a 8-15 cm (3-6 inch) soil core. The 15-122 cm (6-48 inch) cores were cut frozen into 15 cm (6 inch) increments. Soil cores were composited by depth within a replicate to form completed samples.

All soil specimens were placed into freezer storage at about -18°C and remained frozen until processing or analysis of the samples.

3. Description of analytical procedure

Samples were received from the field frozen and were stored at ≤ 0°C. Samples were homogenized at BASF prior to analysis. After homogenization, samples were analyzed using BASF Draft Analytical Method D0004 [*SAHA, M. (2000): "THE DETERMINATION OF BAS 510 F AND ITS METABOLITES, IN SOIL USING LC-MS/MS"*]. The method used for the residue analyses is described below.

The residues of BAS 510 F and M510F47 were extracted from soil by shaking with methanol followed by methanol/water (50:50, v/v). An aliquot (5 mL) of the extract was diluted with a buffer solution (water with 0.1% formic acid and 4 mM ammonium formate; 3 mL) for HPLC-MS/MS determination of BAS 510 F and M510F47. The limit of quantitation (LOQ) for each analyte was 0.010 mg kg^{-1}.

Control samples were fortified either with BAS 510 F or M510F47. A summary of the average procedural recoveries for samples analyzed is provided in section II.2.

Application verification (AV) samples were kept below 0°C during shipping and storage until extraction. Samples were shaken and centrifuged twice with methanol/water. The samples were then serial diluted using 70/30 methanol/buffer water and quantitated using LC-MS/MS. Due to the concentration of the samples, it was not necessary to establish a limit of quantitation for this procedure.

4. Storage stability experiments

The actual length of time between sample collection in the field and sample extraction at the laboratory was recorded on sample residue records. Samples were shipped in freezer trucks to BASF for analysis. The samples were kept frozen during shipment to and storage at BASF. The maximum period any sample was stored before analysis was 495 days (16.3 months). Results revealed that the test item (BAS 510 F) is stable during freezer storage.

In addition, the storage stability of boscalid was investigated in a separate study [*peer-reviewed study BASF DocID 2000/1000136*].

5. Kinetic evaluation

In order to calculate dissipation times (DT_{50}/DT_{75}), the reported residue values were mathematically averaged by replicate to produce a grand mean. In the case where a replicate was analyzed in duplicate or triplicate, the numbers were then averaged to produce a mean for that replicate before averaging with the other replicates. When a replicate sample was below the LOQ or < 0.01 mg kg^{-1}, it was averaged as zero. To determine analyte half-life values, the residues in all soil depths were summed to produce the total residue concentration per sampling period. The summed residue concentrations per sampling period were natural log transformed before analysis by non-linear regression.

The non-linear regression was solved by applying the Gustafson-Holden model (FOMC), using the Origin Scientific Graphics Software (OriginLab Corporation, Northampton, MA, USA; version 6.0, 1999).

6. Residue mobility

To determine if residues had an opportunity to move down through the soil profile with the soil solution, the water content by depth was determined using Time Domain Reflectometry (TDR) was applied. Soil water flux was also calculated at a 30 cm depth based on this method. The TDR system used in this study was a CR10X datalogger (Campbell Scientific Inc., Logan, UT, USA) configured with four CS615 probes located in one of the bare soil subplots. The probes were installed vertically at the 0-30, 30-61, 61-91 and 91-122 cm (0-1, 1-2, 2-3, and 3-4 ft) depths. Since the rods of the CS615 are 30.5 cm (12 inch) in length, vertical installation provides a continuum of volumetric water content measurements from the soil surface to a depth of 122 cm (4 ft).

II. RESULTS AND DISCUSSION

1. Application verification

Application results from the petri dish AV technique ranged from 95.1% to 103.9% (Georgia), from 102.2% to 106.1% (California), and from 76.0% to 82.5% (New York) of the target rate. Actual pass/time and spray equipment calibration data indicated that the target field application rate was precise and accurate. The variance observed in application petri dish AV recoveries was assumed to be a reflection of the technique rather than a demonstration of variance in actual application to plots. Zero-time core recoveries were 78% (Georgia), 122% (California), and 100% (New York) based on the bare soil plot (broadcast application) of the respective field trial site. Differences in calculated application rates to the field and those calculated from zero time cores are probably due to sample handling. More details on the application verification results are given in the study report.

2. Residues in field soil samples

Mean procedural recoveries from control soil samples fortified either with boscalid or M510F47 at a minimum of two levels (0.1 or 0.01 mg kg^{-1}) were generally within the acceptable range of 70-120% (see Table 7.1.2.2.1-33). A summary of the individual procedural recovery results is provided in the study report.

Table 7.1.2.2.1-33: Method procedural recoveries

Analyte	Mean recovery ± RSD [%]		
	Georgia	California	New York
BAS 510 F	96 ± 5.3 (n=70)	97 ± 5.6 (n=83)	104 ± 6.4 (n=43)
M510F47 (2-chloronicotinic acid)	94 ± 16.2 (n=70)	101 ± 12.9 (n=83)	101 ± 13.2 (n=43)

RSD = Relative standard deviation [%]

Field soil samples taken from different depths were analyzed to a maximum of about 360 days after the last of the six applications. The analytical results are summarized in Table 7.1.2.2.1-34 to Table 7.1.2.2.1-38. All residue values presented in these tables are related to the dry weight of the soil and were not corrected for procedural recoveries. Residue concentrations are reported according to soil depth (0-8, 8-15, 15-30, and 30-46 cm, 0-3, 3-6, 6-12, 12-18 inch).

Table 7.1.2.2.1-34: Residues of boscalid and its metabolite M510F47 (2-chloronicotinic acid) [mg kg^{-1} dry weight] in treated soil samples (mean of three replicates) – Georgia site (bare plot; RCN 99509-GA)

Compound [a]	Soil depth [cm]	Targeted days after last application [b]																
		-T1	T1	T1+1	T1+2	T1+3	T1+5	-T2	T2	-T3	T3	-T4	T4	-T5	T5	-T6	T6	1
boscalid	0-8	<0.01	0.15	0.14	0.21	0 23	0.19	0.13	0.37	0.32	0.62	0.44	0.82	0.70	0.99	1.08	1.36	1.21
	8-15	<0.01	<0.01	0.01	<0.01	<0.01	<0.01	<0.01	0.02	0.005	<0.01	<0.01	0.02	0.03	0.03	0.03	0.03	0.03
	15-30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	30-46	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
M510F47	0-8	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	8-15	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	15-30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	30-46	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Compound [a]	Soil depth [cm]	Targeted days after last application [b]												
		2	3	5[c]	7[c]	14[c]	21[c]	30[c]	60	90 (89)[c]	120 (118)[c]	180 (181)[c]	270 (271)[c]	360 (364)[c]
boscalid	0-8	0.93	1.18	1.29	0.89	0.93	1.21	0.76	0.83	0.78	0.90	0.84	0.60	0.31
	8-15	0.01	0.03	0.06	0.007	0.02	0.02	<0.01	0.01	0.003	0.04	0.03	0.09	0.02
	15-30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.005	0.04	0.003
	30-46	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
M510F47	0-8	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	8-15	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	15-30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	30-46	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

T = Application dates

[a] The limit of quantification (LOQ) was 0.01 mg kg^{-1} for each analyte. All residue values were corrected for moisture content and are reported here on a dry weight basis. All value of 0 µg kg^{-1} was used in average calculations for samples where residues were reported as <0.01 mg kg^{-1}.

[b] Sampling dates refer to the targeted sampling dates, the actual sampling dates are given in brackets.

[c] Samples were analyzed down to 46-61 cm (18-24 inch), no residues were found at or above 0.01 ppm.

Table 7.1.2.2.1-35: Residues of boscalid and its metabolite M510F47 (2-chloronicotinic acid) [mg kg^{-1} dry weight] in treated soil samples (mean of three replicates) – Georgia site (cropped plot; RCN 99509-GA)

Compound [a]	Soil depth [cm]	Targeted days after last application [b]																
		-T1	T1	T1+1	T1+2	T1+3	T1+5	-T2	T2	-T3	T3	-T4	T4	-T5	T5	-T6	T6	1
boscalid	0-8	<0.01	0.09	0.04	0.07	0.06	0.05	0.03	0.07	0.11	0.11	0.08	0.22	0.12	0.17	0.21	0.31	0.21
	8-15	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.005	0.08	<0.01	<0.01	<0.01	<0.01
	15-30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	30-46	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
M510F47	0-8	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	8-15	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	15-30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	30-46	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Compound [a]	Soil depth [cm]	Targeted days after last application [b]												
		2	3	5	7	14	21	30	60	90 (89)	120 (118)	180 (181)	270 (271)	360
boscalid	0-8	0.26	0.19	0.31	0.22	0.18	0.32	0.18	0.17	0.18	0.25	0.17	0.18	0.11
	8-15	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	15-30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	30-46	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
M510F47	0-8	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	8-15	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	15-30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	30-46	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

T = Application dates

[a] The limit of quantification (LOQ) was 0.01 mg kg^{-1} for each analyte. All residue values were corrected for moisture content and are reported here on a dry weight basis. All value of 0 µg kg^{-1} was used in average calculations for samples where residues were reported as <0.01 mg kg^{-1}.

[b] Sampling dates refer to the targeted sampling dates, the actual sampling dates are given in brackets.

Table 7.1.2.2.1-36: Residues of boscalid and its metabolite M510F47 (2-chloronicotinic acid) [mg kg^{-1} dry weight] in treated soil samples (mean of three replicates) – California site (bare plot; RCN 99510-CA)

Compound [a]	Soil depth [cm]	Targeted days after last application [b]																
		-T1	T1	T1+1	T1+2	T1+3	T1+5	-T2	T2	-T3	T3	-T4	T4	-T5	T5	-T6	T6	1
boscalid	0-8	<0.01	0.15	0.22	0.17	0 17	0.15	0.17	0.37	0.32	0.51	0.41	0.60	0.50	0.87	0.72	1.11	1.16
	8-15	<0.01	0.01	<0.01	<0.01	0.02	0.005	0.02	<0.01	0.005	0.01	0.08	0.10	0.12	0.07	0.17	0.19	0.14
	15-30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.02	<0.01
	30-46	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
M510F47	0-8	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	8-15	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	15-30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	30-46	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Compound [a]	Soil depth [cm]	Targeted days after last application [b]												
		2	3[c]	5[c]	7[c]	14[c]	21[c]	30	60[d]	90 (91)[d]	120[d]	180 (181)[c]	270 (268)[d]	360[d]
boscalid	0-8	1.00	1.14	1.10	1.04	1.11	0.92	0.68	0.26	0.41	0.43	0.39	0.31	0.28
	8-15	0.14	0.22	0.12	0.20	0.20	0.22	0.23	0.19	0.16	0.26	0.25	0.15	0.04
	15-30	0.003	0.01	0.007	0.02	0.02	0.01	0.01	0.08	0.04	0.03	0.04	0.02	0.04
	30-46	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.02	0.01	<0.01	<0.01	<0.01	0.007
M510F47	0-8	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	8-15	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	15-30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	30-46	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

T = Application dates

[a] The limit of quantification (LOQ) was 0.01 mg kg^{-1} for each analyte. All residue values were corrected for moisture content and are reported here on a dry weight basis. All value of 0 µg kg^{-1} was used in average calculations for samples where residues were reported as <0.01 mg kg^{-1}.

[b] Sampling dates refer to the targeted sampling dates, the actual sampling dates are given in brackets.

[c] Samples were analyzed down to 46-61 cm (18-24 inch), no residues were found at or above 0.01 ppm.

[d] Samples were analyzed down to 61-76 and 76-91 cm (24-30 and 30-36 inch), no residues were found at or above 0.01 ppm.

Table 7.1.2.2.1-37: Residues of boscalid and its metabolite M510F47 (2-chloronicotinic acid) [mg kg^{-1} dry weight] in treated soil samples (mean of three replicates) – California site (cropped plot; RCN 99510-CA)

Compound [a]	Soil depth [cm]	Targeted days after last application [b]																
		-T1	T1	T1+1	T1+2	T1+3	T1+5	-T2	T2	-T3	T3	-T4	T4	-T5	T5	-T6	T6	1
boscalid	0-8	<0.01	0.06	0.05	0.05	0.05	0.05	0.07	0.14	0.11	0.20	0.23	0.25	0.26	0.41	0.30	0.48	0.49
	8-15	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.005	0.006	<0.01	0.02	<0.01	0.03	0.02
	15-30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	30-46	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
M510F47	0-8	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	8-15	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	15-30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	30-46	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Compound [a]	Soil depth [cm]	Targeted days after last application [b]												
		2	3	5	7	14	21	30	60	90 (91)[d]	120[d]	180 (181)[c]	270 (268)[d]	360
boscalid	0-8	0.44	0.48	0.40	0.39	0.42	0.34	0.37	0.27	0.23	0.26	0.29	0.30	0.26
	8-15	0.01	0.03	0.01	0.02	0.02	0.05	0.04	0.03	0.07	0.07	0.02	0.07	0.04
	15-30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.003	<0.01
	30-46	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
M510F47	0-8	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	8-15	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	15-30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	30-46	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

T = Application dates

[a] The limit of quantification (LOQ) was 0.01 mg kg^{-1} for each analyte. All residue values were corrected for moisture content and are reported here on a dry weight basis. All value of 0 µg kg^{-1} was used in average calculations for samples where residues were reported as <0.01 mg kg^{-1}.

[b] Sampling dates refer to the targeted sampling dates, the actual sampling dates are given in brackets.

[c] Samples were analyzed down to 46-61 cm (18-24 inch), no residues were found at or above 0.01 ppm.

[d] Samples were analyzed down to 61-76 and 76-91 cm (24-30 and 30-36 inch), no residues were found at or above 0.01 ppm.

Table 7.1.2.2.1-38: Residues of boscalid and its metabolite M510F47 (2-chloronicotinic acid) [mg kg^{-1} dry weight] in treated soil samples (mean of three replicates) – New York site (bare plot; RCN 99511-NY)

Compound [a]	Soil depth [cm]	Targeted days after last application [b]																
		-T1	T1	T1+1	T1+2	T1+3	T1+5	-T2	T2	-T3	T3	-T4	T4	-T5	T5	-T6	T6	1
boscalid	0-8	<0.01	0.29	0.23	0.17	0 12	0.17	0.17	0.63	0.32	0.58	0.51	0.57	0.69	0.96	0.75	0.98	1.11
	8-15	<0.01	<0.01	<0.01	0.02	0.02	0.02	<0.01	<0.01	<0.01	<0.01	<0.01	0.10	0.04	<0.01	0.03	0.04	0.05
	15-30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	30-46	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
M510F47	0-8	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	8-15	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	15-30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	30-46	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Compound [a]	Soil depth [cm]	Targeted days after last application [b]												
		2	3	5	7	14	21	30 (29)	60	90	120	180 (223)	270 (273)	360 (364)
boscalid	0-8	1.04	0.94	0.98	1.15	0.93	1.03	1.04	1.03	0.66	0.43	0.65	0.94	0.59
	8-15	0.06	0.02	0.05	0.03	<0.01	<0.01	0.008	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	15-30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	30-46	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
M510F47	0-8	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	8-15	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	15-30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	30-46	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

T = Application dates

[a] The limit of quantification (LOQ) was 0.01 mg kg^{-1} for each analyte. All residue values were corrected for moisture content and are reported here on a dry weight basis. All value of 0 µg kg^{-1} was used in average calculations for samples where residues were reported as <0.01 mg kg^{-1}.

[b] Sampling dates refer to the targeted sampling dates, the actual sampling dates are given in brackets.

D. KINETIC EVALUATION

A summary of calculated DT_{50} and DT_{75} values for boscalid and its metabolite M510F47 (where possible) can be found in Table 7.1.2.2.1-39.

Table 7.1.2.2.1-39: Estimated first-order DT_{50} and DT_{75} values of boscalid and its metabolite M510F47 (2-chloronicotinic acid)

Compound	Trial site	DT_{50} [d]	DT_{75} [d]	α	β	r^2
BAS 510 F	Georgia – bare	264	>360	599.03124	$4.38962 * 10^{-6}$	0.68
	Georgia – cropped	282	>360	40.14995	$6.18152 * 10^{-5}$	0.94
	California – bare	150	>360	0.81875	0.00888	0.96
	California – cropped	>360	>360	0.11507	0.44607	0.86
	New York - bare	356	>360	0.27562	0.03195	0.74
M510F47	Georgia – bare	no detect	-	-	-	-
	Georgia – cropped	no detect	-	-	-	-
	California – bare	no detect	-	-	-	-
	California – cropped	no detect	-	-	-	-
	New York - bare	no detect	-	-	-	-

E. RESIDUE MOBILITY

In general, no BAS 510 F residues were found below the 15 cm depth in the cropped plots. One exception to this occurred at the California site where parent compound at LOQ was found at a depth of 15-40 cm (6-12 inch) at 270 DALA (Rep C). Cropped plots reflect real use conditions since the compound will not be applied directly to the soil as in the bare soil plots. Residues in the bare soil plots were confined to the 0-15 cm (0-6 inch) depth at the New York site, 0-30 cm (0-18 inch) depth at the Georgia site and 0-46 cm (0-18 inch) depth at the California site. The deepest residues in the California bare soil plot (30-46 cm depth) were found at three intervals (60 DALA, Reps B and C; 90 DALA, Rep B and 360 DALA, Rep B). No parent residues were found below 46 cm (18 inch) at any site.

Water movement (flux) did occur down through the 122 cm (48 inch) sampling zone. Flux calculations indicate that 1.40, 40.54, and 9.78 cm (0.55, 15.96, and 3.85 inch) of water leached through the 122 cm (48 inch) depth sampling zone at the Georgia, California, and New York sites, respectively. Therefore, the physical conditions (water flux) required to allow compound movement were present at each site.

III. CONCLUSION

Results from this study have shown that BAS 510 F dissipates steadily and does not accumulate when applied under worst case, bare soil conditions.

Report: CA 7.1.2.2.1/8
Jackson S. et al., 2001a
1999 Field dissipation of BAS 510 .. F in turf use patterns
2001/5000833
Guidelines: EPA 164-1
GLP: yes
(certified by United States Environmental Protection Agency)

Executive Summary

This study was designed to support a turf use pattern according to EPA Guideline 164-1. The test substance, BAS 510 .. F, is a broad spectrum fungicide which will be used to control many diseases in field, row crop, orchard, vineyards, turf and other uses. Three field trials were conducted which represent major crop sales regions of the United States (New Jersey, Illinois and Texas).

A single untreated control plot (bare soil), one treated bare soil plot and one treated turf plot were established at each test site. The plots were located on soil typical for the agricultural areas in which they represented. The treated plots received six broadcast applications of the test substance using 561.2 L ha^{-1} (60 gal ac^{-1}) spray volume at fourteen day intervals (targeted). The test substance was applied, beginning in May 1999, at a rate of 302 g a.s. ha^{-1} (0.27 lb a.s. ac^{-1}) for the first three applications then 392 g a.s. ha^{-1} (0.35 lb a.s. ac^{-1}) for the last three applications. Applications were made directly to bare soil plots in an effort to answer guideline questions.

Soil samples were collected to a 122 cm (48 inch) depth using zero contamination sampling equipment. The treated plots were scheduled to be sampled on the following thirty-two sampling events: -T1, T1, T1+2, T1+3, T1+5, T1+7, T1+9, -T2, T2, -T3, T3, -T4, T4, -T5, T5, -T6, T6, 1, 2, 3, 5, 7, 10, 14, 21, 30, 60, 90, 120, 180, 270 and 360 Days After Last Application (DALA). The untreated plots were scheduled to be sampled on the following seven sampling events: -T1 and 1, 5, 30, 90, 180, and 360 DALA intervals. A sampling variance of ± 2 days was acceptable after thirty days. After collection, samples were analyzed using HPLC MS/MS for parent and degradates (2- chloronicotinic acid and M510F49).

Compound mobility was determined by measuring each analyte by depth. If mobility occurred, it was possible to correlate the movement to soil water recharge events. Calculated soil-water recharge events by depth over time and TDR (time domain reflectometry) measurements were used to determine if recharge events had any effect on movement of BAS 510 F or degradate residues from the top 15 cm (6 inch) of soil. In general most residues of BAS 510 F remained in the 0-15 cm (0-6 inch) segments. However, residues were present in the 15-30 cm (6-12 inch) cores through time in the New Jersey bare soil plot and the Illinois turf plot. One core replicate had residues present in the 30-46 cm (12-18 inch) segment (5 DALA) in the bare soil plot at the New Jersey site. Recharge occurred in all plots at the 122 cm (48 inch) depth. Soil water recharge was correlated to the limited residue movement observed based on TDR measurements in bare soil plots.

Based on soil core residue concentrations, half-life values (DT_{50}) were calculated for boscalid and its metabolites M510F47 (2-chloronicotinic acid) and M510F49 where possible. A summary of the calculated boscalid, M510F47 (2-chloronicotinic acid), and M510F49 dissipation times (DT_{50}/DT_{75}) in bare soil are presented below:

Table 7.1.2.2.1-40: Estimated first-order DT_{50} and DT_{75} values of boscalid and its metabolites M510F47 (2-chloronicotinic acid) and M510F49

Compound	Trial site	Bare		Turf	
		DT_{50} [d]	DT_{75} [d]	DT_{50} [d]	DT_{75} [d]
BAS 510 F	New Jersey (poly)	300	-	-	-
	New Jersey	108	>359	44	174
	Illinois (poly)	307	-	-	-
	Illinois	244	>344	155	>344
	Texas (poly)	-	-	194	-
	Texas	143	>316	108	232
M510F47	New Jersey	n.d.	n.d.	< 90 (est.)	-
	Illinois	n.d.	-	< 57 (est.)	-
	Texas	< 1 (est.)	-	< 180	-
M510F49	New Jersey	n.d.	-	24.7	54
	Illinois	< 89 (est.)	-	< 253 (est.)	-
	Texas	< 30 (est.)	-	< 59 (est.)	-

Poly = Polynomial solution
n.d. = Not detected
est. = Estimates

Results from this study have shown that BAS 510 F dissipates steadily under conditions of use.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

Test item (formulation): BAS 510 .. F
Active substance (a.s.): Boscalid (BAS 510 F, Reg. No. 300355)
Chemical name (IUPAC): 2-chloro-N-(4'-chloro-biphenyl-2-yl)nicotinamide
Molar mass: 343.2 g mol^{-1}
Batch No.: AF543-17 (containing 69.6% boscalid)
Type of formulation: WG (wettable granule)

2. Test sites

The dissipation of boscalid under field conditions was investigated at three trial sites representative of major crop producing regions of the U.S. One trial each was performed in Hunterdon County (New Jersey), Clinton County (Illinois), and Waller County (Texas). The site characteristics including the basic soil parameters of the corresponding soil horizons are presented in Table 7.1.2.2.1-41 and Table 7.1.2.2.1-42.

Table 7.1.2.2.1-41: Characteristics of the trial sites used in the field dissipation study (New Jersey and Illinois site)

Trial	**RCN 99512 (NJ)**							
Location	**New Jersey, USA**							
Soil properties	**0-6 in (0-15 cm)**	**6-12 in (15-30 cm)**	**12-18 in (30-46 cm)**	**18-24 in (46-61 cm)**	**24-30 in (61-76 cm)**	**30-36 in (76-91 cm)**	**36-42 in (91-107 cm)**	**42-48 in (107-122 cm)**
Soil class (USDA)	Loam	Loam	Loam	Loam	Loam	Clay loam	Loam	Loam
sand [%]	30	28	32	46	40	34	40	46
silt [%]	50	46	42	28	34	38	36	30
clay [%]	20	26	26	26	26	28	24	24
Organic matter [%]	2.6	0.9	0.3	0.1	0.2	0.1	0.1	0.1
pH [c]	6.3	6.7	6.6	6.3	6.6	5.8	5.4	5.3
CEC [meq $100g^{-1}$]	8.5	7.6	8.6	8.9	7.9	8.1	8.0	8.7
Moisture (gravimetric) at 1/3 bar [%][a]	28.2	24.3	23.0	21.8	21.7	23.0	23.5	22.0
Bulk density [g cm^{-3}] [b]	1.32-1.42	1.47-1.65	1.63-1.75	1.72-1.87	1.73-1.88	1.70-1.83	1.66-1.76	1.59-1.72
Trial	**RCN 99513 (IL)**							
Location	**Illinois, USA**							
Soil properties	**0-6 in (0-15 cm)**	**6-12 in (15-30 cm)**	**12-18 in (30-46 cm)**	**18-24 in (46-61 cm)**	**24-30 in (61-76 cm)**	**30-36 in (76-91 cm)**	**36-42 in (91-107 cm)**	**42-48 in (107-122 cm)**
Soil class (USDA)	Silt loam	Silt loam	Silt loam	Clay	Clay	Clay	Clay loam	Clay loam
sand [%]	27	23	23	21	21	25	21	23
silt [%]	58	58	56	38	32	30	40	42
clay [%]	15	19	21	41	47	45	39	35
Organic matter [%]	1.7	1.2	0.5	0.8	0.7	0.6	0.4	0.2
pH [c]	6.1	5.5	4.9	4.7	4.7	4.8	4.8	4.8
CEC [meq $100g^{-1}$]	10.6	9.5	10.1	23.6	29.4	25.8	22.3	20.1
Moisture (gravimetric) at 1/3 bar [%][a]	29.0	29.8	28.6	38.3	42.8	40.7	37.0	37.2
Bulk density [g cm^{-3}] [b]	1.56-1.67	1.54-1.67	1.55-1.59	1.42-1.58	1.43-1.65	1.31-1.55	1.53-1.74	1.52-1.68

CEC = cation exchange capacity
[a] Measured at six composited cores
[b] Measured at four separate, undisturbed cores
[c] in the US soil pH is commonly measured in H_2O; this is however not explicitly stated in the report

Table 7.1.2.2.1-42: Characteristics of the trial sites used in the field dissipation study (Texas site)

Trial	**RCN 99514 (TX)**							
Location	**Texas, USA**							
Soil properties	**0-6 in (0-15 cm)**	**6-12 in (15-30 cm)**	**12-18 in (30-46 cm)**	**18-24 in (46-61 cm)**	**24-30 in (61-76 cm)**	**30-36 in (76-91 cm)**	**36-42 in (91-107 cm)**	**42-48 in (107-122 cm)**
Soil class (USDA)	Sandy loam	Sandy loam	Sandy loam	Sandy clay loam	Sandy clay loam	Sandy clay	Clay	Sandy clay loam
sand [%]	71	69	63	59	49	45	41	49
silt [%]	22	22	26	20	20	14	18	16
clay [%]	7	9	11	21	31	41	41	35
Organic matter [%]	0.9	0.3	0.3	0.5	0.6	0.5	0.3	0.2
pH [c]	5.9	6.4	6.3	5.8	5.9	6.2	6.6	6.8
CEC [meq $100g^{-1}$]	4.9	4.3	4.8	10.4	14.5	18.4	18.9	17.4
Moisture (gravimetric) at 1/3 bar [%][a]	8.4	9.3	11.1	17.4	22.8	27.8	27.4	23.0
Bulk density [g cm^{-3}] [b]	1.56-1.67	1.62-1.80	1.79-1.91	1.79-1.91	1.65-1.87	1.68-1.87	1.72-1.96	1.90-2.09

CEC = cation exchange capacity
[a] Measured at six composited cores
[b] Measured at four separate, undisturbed cores
[c] in the US soil pH is commonly measured in H_2O; this is however not explicitly stated in the report

The selected fields were representative of major geographic and climatic regions within the United States. Bare soil plots were fallow prior to the study. No product containing boscalid had been used on the test plots in the last three years.

B. STUDY DESIGN

1. Experimental conditions

Each trial area was divided into three plots. One plot was used as control plot (untreated, bare soil), the second (bare soil) and the third plot (turf) were treated with the test item. The treated plot consisted of three sampling sections, each divided into 36-40 equal subplots. For each trial site, the treated plot consisted of six sections with 36-40 subplots each. The control plot consisted of three sampling sections, each comprising 10-40 subplots, and was separated from the treated plot by a buffer zone of at least 35 m width. The size of each subplot was 2.32-3.10 m^2 (25-31.8 sq ft). At each sampling time, three subplots of the control plot and three subplots of the treated plot (one of each sampling section) were randomly selected, sampled, and assigned for replicates A, B, and C.

The product, formulated as a WG (wettable granule), was broadcast applied to bare/cropped soil in six applications at a nominal rate of 302 (0.27 lb a.s. ac^{-1}; applications 1-3) and 392 g a.s. ha^{-1} (0.35 lb a.s. ac^{-1}; applications 4-6). Depending on the trial site, the applications were conducted from end of May-1999 until beginning of Aug-1999 (New Jersey), from beginning of Jun-1999 until mid of Aug-1999 (Illinois), and from beginning of Jul-1999 until mid of Sep-1999 (Texas), using a calibrated boom broadcast sprayer. The actual application rates determined by quantifying the amount of spray discharged ranged from 301 to 352 g a.s. ha^{-1} (0.269-314 lb a.s. ac^{-1}) for applications 1-3 and from 392 to 395 g a.s. ha^{-1} (0.350-0.353 lb a.s. ac^{-1}) for applications 4-6. Details of the application are presented in Table 7.1.2.2.1-43.

Table 7.1.2.2.1-43: Application parameters of field trial sites treated with boscalid

Trial Location	Test item/ Actual content [a]/ Formulation type	Application method	No. of applications	Application rate per treatment			No. of treated replicates	Application date
				Nominal [g a.s. ha^{-1}]	Actual [g a.s. ha^{-1}]	Dose verification [b]		
New Jersey	BAS 510 F 775 - 890 mg a.s. L^{-1} WG	Broadcast spray to planted/bare soil	3	302	306 352 310	101.2-116.1% of nominal rate	3	26-May-99 09-Jun-99 23-Jun-99
	BAS 510 F 1004 mg a.s. L^{-1} WG		3	392	395 394 394	100.5-100.7% of nominal rate	3	07-Jul-99 21-Jul-99 04-Aug-99
Illinois	BAS 510 F 753 - 770 mg a.s. L^{-1} WG	Broadcast spray to planted/bare soil	3	302	301 302 305	99.6-100.6% of nominal rate	3	03-Jun-99 17-Jun-99 02-Jul-99
	BAS 510 F 988 - 1007 mg a.s. L^{-1} WG		3	392	392 392 393	100.0-100.2% of nominal rate	3	15-Jul-99 29-Jul-99 12-Aug-99
Texas	BAS 510 F 776 mg a.s. L^{-1} WG	Broadcast spray to planted/bare soil	3	302	305 306 305	100.7-101.1% of nominal rate	3	07-Jul-99 21-Jul-99 04-Aug-99
	BAS 510 F 1004 - 1006 mg a.s. L^{-1} WG		3	392	394 395 394	100.5-100.8% of nominal rate	3	18-Aug-99 01-Sep-99 15-Sep-99

[a] Values represent the range of test substance concentration in the spray solution (n = 3)
[b] Determined by sprayer calibration/pass time method.

A sprayer calibration/pass time method was used to confirm applications in this study. In addition to the sprayer calibration/pass time method, a petri dish application verification (AV) technique, and a zero-time core recovery method were used to validate application rates.

Verification of application with the petri dish AV technique is simply the amount of test substance found per surface area of the petri dishes adjusted up to represent a value per acre.

The zero-time sample interval is defined as the first sample collected post application to the plot. Normally zero-time calculations are based on the last application in a series, if multiple applications have been made. Zero-time core concentrations (bare soil only) were calculated by subtracting parent residue in the 0-15 cm (0-6 inch) core before the last application was made (-T6) from the parent residue measured immediately after the last application (T6). For calculation purposes, soil weight was based on a furrow acre slice (907184 kg/15 cm; 2,000,000 lb/6 inch).

No tillage was performed during the course of the study from first to last sampling. Turf plots were mowed several times during the course of the study. The plots were kept free of weeds via the application of glyphosate, 2,4-dinitrophenoxyacetic acid, atrazine, s-metolachlor, monosodium methyl arsenate, and paraquat, diquat and simazine.

Seasonal weather data was collected at each trial site for the entire trial period. Precipitation data was collected onsite while daily minimum and maximum air temperatures, wind speed, solar radiation, percent humidity and evapotranspiration (if available) were collected from an on-site or nearby weather station. Irrigation was applied to supplement normal precipitation so that the plots would receive 110% of the historical average rainfall for the study period or crop evapotranspiration. Historical weather data (average monthly minimum and maximum air temperatures and monthly precipitation totals) were submitted for at least a ten-year period from a reliable source located no more than 48 km (30 miles) from the test site.

A summary of monthly weather data (maximum and minimum temperatures, precipitation, as well as volumes of the supplementary irrigation) is presented in Table 7.1.2.2.1-44.

Table 7.1.2.2.1-44: Summary of monthly air temperature (max/min), precipitation, and irrigation at each field trial site

Trial	RCN 99512 (NJ)					RCN 99513 (IL)				
Location	New Jersey, USA					Illinois, USA				
Month/ Year	T_{min} Air [°C]	T_{max} Air [°C]	Precipi-tation [mm]	Irriga-tion [mm]	Total [mm]	T_{min} Air [°C]	T_{max} Air [°C]	Precipi-tation [mm]	Irri-gation [mm]	Total [cm]
May-99	9.5	21.7	0.0 [a]	0.0 [a]	0.0 [a]	-	-	-	-	-
Jun-99	14.7	27.0	18.8	96.5	115.3	18.7	28.3	96.3 [a]	41.9 [a]	138.2 [a]
Jul- 99	18.8	32.1	32.5	149.4	181.9	21.4	32.5	58.9	68.6	127.5
Aug- 99	16.7	28.1	125.0	93.5	218.4	17.0	29.3	55.6	148.6	204.2
Sep- 99	13.8	24.0	424.9	29.2	454.2	11.5	27.7	20.8	182.9	203.7
Oct- 99	4.8	16.9	134.1	35.6	169.7	7.0	21.5	34.0	49.8	83.8
Nov- 99	3.5	13.3	91.7	0.0	91.7	3.8	16.8	24.1	55.1	79.2
Dec- 99	-1.7	6.5	79.0	0.0	78.7	-1.5	7.4	56.9	0.0	56.9
Jan-00	-6.6	2.6	41.9	0.0	41.9	-4.4	4.4	26.2	0.0	26.2
Feb-00	-3.9	5.5	69.1	0.0	69.1	1.2	10.1	101.1	0.0	101.1
Mar-00	1.6	12.8	99.6	0.0	99.6	3.5	15.0	51.1	0.0	51.1
Apr-00	4.7	14.7	102.1	0.0	102.1	10.7	21.5	84.6	0.0	84.6
May-00	10.5	21.8	162.3	38.1	200.4	18.2	27.7	157.2	0.0	157.2
Jun-00	9.5	21.7	117.3	0.0	117.3	17.6	27.3	255.8	11.4	267.2
Jul-00	15.5	26.4	121.7 [a]	38.1 [a]	159.8 [a]	19.3	28.7	117.1 [a]	42.4 [a]	159.5 [a]
Total	-	-	**1620.5**	**480.1**	**2100.6**	-	-	**1140.5**	**602.0**	**1739.9**

Trial	RCN 99514 (TX)				
Location	Texas, USA				
Month/ Year	T_{min} Air [°C]	T_{max} Air [°C]	Precipi-tation [mm]	Irriga-tion [mm]	Total [mm]
Jun-99	22.5	32.1	-	-	-
Jul- 99	22.4	33.6	37.3 [a]	50.0 [a]	87.4 [a]
Aug- 99	22.6	37.6	30.7	103.4	134.1
Sep- 99	17.7	34.2	24.6	98.8	123.4
Oct- 99	12.6	29.4	36.8	85.1	121.9
Nov- 99	8.7	26.4	21.8	50.0	71.9
Dec- 99	3.8	20.3	26.7	16.3	42.9
Jan-00	6.8	19.9	45.2	57.9	103.1
Feb-00	9.6	23.7	30.7	36.1	66.8
Mar-00	12.8	25.4	34.5	55.6	90.2
Apr-00	13.3	26.5	130.6	32.8	163.3
May-00	19.9	31.1	215.6	69.6	285.2
Jun-00	21.8	32.8	70.9	127.0	197.9
Jul-00	21.3	37.3	27.4 [a]	106.2 [a]	133.6 [a]
Total	-	-	**734.1**	**889.0**	**1623.1**

[a] Precipitation totals for the first and last months of each trial reflect active periods only, not totals for the whole month.

2. Sampling

Five replicate cores (0-122 cm) were collected each from three predetermined, randomly selected subplots at each designated sampling interval using a soil corer. A soil depth of up to 122 cm (48 inch) was taken for each soil.

Samples from treated soils were collected from the treated plot on 32 occasions one day prior each of the six applications (-T), on the day of each application (T), between the first and second application (T1+2, T1+3, T1+5, T1+7, T1+9), and 1, 2, 3, 5, 7, 10, 14, 21, 30, 60, 90, 120, 180, 270, 360 days after the last application (DALA). Samples from untreated soils were collected from the control plot on seven occasions one day before the first application (-T1), on the day of application, and 1, 5, 30, 90, 180 and 360 DALA. The specified dates refer to planned sampling dates. Actual sampling dates are given in brackets in the results tables (Table 7.1.2.2.1-46 to Table 7.1.2.2.1-51).

Soil cores from the treated plots were taken to a depth of 122 cm (48 inch) in two stages at each sampling interval. For each sampling event, five 0-15 cm (0-6 inch) and five 15-122 cm (6-48 inch) soil cores were collected from each of three randomly selected subplots, and were designated as replicates A, B, and C. This resulted in a total of fifteen each of the 0-15 cm (0-6 inch) and 15-122 cm (6-48 inch) cores collected at each sampling interval for each treated plot. The 0-15 cm cores were either sampled in two runs or sectioned after sampling to obtain a 0-8 cm (0-3 inch) and a 8-15 cm (3-6 inch) soil core. The 15-122 cm (6-48 inch) cores were cut frozen into 15 cm (6 inch) increments. Soil cores were composited by depth within a replicate to form completed samples.

All soil specimens were placed into freezer storage at about -18°C and remained frozen until processing or analysis of the samples.

3. Description of analytical procedure

Samples were received from the field frozen and were stored at ≤ 0°C. Samples were homogenized at BASF prior to analysis. After homogenization, samples were analyzed using BASF Draft Analytical Method D0004/1 [*already peer-reviewed study BASF DocID 2001/5000881*]. The method used for the residue analyses is described below.

The residues of BAS 510 F, M510F47 (2-chloronicotinic acid), and 2-hydroxy-N-(4'-chlorobiphenyl-2-yl)nicotinamide (M510F49) were extracted from soil by shaking with methanol followed by methanol/water (50:50, v/v). An aliquot (5 mL) of the extract was diluted with a buffer solution (water with 0.1% formic acid and 4 mM ammonium formate; 3 mL) for HPLC-MS/MS determination of BAS 510 F, M510F47, and M510F049. The limit of quantitation (LOQ) for each analyte was 0.01 mg kg^{-1}.

Control samples were fortified either with BAS 510 F, M510F47 (2-chloronicotinic acid), or M510F49. A summary of the average procedural recoveries for samples analyzed is provided in section II.2.

Application verification (AV) samples were kept below 0°C during shipping and storage until extraction. Samples were shaken and centrifuged twice with methanol/water. The samples were then serial diluted using 70/30 methanol/buffer water and quantitated using LC-MS/MS. Due to the concentration of the samples, it was not necessary to establish a limit of quantitation for this procedure.

4. Storage stability experiments

The actual length of time between sample collection in the field and sample extraction at the laboratory was recorded on sample residue records. Samples were shipped in freezer trucks to BASF for analysis. The samples were kept frozen during shipment to and storage at BASF. The maximum period any sample was stored before analysis was 546 days (17.9 months). The detailed stability data are given in the study report. Results revealed that the test item (BAS 510 F) is stable during freezer storage.

In addition, the storage stability of boscalid was investigated in a separate study [*peer-reviewed study BASF DocID 2000/1000136*].

5. Kinetic evaluation

In order to calculate dissipation times (DT_{50}/DT_{75}), the reported residue values were mathematically averaged by replicate to produce a grand mean. In the case where a replicate was analyzed in duplicate or triplicate, the numbers were then averaged to produce a mean for that replicate before averaging with the other replicates. When a replicate sample was below the LOQ or < 0.01 mg kg^{-1}, it was averaged as zero. To determine analyte half-life values, the residues in all soil depths were summed to produce the total residue concentration per sampling period. The summed residue concentrations per sampling period were natural log transformed before analysis by non-linear regression.

The non-linear regression was solved by applying the Gustafson-Holden model (FOMC), using the Origin Scientific Graphics Software (OriginLab Corporation, Northampton, MA, USA; version 6.1, 2000).

6. Residue mobility

To determine if residues had an opportunity to move down through the soil profile with the soil solution, the water content by depth was determined using Time Domain Reflectometry (TDR) was applied. Soil water flux was also calculated at a 30 cm depth based on this method. The TDR system used in this study was a CR10X datalogger (Campbell Scientific Inc., Logan, UT, USA) configured with four CS615 probes located in one of the bare soil subplots. The probes were installed vertically at the 0-30, 30-61, 61-91 and 91-122 cm (0-1, 1-2, 2-3, and 3-4 ft) depths. Since the rods of the CS615 are 30.5 cm (12 inch) in length, vertical installation provides a continuum of volumetric water content measurements from the soil surface to a depth of 122 cm (4 ft).

II. RESULTS AND DISCUSSION

1. Application verification

Application results from the petri dish AV technique ranged from 82% to 98% (New Jersey), from 91% to 125% (Illinois), and from 76% to 88% (Texas) of the target rate. Actual pass/time and spray equipment calibration data indicated that the target field application rate was precise and accurate. The variance observed in application petri dish AV recoveries was assumed to be a reflection of the technique rather than a demonstration of variance in actual application to plots. Zero-time core recoveries were 60% (New Jersey), 46% (Illinois), and 103% (Texas) based on the bare soil plot (broadcast application) of the respective field trial site. Differences in calculated application rates to the field and those calculated from zero time cores are probably due to sample handling. More details on the application verification results are given in the study report.

2. Residues in field soil samples

Mean procedural recoveries from control soil samples fortified either with boscalid, M510F47, or M510F49 at a minimum of two levels (0.1 or 0.01 mg kg^{-1}) were generally within the acceptable range of 70-120% (see Table 7.1.2.2.1-45). A summary of the individual procedural recovery results is provided in the study report.

Table 7.1.2.2.1-45: Method procedural recoveries

Analyte	Mean recovery ± RSD [%]		
	New Jersey	Illinois	Texas
BAS 510 F	95 ± 5.6 (n=79)	95 ± 4.2 (n=71)	95 ± 3.6 (n=71)
M510F47	88 ± 10.9 (n=79)	94 ± 10.6 (n=71)	103 ± 8.5 (n=71)
M510F49	93 ± 5.7 (n=79)	90 ± 5.3 (n=71)	91 ± 4.0 (n=71)

RSD = Relative standard deviation [%]

Field soil samples taken from different depths were analyzed to a maximum of about 360 days after the last of the six applications. The analytical results are summarized in Table 7.1.2.2.1-46 to Table 7.1.2.2.1-51. All residue values presented in these tables are related to the dry weight of the soil and were not corrected for procedural recoveries. Residue concentrations are reported according to soil depth (0-8, 8-15, 15-30, and 30-46 cm).

Table 7.1.2.2.1-46: Residues of boscalid and its metabolites M510F47 (2-chloronicotinic acid) and M510F49 [mg kg^{-1} dry weight] in treated soil samples (mean of three replicates) – New Jersey site (bare plot; RCN 99512-NJ)

Compound [a]	Soil depth [cm]	Targeted days after last application [b]																
		T1	T1+2	T1+3	T1+5	T1+7	T1+9	-T2	T2	-T3	T3	-T4	T4	-T5	T5	-T6	T6	1
boscalid	0-8	0.35	0.35	0.27	0.27	0 19	0.18	0.18	0.57	0.44	0.71	0.46	0.79	0.43	0.92	0.71	0.81	0.92
	8-15	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.01	<0.01	0.003	0.003	<0.01	<0.01	0.003	<0.01	0.01	0.02
	15-30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	30-46	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01[c]	<0.01
M510F47	0-8	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	8-15	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	15-30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	30-46	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
M510F49	0-8	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.003	<0.01	0.008	0.004	0.003	0.01
	8-15	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	15-30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	30-46	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01[c]	<0.01

Compound [a]	Soil depth [cm]	Targeted days after last application [b]										
		2	3	5	7 (8)	14	30	60 (63)	90 (92)	180 (216)	270 (274)	360 (359)
boscalid	0-8	0.84	0.81	0.73	0.67	0.68	0.64	0 56	0.46	0.44	0.66	0.27
	8-15	<0.01	0.01	0.003	0.01	<0.01	0.02	0.04	0.05	0.03	0.09	0.02
	15-30	<0.01	0.003	<0.01	<0.01	<0.01	0.03	0.003	0.01	0.007	<0.01	<0.01
	30-46	0.01	<0.01[d]	0.003[d]	<0.01	<0.01[d]	<0.01[e]	<0.01[e]	<0.01[e]	<0.01	<0.01[c]	<0.01[c]
M510F47	0-8	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	8-15	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	15-30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	30-46	<0.01[c]	<0.01[d]	<0.01[d]	<0.01[c]	<0.01[d]	<0.01[e]	<0.01[e]	<0.01[e]	<0.01	<0.01[c]	<0.01[c]
M510F49	0-8	0.003	0.003	<0.01	<0.01	0.007	0.004	0.006	0.002	0.007	0.008	<0.01
	8-15	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	15-30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	30-46	<0.01[c]	<0.01[d]	<0.01[d]	<0.01	<0.01[d]	<0.01[e]	<0.01[e]	<0.01[e]	<0.01	<0.01[c]	<0.01[c]

T = Application dates

[a] The limit of quantification (LOQ) was 0.01 mg kg^{-1} for each analyte. All residue values were corrected for moisture content and are reported here on a dry weight basis. All value of 0 µg kg^{-1} was used in average calculations for samples where residues were reported as <0.01 mg kg^{-1}.

[b] Sampling dates refer to the targeted sampling dates, the actual sampling dates are given in brackets.

[c] Samples were analyzed down to 46-61 cm (18-24 inches), no residues were found at or above 0.01 mg kg^{-1}.

[d] Samples were analyzed down to 61-76 cm (24-30 inches), no residues were found at or above 0.01 mg kg^{-1}.

[e] Samples were analyzed down to 76-92 cm (30-36 inches), no residues were found at or above 0.01 mg kg^{-1}.

Table 7.1.2.2.1-47: Residues of boscalid and its metabolites M510F47 (2-chloronicotinic acid) and M510F49 [mg kg^{-1} dry weight] in treated soil samples (mean of three replicates) – New Jersey site (turf plot; RCN 99512-NJ)

Compound [a]	Soil depth [cm]	Targeted days after last application [b]															
		-T1	T1	T1+2	T1+3	T1+5	T1+7	T1+9	-T2	T2	-T3	T3	-T4	T4	-T5	T5[c]	-T6	T6[c]
boscalid	0-8	n.a.	0.24	0.29	0.32	0 26	0.31	0.27	0.27	0.67	0.24	0.42	0.78	0.93	0.84	1.14	0.54	1.91
	8-15	n.a.	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.007
	15-30	n.a.	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.003	<0.01	<0.01
	30-46	n.a.	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
M510F47	0-8	n.a.	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.007	0.003	0.007	0.007	0.04
	8-15	n.a.	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	15-30	n.a.	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	30-46	n.a.	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
M510F49	0-8	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.003	<0.01	0.01	0.003	0.02
	8-15	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	15-30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	30-46	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01[c]	<0.01	<0.01	<0.01	<0.01

Compound [a]	Soil depth [cm]	Targeted days after last application [b]											
		1	2	3	5	7 (8)	14	30	60 (63)	90 (92)	180 (216)	270 (274)	360 (359)
boscalid	0-8	0.86	1.47	1.38	0.67	1.24	0.48	1.29	0.58	0.45	0.42	0.29	0.21
	8-15	<0.01	0.007	<0.01	<0.01	0.003	0.007	0.02	0.02	0.02	0.02	0.02	0.01
	15-30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	30-46	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
M510F47	0-8	0.01	0.02	0.02	0.007	0.02	0.007	0.007	0.007	0.02	<0.01	<0.01	<0.01
	8-15	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	15-30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	30-46	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
M510F49	0-8	0.008	0.02	0.02	<0.01	0.02	<0.01	0.02	0.003	0.003	<0.01	<0.01	<0.01
	8-15	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	15-30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	30-46	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

T = Application dates; n.a. = Not analyzed

[a] The limit of quantification (LOQ) was 0.01 mg kg^{-1} for each analyte. All residue values were corrected for moisture content and are reported here on a dry weight basis. All value of 0 µg kg^{-1} was used in average calculations for samples where residues were reported as <0.01 mg kg^{-1}.

[b] Sampling dates refer to the targeted sampling dates, the actual sampling dates are given in brackets.

[c] Samples were analyzed down to 46-61 cm (18-24 inches), no residues were found at or above 0.01 mg kg^{-1}.

Table 7.1.2.2.1-48: Residues of boscalid and its metabolites M510F47 (2-chloronicotinic acid) and M510F49 [mg kg^{-1} dry weight] in treated soil samples (mean of three replicates) – Illinois site (bare plot; RCN 99513-IL)

Compound [a]	Soil depth [cm]	Targeted days after last application [b]															
		-T1	T1	T1+2	T1+3	T1+5	T1+7	T1+9	-T2	T2	-T3	T3	-T4	T4	-T5	T5	-T6	T6
boscalid [c]	0-8	<0.01	0.29	0.18	0.17	0 28	0.23	0.13	0.27	0.47	0.48	0.49	0.39	0.68	0.59	1.12	0.82	0.93
	8-15	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.003	<0.01	<0.01	<0.01	<0.01	0.007	n.a.	n.a.	<0.01
	15-30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	30-46	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
M510F47 [d]	0-8	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	8-15	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	n.a.	n	<0.01
	15-30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	30-46	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
M510F49 [e]	0-8	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.002	<0.01	<0.01	0.003	0.003	0.01	<0.01	0.003
	8-15	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	n.a.	n.a.	<0.01
	15-30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	30-46	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Compound [a]	Soil depth [cm]	Targeted days after last application [b]												
		1	2	3	5	7	14	30 (29)	60	90 (91)	120 (118)	180	270	360 (344)
boscalid	0-8	0.95	0.98	0.67	1.04	0.90	1.02	0.80	0.76	0.82	0.68	0.77	0.65	0.31
	8-15	<0.01	<0.01	0.10	<0.01	<0.01	<0.01	0.003	0.007	<0.01	n.a.	<0.01	<0.01	<0.01
	15-30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	n.a.	<0.01	<0.01	<0.01
	30-46	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
M510F47	0-8	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	n.a.	n.a.
	8-15	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	n.a.	<0.01	n.a.	n.a.
	15-30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	n.a.	<0.01	n.a.	n.a.
	30-46	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	n.a.	n.a.
M510F49	0-8	0.007	0.007	0.003	0.01	0.01	0.003	<0.01	0.003	0.01	0.003	0.003	n.a.	n.a.
	8-15	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	n.a.	<0.01	n.a.	n.a.
	15-30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	n.a.	<0.01	n.a.	n.a.
	30-46	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	n.a.	n.a.

T = Application dates; n.a. = Not analyzed

[a] The limit of quantification (LOQ) was 0.01 mg kg^{-1} for each analyte. All residue values were corrected for moisture content and are reported here on a dry weight basis. All value of 0 µg kg^{-1} was used in average calculations for samples where residues were reported as <0.01 mg kg^{-1}.

[b] Sampling dates refer to the targeted sampling dates, the actual sampling dates are given in brackets.

[c] Samples at T1 and –T2 were analyzed down to 46-61 cm (18-24 inches), no residues were found at or above 0.01 mg kg^{-1}.

[d] Samples at T1 and –T2 were analyzed down to 46-61 cm (18-24 inches) and at 90 DALA , no residues were found at or above 0.01 mg kg^{-1},

[e] Samples at T1, T1+7, T2 and 7 DALA were analyzed down to 46-61 cm (18-24 inches), no residues were found at or above 0.01 mg kg^{-1}.

Table 7.1.2.2.1-49: Residues of boscalid and its metabolites M510F47 (2-chloronicotinic acid) and M510F49 [mg kg^{-1} dry weight] in treated soil samples (mean of three replicates) – Illinois site (turf plot; RCN 99513-IL)

Compound [a]	Soil depth [cm]	Targeted days after last application [b]															
		T1	T1+2	T1+3	T1+5	T1+7	T1+9	-T2	T2	-T3	T3	-T4	T4	-T5	T5	-T6	T6[c]
boscalid	0-8	0.17	0.14	0.18	0.20	0.21	0.19	0.19	0.45	0.44	0 28	0.68	0.73	0.91	1.48	0.30	1 17
	8-15	0.01	<0.01	0.008	<0.01	<0.01	<0.01	<0.01	0.003	0.01	0.02	0.01	0.02	0.01	0.03	0.005	0.06
	15-30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	30-46	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
M510F47	0-8	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.003	<0.01	<0.01
	8-15	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	15-30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	30-46	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
M510F49	0-8	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.003	<0.01	0.01	0.02	<0.01	0.01
	8-15	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	15-30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	30-46	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Compound [a]	Soil depth [cm]	Targeted days after last application [b]												
		1[c]	2	3	5[c]	7	14[c]	30 (29)	60	90 (91)[c]	120 (118)	180	270[c]	360 (344)[c]
boscalid	0-8	1.19	1.24	1.16	1.23	1.15	1.25	0.91	0.82	0.90	0.72	0.45	0.65	0.34
	8-15	0.02	0.03	0.02	0.05	0.01	0.03	0.05	0.03	0.05	n.a.	0.04	0.05	0.03
	15-30	0.003	<0.01	<0.01	0.007	<0.01	<0.01	0.007	0.007	0.01	n.a.	<0.01	0.003	0.003
	30-46	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01[c]	<0.01[c]	<0.01	<0.01
M510F47	0-8	<0.01	<0.01	0.007	<0.01	<0.01	0.003	0.003	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	8-15	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	n.a.	<0.01	<0.01	<0.01
	15-30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	n.a.	<0.01	<0.01	<0.01
	30-46	<0.01	<0.01	<0.01	<0.01	<0.01[c]	<0.01	<0.01[c]	<0.01[c]	<0.01	<0.01	<0.01	<0.01	<0.01
M510F49	0-8	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.01	<0.01	0.01	0.01
	8-15	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	n.a.	<0.01	<0.01	<0.01
	15-30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	n.a.	<0.01	<0.01	<0.01
	30-46	<0.01	<0.01	<0.01	<0.01	<0.01[c]	<0.01	<0.01[c]	<0.01	<0.01	<0.01[c]	<0.01[c]	<0.01	<0.01

T = Application dates
n.a. = Not analyzed
[a] The limit of quantification (LOQ) was 0.01 mg kg^{-1} for each analyte. All residue values were corrected for moisture content and are reported here on a dry weight basis. All value of 0 µg kg^{-1} was used in average calculations for samples where residues were reported as <0.01 mg kg^{-1}.
[b] Sampling dates refer to the targeted sampling dates, the actual sampling dates are given in brackets.
[c] Samples were analyzed down to 46-61 cm (18-24 inches), no residues were found at or above 0.01 mg kg^{-1}.

Table 7.1.2.2.1-50: Residues of boscalid and its metabolites M510F47 (2-chloronicotinic acid) and M510F49 [mg kg^{-1} dry weight] in treated soil samples (mean of three replicates) – Texas site (bare plot; RCN 99514-TX)

Compound [a]	Soil depth [cm]	Targeted days after last application [b]															
		T1	T1+2	T1+3	T1+5	T1+7	T1+9	-T2	T2	-T3	T3	-T4	T4	-T5	T5	-T6	T6
boscalid	0-8	0.28	0.27	0.27	0.18	0.20	0.24	0.23	0.39	0.36	0.61	0.55	0.68	0.67	0.86	0.85	1.12
	8-15	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.007	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	15-30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	30-46	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
M510F47	0-8	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.003	<0.01	0.003
	8-15	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	15-30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	30-46	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
M510F49	0-8	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.003	0.003	0.007	<0.01	0.01	0.008	0.02
	8-15	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	15-30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	30-46	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Compound [a]	Soil depth [cm]	Targeted days after last application [b]											
		1	2	3	5	7	14[c]	30[c]	60 (61)[c]	90	180	270 (271)	360 (316)
boscalid	0-8	1.16	1.21	1.15	1.00	1.19	1.07	0.70	0.98	0.89	0.79	0.45	0.38
	8-15	<0.01	<0.01	<0.01	0.007	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.007	0.01
	15-30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	30-46	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
M510F47	0-8	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	8-15	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	15-30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	30-46	<0.01	<0.01	<0.01	<0.01	<0.01[c]	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
M510F49	0-8	0.02	0.02	0.02	0.01	0.02	0.02	0.01	0.02	0.01	0.01	0.01	0.01
	8-15	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	15-30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	30-46	<0.01	<0.01	<0.01	<0.01[c]	<0.01	<0.01	0.003	<0.01	<0.01	<0.01	<0.01	<0.01

T = Application dates

[a] The limit of quantification (LOQ) was 0.01 mg kg^{-1} for each analyte. All residue values were corrected for moisture content and are reported here on a dry weight basis. All value of 0 µg kg^{-1} was used in average calculations for samples where residues were reported as <0.01 mg kg^{-1}.

[b] Sampling dates refer to the targeted sampling dates, the actual sampling dates are given in brackets.

[c] Samples were analyzed down to 46-61 cm (18-24 inches), no residues were found at or above 0.01 mg kg^{-1}.

Table 7.1.2.2.1-51: Residues of boscalid and its metabolites M510F47 (2-chloronicotinic acid) and M510F49 [mg kg^{-1} dry weight] in treated soil samples (mean of three replicates) – Texas site (turf plot; RCN 99514-TX)

Compound [a]	Soil depth [cm]	Targeted days after last application [b]															
		T1	T1+2	T1+3	T1+5	T1+7	T1+9	-T2	T2	-T3	T3	-T4	T4	-T5	T5[c]	-T6[c]	T6[c]
boscalid	0-8	0.21	0.2	0.17	0.18	0.17	0.21	0.16	0.32	0.28	0 50	0.41	0.68	0.65	0.79	0.89	1.23
	8-15	0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.005	0.01	0.02	0.03	0.02	0.03	0.03	0.05
	15-30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.01	<0.01
	30-46	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
M510F47	0-8	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.01	0.01	0.01	0.02	0.02
	8-15	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	15-30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	30-46	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
M510F49	0-8	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.003	0.003	0.01	0.01	0.01
	8-15	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	15-30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Compound [a]	Soil depth [cm]	Targeted days after last application [b]												
		1	2	3	5	7	14	30	60 (61)	90	120 (121)	180	270 (271)	360 (316)
boscalid	0-8	0.94	1.15	1.12	0.92	0.94	0.76	0.80	0.65	0.61	0.63	0.54	0.27	0.14
	8-15	0.04	0.04	0.03	0.04	0.04	0.04	0.06	0.04	0.04	0.03	0.05	0.03	0.03
	15-30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	30-46	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
M510F47	0-8	0.01	0.02	0.007	<0.01	0.003	0.01	0.007	0.003	<0.01	0.02	0.007	<0.01	<0.01
	8-15	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	15-30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	30-46	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
M510F49	0-8	0.01	0.02	0.02	0.01	0.01	0.01	0.02	0.01	0.01	0.02	0.01	0.01	0.007
	8-15	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	15-30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01[d]	<0.01[d]

T = Application dates; n.a. = Not analyzed

[a] The limit of quantification (LOQ) was 0.01 mg kg^{-1} for each analyte. All residue values were corrected for moisture content and are reported here on a dry weight basis. All value of 0 µg kg^{-1} was used in average calculations for samples where residues were reported as <0.01 mg kg^{-1}.

[b] Sampling dates refer to the targeted sampling dates, the actual sampling dates are given in brackets.

[c] Samples were analyzed down to 46-61 cm (18-24 inches), no residues were found at or above 0.01 mg kg^{-1}.

[d] Samples were analyzed down to 46 cm (18 inches), no residues were found at or above 0.01 mg kg^{-1}.

D. KINETIC EVALUATION

A summary of calculated DT_{50} and DT_{75} values for boscalid and its metabolites M510F47 and M510F49 (where possible) can be found in Table 7.1.2.2.1-52.

Table 7.1.2.2.1-52: Estimated first-order DT_{50} and DT_{75} values of boscalid and its metabolites M510F47 and M510F49

Compound	Trial site	DT_{50} [d]	DT_{75} [d]	α	β	r^2
BAS 510 F	New Jersey – bare	300	-	poly	poly	0.95
	New Jersey – bare	108	> 359	0.214	0.228	0.85
	New Jersey – turf	44	174	0.641	0.044	0.62
	Illinois - bare	307	-	poly	poly	0.92
	Illinois – bare	244	> 344	17.761	$1.629*10^{-4}$	0.67
	Illinois –- turf	155	> 344	0.653	0.012	0.86
	Texas – bare	143	> 316	0.871	0.008	0.85
	Texas – turf	194	-	poly	poly	0.97
	Texas – turf	108	232	4.779	0.001	0.84
M510F47	New Jersey – bare	n.d.	-	-	-	-
	New Jersey – turf	< 90 est.	-	-	-	-
	Illinois – bare	n.d.	-	-	-	-
	Illinois –- turf	< 57 est.	-	-	-	-
	Texas – bare	< 1 est.	-	-	-	-
	Texas – turf	< 180	-	-	-	-
M510F49	New Jersey – bare	no decline	-	-	-	-
	New Jersey – turf	24.7	54	4.507	0.0667	0.87
	Illinois – bare	< 89 (est.)	-	-	-	-
	Illinois –- turf	< 253 (est.)	-	-	-	-
	Texas – bare	< 30 (est.)	-	-	-	-
	Texas – turf	< 59 (est.)	-	-	-	-

Poly = Polynomial solution
n.d. = Not detected
est. = Estimates

E. RESIDUE MOBILITY

In general, no BAS 510 F residues were found below the 15 cm (0-6 inch) depth in the plots. The exception to this was the turf plot at the Illinois site where parent compound was detected in the 15-30 cm sections through the sampling time series. However, considering the application rate, and the number of application, this compound is relatively immobile.

Water movement (flux) did occur down through the 122 cm (48 inch) sampling zone. Flux calculations indicate that 26.21, 16.15, and 10.54 cm (10.32, 6.36, and 4.15 inch) of water leached through the 122 cm (48 inch) depth sampling zone in the bare soil plots (turf at New Jersey) at the New Jersey, Illinois, and Texas sites, respectively. Therefore, the physical conditions (water flux) required to allow compound movement were present at each site.

III. CONCLUSION

Results from this study have shown that BAS 510 F dissipates steadily and does not accumulate when applied under worst case, bare soil conditions.

Report:	CA 7.1.2.2.1/9 Jackson S. et al., 2001b 1999 Field dissipation of BAS 510 .. F in row crop use patterns 2001/5000936
Guidelines:	EPA 164-1
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary

This study was designed to support a row/field crop use pattern according to EPA Guideline 164-1. The test substance, BAS 510 .. F, is a broad spectrum fungicide which will be used to control many diseases in field, row crop, orchard, vineyard and turf. Three field trials were conducted which represent major use areas for the product in the United States (California, Idaho and Florida).

A single untreated control plot (bare soil) and one treated bare soil plot was established at each test site. The plots were located on soil typical for the agricultural areas which they represented. The treated plots received six broadcast applications of the test substance using 374 L ha^{-1} (40 gal ac^{-1}) spray volume at seven day intervals (targeted). The test substance was applied, beginning in June 1999, at a rate of 415 g a.s. ha^{-1} (0.37 lb a.s. ac^{-1}) for the first four applications and at 617 g a.s. ha^{-1} (0.55 lb a.s. ac^{-1}) for the last two applications. Applications were made directly to bare soil plots in an effort to answer guideline questions. However, the bare soil applications greatly exaggerate anticipated residues compared to proper label usage.

Soil samples were collected to a depth of 122 cm (48 inch) using zero contamination sampling equipment. The treated plots were scheduled to be sampled on the following thirty-three sampling events: -T1, T1, T1+1, T1+2, T1+3, T1+5,-T2, T2, -T3, T3, -T4, T4, -T5, T5, -T6, T6 and 1, 2, 3, 5, 7, 10, 14, 21, 30, 60, 90, 120, 180, 270, 360 (all trial sites), and 450 Days After Last Application (DALA; Florida site). The untreated plot was scheduled to be sampled on the following seven sampling events: -T1 and 1, 5, 30, 90, 180, and 360 DALA intervals. A sampling variance of ± 2 days was acceptable after thirty days. After collection, samples were analyzed using HPLC MS/MS for parent and degradates M510F47 (2-chloronicotinic acid) and M510F49.

Compound mobility was determined by measuring each analyte by depth. If mobility occurred, it was possible to correlate the movement to soil water recharge events. Calculated soil-water recharge events by depth over time and TDR (time domain reflectometry) measurements were used to determine if recharge events had any effect on movement of BAS 510 F or degradate residues from the top 15 cm (0-6 inch) of soil. In general most BAS 510 F residues remained in the 0-15 cm (0-6 inch) segments at the California and Idaho sites. However, at the Idaho site, some residues were present in the 15-30 cm (6-12 inch) cores at 14 and 30 DALA (single replicates). Residues at the Florida site were detected in the 30-46 cm (12-18 inch) cores. Residues of the degradates M510F47 and M510F49, if detected, remained in the 0-15 cm (0-6 inch) cores. However, when residue mobility issues are examined, it should be remembered that the product was applied to bare soil plots which were kept totally weed free. The intended target for this product is the crop canopy. Therefore residues are much higher in this study than will be observed with label use (as well as observed compound mobility). Recharge occurred in all plots at the 122 cm (48 inch) depth. Soil water recharge was correlated to the limited residue movement observed based on TDR measurements in the bare soil plots.

Based on soil core residue concentrations, half-life values (DT_{50}) were calculated for boscalid and its metabolites M510F47 and M510F49 where possible. A summary of the calculated boscalid, M510F47 and M510F49 dissipation times (DT_{50}/DT_{75}) in bare soil are presented below:

Table 7.1.2.2.1-53: Estimated first-order DT_{50} and DT_{75} values of boscalid and its metabolites M510F47 (2-chloronicotinic acid) and M510F49

Compound	Trial site	Bare	
		DT_{50} [d]	**DT_{75} [d]**
BAS 510 F	California	76.5	> 329
	Idaho	333	> 345
	Florida	27	> 384
2- chloronicotinic acid	California	16.2	60.1
	Idaho	< 30	< 30
	Florida	n.d.	n.d.
M510F49	California	18.9	102
	Idaho	No decline	No decline
	Florida	11.1	422

n.d. = Not detected

Results from this study have shown that BAS 510 F dissipates steadily under conditions of use.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

Test item (formulation): BAS 510 00 F
Active substance (a.s.): Boscalid (BAS 510 F, Reg. No. 300355)
Chemical name (IUPAC): 2-chloro-N-(4'-chloro-biphenyl-2-yl)nicotinamide
Molar mass: 343.2 g mol^{-1}
Batch No.: AF543-17 (containing 69.6% boscalid)
Type of formulation: WG (wettable granule)

2. Test sites

The dissipation of boscalid under field conditions was investigated at three trial sites representative of major crop producing regions of the U.S. One trial each was performed in Tulare County (California), Payette County (Idaho), and Orange County (Florida). The site characteristics including the basic soil parameters of the corresponding soil horizons are presented in Table 7.1.2.2.1-54 and Table 7.1.2.2.1-55.

Table 7.1.2.2.1-54: Characteristics of the trial sites used in the field dissipation study (California and Idaho site)

Trial	**RCN 99506 (CA)**							
Location	**California, USA**							
Soil properties	**0-6 in (0-15 cm)**	**6-12 in (15-30 cm)**	**12-18 in (30-46 cm)**	**18-24 in (46-61 cm)**	**24-30 in (61-76 cm)**	**30-36 in (76-91 cm)**	**36-42 in (91-107 cm)**	**42-48 in (107-122 cm)**
Soil class (USDA)	Sandy loam	Sandy loam	Sandy loam	Sandy loam	Sandy loam	Sandy loam	Sandy loam	Sandy loam
sand [%]	60	60	60	62	62	64	62	64
silt [%]	32	32	34	30	28	30	30	28
clay [%]	8	8	6	8	10	6	8	8
Organic matter [%]	0.8	0.4	0.2	0.1	0.2	0.1	0.3	0.2
pH [c]	8.9	9.1	9.4	9.7	9.8	9.8	9.6	9.9
CEC [meq $100g^{-1}$]	9.4	10.8	11.3	11.7	12.2	11.7	11.5	11.8
Moisture (gravimetric) at 1/3 bar [%]	14.6	17.4	16.0	14.8	15.4	14.4	14.6	13.9
Bulk density [g cm^{-3}] [a]	1.53-1.57	1.51-1.59	1.46-1.61	1.44-1.56	1.45-1.53	1.45-1.51	1.35-1.51	1.21-1.41
Trial	**RCN 99507 (ID)**							
Location	**Idaho, USA**							
Soil properties	**0-6 in (0-15 cm)**	**6-12 in (15-30 cm)**	**12-18 in (30-46 cm)**	**18-24 in (46-61 cm)**	**24-30 in (61-76 cm)**	**30-36 in (76-91 cm)**	**36-42 in (91-107 cm)**	**42-48 in (107-122 cm)**
Soil class (USDA)	Loam	Loam	Loam	Silt loam	Silt loam	Silt loam	Loam	Clay loam
sand [%]	30	30	30	34	32	40	40	40
silt [%]	46	44	48	60	62	52	40	32
clay [%]	24	26	22	6	6	8	20	28
Organic matter [%]	2.6	1.5	0.6	0.4	0.4	0.3	0.3	0.3
pH [c]	6.4	7.0	7.4	7.6	7.8	7.9	8.1	8.2
CEC [meq $100g^{-1}$]	21.2	21.1	29.5	38.2	35.8	37.8	33.5	31.3
Moisture (gravimetric) at 1/3 bar [%] [a]	33.5	34.9	41.1	43.8	41.1	41.4	40.7	37.1
Bulk density [g cm^{-3}] [b]	1.06-1.29	1.24-1.41	1.21-1.26	1.19-1.45	1.14-1.48	1.40-1.42	1.27-1.43	1.27-1.41

CEC = Cation exchange capacity
[a] Measured at six composited cores
[b] Measured at four separate, undisturbed cores
[c] in the US soil pH is commonly measured in H_2O; this is however not explicitly stated in the report

Table 7.1.2.2.1-55: Characteristics of the trial sites used in the field dissipation study (Florida site)

Trial	**RCN 99508 (FL)**							
Location	**Florida, USA**							
Soil properties	**0-6 in (0-15 cm)**	**6-12 in (15-30 cm)**	**12-18 in (30-46 cm)**	**18-24 in (46-61 cm)**	**24-30 in (61-76 cm)**	**30-36 in (76-91 cm)**	**36-42 in (91-107 cm)**	**42-48 in (107-122 cm)**
Soil class (USDA)	Sand	Sand	Sand	Sand	Sand	Sand	Sand	n.a.
sand [%] silt [%] clay [%]	97 2 1	97 2 1	97 2 1	97 2 1	97 2 1	97 2 1	97 2 1	n.a. n.a. n.a.
Organic matter [%]	1.8	0.8	0.4	0.2	0.2	0.1	0.1	n.a.
pH [c]	7.1	7.3	7.3	7.3	7.2	7.3	7.4	n.a.
CEC [meq $100g^{-1}$]	4.2	2.7	1.5	1.5	1.4	1.4	1.5	n.a.
Moisture (gravimetric) at 1/3 bar [%] [a]	3.0	2.1	1.6	1.7	1.8	1.7	1.8	n.a.
Bulk density [g cm^{-3}] [b]	1.47-1.56	1.62-1.64	1.62-1.67	1.63-1.65	1.63-1.65	1.64-1.67	1.62-1.65	n.a.

CEC = Cation exchange capacity
n.a. = Not available
[a] Measured at six composited cores
[b] Measured at four separate, undisturbed cores
[c] in the US soil pH is commonly measured in H_2O; this is however not explicitly stated in the report

The selected fields were representative of major geographic and climatic regions within the United States. Bare soil plots were fallow prior to the study. No product containing boscalid had been used on the test plots in the last three years.

B. STUDY DESIGN

1. Experimental conditions

Each trial area was divided into two plots. One plot was used as control plot (untreated, bare soil), the second plot (bare soil) was treated with the test item. The treated plot consisted of three sampling sections, each divided into 33-40 equal subplots. The control plot consisted of three sampling sections, each comprising 7-9 subplots, and was separated from the treated plot by a buffer zone of at least 17 m (57 ft) width. The size of each subplot (control and treated) was 4.65 (50 sq ft; California site) or 3.34 m^2 (36 sq ft; Idaho and Florida sites). The size of the control subplots at site California was 9.29 m^2 (100 sq ft). Three subplots of the control plot and three subplots of the treated plot (one of each sampling section) were randomly selected, sampled, and assigned for replicates A, B, and C.

The product, formulated as a WG (wettable granule), was broadcast applied to bare soil in six applications at a nominal rate of 415 (0.37 lb a.s. ac^{-1}; applications 1-4) and 617 g a.s. ha^{-1} (0.55 lb a.s. ac^{-1}; applications 5-6). Depending on the trial site, the applications were conducted from the end of July until the beginning of September 1999 (California), from the middle of July until the middle of August 1999 (Idaho), and from the middle of June until the middle of July 1999 (Florida), using calibrated flat boom broadcast sprayers. The actual application rates determined by quantifying the amount of spray discharged ranged from 408 to 425 g a.s. ha^{-1} (0.364-0.379 lb a.s. ac^{-1}) for applications 1-4 and from 609 to 626 g a.s. ha^{-1} (0.543-0.558 lb a.s. ac^{-1}) for applications 5-6. Details of the application are presented in Table 7.1.2.2.1-56.

Table 7.1.2.2.1-56: Application parameters of field trial sites treated with boscalid

Trial Location	Test item/ Actual content [a]/ Formulation type	Application method	No. of applications	Application rate per treatment			No. of treated replicates	Application date
				Nominal [g a.s. ha^{-1}]	Actual [g a.s. ha^{-1}]	Dose verification [b]		
California	BAS 510 F 1565 - 1627 mg a.s. L^{-1} WG	Broadcast spray to bare soil	4	414	411 415 424 415	99.3-102.4% of nominal rate	3	28-Jul-99 04-Aug-99 11-Aug-99 18-Aug-99
	BAS 510 F 2294 - 2331 mg a.s. L^{-1} WG		2	616	620 608	98.7-100.8% of nominal rate	3	25-Aug-99 01-Sep-99
Idaho	BAS 510 F 1592 mg a.s. L^{-1} WG	Broadcast spray to bare soil	4	414	422 415 410 414	98.9-101.9% of nominal rate	3	13-Jul-99 20-Jul-99 27-Jul-99 03-Aug-99
	BAS 510 F 2370 mg a.s. L^{-1} WG		2	616	616 613	99.5-100.0% of nominal rate	3	10-Aug-99 17-Aug-99
Florida	BAS 510 F 1588 - 1601 mg a.s. L^{-1} WG	Broadcast spray to bare soil	4	414	418 417 418 408	98.3-100.9% of nominal rate	3	08-Jun-99 15-Jun-99 22-Jun-99 29-Jun-99
	BAS 510 F 2361 mg a.s. L^{-1} WG		2	616	625 625	101.5% of nominal rate	3	06-Jul-99 13-Jul-99

[a] Values represent the range of test substance concentration in the spray solution.
[b] Determined by sprayer calibration/pass time method.

A sprayer calibration/pass time method was used to confirm applications in this study. In addition to the sprayer calibration/pass time method, a petri dish application verification (AV) technique, and a zero-time core recovery method were used to validate application rates.

Verification of application with the petri dish AV technique is simply the amount of test substance found per surface area of the petri dishes adjusted up to represent a value per acre.

The zero-time sample interval is defined as the first sample collected post application to the plot. Normally zero-time calculations are based on the last application in a series if multiple applications have been made. Zero-time core concentrations (bare soil only) were calculated by subtracting parent residue in the 0-7.6 cm (0-3 inch) core before the last application was made (-T6) from the parent residue measured immediately after the last application (T6). For calculation purposes, soil weight was based on a furrow acre slice (907184 kg/15 cm; 2,000,000 lb/6 inch).

No tillage was performed during the course of the study from first to last sampling. The plots were kept free of weeds via the application of glyphosate, 2,4-dichloro phenoxy acetic acid, and paraquat.

Seasonal weather data was collected at each trial site for the entire trial period. Precipitation data was collected onsite while daily minimum and maximum air temperatures, wind speed, solar radiation, and percent humidity were collected from an on-site or nearby weather station. Irrigation was applied to supplement normal precipitation so that the plots would receive 110% of the historical average rainfall for the study period or crop evapotranspiration. Historical weather data (average monthly minimum and maximum air temperatures and monthly precipitation totals) were submitted for at least a ten-year period from a reliable source located no more than 48 km (30 miles) from the test site.

A summary of monthly weather data (maximum and minimum temperatures, precipitation, as well as volumes of the supplementary irrigation) is presented in Table 7.1.2.2.1-57.

Table 7.1.2.2.1-57: Summary of monthly air temperature (max/min), precipitation, and irrigation at each field trial site

Trial	RCN 99506 (CA)					RCN 99507 (ID)				
Location	California, USA					Idaho, USA				
Month/ Year	T_{min} Air [°C]	T_{max} Air [°C]	Precipi-tation [mm]	Irriga-tion [mm]	Total [mm]	T_{min} Air [°C]	T_{max} Air [°C]	Precipi-tation [mm]	Irri-gation [mm]	Total [cm]
Jul- 99	16.4	33.2	0.0 [a]	19.1 [a]	19.1 [a]	12.2	34.4	0.0 [a]	124.5 [a]	124.5 [a]
Aug- 99	15.1	31.7	0.0	139.7	139.7	12.8	32.2	0.8	152.4	153.2
Sep- 99	14.2	31.6	1.5	120.7	122.2	5.6	26.7	0.0	50.8	50.8
Oct- 99	9.0	28.1	0.3	127.0	127.3	0.6	20.0	10.2	45.7	55.9
Nov- 99	4.3	19.9	12.4	63.5	75.9	0.0	12.2	24.4	20.3	44.7
Dec- 99	-1.2	15.4	0.0	63.5	63.5	-3.3	1.7	41.7	0.0	41.7
Jan-00	2.4	15.1	31.2	38.1	69.3	-3.3	3.3	68.1	0.0	68.1
Feb-00	5.9	16.9	105.7	0.0	105.7	0.0	9.4	87.1	0.0	87.1
Mar-00	5.8	19.2	61.0	63.5	124.5	0.0	13.3	35.6	0.0	35.6
Apr-00	7.9	24.2	29.2	76.2	105.4	0.0	12.8	32.3	0.0	32.3
May-00	11.8	28.3	4.1	114.3	118.4	7.8	22.8	6.6	53.3	59.9
Jun-00	16.0	33.4	5.6	196.9	202.4	10.0	28.9	8.6	114.3	122.9
Jul-00	14.9	32.9	0.0 [a]	209.6 [a]	209.6 [a]	13.3	33.3	0.0 [a]	132.1 [a]	132.1 [a]
Total	-	-	**251.5**	**1231.9**	**1483.4**	-	-	**315.0**	**693.4**	**1008.4**

Trial	RCN 99508 (FL)				
Location	Florida, USA				
Month/ Year	T_{min} Air [°C]	T_{max} Air [°C]	Precipi-tation [mm]	Irriga-tion [mm]	Total [mm]
Jun-99	21.7	32.2	252.7 [a]	0.0 [a]	252.7 [a]
Jul- 99	22.8	34.4	118.9	189.2	308.1
Aug- 99	22.8	34.4	53.3	215.9	269.2
Sep- 99	21.7	31.7	130.8	228.6	359.4
Oct- 99	18.9	28.3	133.4	109.2	242.6
Nov- 99	13.9	25.6	63.5	119.4	182.9
Dec- 99	10.0	22.2	48.3	66.0	114.3
Jan-00	9.4	22.2	45.7	132.1	177.8
Feb-00	9.4	23.9	2.5	106.7	109.2
Mar-00	13.9	27.8	5.1	195.6	200.7
Apr-00	13.9	28.3	36.8	96.5	133.4
May-00	18.3	32.8	26.7	129.5	156.2
Jun-00	21.1	33.9	154.9	217.2	372.1
Jul-00	21.7	33.3	186.7 [a]	91.4 [a]	278.1 [a]
Total	-	-	**1259.8**	**1897.4**	**3157.2**

[a] Precipitation totals for the first and last months of each trial reflect active periods only, not totals for the hole month.

2. Sampling

Five replicate cores (0-122 cm) were collected each from three predetermined, randomly selected subplots (one per sampling section) at each designated sampling interval using a soil corer. A soil depth of up to 122 cm (48 inch) was taken for each soil.

Samples from treated soils were collected from the treated plot on 32 occasions one day prior each of the six applications (-T), on the day of each application (T), between the first and second application (T1+1, T1+2, T1+3, T1+5), and 1, 2, 3, 5, 7, 10, 14, 21, 30, 60, 90, 120, 180, 270, 360 and (for Florida site only) 450 days after the last application (DALA). Samples from untreated soils were collected from the control plot on seven occasions one day before the first application (-T1) and 1, 5, 30, 90, 180 and 360 (DALA. The specified dates refer to planned sampling dates. Actual sampling dates are given in brackets in the results tables.

Soil cores from the treated plots were taken to a depth of 122 cm (48 inch) in two stages at each sampling interval. For each sampling event, five 0-15 cm (0-6 inch) and five 15-122 cm (6-48 inch) soil cores were collected from each of three randomly selected subplots, and were designated as replicates A, B, and C. This resulted in a total of fifteen each of the 0-15 cm (0-6 inch) and 15-122 cm (6-48 inch) cores collected at each sampling interval for each treated plot. The 0-15 cm cores were either sampled in two runs or sectioned after sampling to obtain a 0-8 cm (0-3 inch) and a 8-15 cm (3-6 inch) soil core. The 15-122 cm (6-48 inch) cores were cut frozen into 15 cm (6 inch) increments. Soil cores were composited by depth within a replicate to form completed samples.

All soil cores were placed into on-site cold storage immediately after sampling. The samples were then transported to the field facility and transferred into walk-in or chest freezers. After sectioning and composition, the larger cores returned to frozen storage. All soil specimens remained frozen until processing or analysis of the samples.

3. Description of analytical procedure

Samples were received from the field frozen and were stored at $\leq 0°C$. Samples were homogenized at BASF prior to analysis. After homogenization, samples were analyzed using BASF Draft Analytical Method D0004/1 [*already peer-reviewed study BASF DocID 2001/5000881*]. The method used for the residue analyses is described below.

The residues of BAS 510 F, M510F47 (2-chloronicotinic acid), and 2-hydroxy-N-(4'-chlorobiphenyl-2-yl)nicotinamide (M510F49) were extracted from soil by shaking with methanol followed by methanol/water (50:50, v/v). An aliquot (5 mL) of the extract was diluted with a buffer solution (water with 0.1% formic acid and 4 mM ammonium formate; 3 mL) for HPLC-MS/MS determination of BAS 510 F, M510F47 and M510F49. The limit of quantitation (LOQ) for each analyte was 0.01 mg kg^{-1}.

Control samples were fortified either with BAS 510 F, M510F47 or M510F49 at a minimum of two levels (0.1 or 0.01 mg kg^{-1}). A summary of the average procedural recoveries for samples analyzed is provided in section II.2.

Application verification (AV) samples were kept below 0°C during shipping and storage until extraction. Samples were shaken and centrifuged twice with methanol/water. The samples were then serial diluted using 70/30 methanol/buffer water and quantitated using LC-MS/MS. Due to the concentration of the samples, it was not necessary to establish a limit of quantitation for this procedure.

4. Storage stability experiments

The actual length of time between sample collection in the field and sample extraction at the laboratory was recorded on sample residue records. Samples were shipped in freezer trucks to BASF for analysis. The samples were kept frozen during shipment to and storage at BASF. The maximum period any sample was stored before analysis was 588 days (19.3 months). Results revealed that the test item (BAS 510 F) is stable during freezer storage.

In addition, the storage stability of boscalid was investigated in a separate study [*peer-reviewed study BASF DocID 2000/1000136*].

5. Kinetic evaluation

In order to calculate dissipation times (DT_{50}/DT_{75}), the reported residue values were mathematically averaged by replicate to produce a grand mean. In the case where a replicate was analyzed in duplicate or triplicate, the numbers were then averaged to produce a mean for that replicate before averaging with the other replicates. When a replicate sample was below the LOQ or < 0.01 mg kg^{-1}, it was averaged as zero. To determine analyte half-life values, the residues in all soil depths were summed to produce the total residue concentration per sampling period. The summed residue concentrations per sampling period were natural log transformed before analysis by non-linear regression.

The non-linear regression was solved by applying the Gustafson-Holden model (FOMC), using the Origin Scientific Graphics Software (OriginLab Corporation, Northampton, MA, USA; version 6.1, 2000).

6. Residue mobility

To determine if residues had an opportunity to move down through the soil profile with the soil solution, the water content by depth was determined using Time Domain Reflectometry (TDR) was applied. Soil water flux was also calculated at a 122 cm (4 ft) depth based on this method. The TDR system used in this study was a CR10X datalogger (Campbell Scientific Inc., Logan, UT, USA) configured with four CS615 probes located in one of the bare soil subplots. The probes were installed vertically at the 0-30, 30-61, 61-91 and 91-122 cm (0-1, 1-2, 2-3, and 3-4 ft) depths. Since the rods of the CS615 are 30.5 cm (12 inch) in length, vertical installation provides a continuum of volumetric water content measurements from the soil surface to a depth of 122 cm (4 ft).

II. RESULTS AND DISCUSSION

1. Application verification

Application results from the petri AV technique ranged from 90% to 101% (California), from 87% to 106% (Idaho), and from 84% to 96% (Florida) of the target rate. Actual pass/time and spray equipment calibration data indicated that the target field application rate was precise and accurate. The variance observed in application petri dish AV recoveries was assumed to be a reflection of the technique rather than a demonstration of variance in actual application to plots. Zero-time core recoveries were 60% (California), 82% (Idaho), and 78% (Florida) based on the bare soil plot (broadcast application) of the respective field trial site. Differences in calculated application rates to the field and those calculated from zero time cores are probably due to sample handling. More details on the application verification results are given in the study report.

2. Residues in field soil samples

Mean procedural recoveries from control soil samples fortified either with boscalid, M510F47 or M510F49 were generally within the acceptable range of 70-120% (see Table 7.1.2.2.1-58). A summary of the individual procedural recovery results is provided in the study report.

Table 7.1.2.2.1-58: Method procedural recoveries

Analyte	Mean recovery ± RSD [%]		
	California	Idaho	Florida
BAS 510 F	100 ± 7.9 (n=49)	92 ± 5.0 (n=60)	96 ± 5.1 (n=63)
M510F47	106 ± 8.8 (n=49)	102 ± 9.6 (n=61)	101 ± 10.3 (n=63)
M510F49	97 ± 7.6 (n=49)	88 ± 4.7 (n=61)	91 ± 4.3 (n=63)

RSD = Relative standard deviation [%]

Field soil samples taken from different depths were analyzed to a maximum of about 360 days after the last of the six applications (384 [nominal 450] days at the Florida site). The analytical results are summarized in Table 7.1.2.2.1-59 to Table 7.1.2.2.1-61. All residue values presented in these tables are related to the dry weight of the soil and were not corrected for procedural recoveries. Residue concentrations are reported according to soil depth (0-8, 8-15, 15-30 (all trial sites), and 30-46 cm (Florida site)).

Table 7.1.2.2.1-59: Residues of boscalid and its metabolites M510F47 (2-chloronicotinic acid) and M510F49 [mg kg^{-1} dry weight] in treated soil samples (mean of three replicates) – California site (bare plot; RCN 99506-CA)

Compound [a]	Soil depth [cm]	Targeted days after last application [b]															
		T1	T1+1	T1+2	T1+3	T1+5	-T2	T2	-T3	T3	-T4	T4	-T5	T5	-T6	T6	1
boscalid	0-8	0.28	0.41	0.31	0.24	0.26	0.24	0.56	0.45	0.84	0.70	1.04	0.85	1.44	1.46	1.78	1.67
	8-15	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.01	<0.01
	15-30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
M510F47	0-8	<0.01	<0.01	<0.01	<0.01	0.003	0.003	0.01	0.007	0.01	0.01	0.02	0.02	0.02	0.03	0.03	0.02
	8-15	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	15-30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
M510F49	0-8	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.007	<0.01	0.01	0.003	0.01	0.003	0.02	0.02	0.02	0.02
	8-15	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	15-30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Compound [a]	Soil depth [cm]	Targeted days after last application [b]												
		2	3	5	7	14	21	30	60 (61)	90	120	180 (181)	270 (272)	360 (329)
boscalid	0-8	1.40	1.26	1.26	1.29	1.28	1.12	1.37	1.04	0.83	0.96	1.20	0.91	0.51
	8-15	<0.01	0.007	<0.01	<0.01	0.10	<0.01	<0.01	0.03	<0.01	0.01	0.007	<0.01	0.003
	15-30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
M510F47	0-8	0.02	0.02	0.02	0.03	0.02	0.04	0.04	<0.01	0.01	0.003	<0.01	0.003	0.007
	8-15	<0.01	<0.01	<0.01	<0.01	0.003	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	15-30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
M510F49	0-8	0.02	0.02	0.02	0.02	0.01	0.007	0.01	0.01	<0.01	0.01	0.01	0.003	<0.01
	8-15	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	15-30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

T = Application dates

[a] The limit of quantification (LOQ) was 0.01 mg kg^{-1} for each analyte. All residue values were corrected for moisture content and are reported here on a dry weight basis. All value of 0 µg kg^{-1} was used in average calculations for samples where residues were reported as <0.01 mg kg^{-1}.

[b] Sampling dates refer to the targeted sampling dates, the actual sampling dates are given in brackets. Samples for all intervals were analyzed at the 30-46 cm (12-18 inch) depth with no residues detected at or above the LOQ.

Table 7.1.2.2.1-60: Residues of boscalid and its metabolites M510F47 (2-chloronicotinic acid) and M510F49 [mg kg^{-1} dry weight] in treated soil samples (mean of three replicates) – Idaho site (bare plot; RCN 99507-ID)

Compound [a]	Soil depth [cm]	Targeted days after last application [b]															
		T1	**T1+1**	**T1+2**	**T1+3**	**T1+5**	**-T2**	**T2**	**-T3**	**T3**	**-T4**	**T4**	**-T5**	**T5**	**-T6**	**T6**	**1**
boscalid	0-8	0.38	0.35	0.33	0.33	0.31	0.35	0.74	0.63	1.06	1.08	1.33	1.30	1.79	1.79	2.23	2.33
	8-15	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.007	<0.01	<0.01	<0.01	<0.01	<0.01	0.003	<0.01	0.007	<0.01
	15-30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
M510F47	0-8	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.003	0.003	0.007	<0.01	<0.01	<0.01	0.007	<0.01	0.01	0.01
	8-15	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	15-30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
M510F49	0-8	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.01	0.01	0.02	0.02	0.02	0.02	0.03	0.03
	8-15	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	15-30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Compound [a]	Soil depth [cm]	Targeted days after last application [b]												
		2	**3**	**5**	**7**	**14**	**21**	**30**	**60**	**90**	**120 (166)**	**180 (186)**	**270 (269)**	**360 (345)**
boscalid	0-8	2.62	2.77	2.27	2.10	2.06	2.29	2.10	1.65	1.67	2.18	1.73	1.57	1.32
	8-15	0.003	0.01	<0.01	0.007	0.003	0.02	0.02	<0.01	<0.01	0.02	0.03	0.03	0.03
	15-30	<0.01	<0.01	<0.01	<0.01	0.01	<0.01	0.02	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
M510F47	0-8	0.01	0.01	0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	8-15	<0.01	<0.01	<0.01	<0.01	<0.01	0.002	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	15-30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
M510F49	0-8	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.02	0.02	0.03	0.02	0.022	0.02
	8-15	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	15-30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

T = Application dates

[a] The limit of quantification (LOQ) was 0.01 mg kg^{-1} for each analyte. All residue values were corrected for moisture content and are reported here on a dry weight basis. All value of 0 µg kg^{-1} was used in average calculations for samples where residues were reported as <0.01 mg kg^{-1}.

[b] Sampling dates refer to the targeted sampling dates, the actual sampling dates are given in brackets. Samples for all intervals were analyzed at the 30-46 cm (12-18 inch) depth with no residues detected at or above the LOQ. At sampling dates 5, 7, 14, 21, 30, 60 and 90 DALA (for 21 DALA boscalid only) samples were also analyzed at the 46-61 cm (18-24 inch) depth with no residues detected at or above the LOQ.

Table 7.1.2.2.1-61: Residues of boscalid and its metabolites M510F47 (2-chloronicotinic acid) and M510F49 [mg kg^{-1} dry weight] in treated soil samples (mean of three replicates) – Florida site (bare plot; RCN 99508-FL)

Compound [a]	Soil depth [cm]	Targeted days after last application [b]																
		T1 [c]	T1+1	T1+2	T1+3	T1+5	-T2 [c]	T2 [c]	-T3	T3 [c]	-T4	T4 [c]	-T5	T5 [c]	-T6	T6 [c]	1	2 [c]
boscalid	0-8	0.25	0.26	0.16	0.16	0 13	0.14	0.38	0.50	0.43	0.44	0.81	0.55	0.88	0.78	1.18	1.26	0.83
	8-15	0.02	0.02	0.03	0.03	0.02	0.04	0.04	0.08	0.05	0.05	0.12	0.12	0.10	0.07	0.10	0.18	0.17
	15-30	<0.01	<0.01	<0.01	<0.01	0.01	<0.01	<0.01	<0.01	<0.01	0.005	<0.01	<0.01	<0.01	<0.01	0.003	<0.01	<0.01
	30-46	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
M510F47	0-8	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	8-15	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	15-30	<0.01	<0.01	<0.01	<0.01	0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
M510F49	0-8	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.01	0.003	0.01	0.01	0.02	0.02	0.01
	8-15	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	15-30	<0.01	<0.01	<0.01	<0.01	0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Compound [a]	Soil depth [cm]	Targeted days after last application [b]												
		3 [c]	5	7 [c]	14 [c]	21 [c]	30 [c]	60 [c]	90 (91)	120 [c]	180 [c]	270 [c]	360 [c]	450 [c] (384)
boscalid	0-8	0.74	0.82	0.61	0.88	0.67	0.47	0.44	0.38	0 32	0.22	0.24	0.14	0.13
	8-15	0.17	0.14	0.08	0.32	0.23	0.13	0.21	0.29	0 17	0.20	0.16	0.54[d]	0.12
	15-30	0.003	<0.01	<0.01	0.01	0.02	0.007	0.03	0.06	0.05	0.06	0.03	0.02	0.05
	30-46	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.003	0.01	<0.01	0.007	<0.01	<0.01	0.007
M510F47	0-8	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	8-15	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	15-30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
M510F49	0-8	0.007	0.01	0.003	0.02	0.01	0.007	0.01	0.01	0.003	0.007	0.01	<0.01	0.003
	8-15	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	15-30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

T = Application dates

[a] The limit of quantification (LOQ) was 0.01 mg kg^{-1} for each analyte. All residue values were corrected for moisture content and are reported here on a dry weight basis. All value of 0 µg kg^{-1} was used in average calculations for samples where residues were reported as <0.01 mg kg^{-1}.

[b] Sampling dates refer to the targeted sampling dates, the actual sampling dates are given in brackets. Samples for all intervals were analyzed at the 30-46 cm (12-18 inch) depth with no residues detected at or above the LOQ.

[c] Samples were also analyzed at the 46-61 cm (18-24 inch) and at the 61-76 cm (24-30 inch) depth with no residues detected at or above the LOQ.

[d] Replicates amounted to 0.07, 0.10, and 1.44 mg kg^{-1}. The 1.44 mg kg^{-1} value is probably an error. The average of 0.54 mg kg^{-1} was plotted but not used to calculate the non-linear dissipation curve.

D. KINETIC EVALUATION

A summary of calculated DT_{50} and DT_{75} values for boscalid and its metabolites M510F47 and M510F49 (where possible) can be found in Table 7.1.2.2.1-62.

Table 7.1.2.2.1-62: Estimated first-order DT_{50} and DT_{75} values of boscalid and its metabolites M510F47 (2-chloronicotinic acid) and M510F49

Compound	Trial site	DT_{50} [d]	DT_{75} [d]	α	β	r^2
BAS 510 F	California	76.5	> 329	0.214	0.322	0.79
	Idaho	333	> 345	0.146	0.342	0.75
	Florida	27	> 384	0.217	0.864	0.78
M510F47	California	16.2	60.1	0.699	0.104	0.80
	Idaho	< 30	< 30	est.	est.	-
	Florida	n.d.	n.d.	-	-	-
M510F49	California	18.9	102	0.469	0.179	0.85
	Idaho	No decline				
	Florida	11.1	422	0.192	3.247	0.39

n.d. = Not detected
est. = Estimates

E. RESIDUE MOBILITY

In general, no BAS 510 F residues remained in the 15 cm (6 inch) segments in the plots. However, at the Idaho site, some residues were present in the 15-30 cm (6-12 inch) cores at 14 and 30 DALA (single replicates). Residues at the Florida site were detected in the 30-46 cm (12-18 inch) cores. Residues of the degradates M510F47 and M510 F49, if detected, remained in the 0-15 cm (0-6 inch) cores.

Water movement (flux) did occur down through the 122 cm (48 inch) sampling zone. Flux calculations indicate that 12.95, 18.80, and 29.72 cm (5.1, 7.4, and 11.7 inch) of water leached through the 122 cm (48 inch) depth sampling zone in the bare soil plots at the California, Idaho, and Florida sites, respectively. Therefore, the physical conditions (water flux) required to allow compound movement were present at each site.

III. CONCLUSION

Results from this study have shown that BAS 510 F dissipates steadily and does not accumulate when applied under worst case, bare soil conditions.

Report: CA 7.1.2.2.1/10
Jackson S. et al., 2001c
1999 Field dissipation of BAS 510 .. F in terrestrial use patterns
2001/5000937
Guidelines: EPA 164-1
GLP: yes
(certified by United States Environmental Protection Agency)

Executive Summary

This study was designed to support a terrestrial use pattern according to EPA Guideline 164-1. The test substance, BAS 510 .. F, is a broad spectrum fungicide which will be used to control many diseases in field, row crop, orchard and vineyard uses as well as turf. Two field trials were conducted in areas which represent major crop producing regions of the United States (North Dakota and Colorado).

A single untreated control plot (bare soil) and one treated bare soil plot was established at each test site. The plots were located on soil typical for the agricultural areas in which they represented. The treated plots received two broadcast applications of the test substance using 374.2 L ha^{-1} (40 gal ac^{-1}) spray volume at six day intervals (targeted). The test substance was applied, beginning in May 1999, at a rate of 561 g a.s. ha^{-1} (0.5 a.s. lb ac^{-1}). Applications were made directly to bare soil plots in an effort to answer guideline questions.

Soil samples were collected to a depth of 122 cm (48 inch) using zero contamination sampling equipment. The treated plots were scheduled to be sampled on the following twenty-four sampling events: -T1, T1, T1+1, T1+2, T1+3, -T2, T2, 1, 2, 3, 5, 7, 10, 14, 21, 30, 60, 90, 120, 180, 270, 360, and (North Dakota site only) 450 Days After Last Application (DALA). The untreated plot was scheduled to be sampled on the following seven sampling events: -T1, 1, 5, 30, 90, 180, and 360 DALA intervals. A sampling variance of ± 2 days was acceptable after thirty days. After collection, samples were analyzed using HPLC MS/MS for parent and degradates [M510F47 (2-chloronicotinic acid, CNA) and M510F49].

Compound mobility was determined by measuring each analyte by depth. If mobility occurred, the movement was correlated to soil water recharge events. Calculated soil-water recharge events by depth over time were used to determine if recharge events had any effect on movement of BAS 510 F or degradate residues from the top 15 cm (6 inch) of soil. In general, residues of BAS 510 F remained in the top 15 cm (6 inch) depth of soil. At the North Dakota site single replicate parent detects were found on 1 and 180 DALA in the 15-30 cm (6-12 inch) depth. Additionally, CNA was detected at 3 and 21 DALA in the 30-46 cm (12-18 inch) depth (but not in the 15-30 cm [6-12 inch] depth). Since appreciable recharge occurred in all plots at the 122 cm (48 inch) depth, it can be concluded that there is little probability that residues are mobile at a level of significance based on TDR water flux measurements in bare soil plots.

Based on soil core residue concentrations, half-life values (DT_{50}) were calculated for boscalid and its metabolites CNA and M510F49 where possible. A summary of the calculated boscalid, CNA, and M510F49 dissipation times (DT_{50}/DT_{75}) in bare soil are presented below:

Table 7.1.2.2.1-63: Estimated first-order DT_{50} and DT_{75} values of boscalid and its metabolites M510F47 (2-chloronicotinic acid) and M510F49

Compound	Trial site	Bare	
		DT_{50} [d]	DT_{75} [d]
BAS 510 F	North Dakota	1	20
	Colorado	119	> 361
M510F47	North Dakota	7.0	< 120
	Colorado	< 21 [a]	< 21 [a]
M510F49	North Dakota	< 1 [a]	< 1 [a]
	Colorado	< 2 [a]	< 2 [a]

[a] Estimates

Results from this study have shown that BAS 510 F dissipates steadily under conditions of use.

I. MATERIAL AND METHODS

A. MATERIALS

Test item (formulation): BAS 510 00 F
Active substance (a.s.): Boscalid (BAS 510 F, Reg. No. 300355)
Chemical name (IUPAC): 2-chloro-N-(4'-chloro-biphenyl-2-yl)nicotinamide
Molar mass: 343.2 g mol^{-1}
Batch No.: AF543-17 (containing 69.6% boscalid)
Type of formulation: WG (wettable granule)

2. Test sites

The dissipation of boscalid under field conditions was investigated at two trial sites representative of major crop producing regions of the U.S.. One trial each was performed in Cass County (North Dakota) and Weld County (Colorado). The site characteristics including the basic soil parameters of the corresponding soil horizons are presented in Table 7.1.2.2.1-64.

Table 7.1.2.2.1-64: Characteristics of the trial sites used in the field dissipation study

Trial	**RCN 99502 (ND)**							
Location	**North Dakota, USA**							
Soil properties	**0-6 in (0-15 cm)**	**6-12 in (15-30 cm)**	**12-18 in (30-46 cm)**	**18-24 in (46-61 cm)**	**24-30 in (61-76 cm)**	**30-36 in (76-91 cm)**	**36-42 in (91-107 cm)**	**42-48 in (107-122 cm)**
Soil class (USDA)	Loam	Loam	Loam	Loam	Loam	Loam	Clay loam	Clay
sand [%]	47	49	47	41	33	29	21	21
silt [%]	32	30	32	32	40	44	48	30
clay [%]	21	21	21	27	27	27	31	49
Organic matter [%]	3.2	2.7	1.4	1.0	0.6	0.5	0.5	0.5
pH [c]	7.9	8.1	8.4	8.7	8.7	8.7	8.5	8.3
CEC [meq $100g^{-1}$]	22.5	21.6	18.6	17.1	17.1	17.9	19.3	24.9
Moisture (gravimetric) at 1/3 bar [%]	25.7	25.2	25.3	25.5	27.3	28.4	31.1	39.0
Bulk density [g cm^{-3}] [a]	1.46-1.62	1.59-1.61	1.58-1.69	1.45-1.69	1.42-1.72	1.51-1.75	1.56-1.72	1.34-1.59
Trial	**RCN 99503 (CO)**							
Location	**Colorado, USA**							
Soil properties	**0-6 in (0-15 cm)**	**6-12 in (15-30 cm)**	**12-18 in (30-46 cm)**	**18-24 in (46-61 cm)**	**24-30 in (61-76 cm)**	**30-36 in (76-91 cm)**	**36-42 in (91-107 cm)**	**42-48 in (107-122 cm)**
Soil class (USDA)	Sandy clay loam	Sandy clay loam	Sandy clay loam	Clay loam	Sandy clay loam	Clay loam	Clay loam	Clay loam
sand [%]	60.8	63.4	57.1	42.0	63.7	28.9	26.5	25.2
silt [%]	14.8	10.7	16.6	22.1	14.9	36.6	42.8	39.3
clay [%]	24.4	25.9	26.3	35.9	21.4	34.5	30.7	35.5
Organic matter [%]	1.3	1.3	0.9	0.9	1.0	0.8	0.5	0.4
pH [c]	8.0	8.2	8.2	8.1	8.2	8.3	8.4	8.3
CEC [meq $100g^{-1}$]	17.0	16.8	17.2	21.5	29.3	32.3	31.0	53.0
Moisture (gravimetric) at 1/3 bar [%] [a]	19.1	18.1	18.1	20.1	26.6	27.6	26.4	28.2
Bulk density [g cm^{-3}] [b]	1.36-1.54	1.42-1.55	1.47-1.75	1.45-1.63	1.42-1.55	1.34-1.51	1.44-1.55	1.49-1.79

CEC = Cation exchange capacity
[a] Measured at six composited cores
[b] Measured at four separate, undisturbed cores
[c] in the US soil pH is commonly measured in H_2O; this is however not explicitly stated in the report

The selected fields were representative of major geographic and climatic regions within the United States. Bare soil plots were fallow prior to the study. No product containing boscalid had been used on the test plots in the last three years.

B. STUDY DESIGN

1. Experimental conditions

Each trial area was divided into two plots. One plot was used as control plot (untreated, bare soil), the second plot (bare soil) was treated with the test item. The treated plot consisted of three sampling sections, each divided into 28-32 equal subplots. The control plot consisted of three sampling sections, each comprising 7-10 subplots, and was separated from the treated plot by a buffer zone of at least 23 m (75 ft) width. The size of each subplot at trial site North Dakota was 1.86 m^2 (20 sq ft; untreated plot) and 2.32 m^2 (25 sq ft; treated plot) and 4.55 m^2 at trial site Colorado (49 sq ft; control and treated plot). At each sampling event, three subplots of the control plot and three subplots of the treated plot (one of each sampling section) were randomly selected, sampled, and assigned for replicates A, B, and C.

The product, formulated as a WG (wettable granule), was broadcast applied to bare soil in two applications at a nominal rate of 561 g a.s. ha^{-1} (0.5 a.s. lb ac^{-1}). Depending on the trial site, the applications were conducted from the middle until the end of May-1999 (North Dakota) and from the end of Jul-1999 until the beginning of Aug-1999 (Colorado), using calibrated flat boom broadcast sprayers. The actual application rates determined by quantifying the amount of spray discharged ranged from 534 to 566 g a.s. ha^{-1} (0.476-0.505 lb a.s. ac^{-1}). Details of the application are presented in Table 7.1.2.2.1-65.

Table 7.1.2.2.1-65: Application parameters of field trial sites treated with boscalid

Trial Location	Test item/ Actual content [a]/ Formulation type	Application method	No. of applications	Application rate per treatment			No. of treated replicates	Application date
				Nominal [g a.s. ha^{-1}]	Actual [g a.s. ha^{-1}]	Dose verification [b]		
North Dakota	BAS 510 F 2155 mg a.s. L^{-1} WG	Broadcast spray to bare soil	2	561	555 566	99.1 and 101.1% of nominal rate	3	24-May-99 31-May-99
Colorado	BAS 510 F 2108 to 2211 mg a.s. L^{-1} WG	Broadcast spray to bare soil	2	561	561 534	95.1 and 100.2% of nominal rate	3	27-Jul-99 02-Aug-99

[a] Values represent the range of test substance concentration in the spray solution.
[b] Determined by sprayer calibration/pass time method.

A sprayer calibration/pass time method was used to confirm applications in this study. In addition to the sprayer calibration/pass time method, a petri dish application verification (AV) technique, and a zero-time core recovery method were used to validate application rates.

Verification of application with the petri dish AV technique is simply the amount of test substance found per surface area of the petri dishes adjusted up to represent a value per acre.
The zero-time sample interval is defined as the first sample collected post application to the plot. Normally zero-time calculations are based on the last application in a series, if multiple applications have been made. Zero-time core concentrations (bare soil only) were calculated by subtracting parent residue in the 0-15 cm (0-6 inch) core before the last application was made (-T2) from the parent residue measured immediately after the last application (T2). For calculation purposes, soil weight was based on a furrow acre slice (907184 kg/15 cm; 2,000,000 lb/6 inch).

No tillage was performed during the course of the study from first to last sampling. The plots were kept free of weeds via the application of glyphosate, tralkoxydim, 2-ethylhexyl ester of 2-methyl-4-chlorophenoxyl-acetic acid, ethalfluralin, and S-metolachlor.

Seasonal weather data was collected at each trial site for the entire trial period. Precipitation data was collected onsite while daily minimum and maximum air temperatures, wind speed, solar radiation, percent humidity and evapotranspiration (if available) were collected from an on-site or nearby weather station. Irrigation was applied to supplement normal precipitation so that the plots would receive 110% of the historical average rainfall for the study period or crop evapotranspiration. Historical weather data (average monthly minimum and maximum air temperatures and monthly precipitation totals) were submitted for at least a ten-year period from a reliable source located no more than 48 km (30 miles) from the test site.

A summary of monthly weather data (maximum and minimum temperatures, precipitation, as well as volumes of the supplementary irrigation) is presented in Table 7.1.2.2.1-66.

Table 7.1.2.2.1-66: Summary of monthly air temperature (max/min), precipitation, and irrigation at each field trial site

Trial	RCN 99502 (ND)					RCN 99503 (CO)				
Location	North Dakota, USA					Colorado, USA				
Month/ Year	T_{min} Air [°C]	T_{max} Air [°C]	Precipi-tation [mm]	Irriga-tion [mm]	Total [mm]	T_{min} Air [°C]	T_{max} Air [°C]	Precipi-tation [mm]	Irri-gation [mm]	Total [cm]
May-99	8.3	20.0	2.0 [b]	0.0 [b]	2.0 [b]	-	-	-	-	-
Jun-99	12.2	25.0	86.1	0.0	86.1	-	-	-	-	-
Jul- 99	14.4	27.2	70.1	55.9	126.0	15.6	29.9	20.3 [b]	2.5 [b]	22.9 [b]
Aug- 99	12.8	26.1	70.9	0.0	70.9	12.6	27.8	86.4	66.0	152.4
Sep- 99	6.1	18.9	131.6	0.0	131.6	5.8	22.1	76.2	0.0	76.2
Oct- 99	-1.1	13.9	6.1	0.0	6.1	-0.6	18.8	17.8	10.2	27.9
Nov- 99	-5.6	10.0	0.0	0.0	0.0	-5.3	15.2	15.2	10.2	25.4
Dec- 99	-12.2	1.1	5.3	0.0	5.3	-7.6	9.3	2.5	0.0	2.5
Jan-00	-18.9	-7.2	5.3	0.0	5.3	-9.2	7.6	5.1	0.0	5.1
Feb-00	-11.1	-0.6	31.5	0.0	31.5	-6.1	11.1	20.3	10.2	30.5
Mar-00	-2.8	6.7	28.7	0.0	28.7	-3.2	11.8	43.2	0.0	43.2
Apr-00	-0.6	12.2	20.6	0.0	20.6	0.4	18.7	27.9	30.5	58.4
May-00	6.7	21.1	56.1	0.0	56.1	6.4	23.4	45.7	35.6	81.3
Jun-00	11.1	23.3	183.4	0.0	183.4	9.7	27.1	20.3	50.8	71.1
Jul-00	14.4	27.2	36.6	0.0	36.6	14.1	31.6	15.2 [b]	30.5 [b]	45.7 [b]
Aug-00	29.4 [a]	13.9 [a]	0.0 [b]	0.0 [b]	0.0 [b]	-	-	-	-	-
Total	-	-	**734.3**	**55.9**	**790.2**	-	-	**396.2**	**246.4**	**642.6**

[a] Average through day of last sampling (08/02/2000)
[b] Precipitation totals for the first and last months of each trial reflect active periods only, not totals for the hole month.

2. Sampling

Five replicate cores (0-122 cm) were collected each from three predetermined, randomly selected subplots (one per sampling section) at each designated sampling interval using a soil corer. A soil depth of up to 122 cm (48 inch) was taken for each soil.

Samples from treated soils were collected from the treated plot on 24 occasions one day prior each of the two applications (-T), on the day of each application (T), between the first and second application (T1+1, T1+2, T1+3), and 1, 2, 3, 5, 7, 10, 14, 21, 30, 60, 90, 120, 180, 270, 360 and (North Dakota site only) 450 days after the last application (DALA). Samples from untreated soils were collected from the control plot on seven occasions one day before the first application (-T1) and 1, 5, 30, 90, 180 and 360 DALA. The specified dates refer to planned sampling dates. Actual sampling dates are given in brackets in the results tables.

Soil cores from the treated plots were taken to a depth of 122 cm (48 inch) in two stages at each sampling interval. For each sampling event, five 0-15 cm (0-6 inch) and five 15-122 cm (6-48 inch) soil cores were collected from each of three randomly selected subplots, and were designated as replicates A, B, and C. This resulted in a total of fifteen each of the 0-15 cm (0-6 inch) and 15-122 cm (6-48 inch) cores collected at each sampling interval for each treated plot. The 0-15 cm cores were sectioned after sampling to obtain a 0-8 cm (0-3 inch) and a 8-15 cm (3-6 inch) soil core. The 15-122 cm (6-48 inch) cores were cut frozen into 15 cm (6 inch) increments. Soil cores were composited by depth within a replicate to form completed samples.

All soil specimens were placed into freezer storage and remained frozen until processing or analysis of the samples.

3. Description of analytical procedure

Samples were received from the field frozen and were stored at ≤ 0°C. Samples were homogenized at BASF prior to analysis. After homogenization, samples were analyzed using BASF Draft Analytical Method D0004/1 [*already peer-reviewed study BASF DocID 2001/5000881*]. The method used for the residue analyses is described below.

The residues of BAS 510 F, M510F47 (2-chloronicotinic acid), and 2-hydroxy-N-(4'-chlorobiphenyl-2-yl)nicotinamide (M510F49) were extracted from soil by shaking with methanol followed by methanol/water (50:50, v/v). An aliquot (5 mL) of the extract was diluted with a buffer solution (water with 0.1% formic acid and 4 mM ammonium formate; 3 mL) for HPLC-MS/MS determination of BAS 510 F, M510F47 and M510F49. The limit of quantitation (LOQ) for each analyte was 0.01 mg kg^{-1}.

Control samples were fortified at four levels (0.01, 0.1, 1.0 or 2.0 mg kg^{-1}) either with BAS 510 F, M510F47 or M510F49. A summary of the average procedural recoveries for samples analyzed is provided in section II.2.

Application verification (AV) samples were kept below 0°C during shipping and storage until extraction. Samples were shaken and centrifuged twice with methanol/water. The samples were then serial diluted using 70/30 methanol/buffer water and quantitated using LC-MS/MS. Due to the concentration of the samples, it was not necessary to establish a limit of quantitation for this procedure.

4. Storage stability experiments

The actual length of time between sample collection in the field and sample extraction at the laboratory was recorded on sample residue records. Samples were shipped in freezer trucks to BASF for analysis. The samples were kept frozen during shipment to and storage at BASF. The maximum period any sample was stored before analysis was 611 days (20.1 months). Results of recovery testing revealed that the test item (BAS 510 F) is stable during freezer storage.

In addition, the storage stability of boscalid was investigated in a separate study [*peer-reviewed study BASF DocID 2000/1000136*].

5. Kinetic evaluation

In order to calculate dissipation times (DT_{50}/DT_{75}), the reported residue values were mathematically averaged by replicate to produce a grand mean. In the case where a replicate was analyzed in duplicate or triplicate, the numbers were then averaged to produce a mean for that replicate before averaging with the other replicates. When a replicate sample was below the LOQ or < 0.01 mg kg^{-1}, it was averaged as zero. To determine analyte half-life values, the residues in all soil depths were summed to produce the total residue concentration per sampling period. The summed residue concentrations per sampling period were natural log transformed before analysis by non-linear regression.

The non-linear regression was solved by applying the Gustafson-Holden model (FOMC), using the Origin Scientific Graphics Software (OriginLab Corporation, Northampton, MA, USA; version 6.1, 2000).

6. Residue mobility

To determine if residues had an opportunity to move down through the soil profile with the soil solution, the water content by depth was determined using Time Domain Reflectometry (TDR) was applied. Soil water flux was also calculated at a 30 cm depth based on this method. The TDR system used in this study was a CR10X datalogger (Campbell Scientific Inc., Logan, UT, USA) configured with four CS615 probes located in one of the bare soil subplots. The probes were installed vertically at the 0-30, 30-61, 61-91 and 91-122 cm (0-1, 1-2, 2-3, and 3-4 ft) depths. Since the rods of the CS615 are 30.5 cm (12 inch) in length, vertical installation provides a continuum of volumetric water content measurements from the soil surface to a depth of 122 cm (4 ft).

II. RESULTS AND DISCUSSION

1. Application verification

Application results from the petri dish AV technique were 87% and 92% (North Dakota) and 85% and 84% (Colorado) of the target rate. Actual pass/time and spray equipment calibration data indicated that the target field application rate was precise and accurate. The variance observed in application petri dish AV recoveries was assumed to be a reflection of the technique rather than a demonstration of variance in actual application to plots. Zero-time core recoveries were 81% (North Dakota) and 109% (Colorado) based on the bare soil plot (broadcast application) of the respective field trial site. Differences in calculated application rates to the field and those calculated from zero time cores are probably due to sample handling. More details on the application verification results are given in the study report.

2. Residues in field soil samples

Mean procedural recoveries from control soil samples fortified either with boscalid, M510F47 or M510F49 were generally within the acceptable range of 70-120% (see Table 7.1.2.2.1-67). A summary of the individual procedural recovery results is provided in the study report.

Table 7.1.2.2.1-67: Method procedural recoveries

Analyte	Mean recovery ± RSD [%]
BAS 510 F	94 ± 10.4 (n=92)
M510F47	94 ± 20.2 (n=88)
M510F49	103 ± 22.2 (n=91)

RSD = Relative standard deviation [%]

Field soil samples taken from different depths were analyzed to a maximum of about 429 days after the last of the two applications. The analytical results are summarized in Table 7.1.2.2.1-68 and Table 7.1.2.2.1-69. All residue values presented in these tables are related to the dry weight of the soil and were not corrected for procedural recoveries. Residue concentrations are reported according to soil depth (0-8, 8-15, 15-30, 30-46 cm).

Table 7.1.2.2.1-68: Residues of boscalid and its metabolites M510F47 (2-chloronicotinic acid) and M510F49 [mg kg^{-1} dry weight] in treated soil samples (mean of three replicates) – North Dakota site (bare plot; RCN 99502-ND)

Compound [a]	Soil depth [cm]	Targeted days after last application [b]										
		-T1 [c]	T1	T1+1	T1+2	T1+3	T2	1	2	3	5 (6)	7
boscalid	0-8	<0.01	0.454	0 224	0.196	0.582	0.859	0.526	0.249	0.652	0.378	0.111
	8-15	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	15-30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
M510F47	0-8	<0.01	0.013	0.009	0.013	0.017	0.022	0.011	<0.01	0.021	0.009	<0.01
	8-15	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	15-30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	30-46	<0.01	0.008	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.004	<0.01	<0.01
M510F49	0-8	<0.01	<0.01	<0.01	<0.01	0.005	0.008	<0.01	<0.01	<0.01	<0.01	<0.01
	8-15	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	15-30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Compound [a]	Soil depth [cm]	Targeted days after last application [b]									
		14	21	30	60	90	120	180 (174)	270 (327)	360 (359)	450 (429)
boscalid	0-8	0.135	0 389	0.194	0.135	0.127	0.110	0.110	0.101	0.177	0.122
	8-15	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.012
	15-30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
M510F47	0-8	<0.01	0.017	0.010	<0.01	0.008	<0.01	<0.01	<0.01	<0.01	<0.01
	8-15	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	15-30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	30-46	<0.01	0.003	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
M510F49	0-8	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	8-15	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	15-30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

T = Application dates

[a] The limit of quantification (LOQ) was 0.01 mg kg^{-1} for each analyte. All residue values were corrected for moisture content and are reported here on a dry weight basis. All value of 0 µg kg^{-1} was used in average calculations for samples where residues were reported as <0.01 mg kg^{-1}.

[b] Sampling dates refer to the targeted sampling dates, the actual sampling dates are given in brackets.

[c] –T1 samples were also analyzed at depths ≥ 46 cm (18 inch) and up to 122 cm (48 inch) with no residues found at or above the LOQ.

Table 7.1.2.2.1-69: Residues of boscalid and its metabolites M510F47 (2-chloronicotinic acid) and M510F49 [mg kg^{-1} dry weight] in treated soil samples (mean of three replicates) – Colorado site (bare plot; RCN 99503-CO)

Compound [a]	Soil depth [cm]	Targeted days after last application [b]										
		-T1 [c]	T1	T1+1	T1+2	T1+3	-T2	T2	1	2	3 (4)	5
boscalid	0-8	<0.01	0.377	0.471	0.345	0.378	0.106	0.653	0.701	0.704	0.575	0.779
	8-15	<0.01	<0.01	<0.01	<0.01	0.161	0.244	0.051	<0.01	<0.01	<0.01	<0.01
	15-30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
M510F47	0-8	<0.01	0.010	<0.01	0.013	0.012	0.003	0.020	0.016	0.020	0.017	0.023
	8-15	<0.01	<0.01	<0.01	<0.01	0.035	0.026	<0.01	<0.01	<0.01	<0.01	<0.01
	15-30	0.013	0.009	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
M510F49	0-8	<0.01	<0.01	<0.01	<0.01	0.005	<0.01	0.008	<0.01	<0.01	<0.01	<0.01
	8-15	<0.01	<0.01	<0.01	<0.01	<0.01	0.013	<0.01	<0.01	<0.01	<0.01	<0.01
	15-30	<0.01	<0.01	<0.01	<0.01	0.009	<0.01	0.013	<0.01	<0.01	<0.01	<0.01

Compound [a]	Soil depth [cm]	Targeted days after last application [b]									
		7	14	21	30 (31)	60	90 (91)	120	180 (217)	270 (269)	360 (361)
boscalid	0-8	0.598	0.708	0.587	0.478	0.529	0.289	0.486	0.350	0.354	0.238
	8-15	0.037	<0.01	<0.01	<0.01	<0.01	0.019	<0.01	0.006	0.012	0.004
	15-30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
M510F47	0-8	0.017	0.016	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	8-15	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	15-30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.003	<0.01
M510F49	0-8	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	8-15	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	15-30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

T = Application dates

[a] The limit of quantification (LOQ) was 0.01 mg kg^{-1} for each analyte. All residue values were corrected for moisture content and are reported here on a dry weight basis. All value of 0 µg kg^{-1} was used in average calculations for samples where residues were reported as <0.01 mg kg^{-1}.

[b] Sampling dates refer to the targeted sampling dates, the actual sampling dates are given in brackets.

[c] –T1 samples were also analyzed at depths ≥ 30-46 cm (18 inch) and up to 122 cm (48 inch) with no residues found at or above the LOQ.

D. KINETIC EVALUATION

A summary of calculated DT_{50} and DT_{75} values for boscalid and its metabolites M510F47 and M510F49 (where possible) can be found in Table 7.1.2.2.1-70.

Table 7.1.2.2.1-70: Estimated first-order DT_{50} and DT_{75} values of boscalid and its metabolites M510F47 (2-chloronicotinic acid) and M510F49

Compound	Trial site	DT_{50} [d]	DT_{75} [d]	α	β	r^2
BAS 510 F	North Dakota	1	20	0.2049	43.266	0.61
	Colorado	119	> 361	0.2195	0.1890	0.80
M510F47	North Dakota	7.0	< 120	0.0634	8000	0.40
	Colorado	< 21	< 21	est.		
M510F49	North Dakota	< 1	< 1	est.		
	Colorado	< 2	< 2	est.		

est. = Estimates

E. RESIDUE MOBILITY

In general, residues of BAS 510 F remained in the top 15 cm (0-6 inch) soil depth. At the North Dakota site single replicate parent detects were found on 1 and 180 DALA in the 15-30 cm (6-12 inch) depth. Additionally, M510F47 was detected at 3 and 21 DALA in the 30-46 cm (12-18 inch) depth (but not in the 15-30 cm [6-12 inch] depth).

Water movement (flux) did occur down through the 122 cm (48 inch) sampling zone. Flux calculations indicate that 14.7 and 17.3 cm (5.8 and 6.8 inch) of water leached through the 122 cm (48 inch) depth sampling zone in the bare soil plots at the North Dakota and Colorado sites, respectively. Therefore, the physical conditions (water flux) required to allow compound movement were present at each site.

Based on the TDR measurements and the resulting flux calculations, it is evident that the test item and its degradates M510F47 and M510F49 were provided opportunity to move down through the soil profile but did not other than the few North Dakota spurious detects.

III. CONCLUSION

Results from this study have shown that BAS 510 F dissipates steadily and does not accumulate when applied under worst case, bare soil conditions.

Report:	CA 7.1.2.2.1/11 Jackson S. et al., 2001d 1999 Field dissipation of BAS 510 .. F in terrestrial use patterns for Canada 2001/5000938
Guidelines:	Agriculture Canada Trade Memorandum T-1-255
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary

This study was designed to support a terrestrial use pattern to satisfy the Health Canada Guideline T-1-255 (Terrestrial Field Dissipation). The test substance, BAS 510 .. F, is a broad spectrum fungicide which will be used to control many diseases in field, row crop, orchard, and vineyard uses as well as on turf. Three field trials were conducted in areas which represent major crop producing regions of Canada (Ontario, Manitoba, and Alberta).

A single untreated control plot (bare soil) and one treated bare soil plot was established at each test site. The plots were located on soil typical for the agricultural areas in which they were located. The treated plots received two broadcast applications of the test substance using 220 liters per hectare spray volume at five day intervals (targeted). The test substance was applied, beginning in July 1999, at a rate of 560 grams active ingredient per hectare. Applications were made directly to bare soil plots in an effort to answer guideline questions.

Soil samples were collected to a 120 cm depth using zero contamination sampling equipment. The treated plots were scheduled to be sampled on the following twenty-four sampling events: -T1, T1, T1+1, T1+2, T1+3, -T2, T2, and 1, 2, 3, 5, 7, 10, 14, 21, 30, 60, 90, 120, 180, 270, 360, 450 and 540 Days After Last Application (DALA). The control plot was scheduled to be sampled on the following seven sampling events: -T1 and 1, 5, 30, 90, 180, and 360 DALA intervals. A sampling variance of ± 2 days was acceptable after thirty days. After collection, samples were analyzed using HPLC-MS/MS for parent and the degradates M510F47 (2-chloronicotinic acid) and M510F49. Based on soil core analysis, the half-life of each analyte was calculated. A summary of the calculated dissipation times (DT) in soil of BAS 510 F and its degradates is presented below:

Table 7.1.2.2.1-71: Estimated first-order DT_{50} and DT_{75} values of boscalid and its metabolites M510F47 (2-chloronicotinic acid) and M510F49

Compound	Trial site	DT_{50} [d]	DT_{75} [d]	α	β	r^2
BAS 510 F	Ontario	30.0	353	0.29174	0.32495	0.79
	Manitoba	316	> 360	0.08935	7.397	0.36
	Alberta [a]	372	Rate constant			0.74
M510F47	Ontario	< 5	< 5	Estimated		
	Manitoba	< 5	< 5	Estimated		
	Alberta	10.5	26.2	1.73167	0.0469	0.73
M510F49	Ontario	< 301	< 301	Estimated		
	Manitoba	Not detected				
	Alberta	No decline				

[a] Simple regression – rate constant

Compound mobility was determined by measuring each analyte by depth. Calculated soil-water recharge events by depth over time and TDR (time domain reflectometry) measurements were used to determine if recharge events occurred and if they had any effect on movement of BAS 510 F or degradate residues from the top 15 cm of soil. If mobility occurred, it was possible to correlate the movement to soil water recharge events. Results from this study indicated that all residues of BAS 510 F remained in the surface 15 cm. Soil water recharge occurred in all plots at the 120 cm depth based on TDR measurements in bare soil plots. Based on calculations of soil-water recharge, residues of BAS 510 F were provided opportunity to move down through the soil profile but did not.

Results from this study have shown that all residues of BAS 510 F dissipate steadily under conditions of use.

I. MATERIAL AND METHODS

A. MATERIALS

Test item (formulation): BAS 510 00 F
Active substance (a.s.): Boscalid (BAS 510 F, Reg. No. 300355)
Chemical name (IUPAC): 2-chloro-N-(4'-chloro-biphenyl-2-yl)nicotinamide
Molar mass: 343.2 g mol^{-1}
Batch No.: AF543-17 (containing 69.6% boscalid)
Type of formulation: WG (wettable granule)

2. Test sites

The dissipation of boscalid under field conditions was investigated at three trial sites representative of major crop producing regions of Canada. One trial each was performed in the regions Ontario, Manitoba, and Alberta. The site characteristics including the basic soil parameters of the corresponding soil horizons are presented in Table 7.1.2.2.1-72 and Table 7.1.2.2.1-73.

Table 7.1.2.2.1-72: Characteristics of the trial sites used in the field dissipation study (Ontario and Manitoba site)

Trial	**RCN 99515 (ON)**							
Location	**Ontario, Canada**							
Soil properties	**0-15 cm (0-6 in)**	**15-30 cm (6-12 in)**	**30-45 cm (12-18 in)**	**45-60 cm (18-24 in)**	**60-75 cm (24-30 in)**	**75-90 cm (30-36 in)**	**90-105 cm (36-42 in)**	**105-120 cm (42-48 in)**
Soil class (USDA)	Loam	Loam	Clay loam	Clay	Clay	Clay loam	Clay loam	Clay loam
sand [%]	34	30	28	22	26	28	26	28
silt [%]	42	44	34	36	28	32	34	40
clay [%]	24	26	38	42	46	40	40	32
Organic matter [%]	2.8	1.9	0.7	0.5	0.5	0.5	0.4	0.2
pH [c]	6.2	6.6	7.3	7.8	8.1	8.2	8.3	8.3
CEC [meq $100g^{-1}$]	12.2	13.7	15.2	16.8	16.8	15.4	15.9	13.3
Moisture (gravimetric) at 1/3 bar [%] [a]	24.1	24.1	25.0	25.8	27.2	26.2	26.3	23.3
Bulk density [g cm^{-3}] [b]	1.15-1.39	1.39-1.57	1.32-1.47	1.37-1.46	1.19-1.52	1.34-1.50	1.37-1.52	1.34-1.50
Trial	**RCN 99516 (MB)**							
Location	**Manitoba, Canada**							
Soil properties	**0-15 cm (0-6 in)**	**15-30 cm (6-12 in)**	**30-45 cm (12-18 in)**	**45-60 cm (18-24 in)**	**60-75 cm (24-30 in)**	**75-90 cm (30-36 in)**	**90-105 cm (36-42 in)**	**105-120 cm (42-48 in)**
Soil class (USDA)	Silt loam	Loam	Clay loam	Clay loam	Clay loam	Loam	Silt loam	Loam
sand [%]	23	27	25	29	29	31	27	29
silt [%]	54	50	44	40	42	46	52	50
clay [%]	23	23	31	31	29	23	21	21
Organic matter [%]	7.0	4.3	2.3	1.3	0.9	0.6	0.5	0.5
pH [c]	7.8	8.4	8.6	8.7	8.6	8.4	8.5	8.3
CEC [meq $100g^{-1}$]	37.5	31.2	26.3	22.8	20.1	16.7	15.8	17.0
Moisture (gravimetric) at 1/3 bar [%] [a]	39.8	38.0	34.5	28.4	28.2	25.3	26.2	27.8
Bulk density [g cm^{-3}] [b]	1.04-1.14	0.99-1.05	1.00-1.16	1.16-1.24	1.18-1.34	1.20-1.43	1.11-1.95	1.29-1.43

CEC = cation exchange capacity
[a] Measured at six composited cores
[b] Measured at four separate, undisturbed cores
[c] in the US soil pH is commonly measured in H_2O; this is however not explicitly stated in the report

Table 7.1.2.2.1-73: Characteristics of the trial sites used in the field dissipation study (Alberta site)

Trial	RCN 99517 (AB)							
	Alberta, Canada							
Soil properties	**0-15 cm (0-6 in)**	**15-30 cm (6-12 in)**	**30-45 cm (12-18 in)**	**45-60 cm (18-24 in)**	**60-75 cm (24-30 in)**	**75-90 cm (30-36 in)**	**90-105 cm (36-42 in)**	**105-120 cm (42-48 in)**
Soil class (USDA)	Loam	Clay loam	Clay loam	Clay	Clay	Clay	Clay	Clay
sand [%]	32	30	22	12	12	12	10	14
silt [%]	42	38	38	36	34	30	28	28
clay [%]	26	32	40	52	54	58	62	58
Organic matter [%]	5.4	1.6	1.2	1.5	1.5	1.6	1.6	1.5
pH [c]	5.5	6.4	7.0	7.8	8.1	8.1	8.1	8.1
CEC [meq $100g^{-1}$]	19.6	17.5	21.4	25.2	24.3	25.7	25.8	24.9
Moisture (gravimetric) at 1/3 bar [%] [a]	27.9	24.2	28.7	32.9	34.6	34.9	35.1	33.8
Bulk density [$g\ cm^{-3}$] [b]	1.28-1.62	1.35-1.73	1.42-1.74	1.42-1.63	1.37-1.71	1.43-1.69	1.48-1.63	1.54

CEC = cation exchange capacity
[a] Measured at six composited cores
[b] Measured at four separate, undisturbed cores
[c] in the US soil pH is commonly measured in H_2O; this is however not explicitly stated in the report

The field trials were conducted in areas of major crop producing regions of Canada. Applications of product were made to bare soil plots under actual field conditions. No product containing boscalid had been used on the test plots in the last three years.

B. STUDY DESIGN

1. Experimental conditions

Each trial area was divided into two plots. One plot was used as control plot (untreated), the second plot (bare soil) was treated with the test item. The treated plot consisted of three sampling sections, each divided into 24-32 equal subplots. The control plot consisted of three sampling sections, each comprising 7-12 subplots. At trial site Ontario, the control plot consisted of one sampling section, comprising 30 subplots. The control plot was separated from the treated plot by a buffer zone of at least 15 m width. The size of each subplot was 4.5-8.0 m^2. Per scheduled sampling interval, five subplots of the control plot and five subplots of the treated plot (five of each sampling section) were randomly selected, sampled, and assigned for replicates 1, 2, and 3.

The product, formulated as a WG (wettable granule), was applied by flat broadcast boom sprayers to bare soil. Two applications at a nominal rate of 560 g a.s. ha^{-1} were conducted. Depending on the trial site, the applications were performed from mid-July 1999 until the end of July 1999. The actual application rates determined by quantifying the amount of spray discharged ranged from 564 to 576 g a.s. ha^{-1}. Details of the application are presented in Table 7.1.2.2.1-74.

Table 7.1.2.2.1-74: Application parameters of field trial sites treated with boscalid

Trial Location	Test item/ Actual content [a]/ Formulation type	Application method	No. of applications	Application rate per treatment			No. of treated replicates	Application date
				Nominal [g a.s. ha^{-1}]	Actual [g a.s. ha^{-1}]	Dose verification [b]		
Ontario	BAS 510 F 3.67 g a.s. L^{-1} WG	Broadcast spray to bare soil	2	560	569 572	101.6-102.1% of nominal rate	3	15-Jul-99 20-Jul-99
Manitoba	BAS 510 F 3.66 g a.s. L^{-1} WG	Broadcast spray to bare soil	2	560	565 576	100.9-102.9% of nominal rate	3	14- Jul-99 19- Jul-99
Alberta	BAS 510 F 3.66-3.71 mg a.s. L^{-1} WG	Broadcast spray to bare soil	2	560	568 564	100.8-101.5% of nominal rate	3	21-Jul-99 28-Jul-99

[a] Values represent the range of test substance concentration in the spray solution (n = 2)
[b] Determined by sprayer calibration/pass time method.

A sprayer calibration/pass time method was used to confirm applications in this study. In addition to the sprayer calibration/pass time method, a petri dish application verification (AV) technique, and a zero-time core recovery method were used to validate application rates.

Verification of application with the petri dish AV technique is simply the amount of test substance found per surface area of the petri dishes adjusted up to represent a value per acre.

The zero-time sample interval is defined as the first sample collected post application to the plot. Normally zero-time calculations are based on the last application in a series, if multiple applications have been made. Zero-time core concentrations (bare soil only) were calculated by subtracting parent residue in the 0-15 cm (0-6 inch) core before the last application was made (-T2) from the parent residue measured immediately after the last application (T2). For calculation purposes, soil weight was based on a furrow acre slice (907185 kg/15 cm; 2,000,000 lb/6 inch).

No tillage was performed during the course of the study from first to last sampling. The plots were kept free of weeds via the application of glyphosate and paraquat.

Seasonal weather data was collected at each trial site for the entire trial period. Precipitation data was collected onsite while daily minimum and maximum air temperatures, wind speed, solar radiation, and percent humidity were collected from an on-site or nearby weather station. Irrigation was applied to supplement normal precipitation so that the plots would receive 110% of the historical average rainfall for the study period. Historical weather data (average monthly minimum and maximum air temperatures and monthly precipitation totals) were submitted for at least a ten-year period from a reliable source located no more than 60 km from the test site.

A summary of monthly weather data (maximum and minimum temperatures, precipitation, as well as volumes of the supplementary irrigation) is presented in Table 7.1.2.2.1-75.

Table 7.1.2.2.1-75: Summary of monthly air temperature (max/min), precipitation, and irrigation at each field trial site

Trial	RCN 99515 (ON)					RCN 99516 (MB)				
Location	Ontario, Canada					Manitoba, Canada				
Month/ Year	T_{min} Air [°C]	T_{max} Air [°C]	Precipi-tation [mm]	Irriga-tion [mm]	Total [mm]	T_{min} Air [°C]	T_{max} Air [°C]	Precipi-tation [mm]	Irri-gation [mm]	Total [mm]
Jul- 99	16.9	28.8	91.2 [a]	35.3 [a]	126.5 [a]	14.4	27.2	29.4 [a]	11.0 [a]	40.4 [a]
Aug- 99	13.4	25.0	56.6	12.0	68.6	11.1	25.6	65.0	46.0	111.0
Sep- 99	10.0	24.3	117.4	24.1	141.5	5.6	18.3	49.2	0	49.2
Oct- 99	4.0	14.9	77.6	0	77.6	-1.1	12.2	27.4	0	27.4
Nov- 99	0.8	8.1	70.6	0	70.6	-5.6	8.3	4.3	0	4.3
Dec- 99	-4.6	2.3	61.8	0	61.8	-13.3	-0.6	18.0	0	18.0
Jan-00	-5.6	-0.6	39.9	0	39.9	-20.6	-8.9	18.4	0	18.4
Feb-00	-3.9	0.9	48.5	0	48.5	-11.7	0.6	72.8	0	72.8
Mar-00	-0.2	8.6	45.0	0	45.0	-5.0	7.2	25.8	0	25.8
Apr-00	1.4	10.5	75.2	0	75.2	-2.2	12.8	9.8	0	9.8
May-00	8.7	19.1	134.4	0	134.4	5.0	20.0	56.6	0	56.6
Jun-00	13.4	23.1	174.2	0	174.2	9.4	21.1	110.8	11.6	122.4
Jul-00	13.3	24.1	81.2 [a]	0 [a]	81.2 [a]	13.9	26.1	137.6 [a]	3.22 [a]	140.8 [a]
Total	-	-	**1073.6**	**71.4**	**1145.0**	-	-	**625.1**	**71.8**	**696.9**

Trial	RCN 99517 (AB)				
Location	Alberta, Canada				
Month/ Year	T_{min} Air [°C]	T_{max} Air [°C]	Precipi-tation [mm]	Irriga-tion [mm]	Total [mm]
Jul- 99	9.1	21.3	15.5 [a]	0 [a]	15.5 [a]
Aug- 99	8.3	24.2	24.9	0.0	24.9
Sep- 99	2.7	18.0	12.6	48.5	61.1
Oct- 99	-0.7	11.0	9.4	0	9.4
Nov- 99	-9.7	1.0	15.5	0	15.5
Dec- 99	-13.6	-0.5	34.1	0	34.1
Jan-00	-22.9	-10.0	26.0	0	26.0
Feb-00	-19.6	-3.5	21.0	0	21.0
Mar-00	-9.I	2.7	11.5	0	11.5
Apr-00	-3.2	9.2	7.3	0	7.3
May-00	1.7	13.0	74.7	4.9	79.6
Jun-00	7.1	19.4	50.9	24.2	75.1
Jul-00	9.7	22.0	101.6 [a]	29.2 [a]	130.8 [a]
Total	-	-	**405.0**	**106.8**	**511.8**

[a] Precipitation totals for the first and last months of each trial reflect active periods only, not totals for the hole month.

2. Sampling

Five replicate cores (0-120 cm) were collected each from three predetermined, randomly selected subplots at each designated sampling interval using a soil corer. A soil depth of up to 120 cm was taken for each soil.

Samples from treated soils were collected from the treated plot on 22 occasions one day prior each of the two applications (-T), on the day of each application (T), between the first and second application (T1+1, T1+2, T1+3), and 1, 2, 3, 5, 7, 10, 14, 21, 30, 60, 90, 120, 180, 270 and 360 days after the last application (DALA). Samples from untreated soils were collected from the control plot on seven occasions one day before the first application (-T1) and 1, 5, 30, 90, 180 and 360 DALA. Actual sampling dates are given in brackets in the results tables.

Soil cores from the treated plots were taken to a depth of 120 cm in two stages at each sampling interval. For each sampling event, five 0-15 cm (0-6 inch) and five 15-120 cm soil cores were collected from each of three randomly selected subplots, and were designated as replicates A, B, and C. This resulted in a total of fifteen each of the 0-15 cm and 15-120 cm cores collected at each sampling interval for each treated plot. The 0-15 cm cores were either sampled in two runs or sectioned after sampling to obtain a 0-7.5 cm and a 7.5-15 cm soil core. The 15-120 cm cores were cut frozen into 15 cm increments. Soil cores were composited by depth within a replicate to form completed samples.

All soil specimens were placed into freezer storage at about -18°C and remained frozen until processing or analysis of the samples.

3. Description of analytical procedure

Samples were received from the field frozen and were stored at $\leq$ 0°C. Samples were homogenized at BASF prior to analysis. After homogenization, samples were analyzed using BASF Draft Analytical Method D0004/1 [*already peer-reviewed study BASF DocID 2001/5000881*] with modifications. The method used for the residue analyses is described below.

The residues of BAS 510 F, M510F47 (2-chloronicotinic acid), and 2-hydroxy-N-(4'-chlorobiphenyl-2-yl)nicotinamide (M510F49) were extracted from soil by shaking with methanol followed by methanol/water (50:50, v/v) and sonication. An aliquot (1 mL) of the extract was diluted with a buffer solution (water or water/methanol 80:20 [v/v] with 0.3% formic acid and 4 mM ammonium formate; 0.5 mL) for HPLC-MS/MS determination of BAS 510 F, M510F47 and M510F49. The limit of quantitation (LOQ) for each analyte was 0.01 mg kg^{-1}.

Control samples were fortified either with BAS 510 F, M510F47 or M510F49. A summary of the average procedural recoveries for samples analyzed is provided in section II.2.

Application verification (AV) samples were kept below 0°C during shipping and storage until extraction. Samples were shaken and centrifuged twice with methanol/water. The samples were then serial diluted using 70/30 methanol/buffer water and quantitated using LC-MS/MS. Due to the concentration of the samples, it was not necessary to establish a limit of quantitation for this procedure.

4. Storage stability experiments

The actual length of time between sample collection in the field and sample extraction at the laboratory was recorded on sample residue records. Samples were shipped in freezer trucks to BASF for analysis. The samples were kept frozen during shipment to and storage at BASF. The maximum period any sample was stored before analysis was 561 days (18.4 months). Results of recovery analyses revealed that the test item (BAS 510 F) is stable during freezer storage.

In addition, the storage stability of boscalid was investigated in a separate study [*peer-reviewed study BASF DocID 2000/1000136*].

5. Kinetic evaluation

In order to calculate dissipation times (DT_{50}/DT_{75}), the reported residue values were mathematically averaged by replicate to produce a grand mean. In the case where a replicate was analyzed in duplicate or triplicate, the numbers were then averaged to produce a mean for that replicate before averaging with the other replicates. When a replicate sample was below the LOQ or < 0.01 mg kg^{-1}, it was averaged as zero. To determine analyte half-life values, the residues in all soil depths were summed to produce the total residue concentration per sampling period. The summed residue concentrations per sampling period were natural log transformed before analysis by non-linear regression.

The non-linear regression was solved by applying the Gustafson-Holden model (FOMC), using the Origin Scientific Graphics Software (OriginLab Corporation, Northampton, MA, USA; version 6.1, 2000).

6. Residue mobility

To determine if residues had an opportunity to move down through the soil profile with the soil solution, the water content by depth was determined using Time Domain Reflectometry (TDR) was applied. Soil water flux was also calculated at a 30 cm depth based on this method. The TDR system used in this study was a CR10X datalogger (Campbell Scientific Inc., Logan, UT, USA) configured with four CS615 probes located in one of the bare soil subplots. The probes were installed vertically at the 0-30, 30-60, 60-90 and 90-120 cm depths. Since the rods of the CS615 are 30 cm in length, vertical installation provides a continuum of volumetric water content measurements from the soil surface to a depth of 120 cm.

II. RESULTS AND DISCUSSION

1. Application verification

Application results from the petri dish AV technique ranged from 72% to 82% (Ontario), from 76% to 85% (Manitoba), and from 95% to 102% (Alberta) of the target rate. Actual pass/time and spray equipment calibration data indicated that the target field application rate was precise and accurate. The variance observed in application petri dish AV recoveries was assumed to be a reflection of the technique rather than a demonstration of variance in actual application to plots. Zero-time core recoveries were 82% (Ontario), 79% (Manitoba), and 95% (Alberta) based on the bare soil plot (broadcast application) of the respective field trial site. Differences in calculated application rates to the field and those calculated from zero time cores are probably due to sample handling. More details on the application verification results are given in the study report.

2. Residues in field soil samples

Mean procedural recoveries from control soil samples fortified either with boscalid, M510F47 or M510F49 at four levels (0.01, 0.1, 1.0 or 2.0 mg kg^{-1}) were generally within the acceptable range of 70-120% (see Table 7.1.2.2.1-76). A summary of the individual procedural recovery results is provided in the study report.

Table 7.1.2.2.1-76: Method procedural recoveries

Mean recovery ± RSD [%]		
BAS 510 F	**M510F47**	**M510F49**
91 ± 9.1 (n=89)	90 ± 17.1 (n=85)	86 ± 10.2 (n=88)

RSD = Relative standard deviation [%]

Field soil samples taken from different depths were analyzed to a maximum of about 360 days after the second of the two applications. The analytical results are summarized in Table 7.1.2.2.1-77 to Table 7.1.2.2.1-79. All residue values presented in these tables are related to the dry weight of the soil and were not corrected for procedural recoveries. Residue concentrations are reported according to soil depth (0-7, 7-15, 15-30, and 30-60 cm).

Table 7.1.2.2.1-77: Residues of boscalid and its metabolites M510F47 (2-chloronicotinic acid) and M510F49 [mg kg^{-1} dry weight] in treated soil samples (mean of three replicates) – Ontario site (bare plot; RCN 99515-ON)

Compound [a]	Soil depth [cm]	Targeted days after last application [b]																		
		-T1	T1	T1+1	T1+2	T1+3	0	1	2	3	5	7	14	21 (22)	30	60 (62)	90 (91)	120 (118)	270 (301)	360 (369)
boscalid	0-7	<0.01	0.373	0.449	0.494	0.451	0.860	0.782	0.728	0.785	0.788	0.485	0.745	0.429	0.251	0 376	0.187	0.396	0.192	0.176
	7-15	<0.01	0.006	0.007	0.038	<0.01	0.005	0.023	0.004	<0.01	0.004	<0.01	0.006	<0.01	0.020	0.019	0.099	0.097	0.022	0.022
	15-30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
M510F47	0-7	<0.01	0.012	<0.01	<0.01	<0.01	0.012	0.013	0.004	0.005	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	7-15	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	15-30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	30-60	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.009	<0.01	<0.01	<0.01	<0.01
M510F49	0-7	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.008	<0.01	0.006	0.003	0.004	0.004	<0.01	<0.01	<0.01	<0.01	0.040	<0.01	<0.01
	7-15	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	15-30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

T = Application dates

[a] The limit of quantification (LOQ) was 0.01 mg kg^{-1} for each analyte. All residue values were corrected for moisture content and are reported here on a dry weight basis. All value of 0 µg kg^{-1} was used in average calculations for samples where residues were reported as <0.01 mg kg^{-1}.

[b] Sampling dates refer to the targeted sampling dates, the actual sampling dates are given in brackets.

Table 7.1.2.2.1-78: Residues of boscalid and its metabolites M510F47 (2-chloronicotinic acid) and M510F49 [mg kg^{-1} dry weight] in treated soil samples (mean of three replicates) – Manitoba site (bare plot; RCN 99516-MB)

Compound [a]	Soil depth [cm]	Targeted days after last application [b]															
		-T1	T1	T1+2	T1+3	T2	1	2	3	5	7	14	21	30 (32)	60 (59)	90 (92)	270 (275)	360
boscalid	0-7	<0.01	0.400	0.143	0.214	0.446	0.602	0.506	0.608	0.325	0 396	0.400	0.344	0.326	0.336	0.421	0.338	0.314
	7-15	<0.01	<0.01	<0.01	<0.01	<0.01	0.004	0.006	0.004	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.006	<0.01
	15-30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
M510F47	0-7	<0.01	0.015	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	7-15	<0.01	0.003	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	15-30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
M510F49	0-7	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	7-15	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	15-30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

T = Application dates

[a] The limit of quantification (LOQ) was 0.01 mg kg^{-1} for each analyte. All residue values were corrected for moisture content and are reported here on a dry weight basis. All value of 0 µg kg^{-1} was used in average calculations for samples where residues were reported as <0.01 mg kg^{-1}.

[b] Sampling dates refer to the targeted sampling dates, the actual sampling dates are given in brackets.

Table 7.1.2.2.1-79: Residues of boscalid and its metabolites M510F47 (2-chloronicotinic acid) and M510F49 [mg kg^{-1} dry weight] in treated soil samples (mean of three replicates) – Alberta site (bare plot; RCN 99517-AB)

Compound [a]	Soil depth [cm]	Targeted days after last application [b]																	
		-T1	T1	T1+1	T1+2	T1+3	0	1	3	5	7 (6)	10 (7)	14	21	30	60 (58)	90 (79)	270 (303)	360 (358)
boscalid	0-7	<0.01	0.449	0.392	0.414	0 312	0.780	0.787	0.727	0.715	0.807	0.770	0.718	0.653	0.755	0.661	0.746	0.397	0.488
	7-15	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	15-30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
M510F47	0-7	0.006	0.019	0.006	0.015	0.006	0.014	0.014	0.013	0.013	0.012	0.014	0.003	<0.01	0.004	<0.01	<0.01	<0.01	<0.01
	7-15	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	15-30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
M510F49	0-7	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.004	0.005	0.009	0.008	0.014	<0.01	0.010
	7-15	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	15-30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

T = Application dates

[a] The limit of quantification (LOQ) was 0.01 mg kg^{-1} for each analyte. All residue values were corrected for moisture content and are reported here on a dry weight basis. All value of 0 µg kg^{-1} was used in average calculations for samples where residues were reported as <0.01 mg kg^{-1}.

[b] Sampling dates refer to the targeted sampling dates, the actual sampling dates are given in brackets.

D. KINETIC EVALUATION

A summary of calculated DT_{50} and DT_{75} values for boscalid and its metabolites M510F47 and M510F49 (where possible) can be found in Table 7.1.2.2.1-80.

Table 7.1.2.2.1-80: Estimated first-order DT_{50} and DT_{75} values of boscalid and its metabolites M510F47 (2-chloronicotinic acid) and M510F49

Compound	Trial site	DT_{50} [d]	DT_{75} [d]	α	β	r^2
BAS 510 F	Ontario	30.0	353	0.29174	0.32495	0.79
	Manitoba	316	> 360	0.08935	7.397	0.36
	Alberta [a]	372	Rate constant			0.74
M510F47	Ontario	< 5	< 5	Estimated		
	Manitoba	< 5	< 5	Estimated		
	Alberta	10.5	26.2	1.73167	0.0469	0.73
M510F49	Ontario	< 301	< 301	Estimated		
	Manitoba	Not detected				
	Alberta	No decline				

[a] Simple regression – rate constant

E. RESIDUE MOBILITY

In general, no BAS 510 F residues were found below the 15 cm depth in the treated bare soil plots.

Water movement (flux) did occur down through the 120 cm sampling zone. Flux calculations indicate that 27.2, 3.1, and 13.3 cm of water leached through the 120 cm depth sampling zone in the bare soil plots at all three sites. Therefore, the physical conditions (water flux) required to allow compound movement were present at each site.

Based on the TDR measurements and the resulting flux calculations, it is evident that the compound and its degradates were provided opportunity to move down through the soil profile but did not.

III. CONCLUSION

Results from this study have shown that BAS 510 F dissipates steadily and does not accumulate when applied under worst case, bare soil conditions.

Report: CA 7.1.2.2.1/12
Jackson S.,White M., 2003a
2000 Field dissipation of BAS 510 .. F in terrestrial use patterns
2002/5004651
Guidelines: EPA 164-1
GLP: yes
(certified by United States Environmental Protection Agency)

Executive Summary

This study was designed to support a terrestrial use pattern according to EPA Guideline 164-1. The test substance BAS 510 .. F is a broad spectrum fungicide which will be used to control many diseases in field, row crop, orchard, and vineyard crops as well as in turf. This study consisted of a single field trial conducted in Northern Texas, and is representative of major crop producing regions in the United States.

A single untreated control plot (bare soil), one treated bare soil plot, and one cropped plot was established at the test site. The plots were located on a soil typical for the area. The treated plots received three broadcast applications of the test substance, using 374 L ha^{-1} (40 gal ac^{-1}) spray volume at fourteen day intervals (targeted). The test substance was applied beginning in August 2000, at a rate of 504 g a.s. ha^{-1} (0.45 lb a.s. ac^{-1}). Applications were made directly to bare soil plots in an effort to answer guideline questions.

Soil samples were collected to a 122 cm (48 inch) depth using zero contamination sampling equipment. The treated plots were scheduled to be sampled on the following sampling events: -T1,T1,T1+2, T1+3, T1+5, T1+7, T1+9, -T2, T2, -T3, T3, 1,2, 3, 5, 7, 10, 14, 21, 30, 60, 90, 120, 180, 270, 360, 450 and 540 Days After Last Application (DALA). The untreated plot was scheduled to be sampled on the following seven sampling events: -T1, 1, 5, 30, 90, 180 and 360 DALA intervals. A sampling variance of ± 2 days was acceptable after thirty days. After collection, samples were analyzed using HPLC-MS/MS for parent and degradates M510F47 (2-chloronicotinic acid) and M510F49. Based on soil core analysis, the half-life of each analyte was calculated. A summary of the calculated dissipation times (DT) in soil for BAS 510 F parent and degradates are presented below:

Table 7.1.2.2.1-81: Estimated first-order DT_{50} and DT_{75} values of boscalid and its metabolites M510F47 (2-chloronicotinic acid) and M510F49

Compound	Site location	DT_{50} [d]	DT_{75} [d]	α	β	r^2
BAS 510 F	Bare soil	133.9	269	70.132	$7.41* 10^{-5}$	0.72
	Crop plot	240	489	20.110	$1.45 * 10^{-4}$	0.53
M510F47	Bare soil	< 30	< 30	Estimate		
	Crop plot	< 30	< 30	Estimate		
M510F49	Bare soil	< 4	< 4	Estimate		
	Crop plot	< 4	< 4	Estimate		

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

Test item (formulation): BAS 510 00 F
Active substance (a.s.): Boscalid (BAS 510 F, Reg. No. 300355)
Chemical name (IUPAC): 2-chloro-N-(4'-chloro-biphenyl-2-yl)nicotinamide
Molar mass: 343.2 g mol^{-1}
Batch No.: AF543-17 (containing 69.6% boscalid)
Type of formulation: WG (wettable granule)

2. Test sites

The dissipation of boscalid under field conditions was investigated at one field trial site in the U.S. The site characteristics including the basic soil parameters of the corresponding soil horizons are presented in Table 7.1.2.2.1-82.

Table 7.1.2.2.1-82: Characteristics of the trial site Texas used in the field dissipation study

Trial	**RCN 99512 (TX)**							
Location	**Texas (TX), USA**							
Soil properties	**0-6 in (0-15 cm)**	**6-12 in (15-30 cm)**	**12-18 in (30-46 cm)**	**18-24 in (46-61 cm)**	**24-30 in (61-76 cm)**	**30-36 in (76-91 cm)**	**36-42 in (91-107 cm)**	**42-48 in (107-122 cm)**
Soil class (USDA)	Loamy sand	Loamy loam	Loamy sand	Sandy loam	Sandy loam	Sandy clay loam	Sandy clay loam	Sandy clay loam
sand [%]	88	87	81	75	65	59	57	51
silt [%]	6	8	10	12	16	16	18	24
clay [%]	6	5	9	13	19	25	25	25
Organic matter [%]	1.4	0.4	0.4	0.5	0.6	0.4	0.4	0.3
pH [c]	6.7	7.7	7.6	7.6	7.8	7.9	7.9	8.1
CEC [meq $100g^{-1}$]	9.3	7.5	9.3	10.4	14.9	17.9	13.0	11.9
Moisture (gravimetric) at 1/3 bar [%][a]	6.3	6.8	9.9	12.8	18.0	22.1	20.6	18.2
Bulk density [g cm^{-3}][b]	1.80	1.85	1.93	1.85	1.81	1.84	1.83	1.77

CEC = Cation exchange capacity
[a] Measured at six composited cores
[b] Measured at undisturbed cores
[c] in the US soil pH is commonly measured in H_2O; this is however not explicitly stated in the report

The field trial was conducted in northern Texas, and is representative of major crop producing regions of the US. Applications of product were made to bare soil and cropped plots under actual field conditions. No product containing boscalid had been used on the test plots in the last three years.

B. STUDY DESIGN

1. Experimental conditions

The trial area was divided into three plots. One plot was used as control plot (untreated), the second (bare soil) and third plot (cropped with peanuts) was treated with the test item. The treated plots as well as the control plot consisted of three sampling sections, each divided into 20 equal subplots. The control plot was separated from the treated plots by a buffer zone of 30.56 m (100 ft) width. The size of each subplot was 2.32 m^2 (25 sq ft).

The product, formulated as a WG (wettable granule), was applied by ground rig spray equipment (tractor-mounted, flat boom sprayers). Three applications at a nominal rate of 504 g a.s. ha^{-1} (0.450 lb a.s. ac^{-1}) were conducted. The applications were performed from mid-August until mid-September 2000. The actual application rates determined by quantifying the amount of spray discharged ranged from 504 to 516 g a.s. ha^{-1} (0.450 – 0.0.460 lb a.s. ac^{-1}). Details of the application are presented in Table 7.1.2.2.1-83.

Table 7.1.2.2.1-83: Application parameters of field trial site treated with boscalid

Test item/ Actual content [a]/ Formulation type	Application method	No. of applications	Application rate per treatment			No. of treated replicates	Application date
			Nominal [g a.s. ha^{-1}]	Actual [g a.s. ha^{-1}]	Dose verification [b]		
BAS 510 F 1.937 g a.s. L^{-1} WG	Broadcast spray to cropped soil	3	504	508 504 504	99.7-100.7% of nominal rate	3	11-Aug-00 25-Aug-00 08-Sep-00
BAS 510 F 1.937 g a.s. L^{-1} WG	Broadcast spray to bare soil	3	504	516 504 504	100.0-101.3% of nominal rate	3	11-Aug-00 25-Aug-00 08-Sep-00

[a] Values represent the range of test substance concentration in the spray solution (n = 3)
[b] Determined by sprayer calibration/pass time method.

A Speedisk™ application verification (AV) technique was used to confirm applications in this study. The Speedisk™ product resembles a short walled Buchner funnel that has C18 material placed in the bottom of the “funnel". The Speedisk™'s were placed randomly in the bare soil plot before each application. The fraction caught in each device was then used to calculate an application rate. In addition to the Speedisk™AV technique, a sprayer calibration/pass time method and zero-time core recovery method were used to validate application rates.

A sprayer calibration/pass time method was used to confirm applications in this study. In addition to the sprayer calibration/pass time method, a petri dish application verification (AV) technique, and a zero-time core recovery method were used to validate application rates.

Verification of application with the petri dish AV technique is simply the amount of test substance found per surface area of the petri dishes adjusted up to represent a value per acre.

The zero-time sample interval is defined as the first sample collected post application to the plot. Normally zero-time calculations are based on the last application in a series if multiple applications have been made. Zero-time core concentrations (bare soil only) were calculated by subtracting parent residue in the 0-15 cm (0-6 inch) core before the last application was made (-T3) from the parent residue measured immediately after the last application (T3). For calculation purposes, soil weight was based on a furrow acre slice (907184 kg/15 cm; 2,000,000 lb/6 inch).

No tillage was performed during the course of the study from first to last sampling. The plots were kept free of weeds via the application of paraquat.

Weather data was collected from weather stations located in Wellington, Texas, approximately eight and five miles away from the test site, respectively. Onsite rain was measured using a NWS rain gauge. Irrigation was applied to supplement normal precipitation so that the plots would receive 110% of the historical average rainfall for the study period or crop evapotranspiration. Historical weather data (average monthly minimum and maximum air temperatures and monthly precipitation totals) were submitted for at least a ten-year period from a reliable source located no more than 13 km (8 miles) from the test site.

A summary of monthly weather data (maximum and minimum temperatures, precipitation, as well as volumes of the supplementary irrigation) is presented in Table 7.1.2.2.1-84.

Table 7.1.2.2.1-84: Summary of monthly air temperature (max/min), precipitation, and irrigation at field trial site

Trial	RCN 99512 (TX)				
Location	Texas, USA				
Month/ Year	T_{min} Air [°C]	T_{max} Air [°C]	Precipitation [mm]	Irrigation [mm]	Total [mm]
Aug-00	19.3	38.1	0.0	180.3	180.3
Sep-00	14.1	35.1	0.0	238.8	2296.2
Oct-00	10.3	23.2	146.1	66.0	212.1
Nov-00	0.5	15.0	62.2	0.0	62.2
Dec-00	-4.4	7.2	35.6	0.0	35.6
Jan-01	-3.2	9.4	17.8	0.0	17.8
Feb-01	-2.9	11.9	53.3	0.0	53.3
Mar-01	0.6	12.7	38.1	0.0	38.1
Apr-01	2.5	26.5	0.0	61.0	61.0
May-01	5.4	29.9	279.4	0.0	279.4
Jun-01	7.1	36.4	97.5	0.0	97.5
Jul-01	22.2	40.3	69.9	0.0	69.9
Aug-01	20.9	37.3	73.7	0.0	73.7
Sep-01	15.4	31.1	88.9	0.0	88.9
Oct-01	6.3	26.8	0.0	68.6	68.6
Nov-01	2.0	16.3	99.1	0.0	99.1
Dec-01	-0.8	14.9	35.6	0.0	35.6
Jan-02	-4.2	13.4	26.7	0.0	26.7
Feb-02	-1.8	13.6	0.0	69.9	69.9
Mar-02	-2.6	17.6	0.0	0.0	0.0
Total	-	-	**1123.7**	**684.5**	**1808.2**

2. Sampling

Samples from treated plots were collected on 28 occasions one day prior each of the six applications (-T), on the day of each application (T), between the first and second application (T1+2, T1+3, T1+5, T1+7, T1+9), and 1, 2, 3, 5, 7, 10, 14, 21, 30, 60, 90, 120, 180, 270, 360, 450 and 540 days after the last application (DALA). Samples from the control plot were collected on seven occasions one day before the first application (-T1) and 1, 5, 30, 90, 180 and 360 DALA. The specified dates refer to planned sampling dates. Actual sampling dates are given in brackets in the results tables.

Soil cores from the treated plots were taken to a depth of 122 cm (48 inch) in two stages at each sampling interval. For each sampling event, five 0-15 cm (0-6 inch) and five 15-122 cm (6-48 inch) soil cores were collected from each of three randomly selected subplots, and were designated as replicates A, B, and C. This resulted in a total of fifteen each of the 0-15 cm (0-6 inch) and 15-122 cm (6-48 inch) cores collected at each sampling interval for each treated plot. The 0-15 cm cores were either sampled in two runs or sectioned after sampling to obtain a 0-8 cm (0-3 inch) and a 8-15 cm (3-6 inch) soil core. The 15-122 cm (6-48 inch) cores were cut frozen into 15 cm (6 inch) increments. Soil cores were composited by depth within a replicate to form completed samples.

All soil specimens were placed into freezer storage at about -18°C and remained frozen until processing or analysis of the samples.

3. Description of analytical procedure

Samples were received from the field frozen and were stored at ≤ 0°C. Samples were homogenized at BASF prior to analysis. After homogenization, samples were analyzed using BASF Draft Analytical Method D0004/1 [*already peer-reviewed study BASF DocID 2001/5000881*]. The method used for the residue analyses is described below.

The residues of boscalid, M510F47 (2-chloronicotinic acid), and 2-hydroxy-N-(4'-chlorobiphenyl-2-yl)nicotinamide (M510F49) were extracted from soil by shaking with methanol followed by methanol/water (50:50, v/v) and sonication. An aliquot of the extract was diluted with a buffer solution (water or water/methanol 80:20 [v/v] with 0.3% formic acid and 4 mM ammonium formate), filtered, and quantitated by HPLC-MS/MS for boscalid, M510F47 and M510F49. The limit of quantitation (LOQ) for each analyte was 0.010 mg kg^{-1}.

Control samples were fortified either with BAS 510 F, M510F47 or M510F49. A summary of the average procedural recoveries for samples analyzed is provided in section II.2.

Application verification (AV) samples were kept below 0°C during shipping and storage until extraction. Samples were shaken and centrifuged twice with methanol/water. The samples were then serial diluted using 70/30 methanol/buffer water and quantitated using LC-MS/MS. Due to the concentration of the samples, it was not necessary to establish a limit of quantitation for this procedure.

4. Storage stability experiments

The actual length of time between sample collection in the field and sample extraction at the laboratory was recorded on sample residue records. Samples were shipped in freezer trucks to BASF for analysis. The samples were kept frozen during shipment to and storage at BASF. The maximum period any sample was stored before analysis was 624 days. Results of recovery tests revealed that the test item (boscalid) is stable during freezer storage.

In addition, the storage stability of boscalid was investigated in a separate study [*peer-reviewed study BASF DocID 2000/1000136]*.

5. Kinetic evaluation

In order to calculate dissipation times (DT_{50}/DT_{75}), the reported residue values were mathematically averaged by replicate to produce a grand mean. In the case where a replicate was analyzed in duplicate or triplicate, the numbers were then averaged to produce a mean for that replicate before averaging with the other replicates. When a replicate sample was below the LOQ or < 0.01 mg kg^{-1}, it was averaged as zero. To determine analyte half-life values, the residues in all soil depths were summed to produce the total residue concentration per sampling period. The summed residue concentrations per sampling period were natural log transformed before analysis by non-linear regression.

The non-linear regression was solved by applying the Gustafson-Holden model (FOMC), using the Origin Scientific Graphics Software (OriginLab Corporation, Northampton, MA, USA; version 7.0, 2000).

6. Residue mobility

To determine if residues had an opportunity to move down through the soil profile with the soil solution, the water content by depth was determined using Time Domain Reflectometry (TDR) was applied. Soil water flux was also calculated at a 122 cm (4 ft) depth based on this method. The TDR system used in this study was a CR10X datalogger (Campbell Scientific Inc., Logan, UT, USA) configured with four CS615 probes located in one of the bare soil subplots. The probes were installed vertically at the 0-30, 30-61, 61-91 and 91-122 cm (0-1, 1-2, 2-3, and 3-4 ft) depths. Since the rods of the CS615 are 30.5 cm (12 inch) in length, vertical installation provides a continuum of volumetric water content measurements from the soil surface to a depth of 122 cm (4 ft).

II. RESULTS AND DISCUSSION

1. Application verification

Application results from the Speedisk™ AV technique ranged from 103.9% to 108.1% of the target rate. Actual pass/time and spray equipment calibration data indicated that the target field application rate was precise and accurate. The variance observed in application Speedisk™ AV recoveries was assumed to be a reflection of the technique rather than a demonstration of variance in actual application to plots. Zero-time core recoveries were 84% based on the bare soil plot (broadcast application) of the respective field trial site. Differences in calculated application rates to the field and those calculated from zero time cores are probably due to sample handling and site specific soil properties. More details on the application verification results are given in the study report.

2. Residues in field soil samples

Mean procedural recoveries from control soil samples fortified with either boscalid, M510F47 or M510F49 at four levels (0.01, 0.1, 1.0 or 2.0 mg kg^{-1}) were generally within the acceptable range of 70-120% (see Table 7.1.2.2.1-85). A summary of the individual procedural recovery results is provided in the study report.

Table 7.1.2.2.1-85: Method procedural recoveries

Trial site	Mean recovery ± RSD [%]		
	BAS 510 F	M510F47	M510F49
Texas	99 ± 12 (n=62)	96 ± 13 (n=62)	92 ± 12 (n=62)

RSD = Relative standard deviation [%]

Field soil samples taken from different depths were analyzed to a maximum of about 540 days after the last of the three applications. The analytical results are summarized in Table 7.1.2.2.1-86 and Table 7.1.2.2.1-87. All residue values presented in these tables are related to the dry weight of the soil and were not corrected for procedural recoveries. Residue concentrations are reported according to soil depth (0-8, 8-15, 15-30, and 30-46 cm, 46-61 cm, 0-3, 3-6, 6-12, 12-18, 18-24 inch).

Table 7.1.2.2.1-86: Residues of boscalid and its metabolites M510F47 (2-chloronicotinic acid) and M510F49 [mg kg-1 dry weight] in treated bare soil samples (mean of three replicates) – Texas site (RCN 99512-TX)

Compound [a]	Soil depth [cm]	Targeted days after last application [b]														
		-T1	T1	T1+2	T1+3	T1+5	T1+7	T1+9	-T2	T2	-T3	T3	1	2	3	5
boscalid	0-8	<0.01	0.22	0 22	0.25	0.28	0.21	0.22	0.17	0.41	0.37	0.70	0.45	0.50	0.49	0.55
	8-15	<0.01	<0.01	0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.02	0.02	0.02	0.02	0.05	0.06
	15-30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	30-46	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	46-61	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
M510F47	0-8	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	8-15	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	15-30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	30-46	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	46-61	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
M510F49	0-8	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.01	<0.01	<0.01	<0.01	<0.01
	8-15	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	15-30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	30-46	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	46-61	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

Compound [a]	Soil depth [cm]	Targeted days after last application [b]												
		7	10	14	21	30	60	90	122 (120)	179 (180)	270	364 (360)	455 (450)	540
boscalid	0-8	0.52	0.45	0.13	0.40	0.59	0.22	0.37	0.24	0.29	0.16	0.08	<0.01	0.07
	8-15	0.03	0.02	0.16	0.05	0.03	0.19	0.14	0.12	0.35	0.07	0.06	0.02	<0.01
	15-30	<0.01	<0.01	<0.01	<0.01	<0.01	0.02	0.01	0.01	<0.01	0.012	0.02	<0.01	<0.01
	30-46	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	46-61	n.a.	n.a.	n.a.	n.a.	n.a.	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
M510F47	0-8	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	8-15	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	15-30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	30-46	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	46-61	n.a.	n.a.	n.a.	n.a.	n.a.	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
M510F49	0-8	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	8-15	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	15-30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	30-46	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	46-61	n.a.	n.a.	n.a.	n.a.	n.a.	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

T = Application dates; n.a. = Not analyzed

[a] The limit of quantification (LOQ) was 0.01 mg kg^{-1} for each analyte. All residue values were corrected for moisture content and are reported here on a dry weight basis. All value of 0 µg kg^{-1} was used in average calculations for samples where residues were reported as <0.01 mg kg^{-1}.

[b] Sampling dates refer to the targeted sampling dates, the actual sampling dates are given in brackets.

Table 7.1.2.2.1-87: Residues of boscalid and its metabolites M510F47 (2-chloronicotinic acid) and M510F49 [mg kg^{-1} dry weight] in treated cropped soil samples (mean of three replicates) – Texas site (RCN 99512-TX)

Compound [a]	Soil depth [cm]	Targeted days after last application [b]														
		-T1	T1	T1+2	T1+3	T1+5	T1+7	T1+9	-T2	T2	-T3	T3	1	2	3	5
boscalid	0-8	<0.01	0.18	0.05	0.05	0.14	0.12	0.20	0.13	0.19	0.26	0.34	0.24	0.32	0.36	0.38
	8-15	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.02	0.02	0.02	0.01	0.01	0.03
	15-30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	30-46	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	46-61	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
M510F47	0-8	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	8-15	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	15-30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	30-46	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	46-61	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
M510F49	0-8	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	8-15	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	15-30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	30-46	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	46-61	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

Compound [a]	Soil depth [cm]	Targeted days after last application [b]												
		7	10	14	21	30	60	90	122 (120)	179 (180)	270	364 (360)	455 (450)	540
boscalid	0-8	0.42	0.37	0.19	0.37	0.33	0.27	0.38	0.43	0.28	0.20	0.09	0.09	0.06
	8-15	0.05	0.02	0.05	0.03	0.02	0.18	0.14	0.10	0.13	0.03	0.05	0.05	<0.01
	15-30	<0.01	<0.01	<0.01	<0.01	<0.01	0.01	0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.02
	30-46	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	46-61	n.a.	n.a.	n.a.	n.a.	n.a.	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
M510F47	0-8	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.01	<0.01
	8-15	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	15-30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.02	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	30-46	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	46-61	n.a.	n.a.	n.a.	n.a.	n.a.	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
M510F49	0-8	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	8-15	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	15-30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	30-46	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	46-61	n.a.	n.a.	n.a.	n.a.	n.a.	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

T = Application dates; n.a. = Not analyzed

[a] The limit of quantification (LOQ) was 0.01 mg kg^{-1} for each analyte. All residue values were corrected for moisture content and are reported here on a dry weight basis. All value of 0 µg kg^{-1} was used in average calculations for samples where residues were reported as <0.01 mg kg^{-1}. [b] Sampling dates refer to the targeted sampling dates, the actual sampling dates are given in brackets.

D. KINETIC EVALUATION

A summary of calculated DT_{50} and DT_{75} values for boscalid and its metabolites M510F47 and M510F49 (where possible) can be found in Table 7.1.2.2.1-88.

Table 7.1.2.2.1-88: Estimated first-order DT_{50} and DT_{75} values of boscalid and its metabolites M510F47 (2-chloronicotinic acid) and M510F49

Compound	Site location	DT_{50} [d]	DT_{75} [d]	α	β	r^2
BAS 510 F	Bare soil	133.9	269	70.132	$7.41* 10^{-5}$	0.72
	Crop plot	240	489	20.110	$1.45 * 10^{-4}$	0.53
M510F47	Bare soil	< 30	< 30	Estimate		
	Crop plot	< 30	< 30	Estimate		
M510F49	Bare soil	< 4	< 4	Estimate		
	Crop plot	< 4	< 4	Estimate		

E. RESIDUE MOBILITY

In general, residues of boscalid remained in the top 15 cm (6 inch) of soil. There were some spurious detect of parent compound and M510F47 at the 15-30 cm (6-12 inch) depth.

Water movement (flux) did occur down through the 122 cm (48 inch) sampling zone. Flux calculations indicate that 711 mm (28 inch) of water leached through the 122 cm (48 inch) depth sampling zone in the bare soil plot. Therefore, the physical conditions (water flux) required to allow compound movement were present at each site.

However, based on the TDR measurements and the resulting flux calculations, it is evident that the compound and its degradates were provided opportunity to move down through the soil profile but did not other than the few spurious detects.

III. CONCLUSION

Results from this study have shown that Boscalid dissipates steadily and does not accumulate when applied under worst case, bare soil conditions.

Report:	CA 7.1.2.2.1/13 Budde E.,Bisharat R., 2013a Kinetic evaluation of five field dissipation studies with BAS 510 F - Boscalid conducted in 1997 and 2007 in Europe: Determination of modeling endpoints according to EFSA 2013/1285541
Guidelines:	FOCUS Kinetics (2006) SANCO/10058/2005 version 2.0, EFSA Guidance to obtain DegT50 values in soil (2010)
GLP:	no

Executive Summary

The dissipation behavior of BAS 510 F – boscalid in soil has been investigated in five field dissipation studies including 14 field trials. The purpose of this evaluation was to analyze the degradation kinetics of boscalid observed in the 14 soils according to the current guidance of the FOCUS workgroup on degradation kinetics, under consideration of the recommendations provided in the EFSA guidance to obtain $DegT_{50}$ values in soil for modeling purposes.

Prior to kinetic evaluation, the sampling intervals of the field studies were normalized to reference conditions regarding soil moisture and temperature according to the time-step normalization technique. Kinetic evaluation was performed on the normalized sampling interval dataset in order to derive degradation parameters that can be used as modeling endpoints.

Modeling endpoints were derived under consideration of the EFSA Guidance as well as recommendations of the FOCUS kinetics working group and were based on a visual and statistical assessments of the kinetic model fits.

Kinetic evaluation of the time-step normalized dataset (20°C, pF2) resulted in normalized field half-lives ($DegT_{50}$) for boscalid between 84.0 and 238.4 days, when all sampling days were included, and between 82.1 and 232.5 days when only data after 10 mm of rainfall had occurred in the study were considered.

I. MATERIAL AND METHODS

Soils

The degradation behavior of boscalid was investigated in five field dissipation studies with 14 field trials in total [*already peer-reviewed studies BASF DocID 2000/1000123, BASF DocID 2000/1013295; new studies CA 7.1.2.2.1/3, BASF DocID 2002/1004283; CA 7.1.2.2.1/6, BASF DocID 2010/1140925; CA 7.1.2.2.1/5, BASF DocID 2010/1126049*]. The trials were situated in typical agricultural regions in Germany (seven trials), France (two trials), Spain (two trials), Sweden (one trial), Italy (one trial) and Denmark (one trial), considering a range of different soils and climatic conditions.

Kinetic modeling

The software package KinGUI version 2.2012.320.1629 was used for parameter fitting [*SCHÄFER et al. (2007)*]. The error tolerance and the number of iterations of the optimization tool (IRLS) were set to the default values of 1×10^{-6} and 100, respectively.

Datasets were prepared for kinetic evaluation as follows:

- Values below the quantification or detection limit were treated as recommended by the FOCUS workgroup [*FOCUS (2006)*]. The LOQ for boscalid reported in the five studies was 0.01 mg kg^{-1}.. A limit of detection (LOD) was not provided in the study reports and was therefore set equal to LOQ. According to FOCUS, values below LOD were set to 0.5 x LOD = 0.005 mg kg^{-1}.
- For each sampling point, the concentration of a compound in the single soil layer given in mg kg^{-1} was transformed to its residue given in g ha^{-1} considering the thickness of the respective segment and undisturbed soil bulk densities for each soil layer. In case soil bulk densities were not provided, a bulk density of 1.5 g cm^{-3} was used. The total residues in the sampled soil core were calculated as the sum of residues of the single soil core segments.

The measured data as well as resulting datasets submitted to kinetic analysis are provided in the original evaluation report.

Normalization procedure

Evaluation of the suitability of field dissipation data for normalization was performed according to the evaluation criteria for normalization compiled by the Dutch regulatory authority (CTB criteria) [*CTB (1999)*].

The time-step normalization procedure was carried out by reducing or increasing day lengths depending on soil temperature and moisture by means of correction factors (f_{temp} and f_{moist}) identical to those used in most regulatory leaching models.

Temperature correction factors (f_{temp}) were determined to account for differences in degradation rates between those at actual daily soil temperatures as calculated by FOCUS-PEARL 4.4.4 and a reference temperature of 20°C using the Q_{10} approach as described in the report of the FOCUS soil modeling working group [see *FOCUS (1997)*]. The Q_{10} response function was applied for temperatures above 0 °C (see Equation 7.1.2.2.1-1 c). Below field temperatures of 0 °C it was assumed that no degradation occurs (Equation 7.1.2.2.1-1 c). For the evaluation, the EFSA opinion on the default Q_{10} value [see *EFSA (2007)*] was followed and a Q_{10} value of 2.58 was used in the assessment.

Moisture correction factors (f_{moist}) were determined to account for differences between actual daily soil moisture as calculated by FOCUS-PEARL 4.4.4 and the reference soil moisture at field capacity (pF 2) (Equation 7.1.2.2.1-1 d).

Normalized day lengths for the kinetic evaluation were derived according to Equation 7.1.2.2.1-1 a. For DAT 0, no normalization was considered and application was assumed to occur at the time point zero. Normalized sampling days (DAT_{norm}) after application were calculated by cumulatively summing up normalized day lengths according to Equation 7.1.2.2.1-1 b.

As outlined by EFSA *[EFSA (2010): Guidance for evaluating laboratory and field dissipation studies to obtain DT_{50} values of plant protection products in soil. EFSA Journal 2010; 8(12):1936. [67 pp.]]* the guidance proposes the splitting of field dissipation studies into two parts viz. before and after at least 10 mm of rain has fallen since application. The kinetic evaluation of data should concentrate on the residues measured after 10 mm of cumulative rain fall in order to guarantee that the results of the evaluation describe the degradation in the soil matrix rather than at the soil surface. A kinetic evaluation was performed for those data sets where an appropriate number of data points remained after the samplings before 10 mm of cumulative rainfall were excluded.

Equation 7.1.2.2.1-2: Calculation of normalized day length based on combination of soil moisture and soil temperature correction factors

a) $$D_{norm} = D * f_{temp} * f_{moisture}$$

b) $$t_i = \sum_{t=1}^{i-1} D_{norm}$$

with:

t_i =	Time from application till sampling at day i	[d]
D_{norm} =	Normalized day length (20°C, pF2)	[d]
i =	Time span between application and sampling	[d]

c) $$f_{temp} = \begin{cases} Q_{10}^{\frac{Tact-Tref}{10}} & \text{for } T_{act} > 0°C \\ 0 & \text{for } T_{act} \leq 0°C \end{cases}$$

d) $$f_{moist} = \begin{cases} \left(\frac{\theta_{act}}{\theta_{ref}}\right)^B & \text{for } \theta_{ref} > \theta_{act} \\ 1 & \text{for } \theta_{ref} \leq \theta_{act} \end{cases}$$

with:

D_{norm} =	normalized day length (temperature and moisture)	
D =	actual day length (1 d)	[days]
f_{temp} =	temperature correction factor	[-]
f_{moist} =	moisture correction factor	[-]
T_{act} =	actual soil temperature (°C)	[C°]
T_{ref} =	reference temperature (20 °C)	[C°]
Q_{10}=	factor of increase of degradation rate with an increase in temperature of 10 °C (Q_{10} = 2.58 [EFSA 2007])	[-]
θ_{act} =	actual soil moisture (vol. water content)	[m^3 m^{-3}]
θ_{ref} =	reference soil moisture at pF2	[m^3 m^{-3}]
B =	exponent of the moisture response function, B = 0.7	[-]

Table 7.1.2.2.1-89 shows the field sampling dates for the trial locations and the normalized (20°C, pF2) day lengths based on soil moisture and soil temperature data as simulated by FOCUS-PEARL 4.4.4.

Table 7.1.2.2.1-89: Time-step normalized (temperature and moisture) sampling days

DU2/15/97		DU3/06/97		ALO/05/98		ALO/06/98		D05/03/98	
DAT	**D_{norm}**	**DAT**	**D_{norm}**	**DAT**	**D_{norm}**	**DAT**	**D_{norm}**	**DAT**	**D_{norm}**
0	0	0	0	0	0	0	0	0	0
13	6.4	12	4.5	14	12.8	15	13.8	14	6.7
30	16.6	28	13.5	30	26.1	30	34.1	30	14.5
57	34.5	61	37.6	60	66.7	63	79.8	59	32.8
93	62.4	97	66.9	98	130.9	99	129.3	97	58.9
176	128.0	179	138.3	182	219.9	182	193.7	181	102.4
365	173.5	367	187.6	349	298.6	356	277.0	357	137.1
449	229.9	452	252.4	-	-	-	-	-	-
544	295.7	545	323.1	-	-	-	-	-	-

HUS/10/98		BKA/666/000/ RES1		BKA/666/000/ RES2		L070706		L070707	
DAT	**D_{norm}**	**DAT**	**D_{norm}**	**DAT**	**D_{norm}**	**DAT**	**D_{norm}**	**DAT**	**D_{norm}**
0	0	7	5.8	7	6.4	0	0	0	0
16	8.3	14	10.0	14	11.8	15	15.9	14	6.6
31	17.2	28	20.1	34	31.6	28	35.8	28	14.8
60	35.1	59	49.2	62	67.1	56	78.6	57	37.8
101	64.3	101	90.3	100	117.1	98	137.3	103	73.9
182	99.3	189	148.6	184	192.5	161	180.9	154	106.4
352	130.3	364	212.7	351	261.5	244	202.2	232	128.2
-	-	-	-	-	-	363	255.6	369	161.3
-	-	-	-	-	-	875	727.5	736	312.4
-	-	-	-	-	-	-	-	824	365.8

DAT = days after treatment
D_{norm} = normalized day (20°C, pF2)

II. RESULTS AND DISCUSSION

The data sets of the 14 trial locations were evaluated for the criterion of 10 mm cumulative rainfall. The evaluation showed that it was possible to perform kinetic analysis for all trial locations, because the number of data points in each of the data sets was still appropriate (between five and nine data points) after excluding sampling dates before 10 mm of cumulative rainfall.

The dissipation behavior of boscalid in the 14 field trials was analyzed in the step-wise approach to derive modeling endpoints proposed in EFSA (2010). A summary of the results of the kinetic evaluation are presented in Table 7.1.2.2.1-90.

Table 7.1.2.2.1-90: Summary of endpoints for use in modeling of boscalid

Field trial	Soil type (DIN)	Kinetic model	All sampling days included		Data > 10 mm rainfall only	
			Rate k [d^{-1}]	$DegT_{50}$ [d]	Rate k [d^{-1}]	$DegT_{50}$ [d]
DU2/15/97 (300 g ha^{-1})	Silty loam	SFO	0.0083	84.0	0.0084	82.1
DU2/15/97 (600 g ha^{-1})	Silty loam	HS	0.0030 [b]	232.5 [c]	0.0030 [b]	232.5 [c]
DU2/15/97 (1200 g ha^{-1})	Silty loam	DFOP	0.0057 [b]	121.6 [c]	0.0057 [b]	121.6 [c]
DU2/15/97			**0.0052**	**133.4**	**0.0052**	**132.4**
DU3/06/97 (300 g ha^{-1})	Silty sand	SFO	0.0038	183.7	0.0035	198.7
DU3/06/97 (600 g ha^{-1})	Silty sand	SFO	0.0038	182.9	0.0035	196.4
DU3/06/97 (1200 g ha^{-1})	Silty sand	SFO	0.0040	171.9	0.0035	195.6
DU3/06/97			**0.0039**	**179.4**	**0.0035**	**196.9**
ALO/05/98	Sandy loam	- [a]	-	-	-	-
ALO/06/98	Sandy loam	SFO	0.0029	238.4	0.0031	225.2
D05/03/98	Loamy sand	SFO	0.0070	98.5	0.0059	117.2
HUS/10/98	Loamy sand	- [a]	-	-	-	-
BKA/666/00/RES1	Medium loamy sand	SFO	0.0045	155.0	0.0045	155.0
BKA/666/00/RES2	Medium loamy sand	HS	0.0041 [b]	168.4 [c]	0.0041 [b]	168.4 [c]
L070706	Lt clay loam	SFO	0.0070	98.3	0.0059	118.0
L070707	Su silty sand	SFO	0.0045	155.3	0.0037	186.0
Geometric mean			**0.0047**	**147.5**	**0.0044**	**158.3**

[a] Field experiment was discarded, because no adequate kinetic model could be derived
[b] Slow rate (k_2)
[c] Calculated from slow rate of biphasic model ($DegT_{50matrix} = ln(2)/k2$))

III. CONCLUSION

Kinetic evaluation of 14 field trials with boscalid, originating from five field dissipation studies, was conducted in order to derive reliable normalized modeling endpoints according to the current guidance of the FOCUS workgroup on degradation kinetics.

Kinetic evaluation of the time-step normalized dataset (20°C, pF2) resulted in normalized field half-lives ($DegT_{50}$) for boscalid between 84.0 and 238.4 days, when all sampling days were included, and between 82.1 and 232.5 days, when only data after 10 mm rainfall had occurred in the study were considered.

Report:	CA 7.1.2.2.1/14 Budde E., 2012a Kinetic evaluation of six field dissipation studies with BAS 510 F - Boscalid conducted between 1999 and 2002 in North America: Determination of modeling endpoints according to EFSA 2012/1189904
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., EFSA Guidance to obtain DT50 values in soil (2010)
GLP:	no

Executive Summary

The dissipation behavior of BAS 510 F – boscalid in soil has been investigated in six field dissipation studies including 15 field trials. The purpose of this evaluation was to analyze the degradation kinetics of boscalid observed in the 15 soils according to the current guidance of the FOCUS workgroup on degradation kinetics, under consideration of the recommendations provided in the EFSA guidance to obtain $DegT_{50}$ values in soil for modeling purposes.

Prior to kinetic evaluation, the sampling intervals of the field studies were normalized to reference conditions regarding soil moisture and temperature according to the time-step normalization technique. Kinetic evaluation was performed on the normalized sampling interval dataset in order to derive degradation parameters that can be used as modeling endpoints.

Modeling endpoints derived under consideration of the EFSA Guidance as well as recommendations of the FOCUS kinetics working group were based on a visual and statistical assessment.

Kinetic evaluation of the time-step normalized dataset (20°C, pF2) resulted in normalized field half-lives ($DegT_{50}$) for boscalid between 51.9 and 243.4 days, when all sampling days were included, and between 62.1 and 300.4 days, when only data with > 10 mm rainfall were considered.

I. MATERIAL AND METHODS

Soils

The degradation behavior of boscalid was investigated in six field dissipation studies with 15 field trials in total [*CA 7.1.2.2.1/10, BASF DocID 2001/5000937; CA 7.1.2.2.1/9, BASF DocID 2001/5000936; CA 7.1.2.2.1/7, BASF DocID 2000/5277; CA 7.1.2.2.1/8, BASF DocID 2001/5000833; CA 7.1.2.2.1/11, BASF DocID 2001/5000938; CA 7.1.2.2.1/12, BASF DocID 2002/5004651*]. The trials were situated in typical agricultural regions in the USA (twelve trials) and Canada (three trials), considering a range of different soils and climatic conditions.

Kinetic modeling

The software package KinGUI version 2.2012.320.1629 was used for parameter fitting [*SCHÄFER, et al. (2007)*]. The error tolerance and the number of iterations of the optimization tool (IRLS) were set to the default values of 1×10^{-6} and 100, respectively.

Datasets were prepared for kinetic evaluation as follows:

- Values below the quantification or detection limit were treated as recommended by the FOCUS workgroup [*FOCUS (2006)*]. The LOQ for boscalid reported in the studies was 0.01 mg kg^{-1}. A limit of detection (LOD) was not provided in the study reports and was therefore set equal to LOQ. According to FOCUS, values below LOD were set to 0.5 x LOD = 0.005 mg kg^{-1}.
- For each sampling point, the concentration of a compound in the single soil layer given in mg kg^{-1} was transformed to its residue given in g ha^{-1} considering the thickness of the respective segment and using the middle point of the reported range of undisturbed soil bulk densities for each soil layer. The total residues in the sampled soil core were calculated as the sum of residues of the single soil core segments.

The measured data as well as resulting datasets submitted to kinetic analysis are provided in the original evaluation report.

Normalization procedure

Evaluation of the suitability of field dissipation data for normalization was performed according to the evaluation criteria for normalization compiled by the Dutch regulatory authority (CTB criteria).

Temperature correction factors (f_{temp}) were determined to account for differences between actual daily soil temperatures as calculated by FOCUS-PEARL 4.4.4 and a reference temperature of 20°C using the Q_{10} approach as described in the report of the FOCUS soil modeling working group [*FOCUS (1997)*]. The Q_{10} response function was applied for temperatures above 0 °C (Equation 7.1.2.2.1-3 c). Below field temperatures of 0 °C it was assumed that no degradation occurs (Equation 7.1.2.2.1-3 c). For the evaluation, the EFSA opinion on the default Q_{10} value [*EFSA (2007)*] was followed and a Q_{10} value of 2.58 was used in the assessment.

The time-step normalization procedure was carried out by reducing or increasing day lengths depending on soil temperature and moisture by means of correction factors (f_{temp} and f_{moist}) identical to those used in most regulatory leaching models.

Moisture correction factors (f_{moist}) were determined to account for differences between actual daily soil moisture as calculated by FOCUS-PEARL 4.4.4 and the reference soil moisture at field capacity (pF 2) (Equation 7.1.2.2.1-3 d).

Normalized day lengths for the kinetic evaluation were derived according to Equation 7.1.2.2.1-3 a. For DAT 0, no normalization was considered and application was assumed to occur at the time point zero. Normalized sampling days (DAT_{norm}) after application were calculated by cumulatively summing up normalized day lengths according to Equation 7.1.2.2.1-3 b.

Equation 7.1.2.2.1-3: Calculation of normalized day length based on combination of soil moisture and soil temperature correction factors

a)
$$D_{norm} = D * f_{temp} * f_{moisture}$$

b)
$$t_i = \sum_{t=1}^{i-1} D_{norm}$$

with:

t_i =	Time from application till sampling at day i	[d]
D_{norm} =	Normalized day length (20°C, pF2)	[d]
i =	Time span between application and sampling	[d]

c)
$$f_{temp} = \begin{cases} Q_{10}^{\frac{Tact-Tref}{10}} & \text{for } T_{act} > 0°C \\ 0 & \text{for } T_{act} \leq 0°C \end{cases}$$

d)
$$f_{moist} = \begin{cases} (\frac{\theta_{act}}{\theta_{ref}})^B & \text{for } \theta_{ref} > \theta_{act} \\ 1 & \text{for } \theta_{ref} \leq \theta_{act} \end{cases}$$

with:

D_{norm} =	normalized day length (temperature and moisture)	
D =	actual day length (1 d)	[days]
f_{temp} =	temperature correction factor	[-]
f_{moist} =	moisture correction factor	[-]
T_{act} =	actual soil temperature (°C)	[C°]
T_{ref} =	reference temperature (20 °C)	[C°]
Q_{10}=	factor of increase of degradation rate with an increase in temperature of 10 °C (Q_{10} = 2.58 [EFSA 2007])	[-]
θ_{act} =	actual soil moisture (vol. water content)	[m^3 m^{-3}]
θ_{ref} =	reference soil moisture at pF2	[m^3 m^{-3}]
B =	exponent of the moisture response function, B = 0.7	[-]

The field sampling dates for the trial locations and the normalized (20°C, pF2) day lengths based on soil moisture and soil temperature data as simulated by FOCUS-PEARL 4.4.4 are presented in Table 7.1.2.2.1-91 to Table 7.1.2.2.1-93.

Table 7.1.2.2.1-91: Time-step normalized (temperature and moisture) sampling days (field trials 99502 – 99508)

99502		99503		99506		99507		99508	
DAT	**D_{norm}**	**DAT**	**D_{norm}**	**DAT**	**D_{norm}**	**DAT**	**D_{norm}**	**DAT**	**D_{norm}**
0	0	0	0	0	0	0	0	0	0
1	0.5	1	1.5	1	1.6	1	1.6	1	1.6
2	1.1	2	3.1	2	2.9	2	2.9	2	3.4
3	1.7	3	4.7	3	4.2	3	3.8	3	5.4
7	4.8	5	7.7	5	6.9	5	6.0	5	9.3
8	5.2	6	8.8	6	8.4	6	6.9	6	11.3
9	5.7	7	9.7	7	9.8	7	7.9	8	15.7
10	6.5	8	10.7	13	17.3	13	15.2	13	24.8
13	9.7	10	12.8	14	18.5	14	16.4	14	26.6
14	10.9	11	14.0	20	25.8	20	25.9	21	40.1
21	17.6	13	16.4	21	27.4	21	27.7	21	40.1
28	22.1	20	24.8	27	37.1	27	35.7	28	53.1
37	30.7	27	33.4	28	38.9	28	37.3	28	53.1
67	64.1	37	46.4	34	51.4	34	43.1	35	67.9
97	93.0	66	66.8	35	52.5	35	44.0	35	67.9
127	110.8	97	80.6	36	53.5	36	45.2	36	70.2
181	124.7	126	88.9	37	54.5	37	46.4	37	72.5
334	135.8	223	104.6	38	55.6	38	47.8	38	74.7
366	151.9	275	122.5	40	57.9	40	50.6	40	78.8
436	213.9	367	214.2	42	61.3	42	52.9	42	83.1
-	-	-	-	49	71.1	49	62.3	49	99.5
-	-	-	-	56	81.2	56	66.7	56	117.2
-	-	-	-	65	94.6	65	72.6	65	137.6
-	-	-	-	96	129.7	95	88.6	95	202.4
-	-	-	-	125	145.9	125	96.8	126	257.5
-	-	-	-	155	155.7	201	104.9	155	292.1
-	-	-	-	216	182.5	221	108.7	215	343.2
-	-	-	-	307	252.4	304	131.0	305	419.7
-	-	-	-	364	348.2	380	200.5	395	569.8
-	-	-	-	-	-	-	-	419	618.8

DAT = days after treatment
D_{norm} = normalized day (20°C, pF2)

Table 7.1.2.2.1-92: Time-step normalized (temperature and moisture) sampling days (field trials 99509 – 99513)

99509		99510		99511		99512		99513	
DAT	**D_{norm}**	**DAT**	**D_{norm}**	**DAT**	**D_{norm}**	**DAT**	**D_{norm}**	**DAT**	**D_{norm}**
0	0	0	0	0	0	0	0	0	0
1	1.7	1	0.8	1	0.4	2	1.4	2	2.6
2	3.4	2	1.7	2	0.8	3	2.1	3	4.1
3	5.2	3	2.4	3	1.4	5	4.3	5	7.6
5	8.8	5	3.5	5	2.8	7	6.6	7	11.3
6	10.5	6	4.0	6	3.7	9	9.2	9	15.4
7	12.3	7	4.7	7	4.6	13	13.4	13	20.6
13	20.1	13	9.3	13	11.8	14	15.0	14	21.4
14	21.7	14	10.2	14	12.7	27	26.6	28	40.7
20	32.6	20	17.7	20	20.4	28	27.5	29	42.3
21	34.7	21	18.6	21	21.8	34	35.9	41	62.7
27	47.9	27	23.3	27	26.3	42	51.1	42	63.9
28	50.3	28	24.0	28	27.4	55	70.2	55	92.1
34	63.4	34	29.6	34	35.3	56	71.8	56	94.1
35	65.4	35	30.7	35	36.4	69	96.3	69	117.0
36	67.5	36	32.0	36	37.4	70	97.8	70	118.5
37	69.6	37	33.1	37	38.5	71	99.1	71	120.3
38	71.6	38	34.7	38	40.0	72	100.5	72	121.8
40	75.7	40	37.3	40	42.5	73	101.8	73	122.9
42	80.3	42	39.7	42	45.2	75	104.5	75	125.0
49	95.1	49	50.8	49	53.5	78	108.0	77	127.9
56	106.3	56	62.6	56	59.0	84	117.8	84	136.6
65	119.6	65	81.6	64	65.8	100	135.3	99	157.2
95	152.1	95	118.3	96	91.1	133	164.3	130	182.6
124	172.1	126	158.9	125	104.0	162	176.7	161	201.6
153	186.5	155	192.7	155	112.3	286	199.0	188	211.2
216	204.9	216	224.3	258	121.5	344	220.4	250	219.2
306	254.3	303	253.7	308	130.5	429	302.6	340	276.5
395	400.0	395	320.6	399	182.3	-	-	414	379.6

DAT = days after treatment
D_{norm} = normalized day (20°C, pF2)

Table 7.1.2.2.1-93: Time-step normalized (temperature and moisture) sampling days (field trials 99514 – 99515 and 2000512)

99514		99515		99516		99517		2000512	
DAT	**D_{norm}**	**DAT**	**D_{norm}**	**DAT**	**D_{norm}**	**DAT**	**D_{norm}**	**DAT**	**D_{norm}**
0	0	0	0	0	0	0	0	0	0
2	4.4	1	1.7	2	1.9	1	0.9	2	2.9
3	6.5	2	3.4	3	2.7	2	1.8	3	4.6
5	10.7	3	5.0	5	4.2	3	2.4	5	8.2
7	14.6	5	7.9	6	5.4	7	4.7	7	12.0
9	18.6	6	9.1	7	6.4	8	5.4	9	15.6
13	27.1	7	9.9	8	7.6	10	6.3	13	22.9
14	29.1	8	11.0	10	9.9	12	7.3	14	24.5
27	58.2	10	13.3	12	12.0	13	7.9	27	50.5
28	60.9	12	15.3	19	17.6	14	8.7	28	52.8
34	76.2	19	22.7	26	22.3	21	15.2	29	54.1
42	97.0	27	28.0	37	29.6	28	20.2	30	55.6
55	131.4	35	34.2	64	44.5	37	26.9	31	57.3
56	133.9	67	56.8	97	53.3	65	40.5	33	60.7
69	162.9	96	68.4	280	66.7	86	45.0	35	63.6
70	164.8	123	75.0	365	102.3	310	65.7	38	67.7
71	166.6	306	100.6	-	-	365	95.1	42	72.8
72	168.1	374	138.9	-	-	-	-	49	77.5
73	169.7	-	-	-	-	-	-	58	86.4
75	173.2	-	-	-	-	-	-	88	103.1
77	176.4	-	-	-	-	-	-	118	107.6
84	187.0	-	-	-	-	-	-	150	109.6
100	210.7	-	-	-	-	-	-	207	118.2
131	237.9	--	-	-	-	-	-	298	171.7
160	259.1	-	-	-	-	-	-	392	329.3
250	319.8	-	-	-	-	-	-	483	388.4
341	442.2	-	-	-	-	-	-	568	403.4
386	546.7	-	-	-	-	-	-	-	-

DAT = days after treatment
D_{norm} = normalized day (20°C, pF2)

II. RESULTS AND DISCUSSION

The data sets of the 15 trial locations were evaluated for the criterion of 10 mm cumulative rainfall [*EFSA (2010)*]. The evaluation showed that it was possible to perform kinetic analysis for all trial locations, because the number of data points in each of the data sets was still appropriate (seven to 16 sampling days) after excluding sampling dates before 10 mm of cumulative rainfall.

The dissipation behavior of boscalid in the 15 field trials was analyzed in a step-wise approach to derive modeling endpoints according to EFSA [*EFSA (2010)*]. A summary of the results of the kinetic evaluation are presented in Table 7.1.2.2.1-94.

Table 7.1.2.2.1-94: Summary of endpoints for use in modeling of boscalid

Field trial	Soil type (USDA)	Kinetic model	All sampling days included		Data > 10 mm rainfall only	
			Rate k [d^{-1}]	$DegT_{50}$ [d]	Rate k [d^{-1}]	$DegT_{50}$ [d]
99502 (ND)	Loam	HS	0.0050[1]	138.6[1]	0.0050[1]	138.6[1]
99503 (CO)	Sandy clay loam	SFO	0.0061	113.2	0.0060	114.6
99506 (CA)	Sandy loam	SFO	0.0031	223.1	0.0025	274.5
99507 (ID)	Loam	SFO	0.0043	161.8	0.0045	155.1
99508 (FL)	Sand	SFO[2]	0.0023	300.4	0.0023	300.4
99509 (GA)	Loamy sand	SFO[2]	0.0030	229.2	0.0028	248.8
99510 (CA)	Sandy loam	SFO	0.0041	167.7	0.0041	168.1
99511 (NY)	Loamy sand	SFO	0.0048	144.5	0.0048	144.5
99512 (NJ)	Loam	SFO	0.0037	185.2	0.0037	186.8
99513 (IL)	Silt loam	SFO	0.0031	222.2	0.0032	214.5
99514 (TX)	Sandy loam	SFO[2]	0.0028	243.4	0.0028	243.4
99515 (ON)	Loam	SFO	0.0134	51.9	0.0112	62.1
99516 (MB)	Silt loam	SFO	0.0055	126.0	-[3]	-[3]
99517 (AB)	Loam	SFO	0.0056	124.4	0.0061	114.1
200512 (TX)	Loamy sand	SFO	0.0046	149.4	0.0046	152.1
Geometric mean			**0.0043**	**160.0**	**0.0041**	**166.8**

[1] slow phase of HS kinetics (rainfall > 10 mm at breakpoint)
[2] outliers excluded
[3] statistically significant degradation parameters could not be derived from trial

III. CONCLUSION

Kinetic evaluation of 15 field trials with boscalid, originating from six field dissipation studies, was conducted in order to derive reliable normalized modeling endpoints according to the current guidance of the FOCUS workgroup on degradation kinetics.

Kinetic evaluation of the time-step normalized dataset (20°C, pF2) resulted in normalized field half-lives ($DegT_{50}$) for boscalid between 51.9 and 243.4 days, when all sampling days were included, and between 62.1 and 300.4 days, when only data after 10 mm rainfall had occurred in the study were considered.

Report: CA 7.1.2.2.1/15
Gooding R., 2001a
Freezer storage stability study of BAS 510 F, 2-chloronicotinic acid, and 2-hydroxy-N-(4-chlorobiphenyl-2-yl)nicotinamide in soil using LC-MS/MS
2001/5000904
Guidelines: EPA 164-1
GLP: yes
(certified by United States Environmental Protection Agency)

Executive Summary

The storage stability of boscalid (BAS 510 F) and its metabolites 2-chloronicotinic acid (M510F47) and 2-hydroxy-N-(4'-chlorobiphenyl-2-yl)nicotinamide (M510F49) in soil was investigated under frozen conditions (≤-5°C).

Control soil samples at two depths, 0-15 cm (0-6") and 30-45 cm (12-18"), from field trial RCN 99510 [*CA 7.1.2.2.1/7, BASF DocID 2000/5277*] were fortified at a level of 0.1 mg kg^{-1} with boscalid and M510F47. Further control soil samples were fortified at a level of 0.1 mg kg^{-1} with M510F49.

The fortified samples were stored frozen (≤ -5°C) and were analyzed after 1, 3 and 6 months (boscalid and M510F47) and after 0 day, 1, and 3 months (M510F49). The results were compared to samples fortified on the day of analysis.

All soil samples were analyzed using BASF Analytical Method D0004/1 [*already peer-reviewed study BASF DocID 2001/5000881*] except for samples that were analyzed at 1 month (samples treated with boscalid and M510F47). Results reveal that after 6 months of freezer storage boscalid and M510F47 remain stable (approximately 90% recovery). Analysis of control samples treated with M510F49 showed a decline in recovery yielding approximately 70% after one month and leveled off after 3 months of storage.

In order to investigate short-term storage stability, additional soil samples were fortified with M510F49 and were analyzed after 7, 14, 21 and 30 days of storage. These results were compared to samples fortified on the day of analysis. All soil samples were analyzed using BASF Analytical Method D0004/1.

The results showed an approximately 15% decline in recovery starting after one week. In an attempt to improve the recovery of M510F49, 14 and 30 day samples were also analyzed by a modified method D0004/1, in which the soil marc was extracted with 1 N sodium hydroxide after the usual extraction procedure. The results were compared to samples fortified on the day of analysis. The results of the sodium hydroxide extraction showed no significant variance in recovery from Method D0004/1.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Materials

Internal code:	BAS 510 F
Common name:	boscalid
Reg. No.:	300355
Chemical name (IUPAC):	2-chloro-N-(4'-chloro-biphenyl-2-yl)nicotinamide
Molar mass:	343.2 g mol^{-1}
Batch No.:	01183-190
Chemical Purity:	99.3%

Internal code:	M510F47
Reg. No.:	107371
Chemical name (IUPAC):	2-chloronicotinic acid
Molecular formula:	$C_6H_4ClNO_2$
Molar mass:	157.6 g mol^{-1}
Batch No.:	01174-232
Purity:	99.8%

Internal code:	M510F49
Reg. No.:	391572
Chemical name (IUPAC):	2-hydroxy-N-(4'-chlorobiphenyl-2-yl)nicotinamide
Molecular formula:	$C_{18}H_{13}ClN_2O_2$
Molar mass:	324.8 g mol^{-1}
Batch No.:	01196-217
Purity:	98.9%

2. Soil

Untreated soil samples from trial site RCN 99510 of a field dissipation study [*CA 7.1.2.2.1/7, BASF DocID 2000/5277*] were used for testing. Both, samples of soil depths 0-15 cm (0-6“) and 30-45 cm (12-18“) were used.

B. STUDY DESIGN

1. Experimental Conditions

Untreated soil samples (duplicates) were weighed individually and placed into screw top glass vials. Each sub sample was fortified at a level of 0.1 mg kg^{-1} with boscalid, M510F47, and M510F49. These fortified sub samples were stored in a freezer (≤ -5°C) along with several unfortified control soil samples which were used later for the control sample and the procedural fortifications. Samples were analyzed at time intervals of 1, 2, 3, and 4 weeks of storage.

2. Sampling

All soil samples remained in freezer storage until analysis. On each day of analysis, duplicate fortified soil samples and three control samples were removed for analysis. Two of the control samples were treated with 0.1 mg kg^{-1} of solution containing boscalid and its metabolites M510F47 and M510F49. These samples served as procedural fortifications. The procedural fortifications were analyzed along with the unfortified control and the two stored fortification samples. The procedural fortifications indicated the efficiency of the method on the day of analysis.

3. Description of analytical procedures

Soil samples were analyzed either by BASF Analytical Methods No. D0004, D0004/1 or a modification of method D0004/1 using sodium hydroxide. The sodium hydroxide modification of D0004/1 was used in the short term stability study of samples fortified only with metabolite M510F49. The limit of quantification (LOQ) was 0.001 mg kg^{-1} for each analyte.

Determination of boscalid and its metabolites M510F47 and M510F49 using method D0004/1
Soil samples were extracted with methanol followed by methanol / water (50:50, v/v). The extracts were combined and diluted with methanol. An aliquot of each sample was diluted with water, containing 4 mM ammonium formate and 0.1% formic acid. The analytes were quantitated individually by LC-MS/MS.

Determination of boscalid metabolite M510F49 using modified method D0004/1 with NaOH extraction
Soil samples were extracted with methanol followed by methanol / water, (v/v, 50:50). The extracts were combined and diluted with methanol.
1 N sodium hydroxide was added to the soil marc. The sample was shaken for one hour and centrifuged. After centrifugation, the extract was decanted and the pH of the alkaline extract was adjusted to ~ 2 with 2 N HCI. Sodium chloride was added. Afterwards, the aqueous layer was extracted with ethyl acetate. The combined ethyl acetate layers were evaporated to dryness. The dry residue was dissolved in the combined methanol / water extract from the previous methanol / water extraction.

II. RESULTS AND DISCUSSION

A summary of the recovery results after storage is presented in Table 7.1.2.2.1-95 to Table 7.1.2.2.1-97. Average recoveries were corrected for the average procedural recovery obtained on the day of analysis. Stored recoveries were corrected for procedural recoveries. All recoveries were corrected for control residue.

Results revealed that concentrations of boscalid and its metabolite M510F47 remained stable after 6 months of freezer storage, expressed by recoveries of > 90%.

The recovery of metabolite M510F49 declined to 70-80% after 3 months of freezer storage. The short term storage stability study showed that in one week the recovery of M510F49 declined to 80%. Supplementing the extraction method with a 1 N sodium hydroxide extraction showed little improvement in the recoveries. The extraction with 1 N sodium hydroxide at higher temperature was not conducted due to the potential hydrolysis of the metabolite.

Table 7.1.2.2.1-95: Storage stability of boscalid and its metabolites M510F47 and M510F49 in frozen soil

Soil depth	Analytes	Storage interval [months]	Recovery (not corrected) [%]	Procedural recovery [%]	Procedural recovery average	Recovery- (corrected) [%]
0-15 cm (0-6'')	boscalid	0	104.0	100.5	97	103
		1	105.5	100.5		105
		3	91.0	89.5		102
		6	91.0	96.0		95
	M510F47	0	88.5	92.0	90	96
		1	91.5	92.0		99
		3	81.0	82.5		98
		6	87.0	92.5		94
	M510F49	0	96.0	96.5	95	99
		1	73.5	96.5		76
		3	76.0	90.5		84
30-45 cm (12-18")	boscalid	0	100.0	99.5	97	101
		1	100.5	99.5		101
		3	90.0	92.5		97
		6	84.0	95.0		88
	M510F47	0	102.5	104.5	103	98
		1	106.5	104.5		102
		3	96.0	96.5		100
		6	98.5	107.0		92
	M510F49	0	100.5	91.0	92	111
		1	64.0	91.0		70
		3	69.0	92.5		75

Table 7.1.2.2.1-96: Short term storage stability of metabolite M510F49 in frozen soil

Soil depth	Analytes	Storage interval [months]	Recovery (not corrected) [%]	Procedural recovery [%]	Procedural recovery average	Recovery- (corrected) [%]
0-15 cm (0-6'')	M510F49	1	74.5	89.5	90	83
		2	75.5	89.5		84
		3	72.5	90.0		81
		4	75.5	91.5		83

Table 7.1.2.2.1-97: Short term storage stability of metabolite M510F49 in frozen soil according to modified method D0004/1

Soil depth	Analytes	Storage interval [months]	Recovery (not corrected) [%]	Procedural recovery [%]	Procedural recovery average	Recovery- (corrected) [%]
0-15 cm (0-6'')	M510F49	1	84.5	98.0	96	86
		4	86.0	94.5		91

III. CONCLUSION

This study demonstrates that boscalid (BAS 510 F) and its metabolite M510F47 (2-chloronicotinic acid) are stable in soil when stored at ≤ -5°C for 6 months. The recovery of metabolite M510F49 (2-hydroxy-N-(4'-chlorobiphenyl-2-yl)nicotinamide) declined slightly, but remained stable at approximately 70-80% after 3 months.

Report:	CA 7.1.2.2.1/16 Gooding R., 2003a Freezer storage stability study of BAS 510 F, 2-chloronicotinic acid, and 2-hydroxy-N-(4-chlorobiphenyl-2-YL)nicotinamide in soil using LC-MS/MS 2003/5000055
Guidelines:	EPA 164-1
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary

The storage stability of boscalid (BAS 510 F) and its metabolites 2-chloronicotinic acid (M510F47) and 2-hydroxy-N-(4‘-chlorobiphenyl-2-yl)nicotinamide (M510F49) in soil was investigated under frozen conditions (≤-5°C).

Control soil samples at two depths, 0-15 cm (0-6“) and 30-45 cm (12-18“), from field trial RCN 99510 [*CA 7.1.2.2.1/7, BASF DocID 2000/5277*] were fortified at a level of 0.1 mg kg^{-1} with boscalid and M510F47. Further control soil samples were fortified at a level of 0.1 mg kg^{-1} with M510F49.

The fortified samples were stored frozen (≤ -5°C) and were analyzed after 1, 3, 6, 9, 12, 15, 18 and 24 months and additionally at 0 days for soils samples fortified with M510F49 only. The results were compared to samples fortified on the day of analysis.

All soil samples were analyzed using BASF Analytical Method D0004/1 [*already peer-reviewed study BASF DocID 2001/5000881*] except for samples that were analyzed at 1 month (samples treated with boscalid and M510F47).

Results reveal that after 24 months of freezer storage boscalid and M510F47 remain stable (approximately 90% recovery).

Analysis of samples treated with M510F49 showed a decline in recovery yielding approximately 70% after one month and leveled off after 3 months of storage.

In order to investigate short-term storage stability, additional soil samples were fortified with M510F49 and were analyzed after 7, 14, 21 and 30 days of storage. These results were compared to samples fortified on the day of analysis. All soil samples were analyzed using BASF Analytical Method D0004/1.

The results showed an approximately 15% decline in recovery starting after one week. In an attempt to improve the recovery of M510F49, 14 and 30 day samples were also analyzed by a modified method D0004/1, in which the soil marc was extracted with 1 N sodium hydroxide after the usual extraction procedure. The results were compared to samples fortified on the day of analysis. The results of the sodium hydroxide extraction showed no significant variance in recovery from Method D0004/1.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Materials

Internal code:	BAS 510 F
Common name:	boscalid
Reg. No.:	300355
Chemical name (IUPAC):	2-chloro-N-(4'-chloro-biphenyl-2-yl)nicotinamide
Molecular formula:	$C_{18}H_{12}Cl_2N_2O$
Molar mass:	343.2 g mol^{-1}
Batch No.:	01183-190
Chemical Purity:	99.3%

Internal code:	M510F47
Reg. No.:	107371
Chemical name (IUPAC):	2-chloronicotinic acid
Molecular formula:	$C_6H_4ClNO_2$
Molar mass:	157.6 g mol^{-1}
Batch No.:	01174-232
Purity:	99.8%

Internal code:	M510F49
Reg. No.:	391572
Chemical name (IUPAC):	2-hydroxy-N-(4'-chlorobiphenyl-2-yl)nicotinamide
Molecular formula:	$C_{18}H_{13}ClN_2O_2$
Molar mass:	324.8 g mol^{-1}
Batch No.:	01196-217
Purity:	98.9%

2. Soil

Untreated soil samples from trial site RCN 99510 of a field dissipation study [*CA 7.1.2.2.1/7, BASF DocID 2000/5277*] were used for testing. Both, samples of soil depths 0-15 cm (0-6“) and 30-45 cm (12-18“) were used.

B. STUDY DESIGN

1. Experimental Conditions

Untreated soil samples (duplicates) were weighed individually and placed into screw top glass vials. Each sub sample was fortified at a level of 0.1 mg kg^{-1} with boscalid, M510F47, and M510F49. These fortified sub samples were stored in a freezer (≤ -5°C) along with several unfortified control soil samples which were used later for the control sample and the procedural fortifications. Samples were analyzed at time intervals of 1, 2, 3, and 4 weeks of storage.

2. Sampling

All soil samples remained in freezer storage until analysis. On each day of analysis, duplicate fortified soil samples and three control samples were removed for analysis. Two of the control samples were treated with 0.1 mg kg^{-1} of solution containing boscalid and its metabolites M510F47 and M510F49. These samples served as procedural fortifications. The procedural fortifications were analyzed along with the unfortified control and the two stored fortification samples. The procedural fortifications indicated the efficiency of the method on the day of analysis.

3. Description of analytical procedures

Soil samples were analyzed either by BASF Analytical Methods No. D0004, D0004/1 or a modification of method D0004/1 using sodium hydroxide. The analysis of 0 day and 1 month samples were conducted using Method D0004 without determination of metabolite 1-(4-Chlorophenyl)-2-aminobenzene.) The sodium hydroxide modification of D0004/1 was used in the short term stability study of samples fortified only with 2-hydroxy-N-(4‘- chlorobiphenyl-2-yl)nicotinamide.

<u>Determination of boscalid and its metabolites M510F47 and M510F49 using method D0004 and D0004/1</u>
Soil samples were extracted with methanol followed by methanol / water (50:50, v/v). The extracts were combined and diluted with methanol. An aliquot of each sample was diluted with water, containing 4 mM ammonium formate and 0.1% formic acid. The analytes were quantitated individually by LC-MS/MS.

Determination of boscalid metabolite M510F49 using modified method D0004/1 with NaOH extraction

Soil samples were extracted with methanol followed by methanol / water, (v/v, 50:50). The extracts were combined and diluted with methanol.

1 N sodium hydroxide was added to the soil marc. The sample was shaken for one hour and centrifuged. After centrifugation, the extract was decanted and the pH of the alkaline extract was adjusted to ~ 2 with 2 N HCI. Sodium chloride was added. Afterwards, the aqueous layer was extracted with ethyl acetate. The combined ethyl acetate layers were evaporated to dryness. The dry residue was dissolved in the combined methanol / water extract from the previous methanol / water extraction.

An aliquot of each sample was diluted with water, containing 4 mM of ammonium formate and 0.1% formic acid. The analytes were quantitated by LC-MS/MS.

Limit of quantification (LOQ)

The LOQ for boscalid and its metabolites in soil using Method 00004/1 was 0.01 mg kg^{-1}. In this study, however, the lowest fortification level was 0.1 mg kg^{-1}. Fortification at this level produces a more accurate quantification if an analyte declines in recovery.

II. RESULTS AND DISCUSSION

A summary of the recovery results after storage is presented in Table 7.1.2.2.1-98 to Table 7.1.2.2.1-101. Average stored recoveries were corrected for the average procedural recovery obtained on the day of analysis. Stored recoveries were corrected for procedural recoveries. All recoveries were corrected for control residue.

Table 7.1.2.2.1-98: Storage stability of boscalid and its metabolites M510F47 and M510F49 in frozen soil (0-15 cm; 0-6")

Analytes	Storage interval [months]	Stored recovery (not corrected) [%]	Procedural recovery [%]	Procedural average [%]	Stored recovery-(corrected) [%]
boscalid	0	104.0	100.5	92	103
	1	105.5			105
	3	91.0	89.5		102
	6	91.0	96.0		95
	9	88.5	93.0		95
	12	95.0	90.0		106
	15	87.0	91.5		95
	18	94.5	92.0		103
	24	81.5	79.5		103
M510F47	0	88.5	92.0	90	96
	1	91.5			99
	3	81.0	82.5		98
	6	87.0	92.5		94
	9	93.2	92.5		101
	12	102.0	96.5		106
	15	94.5	97.0		97
	18	94.0	95.0		99
	24	78.5	75.5		104
M510F49	0	96.0	96.5	84	99
	1	73.5			76
	3	76.0	90.5		84
	6	73.5	85.5		86
	9	74.0	78.5		94
	12	68.0	73.5		93
	15	80.5	88.0		91
	18	77.0	90.0		86
	24	71.5	71.0		101

Table 7.1.2.2.1-99: Storage stability of boscalid and its metabolites M510F47 and M510F49 in frozen soil (30-45 cm; 12-18")

Analytes	Storage interval [months]	Stored recovery (not corrected) [%]	Procedural recovery [%]	Procedural average [%]	Stored recovery-(corrected) [%]
Boscalid	0	100.0	99.5	93	101
	1	100.5			101
	3	90.0	92.5		97
	6	84.0	95.0		88
	9	83.5	91.0		92
	12	91.5	94.5		97
	15	92.0	90.0		102
	18	89.0	94.0		95
	24	83.0	86.5		96
M510F47	0	102.5	104.5	98	98
	1	106.5			102
	3	96.0	96.5		100
	6	98.5	107.0		92
	9	101.5	108.0		94
	12	110.5	104.5		106
	15	84.5	82.0		103
	18	101.5	99.0		103
	24	81.5	78.5		104
M510F49	0	100.5	91.0	88	111
	1	64.0			70
	3	69.0	92.5		75
	6	61.0	87.0		70
	9	65.0	88.0		74
	12	68.5	89.0		77
	15	63.0	92.5		68
	18	64.5	86.0		75
	24	63.0	81.0		78

Table 7.1.2.2.1-100: Short term storage stability of boscalid metabolite M510F49 in frozen soil (0-15 cm; 0-6")

Storage interval [months]	Stored recovery [%]	Procedural recovery [%]	Procedural average [%]	Stored recovery-corrected [%]
1	74.5	89.5	90	83
2	75.5	89.5		84
3	72.5	90.0		81
4	75.5	91.5		83

Table 7.1.2.2.1-101: Short term storage stability of boscalid metabolite M510F49 in frozen soil (0-15 cm; 0-6") with modified method D0004/1

Storage interval [months]	Stored recovery (not corrected) [%]	Procedural recovery [%]	Procedural average [%]	Stored recovery-(corrected) [%]
1	84.5	98.0	96	86
4	86.0	94.5		91

III. CONCLUSION

Results showed that after 24 months of freezer storage the concentrations of boscalid and its metabolite M510F47 (2-chloronicotinic acid) in fortified soil samples remained stable, expressed by recoveries of > 90%.
The recovery of boscalid metabolite M510F49 (2-hydroxy-N-(4'-chlorobiphenyl-2-yl)nicotinamide) declined during storage to 70-80% within 3 months, remaining constant at 70-80% up to 24 months. The short term storage stability study showed that in one week the recovery of M510F49 declined to 80%. Supplementing the extraction method with a 1N sodium hydroxide extraction showed little improvement in the recoveries. The extraction with 1 N sodium hydroxide at higher temperature was not conducted due to the potential hydrolysis of the metabolite.
This study demonstrates that boscalid (BAS 510 F) and its metabolite M510F47 are stable in soil stored at ≤ -5°C for 24 months. The recovery of metabolite M510F49 declined slightly, but remained constant at approximately 70-80% up to 24 months.

CA 7.1.2.2.2 Soil accumulation studies

Two experimental studies on the potential accumulation of residues of boscalid following typical application to grapes and vegetables were submitted for the previous Annex I listing. The related reports are summarized in the following table [Table 7.1.2.2.2-1].

Table 7.1.2.2.2-1: Studies on terrestrial field accumulation of boscalid

Reference	BASF DocID	Sites	Application rate [kg ha^{-1}]	Crop	Incubation period [years]	Remark
Kellner O., Grote C., 2001a;	2000/1017039	Grünstadt/ Germany	2.1 per year (1998 - 2000)	grapes	3	Report out of date, superseded by 2004/1003851
Kellner O. et al., 2004a	2004/1003851	Grünstadt/ Germany	2.1 per year (1998 – 2003)	grapes	5	
Kellner O., Grote C., 2001b;	2000/1017040	Studernheim/ Germany	1998: 2.1 1999: 1.7 2000: 0.0	lettuce, green beans, carrots	3	Report out of date, superseded by 2005/1013964
Grote C., Platz K., 2005a	2005/1013964	Studernheim/ Germany	1998: 2.1 1999: 1.7 2000: 0.0 2001: 2.1 2002: 1.7 2003: 0.0 2004: 2.1	lettuce, green beans, carrots, cauliflower	7	

Table 7.1.2.2.2-2: Kinetic evaluation of terrestrial field accumulation studies with boscalid

Reference	BASF DocID	Sites	Evaluation	Remark
Platz K., 2001b	2000/1017045	Grünstadt/ Germany	Experimental data from 2000/1017039, estimation of accumulation plateau	Report out of date, superseded by 2004/1003851
Platz K., 2001c	2000/1017046	Studernheim/ Germany	Experimental data from 2000/1017040, estimation of accumulation plateau	Report out of date, superseded by 2005/1013964

Another study was included in the previous Annex I dossier that showed the predicted accumulation levels and predicted environmental concentrations following multi-year application of boscalid to grapes and vegetables according to Good Agricultural Practice [*already submitted study BASF DocID 2000/1017050*]. The report was superseded by the final report on the accumulation study in grapes [*already submitted, BASF DocID 2004/1003851*] and the second interim report on the accumulation study in vegetables [*already submitted, BASF DocID 2005/1013964*].

The final report on the accumulation study in grapes shows that the estimated residue plateau due to multi-year application of boscalid reached 2 kg/ha which equals 95 % of the total yearly application rate that was tested in the study.

The final report on the accumulation study in vegetables is included in the current dossier under [*CA 7.1.2.2.2/1, BASF DocID 2009/1070939*] and supersedes the interim report *BASF DocID 2005/1013964*. The estimated residue plateau level due to multi-year application of boscalid reached 1.5 kg/ha or 118 % of the average total yearly application rate that was tested in the study.

A new soil accumulation study was initiated for supplemental information on accumulation behaviour of boscalid and the related interim report is included in the current dossier under [*CA 7.1.2.2.2/2, BASF DocID 2015/1178191*]. As the study was not complete at the time of submission a modelling approach was applied to estimate the ultimate plateau concentration on the basis of the data available so far [*see CA 7.1.2.2.2./3, BASF DocID 2015/1197308*]. The estimated residue plateau level due to multi-year application of boscalid reached 2.676 kg/ha or 167 % of the average total yearly application rate that was tested in the study.

Report:	CA 7.1.2.2.2/1 Penning H. et al., 2009a Accumulation behaviour of BAS 510 F in soil under field conditions over several years after application onto vegetables 2009/1070939
Guidelines:	BBA VI 4-1 (December 1986), SETAC Procedures for assessing the environmental fate and ecotoxicity for pesticides (March 1995), IVA Guideline for residue analysis part V (1993)
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

The accumulation behavior of boscalid under field conditions was investigated over an eleven-year-period from 1998 to 2009. The aim of the study was to determine the residue level of boscalid in soil at steady state after multi-year application.

The trial was conducted in a typical vegetable growing area in Rhineland-Palatinate, Germany, on a loamy sand soil with a pH value of 7.8.

Starting in 1998, the trial site was cultivated with vegetable crops in two consecutive years and cereals in the third year according to Good Agricultural Practice of vegetable growing in Germany. The triennial cultivation scheme was repeated four times until 2009. Boscalid was applied to the vegetable crops only. Nominal application rates of boscalid in the first vegetable year of each cultivation cycle were 2 x 300 g a.s. ha^{-1} to lettuce and 3 x 500 g a.s. ha^{-1} to green beans. The nominal application rates in the second vegetable year of each cycle were 3 x 300 g a.s. ha^{-1} to carrots and 2 x 400 g a.s. ha^{-1} to cauliflower. The actual amounts of boscalid applied to the field as determined by spray broth calculations differed only slightly from the nominal rates. The total amount of boscalid applied in the first and second vegetable year of each cultivation cycle was 2100 g and 1700 g, respectively, and represents a reasonable worst-case application scheme of boscalid in crop rotation.

The trial area was subdivided into four plots or treatments. Treatment 1 was used as untreated control plot, whereas treatments 2, 3 and 4 were identically treated plots. Soil samples were taken from all untreated and treated plots twice a year, once in spring before the first application of the year and once after harvest of the (last) crop. Starting in 2006 an additional sampling per year was performed.

Samples were analyzed for boscalid. The measured concentrations were not corrected, neither for recoveries nor blanks, and all results were referred to dry soil weight.

Samples from the control plot showed no boscalid residues above the determination limit of 0.01 mg kg^{-1} from the first sampling in April 1998 until sampling No. 15 in April 2005. These data demonstrate that no interferences of the sample material with the analytical procedure occurred and that the control plots were free of residues of boscalid during that period. However, boscalid was detected in increasing levels in the control plot beginning in November 2005. The increase of residues on the control plot in the last years of the accumulation study hints to several partly excessive applications of boscalid that were not in line with the application scheme of the study.

Residues measured in the treated plots showed regular behavior until sampling No. 15 in April 2005. Concentrations in soil increased after the annual application periods followed by a period of degradation with decreasing residues. After application in the growing season, significant residues of boscalid were detected in soil in the spring of the following year and were distributed also to deeper soil layers. This was caused by soil treatments like tillage or ploughing to a maximum depth of 35 cm. However, the highest amounts of residues were detected from 0 to 30 cm depth. In the treated plots, residue levels after the application period increased compared to residue levels before that period by 106% to 127% of the nominal yearly application rate. A comparison showed that the residue development after 2005 became very heterogeneous among the three treated plots (i.e., increase of residue levels in one plot and decrease at the same time in another plot). The extraordinary increase of residues and the variability between the three treated replicates give indication that the residue situation cannot be explained by the regular planned applications, which were verified via Petri dish analysis. From these results and additional investigations it was concluded that additional irregular applications must be assumed. However, further details of these additional applications could not be elucidated.

A compartment model was set up to estimate the residue level of boscalid in soil at steady state after multi-year use. Dissipation behavior was described using single first-order kinetics and a site-specific DT_{50} value that was estimated from the residues measured on the treated plots during eight individual dissipation periods over the course of the study. The compartment model was fitted to the residues observed in samples up to 2005 only. Observed residues from later samplings were not included, because the irregular increase of residues starting with sampling event 16 was considered not to result from the regular application scheme of the accumulation study and, therefore, not to reflect adequately the accumulation behavior of boscalid.

The evaluation of the model results showed that predicted minimum and maximum values in the third and fourth application cycle were comparable, indicating that the model curve approximated steady state conditions in the third application cycle. The plateau level (i.e., the maximum of predicted residue levels before the application period of the second vegetable year, n = 4) was predicted in year 8 of the eleven-year accumulation study. The predicted plateau amounted to 1.50 kg ha^{-1} or 118 % of the average yearly application rate of the study. Assuming a soil bulk density of 1500 kg m^{-3} and a soil layer of 0.3 m as a realistic depth of soil cultivation in vegetable crops, the predicted plateau corresponds to 0.333 mg kg^{-1}. The peak level (i.e. the maximum of predicted residue levels in autumn of the second vegetable year, n = 4) was predicted in year 11 of the study and amounted to 2.06 kg ha or 162% of the average yearly application rate of the study. Assuming a soil bulk density of 1500 kg m^{-3} and a soil layer of 0.3 m, the predicted peak level corresponds to 0.457 mg kg^{-1}.

I. MATERIALS AND METHODS

1. Test material

Treatment No. 1-5:

Test item (formulation):	BAS 510 KA F
Active substance (a.s.):	BAS 510 F
Type of formulation:	SC
Batch no.:	2008 CP031513
Content of active substance:	500 g L^{-1} (nominal)

Treatment No. 6 -40:

Test item (formulation):	BAS 510 01 F
Active substance (a.s.):	BAS 510 F
Type of formulation:	WG
Batch no.:	99-3 (applied 1999), 2001-1 (applied 2001, 2002), 2000-1 (2004), 1302 (2005), 1453 (2007, 2008)
Content of active substance:	50% (nominal)

2. Test site

The accumulation behavior of boscalid under field conditions was investigated over an eleven-year-period from 1998 to 2009. The trial was conducted in a typical vegetable growing area in Rhineland-Palatinate, Germany on a loamy sand soil with an organic carbon content of 1.0%, a pH value ($CaCl_2$) of 7.8, cation exchange capacity of 13 mVal 100 g^{-1} dry soil and a maximum water holding capacity of 43 g water 100 g^{-1} dry soil.

3. Experimental treatments

Starting in 1998, the trial site was cultivated with vegetable crops in two consecutive years and cereals in the third year according to Good Agricultural Practice of vegetable growing in Germany. The triennial cultivation scheme was repeated four times until 2009. Boscalid was applied to the vegetable crops, but the cereals that were grown every third year were not treated with the active ingredient (a.s.). In summary, 15200 g boscalid were applied in the course of the study; 8400 g ha^{-1} boscalid were applied to lettuce/green beans (4 years with 2100 g a.s. ha^{-1} a^{-1}) and 6800 g ha^{-1} (4 years with 1700 g a.s. ha^{-1} a^{-1}) were applied to onto carrots/cauliflower. The application parameters including dates of applications, formulation, crops, growth stages and product and spray mixture applied are given in the final report of the study.

Application verification
To determine the quantity of spray mixture and active ingredient actually applied to the trial area, the quantity of spray mixture discharged prior to the application and the quantity remaining in the equipment after application were determined by analyzing Petri dishes laid out on the treated plots and subtracted from the prepared spray mixture.

Agronomic measures
Details to previous crops, fertilization and crop protection measures can be found in the study report. In terms of molecular structure the additionally applied pesticides were not similar to BAS 510 F.
The cultivation of the soils was mostly not deeper than 20 cm. Only the plowing was down to a depth of maximum 35 cm. The crops were harvested according to good agricultural practice.
Air temperature, soil temperature, relative humidity, condition of soil surface and wind velocity were recorded at the trial site during applications (presented in the study report) and samplings (except wind velocity; shown in Table 7.1.2.2.2-3).

Table 7.1.2.2.2-3: Summary of climatic conditions during soil sampling at the trial site in Studernheim, Germany used to investigate the accumulation behavior of boscalid

Sampling date	T_{air} [°C]	T_{soil} [°C]	Rel. humidity [%]	Soil surface	Sampling date	T_{air} [°C]	T_{soil} [°C]	Rel. humidity [%]	Soil surface
30/04/1998	14	12	63	Humid	21/10/2004	18	12	75	Humid
12/10/1998	12	12	70	Humid	04/04/2005	14	11	65	Humid
08/03/1999	7	4	62	Humid	17/11/2005	6	5	65	Humid
03/11/1999	10	8	83	Humid	28/03/2006	12	7	68	Humid
13/03/2000	14	9	50	Dry	23/08/2006	23	19	68	Humid
21/08/2000	20	20	64	Humid	08/11/2006	9	8	84	Humid
04/04/2001	11	7	72	Dry	02/04/2007	14	9	58	Dry
06/11/2001	7	6	85	Humid	26/07/2007	29	26	52	Humid
28/02/2002	5	5	75	Humid	28/11/2007	2	1	90	Dry
19/11/2002	10	9	80	Humid	25/04/2008	18	15	58	Dry
17/03/2003	15	4	65	Dry	18/08/2008	28	21	58	Dry
21/08/2003	26	22	64	Dry	27/11/2008	7	5	72	Humid
15/03/2004	10	4	67	Humid	02/04/2009	17	10	60	Dry

4. Sampling and storage

Soil samples were taken in spring before seeding or planting the first crop and in autumn after harvest of the last crop, before the soil was ploughed. Starting in 2006, an additional sampling per year was performed. In this year the additional sampling was conducted in fall before ploughing (November), in 2007 and 2008 the additional sampling was performed after harvest of the first crop before planting/seeding the second crop.

A total of 10 soil cores to a depth of 50 cm was taken per sampling in each plot/treatment. This means that 10 soil cores from the untreated plot and 3 x 10 soil cores from treated plots were sampled. As an exemption, sampling no. 25 was conducted with 20 cores from each of the four plots to get a definitive representative sample from the field cultivated with cauliflower. Each set of cores was regarded as one field sample and processed together. Thus, 3 replicates of treated plots were available.
The soil cores were divided into 0 – 10, 10 – 25 and 25 – 50 cm core segments in deep frozen state. Beginning with 2001 (April - sampling no. 7), the soil increments for analysis were changed to 0 - 10, 10 - 20, 20 - 30, 30 - 40 and 40 - 50 cm to give a more detailed overview of the distribution of the residues within the soil layers. Beginning with the fall sampling in 2006 (August - sampling no. 18), the segmentation pattern was changed to 0 - 5, 5 - 10, 10 - 20, 20 - 30, 30 - 40 and 40 - 50 cm again to improve residue resolution.

The core segments of the same soil depth from one field sample were ground up together with dry ice in frozen state by different mills (hammer or stephan mill) producing one homogenized laboratory sample. Starting with 2006 (March - sampling 17), prior to the processing of the soil segments, the weight and the moisture of the soil segments were determined.

Representative aliquots of the homogenized field sample were stored in plastic containers at temperatures around or below -18°C. After taking aliquot samples of soil for analysis, the remaining amounts of soil were stored again at temperatures around or below -18°C.
The soil of the Petri dishes was not further processed. Each dish with soil represented one laboratory sample and the soil was directly analyzed.

5. Analytical procedure

The soil samples were analyzed with BASF method 408/1 for concentrations of the parent compound boscalid. Beginning with 2005, BASF method D0004, which applies LC-MS/MS for quantification, was used for analysis with slight modifications.

Principle of method 408/1
A 25 g aliquot of the soil sample is extracted twice with methanol (100 mL + 50 mL). For extraction, the soil sample is shaken each time on a mechanical shaker for one hour. The extraction mixture is then centrifuged and the methanol extract is removed. Both extracts are combined and diluted to 200 mL with methanol. An aliquot of the methanol extract is evaporated to dryness. The residue is dissolved in n-hexane and cleaned up on a SPE-silica gel column. The analyte is eluted from the silica gel column using (n-hexane/ethyl acetate, 1/1, v/v). After evaporation of the eluate to dryness, the residue is dissolved in acetonic internal standard solution for quantitative determination by GC/MS.

Principle of method D0004
A 5 g soil sample aliquot is extracted with methanol followed by methanol/water (50/50, v/v). An aliquot of the extract is diluted with methanol/water (80/20, v/v) and analyzed for Boscalid by HPLC-MS/MS.
For analysis of application verifications (Petri dishes filled with 50 g soil), the total amount of soil (50 g) is transferred to a glass bottle and the Petri dish is rinsed with methanol. The soil is then extracted in a total volume of 100 mL methanol (including the rinsing volume). The supernatant is diluted and analyzed as above.

The results of the validation study of BASF method 408/1 (*already peer-reviewed study BASF DocID 1998/11314*) and BASF method D0004 (*already peer-reviewed study BASF DocID 2001/5000881*) as well as the procedural recovery results obtained within this study demonstrate that BAS 510 F residues in soil can be accurately determined with a limit of determination of 0.01 mg kg^{-1}.
Results were calculated on a wet soil basis and uncorrected for procedural recovery.

Procedural recovery experiments were conducted using untreated specimens. Fortification levels of boscalid were at of 0.01, 0.1 and 1.0 mg kg^{-1}.

6. Estimation of the plateau residue level of boscalid in soil after multi-year application

Conceptual approach
The conceptual approach to derive the plateau residue level of boscalid in soil that was considered most appropriate to account for the observed accumulation pattern with its marked change in residue development was:

- to gain information about the dissipation behavior over the course of the study period gain observations from all sampling events,
- to optimize the model to predict residues of boscalid in soil over time based on observations 2 to 15 only, because the increase in residues of more than 100 % of the nominal application rate from sampling event 16 on implies that including those observation may lead to a biased model fit and consequently biased prediction of the residue plateau level of boscalid in soil,
- to run the optimized model for the eleven-year period of the study and to derive the plateau residue level from that period of the modeled time series where steady state conditions (equilibrium of application and dissipation) are approximated.

Model overview
Residues in soil of boscalid were predicted using a compartment model that considers the following processes:

- application of the a.s. at individual events
- crop interception of the a.s. at individual application events
- deposition of intercepted a.s. on the soil surface (e.g. via falling leaves) at any time after application (e.g. during harvest)
- dissipation in soil of the a.s. between application events with regard to soil temperature and moisture.

The soil compartment receives input from individual application events that reflect the combined effect of the processes of application, crop interception, and deposition of intercepted a.s. on soil surface. Interception and deposition are considered as crop-specific parameters, i.e. individual values of interception and deposition were used for each crop. It is assumed that the process of crop interception and the process of deposition of the intercepted a.s. occur at the same time to avoid over-parameterization of the model. The dissipation of residues from the soil compartment is described using single-first order (SFO) kinetics.

Estimation of dissipation times
The dissipation time DT_{50} at reference conditions was calculated from site-specific DT_{50} values that were estimated for individual dissipation periods over the course of the study.

Dissipation periods extend from the end of one application period to the beginning of the next, i.e. from the last application in autumn to the next application in spring. Regarding the repeated triennial cultivation pattern in the present study (1st year: vegetables; 2nd year vegetables; 3rd year: cereals; repeated), typical dissipation periods extend over about 7 months (two consecutive vegetable cultivation years) or over about 7+12 months (cereals cultivation year before next vegetable cultivation year). Eight individual dissipation periods occurred during the study and the development of residue levels was characterized by two to five soil samples per period. For each dissipation periods, the SFO kinetic model was fitted to the observed residues in order to estimate the parameters $C_{initial}$ and DT_{50}.

Model optimization and modeling endpoints
The optimization procedure was based on observed residues from sampling events 2 to 15. Observations from sampling event 16 on were excluded, because increases of the residues of >100 % of the nominal application rate (despite dissipation processes) in the treated plots coincided with residue detection in the control plot. This strongly implies applications of boscalid that were not in line with the scheduled application pattern of the study.

The fitted model curve was used to estimate the plateau level and the peak level of residues of boscalid in soil at steady state after multi-year application. The plateau level reflects the typical minimum residue level in soil before annual application in spring and the peak level reflects the typical maximum residue level in soil after annual application in autumn.

In order to obtain representative estimates of the residue level of boscalid in soil at steady state after multi-year application, both the plateau level and the peak level were derived from predicted residues of the second "vegetable year" in each application cycle:

- the maximum of the predicted spring residues (n = 4) can be considered as adequate estimate of the plateau level of residues of boscalid in soil
- the maximum of the predicted autumn residues (n =4) can be considered as adequate estimate of the peak level of residues of boscalid in soil

II. RESULTS AND DISCUSSION

A. METHOD AND APPLICATION VERIFICATION

Residues in the soil samples of the Petri dishes (2007 and 2008)
The total amount of soil present in each Petri dish was extracted and analyzed for boscalid. A correlation of these measured data with the nominal application rate obtained from calculation of spray broth depletion during application confirms the excellent performance of the applications (see Table 7.1.2.2.2-4).

The application verification derived from sprayed amount and from application monitors (Petri dishes) is summarized in Table 7.1.2.2.2-4.

Table 7.1.2.2.2-4: Application verification of boscalid

Application No.	Date	DAFT	Formulation	Nominal application rate [g a.s. ha^{-1}]	Actual application rate [g a.s. ha^{-1}]	Application rate (Petri dishes) [g a.s. ha^{-1}]
31	22.05.2007	3295	WG	300	310	330
32	05.06.2007	3309	WG	300	290	255
33	13.09.2007	3409	WG	500	480	411
34	24.09.2007	3420	WG	500	485	428
35	09.10.2007	3435	WG	500	495	489
36	30.06.2008	3700	WG	300	290	287
37	14.07.2008	3714	WG	300	300	354
38	01.08.2008	3732	WG	300	305	323
39	11.09.2008	3773	WG	400	390	370
40	14.10.2008	3806	WG	400	390	374

DAFT = day after first treatment

B. FINDINGS

Procedural recovery and application verification
To demonstrate the efficiency of the analytical procedures for routine analyses within this study, procedural recovery experiments with the used field soils were performed. The mean recovery obtained for boscalid was 95.9 ± 9.1% (n = 112).
Application verification by means of Petri dish analysis demonstrated, that the application rates calculated from measured concentrations are in agreement with the target rates confirming excellent performance of all scheduled applications.

Residue levels in the control plots
Until sampling No. 15, the residues in the control plot and the treated plots showed regular behavior. Starting with sampling 16 and lasting until the end of the study, residues in the control plot were observed at varying concentrations. At the same time, unexpected high and also varying concentrations in all three treated plots were observed. In addition, the variability between the treated plots increased. Investigations were undertaken in order to explain this development. As a result, additional irregular applications on both, the control and treated plots must be assumed. A summary of the residue data in the control plots is given in Table 7.1.2.2.2-5. Detailed results of the residue analysis of the soil samples of different depths of the untreated control plot are given in the study report.

Residue levels in the treated plots
Detailed results of the residue analysis of the soil samples of different depths of the untreated control plot (treatment 1) and the treated plots (treatments 2, 3, 4) are given in the study report.

Residues measured in the treated plots showed regular behavior until sampling No. 15 in April 2005. Concentrations in soil increased after each annual application period followed by a period of degradation with decreasing residues. After application in the growing season, significant residues of boscalid were detected in soil in the spring of the following year and were distributed also to deeper soil layers. This was caused by soil treatments like tillage or ploughing to a maximum depth of 35 cm. However, the highest amounts of residues were detected from 0 to 30 cm depth.

From sampling no. 16 on, an unexpected development of the soil residues was observed that coincided with the beginning of residue detection in the control plot.

In the treated plots, residue levels after the application period increased compared to residue levels before that period by 106 % to 127 % of the nominal yearly application rate of boscalid. An increase in residues of 127 % of the yearly application rate was observed at sampling event 22 in 2007 when also extraordinary high residues were detected in the control plot. Assuming treatment of the plots according to the application scheme of the study, an increase in residues of more than 100 % of the nominal yearly application rate is not possible, especially since dissipation processes reduce the amount of residues over the year.

A comparison showed that the residue behavior was very heterogeneous among the three treated plots. In autumn 2007 (sampling 22) residue levels increased compared to the preceding spring (sampling 21) by about 2 kg ha^{-1} to 2.7 kg ha^{-1} to 2.1 kg ha^{-1} for plots 2, 3 and 4 with applied amounts of only 1.5 kg ha^{-1} (3 x 0.5 kg ha^{-1}) in between.

Between April and August 2008 (samplings 23 and 24), amounts of 0.9 kg boscalid ha^{-1} (3 x 0.3 kg ha^{-1}) were applied to plot 2, 3 and 4. In spite of this, boscalid residues in plot 3 and 4 decreased, while in plot 2 an increase by 2.2 kg ha^{-1} was observed. In November 2008 (sampling 25), after application of 0.8 kg ha^{-1} (2 x 400 g ha^{-1}) in between, amounts in plot 2 decreased, while in plot 3 and 4 the residue levels increased by 2.8 and 1.7 kg ha^{-1}, respectively.

As a result, the variability between the three treated replicates is very high at sampling 22, 24 and 25 which is a further indication that the residue situation cannot be explained by the regular planned applications, which were verified via Petri dish analysis.

From these results and additional investigations it was concluded that additional irregular applications must be assumed. Further details of these additional applications could not be elucidated. A summary of the residue data is given in Table 7.1.2.2.2-5.

Table 7.1.2.2.2-5: Measured residues of boscalid in the plots of the vegetable accumulation study

Sampling event			Crop cultivation year	Residues measured in 0-50 cm [a] [kg ha^{-1}]						
				Control plot		Treated plots				
No.	Date	DAFT [d]		Treatment 1	Increase [b] [%]	Treatment 2	Treatment 3	Treatment 4	Mean	Increase [b] (Mean) [%]
1	30.04.1998	- 14	Vegetables		n.c.	<0.01	<0.01	<0.01	<0.01	66
2	12.10.1998	151		-		0.630	1.017	2.483	1.377	
3	08.03.1999	298	Vegetables	-		0.623	1.029	0.491	0.714	48
4	03.11.1999	538		-		1.621	1.871	1.085	1.526	
5	13.03.2000	669	Cereals	-		1.088	0.930	0.821	0.946	n.c.
6	21.08.2000	830		-		1.219	1.084	0.941	1.081	
7	04.04.2001	1056	Vegetables	-		0.479	0.628	0.555	0.554	54
8	06.11.2001	1272		-		1.523	1.893	1.638	1.685	
9	28.02.2002	1386	Vegetables	-		1.193	1.352	1.274	1.273	75
10	19.11.2002	1650		-		2.703	2.429	2.526	2.553	
11	17.03.2003	1768	Cereals	-		0.822	1.008	0.771	0.867	n.c.
12	21.08.2003	1925		-		1.685	2.285	1.694	1.888	
13	15.03.2004	2132	Vegetables	-		1.025	1.147	1.124	1.099	43
14	21.10.2004	2352		-		2.155	2.030	1.844	2.010	
15	04.04.2005	2517	Vegetables	-	17	1.588	-	1.063	1.326	112
16	17.11.2005	2744		0.294		3.018	3.265	3.388	3.224	
17	28.03.2006	2875	Cereals	0.095	n.c.	2.201	2.128	1.848	2.059	n.c.
18	23.08.2006	3023		0.194		2.383	2.204	1.728	2.105	
19	08.11.2006	3100		0.248		1.675	2.069	1.949	1.898	
20	02.04.2007	3245	Vegetables	0.084	54	1.202	1.123	1.654	1.326	127
21	26.07.2007	3360		0.334		1.684	2.158	1.435	1.759	
22	28.11.2007	3485		1.223		3.635	4.830	3.530	3.998	
23	25.04.2008	3634	Vegetables	0.376	34 [c]	1.472	2.100	2.141	1.904	106
24	18.08.2008	3749		1.255		3.652	1.791	1.408	2.284	
25	27.11.2008	3850		0.956 [c]		3.326	4.612	3.181	3.706	
26	02.04.2009	3976	Cereals	0.616		2.149	1.926	2.226	2.100	

DAFT = days after first treatment
- = no detection of boscalid
n.c. = not calculated
[a] The sum of residues in kg ha^{-1} that is calculated from concentrations measured in 0–50 cm with a default bulk density of 1.5 g cm^{-3} for samplings 1 to 15 and individual field bulk density values for samplings 16 to 26
[b] Difference between measured residue level in spring and subsequent autumn sample expressed as percentage of nominal application rate of the respective application season [%]
[c] additional high residue detected in one layer but not considered for summarized residue

Dissipation time

A total of eight individual dissipation periods in the course of the study were used to estimate a representative DT_{50} value for modeling the plateau residue level of boscalid in soil. The dissipation periods include data measured from 2005 onward, because the observed residue decline in dissipation periods before and after 2005 did not differ (see Table 7.1.2.2.2-6), although residues began to increase unexpectedly in 2005 as described before. The estimated DT_{50} values ranged from 23 to 264 d and were in the same order of magnitude as DT_{50} values obtained from various dissipation trials in the field. As a conservative approach, the maximum DT_{50} value of 264 d was used to model the residue level in soil of boscalid.

Table 7.1.2.2.2-6: Residue levels in the treated plots over the course of the study

Sampling event	Sampling date	Days after first treatment [d]	Crop cultivation year	Average sum of residues measured in 0-50 cm [kg a.s. ha^{-1}]	Residues in spring [a] [% of residues in autumn]	Dissipation periods
1	30.04.1998	- 14	Vegetables	0.000	N/A	N/A
2	12.10.1998	151		1.377	52	1
3	08.03.1999	298	Vegetables	0.714		
4	03.11.1999	538		1.526	62	2
5	13.03.2000	669	Cereals	0.946		
6	21.08.2000	830		1.081	51	
7	04.04.2001	1056	Vegetables	0.554		
8	06.11.2001	1272		1.685	76	3
9	28.02.2002	1386	Vegetables	1.273		
10	19.11.2002	1650		2.553	34	4
11	17.03.2003	1768	Cereals	0.867		
12	21.08.2003	1925		1.888	58	
13	15.03.2004	2135	Vegetables	1.099		
14	21.10.2004	2352		2.010	66	5
15	04.04.2005	2517	Vegetables	1.326		
16	17.11.2005	2744		3.224	64	6
17	28.03.2006	2875	Cereals	2.059		
18	23.08.2006	3023		2.105	-	
19	08.11.2006	3100		1.898	70	
20	02.04.2007	3245	Vegetables	1.327		
21	26.07.2007	3360		1.759	-	
22	28.11.2007	3485		4.000	47	7
23	25.04.2008	3634	Vegetables	1.897		
24	18.08.2008	3749		2.284	-	
25	27.11.2008	3850		3.706	57	8
26	02.04.2009	3976	Cereals	2.100		
				Mean value	**58**	

[a] residue level measured in spring sample expressed as percentage of residue level measured in previous autumn sample
N/A not applicable

Model fit

Residue levels of boscalid in soil were modeled using a DT_{50} value of 264 d. The model was fitted to field observations from sampling events 2 to 15 by optimizing the deposition parameter $f_{deposit}$. Observations from sampling event 16 on were not considered for fitting, because increasing residues of > 100% of the nominal application rate in the treated plots coincided with residue detection in the control plot indication applications of boscalid out of the scheduled application pattern of the study.

The model curve obtained by the optimization procedure is given in Figure 7.1.2.2.2-1.

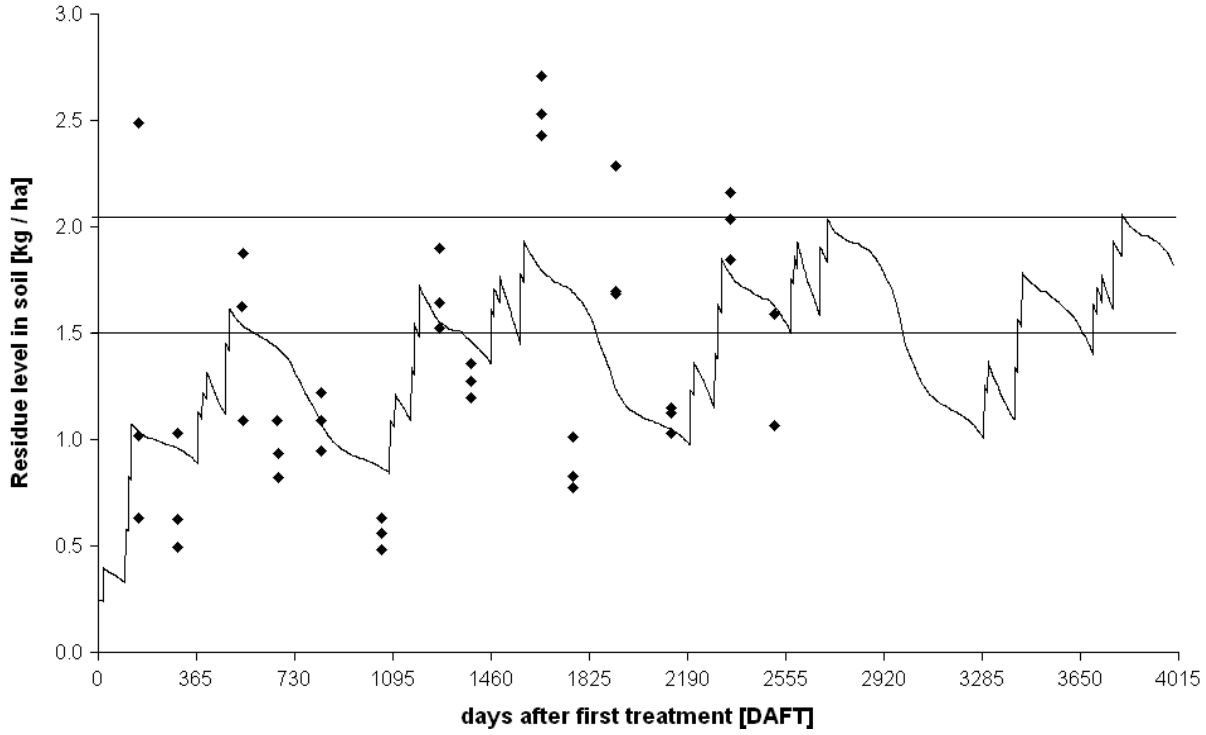


Figure 7.1.2.2.2-1: Observed soil residues of boscalid during multi-year application [sampling events 2 to 15] and fitted model curve (optimised $f_{deposit}$)

Figure 7.1.2.2.2-1 presents measured residue levels during the first three application cycles of the study (sampling events 2 to 15, black dots) and the curve of the modeled residues. In each of the four triennial application cycles, predictions oscillate between minimum and maximum values. Both minimum and maximum values in the third and fourth application cycle are comparable, indicating that the model curve approximates steady state conditions in the third application cycle. Visual assessment shows that modeled residues match observations fairly well given the variability in replicate samples.

In Figure 7.1.2.2.2-2, modeled residue levels are plotted a) with field observations from sampling events 2 to 15 that were used to fit the model and b) with field observations from sampling events 16 to 26. The latter were not used for fitting because of the quantifiable contamination of control samples and the observed increase in average residue level on the treated plots from spring to autumn that was 106% to 127% of the nominal yearly application rate despite dissipation processes. This coincidence of increases of residues of > 100% of the nominal application rate (despite dissipation processes) in the treated plots and the detection of residues in the control plot strongly implies applications of boscalid that were not in line with the scheduled application pattern of the study and hints to at least three additional applications at high rate between sampling events 15 and 16, 20 and 22, and 23 and 25.

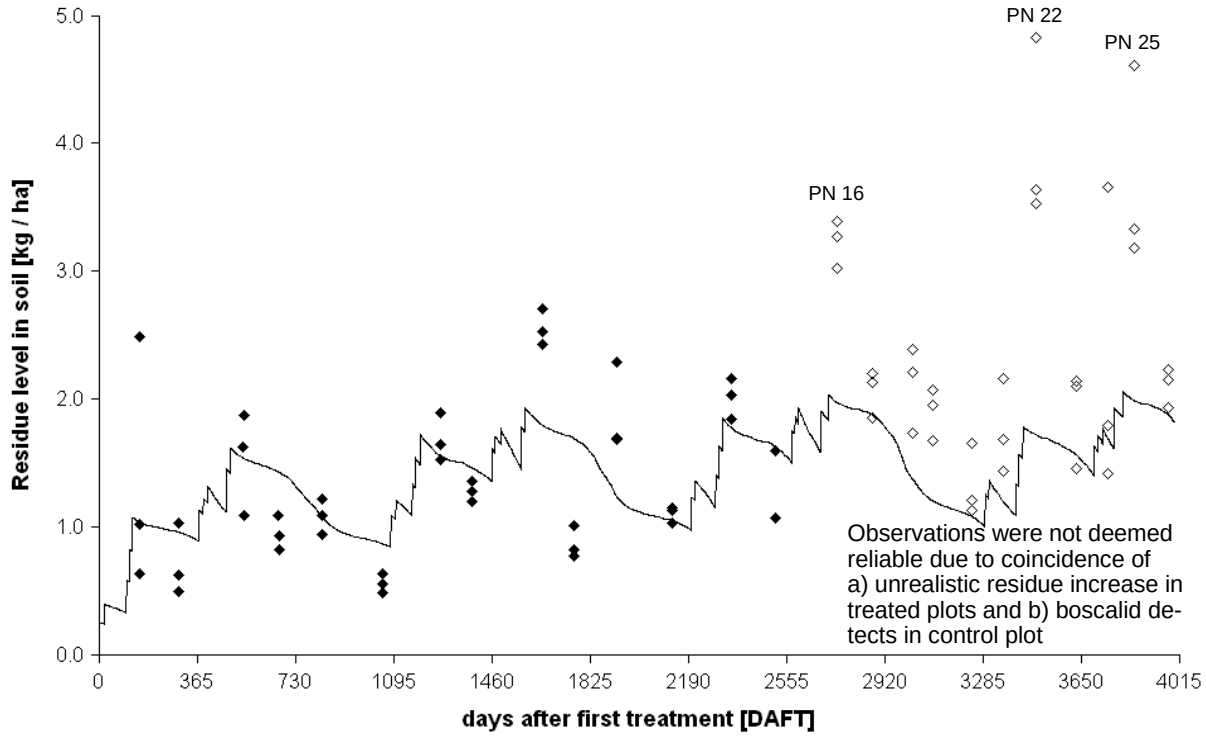


Figure 7.1.2.2.2-2: Observed soil residues of boscalid during multi-year application and fitted model curve (deposition parameter optimised using sampling events 2 to 15)

Plateau level and peak level in soil at steady state

Plateau level and peak level in soil at steady state, i.e., typical minimum and maximum residues after multi-year use, were derived from the modeled residue data presented in Figure 7.1.2.2.2-1.

The residue plateau (i.e., the maximum of predicted residue levels in spring of the second vegetable year, n = 4) was predicted in year 8 of the study and amounted to 1.50 kg ha^{-1}. The peak level (i.e., the maximum of predicted residue levels in autumn of second vegetable year, n = 4) was predicted in year 11 of the study and amounted to 2.06 kg ha^{-1}. These values correspond to 0.333 mg kg^{-1} and 0.457 mg kg^{-1} assuming a soil bulk density of 1500 kg m^{-3} and a soil layer of 0.3 m that is deemed a realistic depth of soil cultivation in vegetable crops.

The predicted plateau and peak level correspond to an average yearly application rate of boscalid of 1.27 kg ha^{-1} onto vegetable crops (average rate considering application and no-application years) and can be expressed as 118% and 162% of the average yearly application rate.

III. CONCLUSIONS

The plateau level of residues of boscalid in soil at steady state after multi-year application was predicted in year 8 of the eleven-year accumulation study. The predicted plateau amounted to 1.5 kg ha^{-1} or 118 % of the average yearly application rate of the study. Assuming a soil bulk density of 1500 kg m^{-3} and a soil layer of 0.3 m, the predicted plateau corresponds to 0.333 mg kg^{-1}.

The peak level was predicted in year 11 of the study and amounted to 2.06 kg ha^{-1} or 162% of the average yearly application rate of the study. Assuming a soil bulk density of 1500 kg m^{-3} and a soil layer of 0.3 m, the predicted peak level corresponds to 0.457 mg kg^{-1}.

Predictions were based on a field DT_{50} that was derived from residues observed during the entire course of the study. The model to estimate peak and plateau level was fitted to observed residues in samples from the first eight years of the study (sampling events 1 to 15) only, because observed residues from sampling event 16 on were not considered reliable. Increases of residues of > 100 % of the nominal application rate (despite dissipation processes) in the treated plots coincided with residue detection in the control plot. This strongly implies applications of boscalid that were not in line with the scheduled application pattern of the study and hints to at least three additional applications at high rate between sampling events 15 and 16, 20 and 22, and 23 and 25.

Report:	CA 7.1.2.2.2/2 Richter T.,Kuhnke G., 2015 a Interim Report - Accumulation and dissipation behavior of BAS 510 F (Boscalid) in soil under field conditions in Northern Italy following repeated application onto vegetables over several years 2015/1178191
Guidelines:	SANCO/3029/99 rev. 4 (11 July 2000), EEC 95/36, SETAC, BBA IV 4-1, ECPA Guidance Document on Field Soil Dissipation Studies Aug. 1997, EEC 7029/VI/95 rev. 5 (July 22 1997), IVA Guideline Residue Studies Part V (1992)
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

The accumulation behaviour of boscalid is investigated and reported here at a field site near Poggio Renatico, Italy in alternating crops of lettuce and cabbage (every first year) and green beans and carrots (every second year). It is an ongoing study, which was started in May 2009 and is scheduled to last for several years. Data available up to now cover the field work started in 2009 until 2014 and the analytical results up to and including sampling no. Y6_S3 (November 2014).

The trial site represents a typical region of agricultural practice and was already cropped with lettuce when starting the study in 2009. It consists of an untreated area for control purposes and a treated area intended for applications.

The product BAS 516 07 F (formulated as WG) was broadcast applied at four times each year on the treated plot which was three-fold replicated with a subplot size each of 240 m². The nominal rate for each application was 400 g boscalid (BAS 510 F) per hectare and the target water volume was 400, respectively 500 L ha^{-1}. The test item was first applied to lettuce at BBCH growth stage 15-41 and repeated after 7-8 days, application to cabbage was first at BBCH growth stage 41 and was repeated after 14 days. Application to green beans started at BBCH growth stage 51-55 and was repeated after 7-8 days, application to carrots started at BBCH growth stage 41-42 and was repeated at 8-13 days.

The actual application rates, determined by quantifying the amount of spray discharged, ranged from 390 to 402 g a.s. ha^{-1} for each application. Dose verification conducted via application monitors yielded average application rated between 353 and 484 g ha^{-1} equivalent to 88, respectively 121% of the intended rate across all applications.

During the course of the study, the field was subjected to usual agronomic measures in line with good agricultural practice. Grubbing of the soil was not deeper than 40 cm. The trial site was irrigated according to GAP (crop need) by sprinkler irrigation every 14 days matching the amount evaporated during this period (Non-GLP). Climatic conditions were recorded by means of an on-site weather station.

Soil samples were taken to a depth of 50 cm three times a year. The first sampling each year was after soil preparation before planting/seeding of the first crop, the second sampling after harvest of the first crop before soil preparation, and the third sampling was after harvest of the second crop before soil preparation. The soil cores were cut into 10 cm sections immediately after sampling, samples from 0-10 cm depth were cut into 5 cm sections. Soil segments of the same depth were pooled for the control plot and each treated replicate subplot separately. All soil specimens were stored deep frozen at ≤ -18°C within less than six hours and were kept at this temperature until shipment in frozen state to BASF SE, Limburgerhof, Germany. The frozen soil segments were ground up together with dry ice and representative aliquots of the homogenized soil specimens were taken for residue analysis. All soil specimens remained frozen at ≤ -18°C until final analysis.

Field soil specimens were analysed for boscalid using BASF method L0096/01. The analytical method involved extraction with methanol/acetate buffer 80 + 20 (v/v) and subsequent determination of BAS 510 F by LC-MS/MS. The limit of quantification (LOQ) was 0.01 mg kg^{-1}. Application monitors (Petri dish specimens) were analysed for boscalid using the same method with minor adaptations accounting for the larger quantity of soil. The validity of the analytical method was proven within the present study by analysis of untreated and fortified samples.

After a period of decreasing yearly rate contributions resulting in a flattening of the cumulated concentration levels for this first 6 years of the study, this trend is an indicator for approaching a plateau concentration soon.

I. MATERIAL AND METHODS

1. Test material

Test item (formulation):	BAS 516 07 F
Active substance (a.s.):	BAS 510 F
<u>Test item used in 2009</u>	
Batch no.:	11041
Content of active substance:	267 g/kg BAS 510 F (nominal content 269 g/kg) 65 g/kg BAS 500 F (nominal content 67 g/kg)
<u>Test item used in 2010</u>	
Batch no.:	08-120050
Content of active substance:	266 g/kg BAS 510 F (nominal content 267 g/kg) 69 g L^{-1} BAS 500 F (nominal content 67 g/L)

Test item used in 2011/2012
Batch no.: 08-120050
Content of a.s.: 266 g/kg BAS 510 F (nominal content 267 g/kg)
69 g/kg BAS 500 F (nominal content 67 g/kg)

Test item used in 2013/2014
Batch no.: 12-000110
Content of active substance: 271 g/kg BAS 510 F (nominal content 267 g/kg)
67 g/kg BAS 500 F (nominal content 67 g/kg)

2. Test site

The accumulation behaviour of boscalid under field conditions was investigated starting from May 2009. The selected site is located within a typical agricultural area for growing crops, especially vegetables, and has been under cultivation for years before the start of the trial. The trial is located in Italy, near Poggio Renatico in the Emilia Romagna Region (Ferrara Province) on a sandy silt soil with an organic carbon content of 1.2%, a pH value ($CaCl_2$) of 7.5, cation exchange capacity of 16.3 mVal 100 g^{-1} dry soil and a maximum water holding capacity of 37.0 g water 100 g^{-1} dry soil (top soil).

3. Experimental treatments

Starting in 2009, the trial area was alternatingly cropped with lettuce, cabbage in the first, third and fifth year, and with green bean and carrot in the second, fourth and sixth year. The product was broadcast applied onto the crop four times a year using a calibrated boom sprayer. The formulation BAS 516 07 F (product name “Signum”) was used. The formulation is an WG formulation with a nominal content of 267 g boscalid/kg. BAS 516 07 F additionally contained the active substance BAS 500 F at a nominal content of 67 g a.s./kg. The nominal rate for each application was 400 g boscalid ha^{-1}.

The test item was first applied to lettuce at BBCH growth stage 15-41 and repeated after 7-8 days. Application to cabbage was first at BBCH growth stage 41 and was repeated after 14 days. Application to green beans, second year, started at BBCH growth stage 51-55 and was repeated after 7-8 days. Application to carrots started at BBCH growth stage 41-42 and was repeated after 8-13 days. In 2011 and 2013, the first application to lettuce was delayed due to unfavourable weather conditions.

At each application, a separate spray mixture was prepared for each treated subplot, and the test item was applied to each subplot separately. The actual application rates, determined by quantifying the amount of spray discharged, ranged from 390.3 to 401.7 g a.s. ha^{-1} averaged over the three replicate subplots.

Application verification
In addition, the dose was verified by means of sampling Petri dishes filled with standard soil. The petri dishes were placed on the treated plots (5 in each subplot) before each application. On completion of the application, the petri dishes were stored in a freezer at ≤ -18°C.

Agronomic measures
Details to previous crops, fertilization and crop protection measures can be found in the interim study report. In terms of molecular structure, the additionally applied pesticides were not similar to boscalid.
The grubbing of the soil was not deeper than 40 cm. The crops were harvested according to good agricultural practice.
Air temperature, soil temperature, air humidity, precipitation, evapotranspiration, wind velocity and global radiation were recorded daily at an on-site weather station. Air temperatures were measured at 2 m height, soil temperatures at 10 cm depth. Details can be found in the Interim Study Report or the Interim Field Phase Report.

4. Sampling and storage

In addition to sampling the application monitors (Petri dish samples) after each application, soil samples were taken to a depth of 50 cm three times a year. The first sampling each year was performed after soil preparation before the planting/seeding of the first crop, the second sampling after harvest of the first crop before soil preparation, and the third sampling was performed after harvest of the second crop before soil preparation. Details are summarised in the interim study report.

Soil specimens were taken from ten points (within a defined sub-subplot [SSP]) of each of the three treated subplots (coded as replicate 2, 3, 4) as well as from the untreated plot (coded as replicate 1). Already sampled SSPs were not sampled again.

From study start, including first soil sampling in 2011, soil cores were taken manually from 0 - 30 cm depth and 5 cm diameter in one step fitted with acetate liners. Sampling of deeper layers is described below.

From second sampling in 2011 on, soil segments from 0 – 10 cm depth were taken separately using flange metal tubes pressed into the ground. The soil within this flange was collected through a manual soil corer fitted with acetate liners. The metal flange remained in the soil until completion of soil samplings. Soil segments from 10 – 30 cm and 30 – 50 cm depth were taken separately using a manually soil corer fitted with acetate liners. Sampling for the soil cores at 10 – 30 cm depth and 5 cm diameter was started at the bottom of the 10 cm hole originating from sampling of the 0 – 10 cm depth described above. Sampling for the soil cores at 30 – 50 cm depth with 2.5 cm diameter was started at the bottom of the 30 cm hole originating from sampling of the 10 - 30 cm depth described above.

The earlier taken, 0 - 30 cm field soil cores were cut after sampling and before freezing in 0 - 5, 5 - 10, 10 - 20 and 20 - 30 cm segments with an electric saw.

The 0 - 10 cm main sample cores taken separately were cut either immediately after each sampling and before freezing into 5 cm segments with an electric saw.

Furthermore and as well immediately after sampling and before freezing, the field fresh soil cores from 10 – 30 cm and 30 – 50 cm soil cores from the main samples were cut into 10 cm segments with an electric saw.

Generally, soil core segments of each individual replicate subplot were pooled according to their soil depth in a polyethylene bag, double bagged and a unique sample no. was assigned to the resulting pool sample. The remains of the plastic liners were disposed of.

All soil specimens were deep frozen at a temperature around or below -18°C within less than 6 hours time. Additional to the main samples, double samples (= retain samples) were taken at all sampling times except for the application verification samples (Petri dish samples). They were taken in the same way, the same quantity, and from the same sub-subplots as the main samples. The double samples were not segmented but placed directly into freezer storage at the field test site as whole cores in the plastic liners.

5. Analytical procedure

Field soil specimens were analysed for Boscalid using BASF method L0096/01. The analytical method involved extraction with methanol/acetate buffer 80 + 20 (v/v) and subsequent determination of Boscalid by LC-MS/MS without further clean up or concentration steps. The limit of quantification (LOQ) was 0.01 mg kg^{-1} based on dry weight.

Petri dish specimens were analysed for Boscalid using the same method with minor adaptations to account for the larger quantity of soil to be extracted with the same LOQ.

Analysis of field soil specimens originating from the treated plots was conducted down to a maximum soil depth of 50 cm.

The validity of the analytical methods was demonstrated within the present study by analysis of untreated and fortified samples.

II. RESULTS AND DISCUSSION

A. APPLICATION VERIFICATION

Residue levels of Boscalid achieved on extraction and analysis of the application monitors (Petri dishes filled with standard soil) were converted into area concentration (in g ha^{-1}) taking into account the area of the Petri dishes (91.6 cm^2).

As a result, the obtained rates for the individual applications ranged from 353 to 484 g ha^{-1}, representing 88-121% of the target application rate with a mean value of 421 g ha^{-1} (105% of target rate), respectively.

The results show low variability of residues and are in excellent agreement with intended application rates. Results are summarised in Table 7.1.2.2.2-7.

Table 7.1.2.2.2-7: Application verification of boscalid

Application No.	Date	DAFT	Nominal application rate [g as/ha]	Actual application rate [g as ha^{-1}] [a]	Application rate (Petri dishes) [g as ha^{-1}] [b]	% of nominal application rate
1	18.06.09	0	400	400.0	414.8	104
2	26.06.09	8	400	390.3	400.3	100
3	05.10.09	109	400	399.0	403.9	101
4	19.10.09	123	400	399.7	458.5	115
5	06.07.10	383	400	397.0	414.8	104
6	14.07.10	391	400	398.0	423.9	106
7	30.09.10	469	400	399.0	402.1	101
8	08.10.10	477	400	397.0	413.0	103
9	13.06.11	725	400	398.0	376.6	94
10	20.06.11	732	400	391.3	464.0	116
11	10.10.11	844	400	395.3	447.6	112
12	24.10.11	858	400	397.0	436.7	109
13	20.06.12	1098	400	399.3	476.7	119
14	27.06.12	1105	400	401.0	478.5	120
15	25.10.12	1225	400	401.3	449.4	112
16	07.11.12	1238	400	398.0	418.5	105
17	17.06.13	1460	400	397.0	405.7	101
18	25.06.13	1468	400	401.7	484.0	121
19	14.10.13	1579	400	399.3	433.0	108
20	28.10.13	1593	400	393.0	358.4	90
21	13.06.14	1821	400	394.0	400.3	100
22	20.06.14	1828	400	401.3	353.0	88
23	20.10.14	1950	400	395.3	378.5	95
24	29.10.14	1959	400	398.3	407.6	102

DAFT = Days after first treatment

[a] Determined by measurement of spray liquid applied (average of three replicates)

[b] Petri dishes were placed directly on the soil surface at the border between subplots at positions that were free of plant foliage; calculation: application rate [g/ha] = residue petri dish [mg/kg] * 10^5 / 20 * 91.6 [cm^2], with: 10^5: conversion factor including conversion of [ha] to [$cm^{2]}$ and [mg] residue to [g]; 20: conversion residue from kg to 50 g soil weight; 91.6 cm^2: area Petri dish; example: subplot 2, application 2009-1: petri dish mean residue: 7.6 mg/kg * 10^5 / 20 * 91.6 = 7.6 * 10^5 / 1832 = 414.8 g/ha

B. FINDINGS

Procedural recoveries

Procedural recovery experiments performed with untreated field soil specimens spiked with boscalid at concentration levels of 0.01, 0.1 and 1 mg kg^{-1} yielded overall mean recovery rates between 96.7 and 97.6%, confirming the validity of the analytical method used in this study. Detailed results are summarised in Table 7.1.2.2.2-8.

Table 7.1.2.2.2-8: Method procedural recoveries for boscalid

Analyte	Fortification levels [mg kg^{-1}]	Average recovery [%] ± RSD	n
boscalid	0.01	96.7 ± 7.0	42
boscalid	0.1	96.7 ± 5.8	28
boscalid	1	97.6 ± 7.6	38
	Mean value	97.0 ± 6.9	108

RSD = Relative Standard Deviation

The data prove that the analytical method applied is suitable to accurately determine residues of boscalid in soil down to a concentration of 0.01 mg kg^{-1} (LOQ).

Residue levels in the control plots

Untreated field soil specimens of the respective soil depths from the control plot were analysed for residues of boscalid. No residues above 30% of the LOQ were detected in any of the control samples proving that there were no interferences of the untreated soil material with the analytical procedures used.

Residue levels in the treated plots

The analytical average results of boscalid for the individual replicates 2, 3, and 4 of the treated plot are summarised in Table 7.1.2.2.2-9 to Table 7.1.2.2.2-11.

All residue values presented in this table are related to the dry weight of the soil and were not corrected for procedural recoveries. Residue levels of boscalid in µg kg^{-1} dry soil (Table 7.1.2.2.2-9) were converted to residue rates in g ha^{-1} taking into account the standard soil density of 1.5 g cm^{-3} (Table 7.1.2.2.2-10) and the actual soil density (Table 7.1.2.2.2-11) and were summed up for all depths between 0 and 50 cm analysed.

For the evaluation of the development of the g ha^{-1} rates by time, both either related to standard density and to actual density are plotted against the corresponding sampling events and graphically evaluated. The graphical plots are given below in Figure 7.1.2.2.2-1. After a period of decreasing yearly rate contributions resulting in a flattening of the graph for the first 6 years of the study, this trend is an indicator for approaching a plateau concentration soon. A detailed kinetic evaluation conducted in parallel resulted in a separate modelling report [*CA 7.1.2.2.2./3, BASF DocID 2015/1197308*].

Beside the development by time, the development by depth has additionally to be addressed. The rare and very low residues found in the 40-50 cm soil layer are explained by grubbing down to a depth of 40 cm during field cultivation. Related to the cumulated concentrations of the respective sampling, they are however not significant.

Table 7.1.2.2.2-9: Boscalid concentration (µg kg^{-1}) in soil of treated plot 2, 3 and 4 (years 2009 – 2014)

Sampling No	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Sampling event	Y1 S1	Y1 S2	Y1 S3	Y2 S1	Y2 S2	Y2 S3	Y3 S1	Y3 S2	Y3 S3	Y4 S1	Y4 S2	Y4 S3	Y5 S1	Y5 S2	Y5 S3	Y6 S1	Y6 S2	Y6 S3
DAFT (actual)	-30	26	216	313	405	586	698	763	881	1049/ 1050	1132	1280/ 1281	1440/ 1441	1489/ 1490	1623/ 1624	1783/ 1784	1860/ 1861	1986/ 1987
Depth [cm]	**Residues of boscalid in subplot 2 [µg kg^{-1}]**																	
0 – 5	0	616	1001	838	1443	1536	693	1218	1467	1257	1689	1998	1032	1854	2166	1371	1882	1995
5 - 10	0	0	188	262	291	618	792	392	661	1023	634	1012	1098	937	1104	1177	1124	924
10 – 20	0	0	22.3	30.9	12.8	124	483	115	127	419	355	371	304	248	309	354	285	198
20 – 30	-	-	0	0	0	11.0	37.3	26.9	20.5	101	54.3	26.5	37.1	25.8	22.7	59.2	55.8	67.9
30 – 40	-	-	0	0 [a]	0 [a]	0	0	0	0	16.9	46.3	24.8	26.8	54.3	0	21.7	45.4	21.6
40 – 50	-	-	-	0	0	0	0	0	0	0	0	0	14.8	0	0	13.7	19.1	31.3
cumulated to 0– 10 cm [µg kg^{-1}]	**0**	**308**	**617**	**581**	**880**	**1212**	**1262**	**947**	**1211**	**1677**	**1617**	**1928**	**1448**	**1724**	**1967**	**1723**	**1908**	**1779**
Depth [cm]	**Residues of boscalid in subplot 3 [µg kg^{-1}]**																	
0 – 5	0	959	792	1005	1345	1254	751	1619	1650	1349	1660	2301	1477	2089	2560	1905	2063	2066
5 - 10	0	0	229	559	308	594	722	796	654	989	981	870	1236	1150	1193	1021	1189	931
10 – 20	0	0	16.8	89.0	46.9	80.7	180	242	135	206	565	297	292	402	248	348	217	212
20 – 30	-	-	0	0	14.5	10.8	24.7	38.2	24.5	15.4	73.2	25.9	39.1	82.9	51.0	52.3	56.4	78.9
30 – 40	-	-	11.0 [b]	0 [a]	0 [a]	0	18.6	0	15.2	0	25.0	20.7	17.3	58.6	31.7	29.7	20.4	93.9
40 – 50	-	-	0	0	11.4	0	0	0	11.7	0	0	12.8	0	13.1	20.4	12.4	0	70.7
cumulated to 0– 10 cm [µg kg^{-1}]	**0**	**480**	**539**	**871**	**899**	**1016**	**959**	**1487**	**1338**	**1390**	**1984**	**1942**	**1705**	**2176**	**2228**	**1905**	**1920**	**1954**
Depth [cm]	**Residues of boscalid in subplot 4 [µg kg^{-1}]**																	
0 – 5	0	674	635	1100	1512	1391	654	1529	2161	1148	1738	2065	1313	1918	1841	1547	2122	2154
5 - 10	0	0	143	415	406	607	558	299	861	819	772	1056	1255	1089	1276	1138	1122	1144
10 – 20	0	0	18.0	40.5	135	194	177	81.2	188	157	377	246	441	310	364	307	310	349
20 – 30	-	-	0	0	14.3	17.3	12.8	18.2	23.2	20.3	57.2	30.0	36.2	47.2	26.0	37.8	35.5	30.3
30 – 40	-	-	0	0 [a]	0 [a]	0	0	12.2	0	0	21.9	23.0	11.4	33.5	0	0	23.2	22.4
40 – 50	-	-	-	0	0	0	0	0	0	0	0	0	0	0	0	0	16.0	0
cumulated to 0– 10 cm [µg kg^{-1}]	**0**	**337**	**407**	**798**	**1109**	**1211**	**795**	**1026**	**1722**	**1161**	**1711**	**1859**	**1773**	**1894**	**1949**	**1687**	**2006**	**2051**

DAFT = Days after first treatment - = sample not analysed "0" = value for residue < LOQ (limit of quantification)

[a] Measured values (30 – 40 cm), Y2_S1/Y2_S2: 51.0/346 µg kg^{-1} (Replicate 2), 54.1/192 µg/kg (Replicate 3), 77.9/190 µg kg^{-1} (Replicate 4);
Samples assumed to be contaminated from top soil due to sampling procedures at the field; from surrounding layers, residues supposed to be below LOQ; sampling procedure was changed to top soil sampling with a flange remaining in soil for deeper soil samplings.

[b] Due to measuring uncertainty, residues around LOQ could appear above LOQ and fit therefore not always in the vertical trend

Table 7.1.2.2.2-10: Boscalid concentration in soil of treated plot 2, 3 and 4 (years 2009 – 2014), converted to g ha^{-1} based on standard dry soil density of 1.5 g cm^{-3} for individual soil layers [a]

Sampling No	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Sampling event	Y1_S1	Y1_S2	Y1_S3	Y2_S1	Y2_S2	Y2_S3	Y3_S1	Y3_S2	Y3_S3	Y4_S1	Y4_S2	Y4_S3	Y5_S1	Y5_S2	Y5_S3	Y6_S1	Y6_S2	Y6_S3
DAFT (actual)	-30	26	216	313	405	586	698	763	881	1049/ 1050	1132	1280/ 1281	1440/ 1441	1489/ 1490	1623/ 1624	1783/ 1784	1860/ 1861	1986/ 1987
Depth [cm]	**Residues of boscalid in subplot 2 [g ha^{-1}] – calculated**																	
0 – 5	0	462	750	629	1082	1152	520	913	1100	943	1267	1499	774	1391	1625	1028	1412	1496
5 - 10	0	0	141	197	218	464	594	294	496	768	475	759	824	703	828	883	843	693
10 – 20	0	0	33.5	46.4	19.2	186	724	173	190	629	532	557	456	372	464	531	427	297
20 – 30	-	-	0	0	0	16.5	56.0	40.4	30.8	151	81.5	39.8	55.7	38.7	34.1	88.8	83.6	102
30 – 40	-	-	0	0	0	0	0	0	0	25.4	69.5	37.2	40.2	81.5	0	32.5	68.1	32.3
40 – 50	-	-	-	0	0	0	0	0	0	0	0	0	22.2	0	0	20.6	28.7	46.9
total residues in soil profile [g ha^{-1}]	**0**	**462**	**925**	**872**	**1320**	**1818**	**1894**	**1420**	**1817**	**2515**	**2425**	**2892**	**2172**	**2585**	**2950**	**2584**	**2862**	**2668**
Depth [cm]	**Residues of boscalid in subplot 3 [g ha^{-1}] – calculated**																	
0 – 5	0	720	594	754	1009	941	563	1214	1238	1012	1245	1726	1108	1567	1920	1429	1548	1550
5 - 10	0	0	172	419	231	446	541	597	490	742	736	652	927	863	895	766	892	698
10 – 20	0	0	25.2	134	70.4	121	270	363	202	309	848	446	438	603	372	522	326	318
20 – 30	-	-	0	0	21.8	16.2	37.1	57.3	36.8	23.1	109.8	38.9	58.7	124.4	76.5	78.5	84.7	118
30 – 40	-	-	16.5	0	0	0	27.9	0	22.8	0	37.5	31.1	26.0	87.9	47.6	44.5	30.7	141
40 – 50	-	-	0	0	17.1	0	0	0	17.6	0	0	19.2	0	19.7	30.6	18.7	0	106
total residues in soil profile [g ha^{-1}]	**0**	**720**	**808**	**1306**	**1349**	**1524**	**1439**	**2231**	**2007**	**2085**	**2976**	**2912**	**2557**	**3264**	**3341**	**2858**	**2881**	**2931**
Depth [cm]	**Residues of boscalid in subplot 4 [g ha^{-1}] – calculated**																	
0 – 5	0	506	476	825	1134	1043	490	1147	1621	861	1304	1549	985	1439	1381	1160	1591	1616
5 - 10	0	0	107	311	305	455	419	224	646	614	579	792	941	817	957	853	841	858
10 – 20	0	0	27.0	60.8	203	291	265	122	282	236	565	369	662	465	546	460	465	524
20 – 30	-	-	0	0	21.5	26.0	19.2	27.3	34.8	30.5	85.8	45.0	54.3	70.8	39.0	56.7	53.3	45.4
30 – 40	-	-	0	0	0	0	0	18.3	0	0	32.9	34.5	17.1	50.3	0	0	34.7	33.7
40 – 50	-	-	-	0	0	0	0	0	0	0	0	0	0	0	0	0	23.9	0
total residues in soil profile [g ha^{-1}]	**0**	**506**	**610**	**1197**	**1663**	**1816**	**1193**	**1539**	**2583**	**1742**	**2566**	**2789**	**2659**	**2841**	**2923**	**2531**	**3009**	**3077**

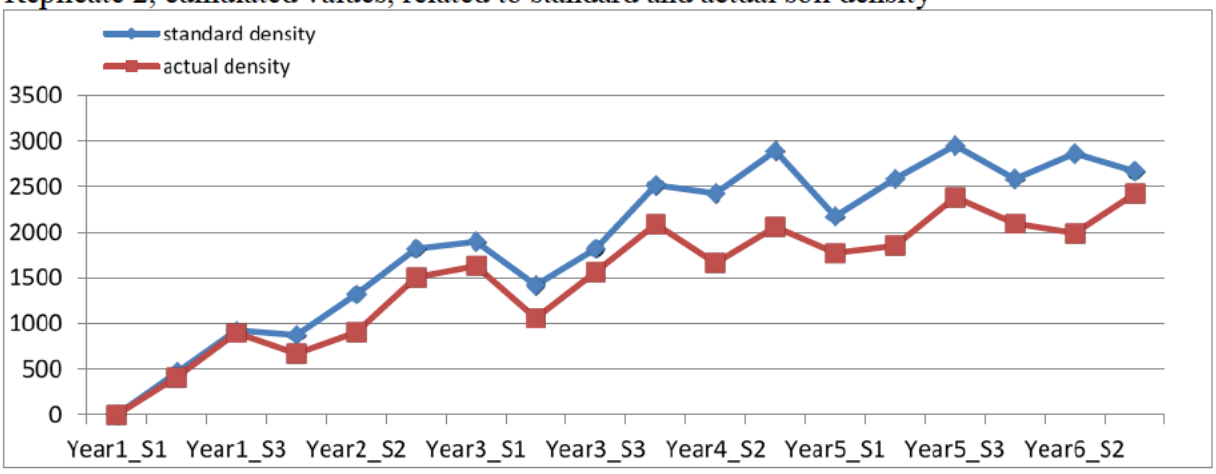
DAFT = Days after first treatment
- Sample not analysed
[a] Calculated assuming standard soil density of 1.5 g cm^{-3} for individual soil layers; residue values < 0.01 mg kg^{-1} (< LOQ; limit of quantification) were treated as 0 g ha^{-1}

Table 7.1.2.2.2-11: Boscalid concentration in soil of treated plot 2, 3 and 4 (years 2009 – 2014), converted to g ha^{-1} based on actual soil density for individual soil layers [a]

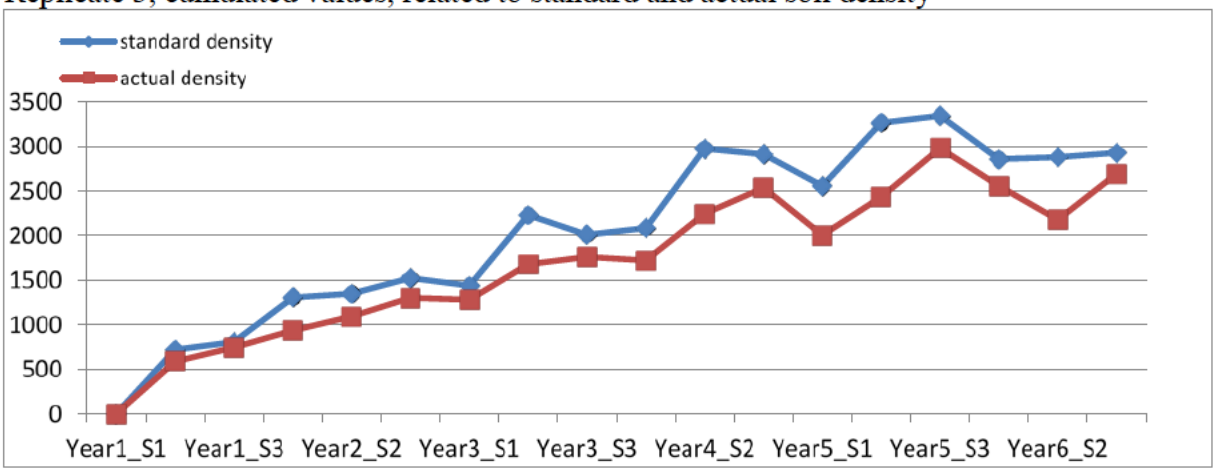
Sampling No	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Sampling event	Y1 S1	Y1 S2	Y1 S3	Y2 S1	Y2 S2	Y2 S3	Y3 S1	Y3 S2	Y3 S3	Y4 S1	Y4 S2	Y4 S3	Y5 S1	Y5 S2	Y5 S3	Y6 S1	Y6 S2	Y6 S3
DAFT (actual)	-30	26	216	313	405	586	698	763	881	1049/ 1050	1132	1280/ 1281	1440/ 1441	1489/ 1490	1623/ 1624	1783/ 1784	1860/ 1861	1986/ 1987
Depth [cm]	**Residues of boscalid in subplot 2 [g ha^{-1}] – calculated**																	
0 – 5	0	412	727	439	791	886	337	619	899	679	692	817	519	918	1211	737	901	1336
5 - 10	0	0	137	184	94.5	424	522	242	464	646	338	662	699	483	722	773	579	638
10 – 20	0	0	32.6	45.4	18.1	178	715	162	172	597	495	507	444	343	418	461	363	290
20 – 30	-	-	0	0	0	15.7	53.4	35.8	28.8	144	78.9	37.0	53.6	35.3	31.0	72.6	64.4	87.0
30 – 40	-	-	0	0	0	0	0	0	0	22.7	57.9	32.9	36.8	71.8	0	30.8	58.4	29.4
40 – 50	-	-	-	0	0	0	0	0	0	0	0	0	20.3	0	0	19.8	26.1	39.4
total residues in soil profile [g ha^{-1}]	**0**	**412**	**897**	**669**	**904**	**1504**	**1628**	**1058**	**1564**	**2089**	**1661**	**2056**	**1774**	**1851**	**2382**	**2095**	**1991**	**2421**
Depth [cm]	**Residues of boscalid in subplot 3 [g ha^{-1}] – calculated**																	
0 – 5	0	590	544	500	802	777	440	753	1000	729	760	1365	725	985	1639	1173	1098	1407
5 - 10	0	0	163	320	191	397	513	515	480	666	550	651	799	685	847	773	676	624
10 – 20	0	0	24.5	116	66.0	113	266	356	207	305	799	434	402	545	350	480	301	320
20 – 30	-	-	0	0	19.1	14.5	35.8	54.8	37.2	21.8	103	40.0	53.6	117	70.2	70.8	74.9	110
30 – 40	-	-	15.0	0	0	0	26.1	0	20.6	0	33.6	26.5	25.0	81.2	44.1	42.8	27.8	131
40 – 50	-	-	0	0	16.1	0	0	0	15.9	0	0	18.2	0	18.1	28.8	17.7	0	101
total residues in soil profile [g ha^{-1}]	**0**	**590**	**747**	**936**	**1094**	**1302**	**1281**	**1679**	**1761**	**1722**	**2245**	**2535**	**2005**	**2430**	**2979**	**2558**	**2178**	**2693**
Depth [cm]	**Residues of boscalid in subplot 4 [g ha^{-1}] – calculated**																	
0 – 5	0	386	464	574	789	840	364	872	1252	667	834	1311	693	1076	1140	891	1167	1470
5 - 10	0	0	104	252	233	411	371	193	621	520	473	746	868	649	898	740	745	719
10 – 20	0	0	24.0	58.8	189	285	246	118	274	229	538	337	640	404	532	431	410	474
20 – 30	-	-	0	0	19.5	25.6	17.5	26.2	33.4	29.2	57.5	44.9	54.0	67.2	36.8	51.2	44.3	37.5
30 – 40	-	-	0	0	0	0	0	15.3	0	0	29.0	28.6	15.6	45.9	0	0	28.8	30.2
40 – 50	-	-	-	0	0	0	0	0	0	0	0	0	0	0	0	0	21.5	0
total residues in soil profile [g ha^{-1}]	**0**	**386**	**591**	**884**	**1231**	**1561**	**999**	**1224**	**2180**	**1446**	**1931**	**2468**	**2271**	**2242**	**2607**	**2113**	**2417**	**2731**

DAFT = Days after first treatment
- = Sample not analysed
[a] Calculations are based on actual dry soil density for individual soil layers; residue values < 0.01 mg kg^{-1} (<LOQ) were treated as 0 g ha^{-1}

Replicate 2, cumulated values, related to standard and actual soil density



Replicate 3, cumulated values, related to standard and actual soil density



Replicate 3, cumulated values, related to standard and actual soil density

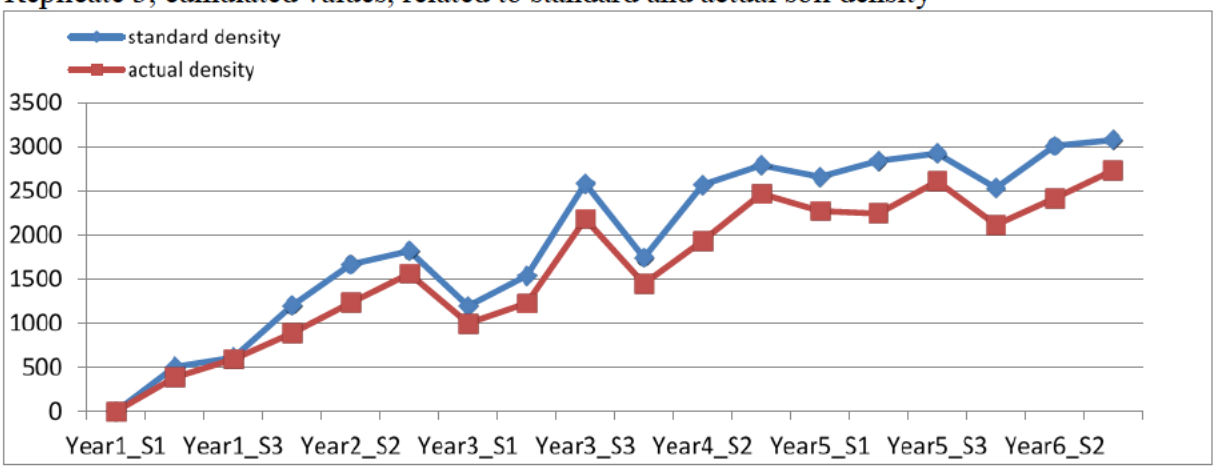


Figure 7.1.2.2.2-3: Observed soil residues of boscalid during multi-year application

III. CONCLUSION

In spring 2009, a field soil accumulation study was initiated at a trial site in Italy with alternating crops cycles of lettuce and cabbage planted at first year and green beans and carrots at second year. The data presented in this interim report cover the study period from start in May 2009 until end of 2014 (analytical results until sampling Y6_S3 in November 2014).

Applications during the investigated period are in agreement with the intended rates with very low variability.

Analytical methodology was well applicable for analysing residues of Boscalid in soil, either taken from the field and in application monitors (petri dishes).

Residues of boscalid follow a clear trend during the course of the study with very limited variations. All three treated replicate plots are in agreement especially when considering experimental procedures at the field which are determined to increase residue variability (e. g. applications onto crops, choice of soil sampling spots and soil cultivation).

Decreasing yearly rate contributions are observed during this first 6 years of the study resulting in a flattening of cumulated residues for that period. This trend is considered as an indicator for approaching a plateau concentration soon.

Residue contributions in 40-50 cm layer are due to deep ploughing. They are rare, very low and therefore of no significance for the accumulation behaviour of Boscalid.

The study will be continued until a final assessment can be made.

Report: CA 7.1.2.2.2/3
Platz K., 2015a
The plateau value in soil following long-term application of BAS 510 F - Boscalid derived from a field accumulation study in Northern Italy
2015/1197308
Guidelines: none
GLP: no

Note: This study was not listed in the application submitted for renewal of approval, but was subsequently conducted for the evaluation of the study CA 7.1.2.2.2/2.

Executive Summary

The accumulation behavior of boscalid (BAS 510 F) in soil has been investigated in a vegetable field accumulation study conducted in Northern Italy. An interim study report provides experimental data for a study period of five years. The purpose of this kinetic evaluation is to estimate the plateau value in spring before the first yearly application event on the basis of the experimental results. In a conservative approach, this value can be used for PEC calculation to cover the background soil concentration before the yearly treatments.

A SFO kinetic modeling approach was established with the optimization tool ModelMaker to calculate the plateau value at steady state under consideration of the increase of the sampled soil residues each year in spring, before the yearly application events. The estimated parameters of the modeling approach are the "(non-normalized) dissipation rate constant k" and the "yearly compound rate D that contributes to accumulation". As k was not corrected (normalized) to reference soil temperature and moisture conditions the modeled curve does not describe the actual daily dissipation behavior but the average annual dissipation behavior of the compound. The parameter D is influenced by multiple processes like the application rate, crop interception, wash-off events etc. The annotation "that contributes to accumulation" was added as the fraction of the soil load that undergoes short-term dissipation processes like photolytic degradation, volatilization, non-equilibrium sorption etc. does not contribute to accumulation in soil.

A DT_{50} value of 759.9 d was calculated under consideration of the estimated "dissipation rate constant k". The estimated "yearly compound rate D that contributes to accumulation" is 995.3 g ha^{-1} from a total application amount of 1600 g ha^{-1}. The appropriateness of the SFO kinetic model to describe the accumulation behavior and the reliability of the estimated parameters were tested with statistical indices recommended by FOCUS Kinetics. The t-test of the "dissipation rate constant k" showed that k was estimated significantly different from zero at P=0.05. The chi^2-error value is very low with 1.2% indicating a good fit of the observations by the applied SFO kinetic model. The residual plot that shows that the residuals are randomly scattered around zero also confirms the kinetic modeling approach. Hence, it can be concluded that the extrapolated plateau value at steady state is based on reliable basic assumptions.

The plateau value at steady state of the soil accumulation study boscalid conducted in vegetable crops was extrapolated with 2676 g ha^{-1}. Under consideration of the experimental total yearly application rate of 1600 g ha^{-1} the plateau can be expressed as accumulation factor of 1.67 of yearly total applied compound.

I. MATERIAL AND METHODS

The description and the experimental setup of the investigated soil accumulation trial is summarized in CA 7.1.2.2.2/2 [*Richter T. et al. (2015) - DocID 2015/1178191*].

SFO kinetic fit approach

A simplified SFO (single first order) kinetic modeling approach was established to calculate the plateau value under consideration of the field sampling data. The estimated model parameters are the “dissipation rate constant k” and the “yearly compound rate that contributes to accumulation D”. Further model parameters were not included in order to prevent an overparameterization of the model.

Dissipation rate constant (k):
Regular irrigation events onto the vegetable crops lead to balanced moisture conditions during the growing period in the different years. The yearly average soil temperatures measured in 10 cm depth are also comparable between study years. Furthermore, the same cultivation practices are applied in each study year. Hence, comparable average dissipation conditions and consequently comparable dissipation rates can be assumed for the individual years.

A SFO kinetic fit approach of the observations was applied that is characterized by the dissipation rate constant k. As k was not corrected (normalized) to reference soil temperature and moisture conditions the modeled curve does not describe the actual daily dissipation behavior but the average annual dissipation behavior of the compound.

Yearly compound rate D that contributes to accumulation (D):
Furthermore, because of regular crop cycles and the same application pattern being maintained during the study, one characteristic “yearly compound rate D that contributes to accumulation” was assumed for the modeling approach.

The “yearly compound rate D that contributes to accumulation” (hereafter “yearly compound rate D”) is influenced by multiple processes like the application rate, crop interception, wash-off events etc. The annotation “that contributes to accumulation” was added as the fraction of soil load that undergoes short-term dissipation processes like photolytic degradation, volatilization, non-equilibrium sorption etc. would not contribute to accumulation in soil.

The dependency of the plateau value in spring before the yearly application following long-term application of a compound from the “yearly compound rate D” and the “dissipation rate constant k” assuming SFO kinetics is shown in Equation 7.1.2.2.2-1.

Equation 7.1.2.2.2-1: Plateau value at steady state (A_{ss})

$$A_{ss} = D * \frac{e^{-k*t}}{(1 - e^{-k*t})}$$

where

D	yearly compound rate that contributes to accumulation	[g ha^{-1}]
k	dissipation rate constant	[1 d^{-1}]
t	yearly application interval (365 d)	

The amount of the compound in spring before the yearly application event following the year of N^{th} application is simply.

Equation 7.1.2.2.2-2: Compound amount following the year of N^{th} application (A_N)

$$A_N = D * \frac{(1 - (e^{-k*t})^N) * e^{-k*t}}{(1 - e^{-k*t})}$$

where

D	yearly compound rate that contributes to accumulation	[g ha^{-1}]
k	dissipation rate constant	[1 d^{-1}]
t	yearly application interval (365 d)	
N	application no.	

Sampling data used for modeling

The “dissipation rate constant k” and the “yearly compound rate D” were estimated under consideration of the yearly increase of the soil residues sampled in spring before the yearly applications (i.e. the first yearly sampling). Replicate values were taken into account following the FOCUS guidance [*FOCUS (2006)*].

The second and third yearly samplings were not included into the fit as they can be assumed to be affected by short-term processes and fluctuations in the dissipation behavior due to daily temperature and moisture conditions that are not covered by the simplified SFO kinetic model.

The residue data that were considered for the fit approach are given in Table 7.1.2.2.2-12.

Table 7.1.2.2.2-12: Experimental residue data used as model data

DAFT [a]	Boscalid [g ha^{-1}]
313.00001	669
313.00002	936
313.00003	884
698.00001	1628
698.00002	1281
698.00003	999
1049 [b]	2089
1050.00001 [b]	1722
1050.00002 [b]	1446
1440 [c]	1774
1441.00001 [c]	2005
1441.00002 [c]	2271
1783 [d]	2095
1784.00001 [d]	2558
1784.00002 [d]	2113

[a] Days after first treatment, in some cases an offset was applied for technical reasons
[b,c,d] Values were considered as replicates

Modeling Tool ModelMaker and Optimization Settings

As the yearly multiple application pattern of the accumulation study cannot be described by Equation 7.1.2.2.2-2, the modeling was carried out with the parameter estimation tool ModelMaker v.3 patch 3.0.4 [*Walker, A., Crout, N. (2007) ModelMaker, User Manual, Version 3.- Cherwell Scientific Publishing Limited, Oxford*].

The different experimental treatments were simulated in the model by introducing application events at the actual single application times (DAFT) with rates that were expressed as "yearly compound rate D" divided by the number of yearly single application events (n = 4).

The Marquardt procedure (option least squares) was used for optimizing the estimated parameters.

The t-test and the χ^2 test were performed as described in the DEGKIN EXCEL sheet recommended by FOCUS [*FOCUS (2007): FOCUS DEGKIN v2*].

The goodness-of-fit of the kinetic model was assessed by visual inspection and statistical measures, as recommended by the FOCUS Kinetics guidance [*FOCUS (2006)*].

Extrapolation of the Plateau Value at Steady State

The plateau value at steady state was extrapolated using the kinetic model and the estimated parameters. For the extrapolation, repeating periodic application events were added at 365 day intervals following the single treatments of the last experimental year, so maintaining the cycle of four applications per year.

II. RESULTS AND DISCUSSION

SFO kinetic fit of the soil residue data

The results of the estimated parameters resulting from SFO kinetics are given in Table 7.1.2.2.2-13.

Table 7.1.2.2.2-13: Estimated parameters of SFO kinetics

D [g ha^{-1}]	k [1 d^{-1}]	t-test [-]	χ^2 error value [%]	DT_{50} [d]
995.3	0.0009122	0.002	1.2	759.9

D Yearly compound rate that contributes to accumulation

The DT_{50} value of 759.9 days was calculated under consideration of the estimated dissipation rate constant k.

Under consideration of the “average yearly DT_{50} value” and the “average yearly compound rate D” one can assume that after the 5 year test period about 80% of the minimal plateau value is reached. One can further assume that 95% and 99% of the plateau value would be reached after a study duration of about 9 years and 13 years, respectively.

Based on the estimated parameters, it was concluded that the extrapolated plateau value at steady state given below is based on reliable basic assumptions.

Extrapolation of the plateau value

The graph that describes the model data to the experimental observations and the extrapolated plateau value is given in Figure 7.1.2.2.2-4.

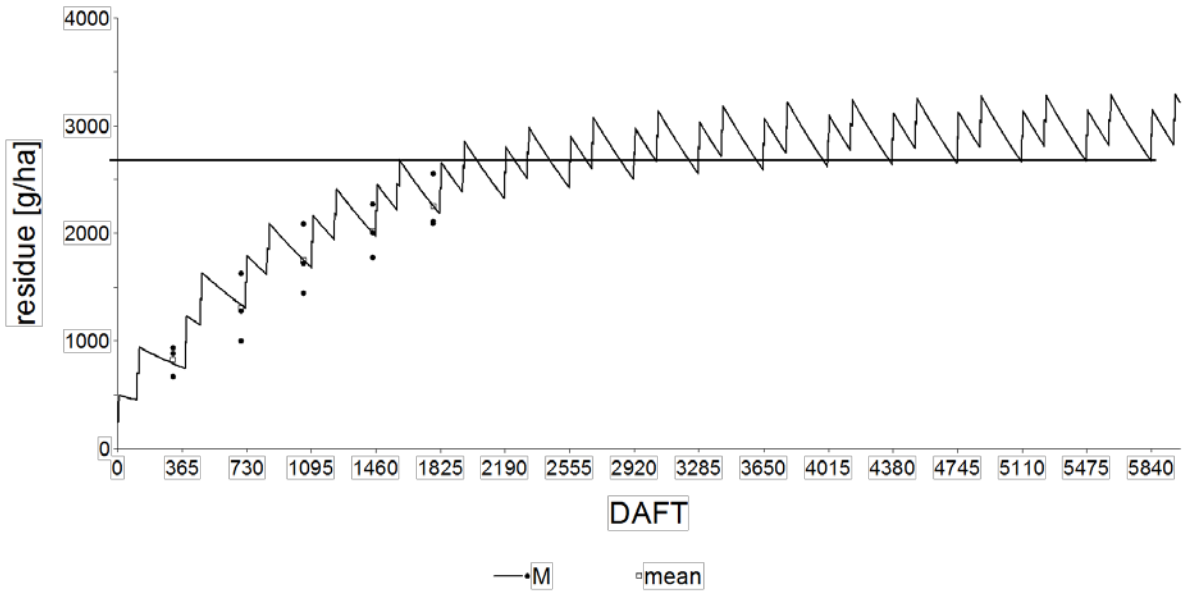


Figure 7.1.2.2.2-4: Modeled curve and plateau value at steady state

The extrapolated plateau value expressed as "g ha^{-1}" and as "fraction of yearly total applied compound" (accumulation factor) is given in Table 7.1.2.2.2-14.

Table 7.1.2.2.2-14: Plateau value at steady state

Plateau value [g ha^{-1} (at 5835 DAFT)]	Yearly total applied compound [g ha^{-1}]	Accumulation factor [-]
2676	1600	1.67

III. CONCLUSION

The plateau value at steady state of the soil accumulation study of boscalid conducted in vegetable crops was extrapolated with 2676 g ha^{-1}. Under consideration of the total yearly application rate of 1600 g ha^{-1} in the study the plateau value can be expressed as accumulation factor of 1.67 of the yearly total applied compound.

Summary of degradation endpoints for boscalid and its metabolites in various soils under laboratory conditions

Table 7.1.2.2.2-15: Summary table of best-fit degradation endpoints of boscalid obtained in standard laboratory soil studies

BASF DocID	Soil / Soil type	pH ($CaCl_2$)	Org. C [%]	Temp. [°C]	Moisture [% MWHC]	Best-fit $DegT_{50}$ / $DegT_{90}$ [d]	Kinetic model	χ^2 error
Aerobic degradation								
1999/11807	Bruch West Sandy loam (d),(p)	7.4	1.3	20	40	107 / 360	1st order	-
2000/1013279	Li35b Loamy sand (d)	6.6	1.0	20	40	322 / not reported	1st order	-
	Lufa 2.2 Loamy sand (d)	5.6	2.5	20	40	384 / not reported	1st order	-
	US soil Loamy sand (d)	7.0	0.6	20	40	376 / not reported	1st order	-
	Minto (Canada) Loam (d)	7.7	3.1	20	40	133 / 422	1st order	-
2002/5002772	California Clay loam (p)	7.8 [a]	2.7	27	75 (at 0.33 bar)	159 / not reported	Linear regression	-
	Idaho Clay loam (p)	6.8 [a]	2.0	27	75 (at 0.33 bar)	573 / not reported	Linear regression	-
	Illinois Silt loam (p)	6.5 [a]	1.3	27	75 (at 0.33 bar)	378 / not reported	Linear regression	-
	North Dakota Loam (p)	7.7 [a]	2.1	27	75 (at 0.33 bar)	277 / not reported	Linear regression	-
Soil photolysis								
2000/1014989	Bruch West Loamy sand (p)	1.9	7.3	22	40	135 / not reported	1st order	-
Anaerobic degradation								
2000/1014986	Sandy loam (d)	1.6	7.2	20	flooded	261 / not reported	1st order	-
2000/1014990	Sandy loam (p)	1.7	7.5	20	flooded	345 / not reported	1st order	-

(d), (p) – diphenyl-, or pyridine-labeled test item used
MWHC maximum water holding capacity
[a] no method/medium stated in original study report

Table 7.1.2.2.2-16: Summary table of best-fit degradation endpoints of boscalid obtained in laboratory soil studies – non-standard conditions

BASF DocID	Soil / Soil type	pH ($CaCl_2$)	Org. C [%]	Temp. [°C]	Moisture [% MWHC]	Best-fit $DegT_{50}$ / $DegT_{90}$ [d]	Kinetic model	χ^2 error
Aerobic degradation – various incubation conditions								
2000/1013279	Lufa 2.2 Loamy sand (d)	5.6	2.5	5	40	stable	1st order	-
	Lufa 2.2 Loamy sand (d)	5.6	2.5	30	40	365 / not reported	1st order	-
	Lufa 2.2 Loamy sand (d)	5.6	2.5	20	20	stable	1st order	-
	Lufa 2.2 sterile Loamy sand (d)	5.6	2.5	20	40	stable	1st order	-
Aerobic degradation – influence of pretreatment								
1998/10607	Limburgerhof I (pretreated) Loamy sand (d)	5.9	1.4	20	50	>240 / not reported	Square root 2nd order	-
	Limburgerhof II (pretreated) Loamy sand (d)	5.9	1.4	20	50	>240 / not reported	Square root 2nd order	-
	Limburgerhof III (pretreated) Loamy sand (d)	5.9	1.4	20	50	>240 / not reported	Square root 1st order	-
	Limburgerhof IV (control) Loamy sand (d)	5.9	1.4	20	50	>240 / not reported	Square root 1st order	-
	Edesheim V (pretreated) Loam (d)	6.9	1.6	20	50	141 / not reported	1st order	-
	Edesheim VI (pretreated) Loam (d)	6.9	1.6	20	50	155 / not reported	1st order	-
	Edesheim VII (control) Loam (d)	6.9	1.6	20	50	201 / not reported	1st order	-
Degradation of aged and non-aged residues								
2008/1013108	Studernheim Loam Aged residues	7.5	2.0	20	40	746 / >1000	SFO	4.4
	Studernheim Loam Freshly applied	7.5	2.0	20	40	336 / >1000	SFO	7.0

(d), (p) – diphenyl-, or pyridine-labeled test item used
MWHC maximum water holding capacity

Table 7.1.2.2.2-17: Summary table of degradation endpoints for modeling of boscalid obtained in standard laboratory soil studies (normalized to 20°C, pF2)

BASF DocID	Soil / Soil type	pH ($CaCl_2$)	Org. C [%]	Temp. [°C]	Moisture [% MWHC]	$DegT_{50}$ at study conditions [d]	$DegT_{50}$ normalized to 20°C, pF2 [d]	Kinetic model	χ^2 error
Aerobic degradation									
1999/11807 2014/1261100	Bruch West Sandy loam (d),(p)	7.4	1.3	20	40	300.2	280.0	DFOP	5.3
2000/1013279 2014/1261100	Li35b Loamy sand (d)	6.6	1.0	20	40	430.7	413.3	SFO	3.9
	Lufa 2.2 Loamy sand (d)	5.6	2.5	20	40	418.0	418.0	SFO	4.1
	US soil Loamy sand (d)	7.0	0.6	20	40	526.4	372.7	SFO	3.6
	Minto (Canada) Loam (d)	7.7	3.1	20	40	212.2 [b]	163.3	DFOP	4.1
2002/5002772 2014/1261100	California Clay loam (p)	7.8 [a]	2.7	27	75 (at 0.33 bar)	305.8 [b]	437.0	DFOP	2.3
	Idaho Clay loam (p)	6.8 [a]	2.0	27	75 (at 0.33 bar)	645.4 [b]	1214.4	DFOP	2.3
	Illinois Silt loam (p)	6.5 [a]	1.3	27	75 (at 0.33 bar)	613.4 [b]	1081.3	DFOP	3.1
	North Dakota Loam (p)	7.7 [a]	2.1	27	75 (at 0.33 bar)	531.2 [b]	869.0	DFOP	3.6
Geometric mean (n=9)							**484.4**		
Soil photolysis									
2000/1014989 2014/1261102	Bruch West Loamy sand (p)	1.9	7.3	22	40	126.8	Not calc.	SFO	0.9
Anaerobic degradation									
2000/1014986 2014/1261101	Sandy loam (d)	1.6	7.2	20	flooded	477.4 [b]	Not calc.	DFOP	0.7
2000/1014990 2014/1261101	Sandy loam (p)	1.7	7.5	20	flooded	594.5 [b]	Not calc.	DFOP	0.9

(d), (p) – diphenyl-, or pyridine-labeled test item used
MWHC maximum water holding capacity
[a] no method stated in original study report
[b] calculated from slow phase of DFOP model ($DT_{50} = ln(2)/k2$)

Table 7.1.2.2.2-18: Summary table on degradation endpoints for modeling of boscalid obtained in laboratory soil studies – non-standard conditions

BASF DocID	Soil / Soil type	pH ($CaCl_2$)	Org. C [%]	Temp. [°C]	Moisture [% MWHC]	$DegT_{50}$ at study conditions [d]	$DegT_{50}$ normalized to 20°C, pF2 [d]	Kinetic model	χ^2 error
Aerobic degradation – various incubation conditions									
2000/1013279 2014/1261100	Lufa 2.2 Loamy sand (d)	5.6	2.5	5	40	Not calc.	Not calc.	-	-
	Lufa 2.2 Loamy sand (d)	5.6	2.5	30	40	358.4	Not calc.	SFO	1.1
	Lufa 2.2 Loamy sand (d)	5.6	2.5	20	20	Not calc.	Not calc.	-	-
	Lufa 2.2 sterile Loamy sand (d)	5.6	2.5	20	40	Not calc.	Not calc.	-	-
Aerobic degradation – influence of pretreatment									
1998/10607 2014/1261100	Limburgerhof I (pretreated) Loamy sand (d)	5.9	1.4	20	50	No reliable endpoint	Not calc.	DFOP	1.9
	Limburgerhof II (pretreated) Loamy sand (d)	5.9	1.4	20	50	889.8 [a]	Not calc.	DFOP	1.1
	Limburgerhof III (pretreated) Loamy sand (d)	5.9	1.4	20	50	605.9 [a]	Not calc.	DFOP	1.2
	Limburgerhof IV (control) Loamy sand (d)	5.9	1.4	20	50	418.6 [a]	Not calc.	DFOP	1.8
	Edesheim V (pretreated) Loam (d)	6.9	1.6	20	50	139.8	Not calc.	SFO	2.0
	Edesheim VI (pretreated) Loam (d)	6.9	1.6	20	50	152.4	Not calc.	SFO	3.0
	Edesheim VII (control) Loam (d)	6.9	1.6	20	50	194.4	Not calc.	SFO	1.6

(d), (p) – diphenyl-, or pyridine-labeled test item used
MWHC maximum water holding capacity
[a] calculated from slow phase of DFOP model (DT_{50} = ln(2)/k2)

Table 7.1.2.2.2-19: Summary table of best-fit degradation endpoints of boscalid metabolites obtained in standard laboratory soil studies

BASF DocID	Soil / Soil type	pH ($CaCl_2$)	Org. C [%]	Temp. [°C]	Moisture [% MWHC]	Best-fit $DegT_{50}$ / $DegT_{90}$ [d]	Kinetic model	χ^2 error
Aerobic soil degradation of M510F47								
2000/1013280 (labeled M510F47)	Bruch West Sandy loam	7.6	1.9	20	40	Not calc.	-	-
2013/1341957 (unlabeled M510F47)	Li10 Loamy sand	6.4	0.84	20	40	15.8 / 52.3	SFO	12.2
	Lufa 2.2 Sandy loam	5.4	1.47	20	40	9.69 / 32.2	SFO	8.7
	Lufa 5M Loamy sand	7.2	2.03	20	40	10.7 / 35.6	SFO	10.6
2002/5002772 (labeled boscalid)	California Clay loam (p)	7.8 [a]	2.7	27	75 (at 0.33 bar)	Metabolite not detected	-	-
	Idaho Clay loam (p)	6.8 [a]	2.0	27	75 (at 0.33 bar)	Metabolite detected only in one sample	-	-
	Illinois Silt loam (p)	6.5 [a]	1.3	27	75 (at 0.33 bar)	Metabolite data too scattered for kinetic evaluation	-	-
	North Dakota Loam (p)	7.7 [a]	2.1	27	75 (at 0.33 bar)			
Anaerobic soil degradation of M510F47								
2000/1014990 (labeled boscalid)	Sandy loam (p)	1.7	7.5	20	flooded	Not calc.	-	-
Aerobic soil degradation of M510F49								
2002/5002772 (labeled boscalid)	California Clay loam (p)	7.8 [a]	2.7	27	75 (at 0.33 bar)	Not calc.	-	-
	Idaho Clay loam (p)	6.8 [a]	2.0	27	75 (at 0.33 bar)	Not calc.	-	-
	Illinois Silt loam (p)	6.5 [a]	1.3	27	75 (at 0.33 bar)	Not calc.	-	-
	North Dakota Loam (p)	7.7 [a]	2.1	27	75 (at 0.33 bar)	Not calc.	-	-
2014/1049139 (M510F49)	Li10 Loamy sand	6.4	0.84	20	40	>240 / >240	FOMC & DFOP	< 5
	Lufa 2.2 Sandy loam	5.4	1.47	20	40	>240 / >240	FOMC & DFOP	< 5
	Lufa 5M Loamy sand	7.2	2.03	20	40	>240 / >240	FOMC & DFOP	< 5

(p) – pyridine-labeled test item used
MWHC maximum water holding capacity
[a] no method/medium stated in original study report

Table 7.1.2.2.2-20: Summary table on degradation endpoints for modeling of boscalid metabolites obtained in standard laboratory soil studies (normalized to 20°C, pF2)

BASF DocID	Soil / Soil type	pH ($CaCl_2$)	Org. C [%]	Temp. [°C]	Moisture [% MWHC]	Kinetic model	χ^2 error	$DegT_{50}$ at study conditions [d]	$DegT_{50}$ normalized to 20°C, pF2 [d]	Formation fraction
Aerobic soil degradation of M510F47										
2000/1013280 (labeled M510F47) 2014/1261100	Bruch West Sandy loam	7.6	1.9	20	40	SFO	12.1	3.3	2.9	n.a.
2013/1341957 (unlabeled M510F47)	Li10 Loamy sand	6.4	0.84	20	40	SFO	12.2	15.8	12.1	n.a.
	Lufa 2.2 Sandy loam	5.4	1.47	20	40	SFO	8.7	9.69	7.4	n.a.
	Lufa 5M Loamy sand	7.2	2.03	20	40	SFO	10.6	10.7	8.2	n.a.
Geometric mean (n=4)									**6.8**	
Anaerobic soil degradation of M510F47										
2000/1014990 (labeled boscalid) 2014/1261101	Sandy loam (p)	1.7	7.5	20	flooded	SFO	13.4	No reliable endpoint		0.373
Aerobic soil degradation of M510F49										
2002/5002772 (labeled boscalid) 2014/1261100	California Clay loam (p)	7.8 [a]	2.7	27	75 (0.33 bar)	SFO [b]	18.9	248.9	355.7	n.a.
	Idaho Clay loam (p)	6.8 [a]	2.0	27	75 (0.33 bar)	SFO [c]	15.8	No reliable endpoint		0.100
	Illinois Silt loam (p)	6.5 [a]	1.3	27	75 (0.33 bar)	SFO [c]	8.9	No reliable endpoint		0.291
	North Dakota Loam (p)	7.7 [a]	2.1	27	75 (0.33 bar)	No acceptable fit derived				
2014/1049139 (unlabeled M510F49)	Li10 Loamy sand	6.4	0.84	20	40	FOMC & HS	< 5	>240	Not calc.	n.a.
	Lufa 2.2 Sandy loam	5.4	1.47	20	40	FOMC & HS	< 5	>240	Not calc.	n.a.
	Lufa 5M Loamy sand	7.2	2.03	20	40	FOMC & HS	< 5	>240	Not calc.	n.a.

(p) – pyridine-labeled test item used
MWHC maximum water holding capacity
n.a. not applicable
[a] no method/medium stated in original study report
[b] Metabolite decline fit
[c] DFOP kinetics for parent

Summary of degradation rates for boscalid in field dissipation studies

Table 7.1.2.2.2-21: Summary table of best-fit field half-lives of boscalid obtained in terrestrial field dissipation studies conducted in Europe

BASF DocID	Trial / Location	Soil type	pH ($CaCl_2$)	Org. C [%]	Best-fit $DisT_{50}$ / $DisT_{90}$ [d]	Kinetic model	χ^2 error
2000/1000123 2015/1018173	DU2/15/97 (300 g ha^{-1}) Stetten, Germany	Silty loam	7.5	0.8	102.0 / 794.7	FOMC	11.3
	DU2/15/97 (600 g ha^{-1}) Stetten, Germany	Silty loam	7.5	0.8	46.1 / 519.2	FOMC	13.1
	DU2/15/97 (1200 g ha^{-1}) Stetten, Germany	Silty loam	7.5	0.8	26.3 / 527.0	DFOP	7.0
	DU2/15/97 Stetten, Germany	**Silty loam**	**7.5**	**0.8**	**49.8 / 601.3**	**Geometric mean**	
	DU3/06/97 (300 g ha^{-1}) Schifferstadt, Germany	Silty sand	5.4	0.7	228.3 / >1000	FOMC	6.4
	DU3/06/97 (600 g ha^{-1}) Schifferstadt, Germany	Silty sand	5.4	0.7	194.5 / >1000	FOMC	8.7
	DU3/06/97 (1200 g ha^{-1}) Schifferstadt, Germany	Silty sand	5.4	0.7	175.3 / >1000	FOMC	12.5
	DU3/06/97 Schifferstadt, Germany	**Silty sand**	**5.4**	**0.7**	**198.2 / >1000**	**Geometric mean**	
2000/1013295 2015/1018173	ALO/05/98 Manzanilla, Spain	Sandy loam	7.4	0.6	73.4 [c] / not calc.	DFOP	28.9
	ALO/06/98 Alcala del Rio, Spain	Sandy loam	7.7	0.9	No visually acceptable fit	-	-
	D05/03/98 Grossharrie, Germany	Loamy sand	6.1	1.2	140.5 / >1000	FOMC	10.3
	HUS/10/98 Bjärred, Sweden	Loamy sand	5.9	1.0	No visually acceptable fit	-	-
2002/1004283 2014/1086103	BKA/666/00/RES 1 Loire Valley, Northern France	Medium loamy sand [a]	7.2 [b]	0.6	227.4 / 755.2	SFO	10.8
	BKA/666/00/RES 2 Languedoc-Roussillon, Southern France	Silty loamy sand [a]	5.4 [b]	0.7	83.2 / 647.3	FOMC	10.7
2010/1126049	L070707 Middelfart, Denmark	Loamy sand	5.6	0.92	196 / >1000	DFOP	5.5
2010/1140925	L070706 Budrio, Italy	Silty clay loam	7.5	0.40	43.6 / not calc.	DFOP	12.9

[a] Soil class according to DIN 4220
[b] No information on method available
[c] DFOP fit not statistically acceptable, but estimated DT_{50} value is considered to be reliable trigger endpoint due to visual quality of fit

Table 7.1.2.2.2-22: Summary table of best-fit field half-lives of boscalid obtained in terrestrial field dissipation studies conducted in the USA and Canada [a]

BASF DocID	Trial / Location	Soil type	pH [b, c]	Org. C [%] [c]	Best-fit $DisT_{50}$ / $DisT_{75}$ [d] [d]	Kinetic model	χ^2 error
2001/5000937	99502 North Dakota	Loam	7.9	1.9	1 / 20	1st order	-
	99503 Colorado	Sandy clay loam	8.0	0.8	119 / >361	1st order	-
2001/5000936	99506 California	Sandy loam	8.9	0.5	76.5 / >329	1st order	-
	99507 Idaho	Loam	6.4	1.5	333 / >345	1st order	-
	99508 Florida	Sand	7.1	1.0	27 / >384	1st order	-
2000/5277	99509 Georgia	Loamy sand	6.6	0.8	264 / >360	1st order	-
	99510 California	Sandy loam	7.0	1.0	150 / >360	1st order	-
	99511 New York	Loamy sand	6.1	2.7	356 / >360	1st order	-
2001/5000833	99512 New Jersey	Loam	6.3	1.5	108 / >359	1st order	-
	99513 Illinois	Silt loam	6.1	1.0	244 / >344	1st order	-
	99514 Texas	Sandy loam	5.9	0.5	143 / >316	1st order	-
2001/5000938	99515 Ontario	Loam	6.2	1.6	30 / 353	1st order	-
	99516 Manitoba	Silt loam	7.8	4.1	316 / >360	1st order	-
	99517 Alberta	Loam	5.5	3.1	372 / not calc.	Rate constant [e]	-
2002/5004651	200512 Texas	Loamy sand	6.7	0.8	133.9 / 269	1st order	-

[a] In case of cropped and bare soil study sites, results are given for bare soil trials only
[b] No information on method available
[c] Refers to the top soil layer (0-15 cm), calculated as content of organic matter OM (%) / 1.724
[d] In all studies, $DisT_{75}$ instead of $DisT_{90}$ values are reported
[e] Simple regression

Table 7.1.2.2.2-23: Summary table of best-fit field half-lives of boscalid metabolite M510F47 obtained in terrestrial field dissipation studies conducted in the USA and Canada [a]

BASF DocID	Trial / Location	Soil type	pH [b, c]	Org. C [%] [c]	Best-fit $DisT_{50}$ / $DisT_{75}$ [d] [d]	Kinetic model	χ^2 error
2001/5000937	99502 North Dakota	Loam	7.9	1.9	7.0 / <120	1st order	-
	99503 Colorado	Sandy clay loam	8.0	0.8	<21 / <21	estimated	-
2001/5000936	99506 California	Sandy loam	8.9	0.5	16.2 / 60.1	1st order	-
	99507 Idaho	Loam	6.4	1.5	<30 / <30	estimated	-
	99508 Florida	Sand	7.1	1.0	Not detected	-	-
2000/5277	99509 Georgia	Loamy sand	6.6	0.8	Not detected	-	-
	99510 California	Sandy loam	7.0	1.0	Not detected	-	-
	99511 New York	Loamy sand	6.1	2.7	Not detected	-	-
2001/5000833	99512 New Jersey	Loam	6.3	1.5	Not detected	-	-
	99513 Illinois	Silt loam	6.1	1.0	Not detected	-	-
	99514 Texas	Sandy loam	5.9	0.5	<1 / -	estimated	-
2001/5000938	99515 Ontario	Loam	6.2	1.6	<5 / <5	estimated	-
	99516 Manitoba	Silt loam	7.8	4.1	<5 / <5	estimated	-
	99517 Alberta	Loam	5.5	3.1	10.5 / 26.2	1st order	-
2002/5004651	200512 Texas	Loamy sand	6.7	0.8	<30 / <30	estimated	-

[a] In case of cropped and bare soil study sites, results are given for bare soil trials only
[b] No information on method available
[c] Refers to the top soil layer (0-15 cm), calculated as content of organic matter OM (%) / 1.724
[d] In all studies, $DisT_{75}$ instead of $DisT_{90}$ values are reported

Table 7.1.2.2.2-24: Summary table of best-fit field half-lives of boscalid metabolite M510F49 obtained in terrestrial field dissipation studies conducted in the USA and Canada [a]

BASF DocID	Trial / Location	Soil type	pH [b, c]	Org. C [%] [c]	Best-fit $DisT_{50}$ / $DisT_{75}$ [d] [d]	Kinetic model	χ^2 error
2001/5000937	99502 North Dakota	Loam	7.9	1.9	<1 / <1	estimated	-
	99503 Colorado	Sandy clay loam	8.0	0.8	<2 / <2	estimated	-
2001/5000936	99506 California	Sandy loam	8.9	0.5	18.9 / 102	1st order	-
	99507 Idaho	Loam	6.4	1.5	No decline	-	-
	99508 Florida	Sand	7.1	1.0	11.1 / 422	1st order	-
2001/5000833	99512 New Jersey	Loam	6.3	1.5	Not detected	-	-
	99513 Illinois	Silt loam	6.1	1.0	<89 / -	estimated	-
	99514 Texas	Sandy loam	5.9	0.5	<30 / -	estimated	-
2001/5000938	99515 Ontario	Loam	6.2	1.6	<301 / <301	estimated	-
	99516 Manitoba	Silt loam	7.8	4.1	Not detected	-	-
	99517 Alberta	Loam	5.5	3.1	No decline	-	-
2002/5004651	200512 Texas	Loamy sand	6.7	0.8	<4 / <4	estimated	-

[a] In case of cropped and bare soil study sites, results are given for bare soil trials only
[b] No information on method available
[c] Refers to the top soil layer (0-15 cm), calculated as content of organic matter OM (%) / 1.724
[d] In all studies, $DisT_{75}$ instead of $DisT_{90}$ values are reported

Table 7.1.2.2.2-25: Summary table on normalized field half-lives of boscalid derived from terrestrial field dissipation studies conducted in the EU - for use as modelling endpoints

BASF DocID	Trial / Location	Soil type	pH ($CaCl_2$)	Org. C [%]	$DegT_{50}$ (20°C, pF2) [d]	Kinetic model	χ^2 error
2000/1000123 2013/1285541	DU2/15/97 (300 g ha^{-1}) Stetten, Germany	Silty loam	7.5	0.8	82.1	SFO	11.9
	DU2/15/97 (600 g ha^{-1}) Stetten, Germany	Silty loam	7.5	0.8	232.5 [c]	HS	9.7
	DU2/15/97 (1200 g ha^{-1}) Stetten, Germany	Silty loam	7.5	0.8	121.6 [c]	DFOP	6.1
	DU2/15/97 Stetten, Germany	**Silty loam**	**7.5**	**0.8**	**132.4**	**Geometric mean**	
	DU3/06/97 (300 g ha^{-1}) Schifferstadt, Germany	Silty sand	5.4	0.7	198.7	SFO	6.3
	DU3/06/97 (600 g ha^{-1}) Schifferstadt, Germany	Silty sand	5.4	0.7	196.4	SFO	9.7
	DU3/06/97 (1200 g ha^{-1}) Schifferstadt, Germany	Silty sand	5.4	0.7	195.6	SFO	12.9

BASF DocID	Trial / Location	Soil type	pH ($CaCl_2$)	Org. C [%]	$DegT_{50}$ (20°C, pF2) [d]	Kinetic model	χ^2 error
	DU3/06/97 Schifferstadt, Germany	**Silty sand**	**5.4**	**0.7**	**196.9**	**Geometric mean**	
2000/1013295 2013/1285541	ALO/05/98 Manzanilla, Spain	Sandy loam	7.4	0.6	No acceptable model fit [d]	-	-
	ALO/06/98 Alcala del Rio, Spain	Sandy loam	7.7	0.9	225.2	SFO	14.3
	D05/03/98 Grossharrie, Germany	Loamy sand	6.1	1.2	117.2	SFO	10.1
	HUS/10/98 Bjärred, Sweden	Loamy sand	5.9	1.0	No acceptable model fit [d]	-	-
2002/1004283 2013/1285541	BKA/666/00/RES 1 Loire Valley, Northern France	Medium loamy sand [a]	7.2 [b]	0.6	155.0	SFO	11.4
	BKA/666/00/RES 2 Languedoc-Roussillon, Southern France	Silty loamy sand [a]	5.4 [b]	0.7	168.4 [c]	HS	9.9
2010/1126049 2013/1285541	L070707 Middelfart, Denmark	Loamy sand	5.6	0.92	118.0	SFO	18.8
2010/1140925 2013/1285541	L070706 Budrio, Italy	Silty clay loam	7.5	0.40	186.0	SFO	5.7
Geometric mean (n=8)					**158.3**		

[a] Soil class according to DIN 4220
[b] No information on method available
[c] Calculated from slow rate of biphasic model ($DegT_{50matrix}$ = ln(2)/k2)
[d] $DegT_{50}$ was not derived, because SFO, DFOP and HS were not appropriate to achieve both visually and statistically acceptable fits

Table 7.1.2.2.2-26: Summary table of normalized field half-lives of boscalid derived from terrestrial field dissipation studies conducted in the USA and Canada [a]

BASF DocID	Trial / Location	Soil type	pH [b, c]	Org. C [%] [c]	$DegT_{50}$ (20°C, pF2) [d]	Kinetic model	χ^2 error
2001/5000937 2012/1189904	99502 North Dakota	Loam	7.9	1.9	138.6 [d]	HS	38.6
	99503 Colorado	Sandy clay loam	8.0	0.8	114.6	SFO	10.7
2001/5000936 2012/1189904	99506 California	Sandy loam	8.9	0.5	274.5	SFO	9.9
	99507 Idaho	Loam	6.4	1.5	155.1	SFO	8.4
	99508 Florida	Sand	7.1	1.0	300.4	SFO [e]	17.9
2000/5277 2012/1189904	99509 Georgia	Loamy sand	6.6	0.8	248.8	SFO [e]	13.0
	99510 California	Sandy loam	7.0	1.0	168.1	SFO	11.2
	99511 New York	Loamy sand	6.1	2.7	144.5	SFO	12.3
2001/5000833 2012/1189904	99512 New Jersey	Loam	6.3	1.5	186.8	SFO	11.1
	99513 Illinois	Silt loam	6.1	1.0	214.5	SFO	8.7
	99514 Texas	Sandy loam	5.9	0.5	243.4	SFO [e]	4.4
2001/5000938 2012/1189904	99515 Ontario	Loam	6.2	1.6	62.1	SFO	22.6
	99516 Manitoba	Silt loam	7.8	4.1	No acceptable model fit [f]	-	-
	99517 Alberta	Loam	5.5	3.1	114.1	SFO	10.5
2002/5004651 2012/1189904	200512 Texas	Loamy sand	6.7	0.8	152.1	SFO	20.1
Geometric mean (n=14)					**166.8**		

[a] In case of cropped and bare soil study sites, results are given for bare soil trials only
[b] No information on method available
[c] Refers to the top soil layer (0-15 cm), calculated as content of organic matter OM (%) / 1.724
[d] Slow phase of HS kinetics (rainfall >10 mm at breakpoint)
[e] Outliers excluded
[f] DegT50matrix could not be derived due to the scatter of data

Overall summary of degradation in soil

The degradation of boscalid in soil has been investigated in laboratory and field studies.

The laboratory studies showed slow degradation under aerobic conditions (best-fit $DegT_{50}$ = 133 - 537 days). Degradation was also slow under anaerobic conditions (best-fit $DegT_{50}$ = 261 - 345 days), but results from the soil photolysis study indicate that light might slightly enhance the degradation of boscalid in soil (best-fit $DegT_{50}$ = 135 days).

Metabolite M510F47 was detected under aerobic and anaerobic conditions. Degradation was fast under aerobic conditions (best-fit DT_{50} < 16 days), but slowed down under anaerobic conditions leading to detection levels > 5% TAR. Formation fractions under aerobic conditions could not be estimated, since the metabolite was not detected in the parent studies or only in one sample or metabolite data from parent studies were too scattered for evaluation. The estimated formation fraction under anaerobic conditions was 0.373.

Metabolite M510F49 was detected at levels > 10 % under aerobic conditions and degraded slowly under laboratory conditions (best-fit DT_{50} > 240 days). Estimated formation fractions were < 0.3.

Terrestrial field dissipation studies at European and US sites showed faster degradation of boscalid compared to the laboratory. Half-lives normalized to 20°C and pF2 were on average similar in both regions (geometric mean $DegT_{50}$ of 158.3 and 166.8 days, respectively).

Metabolites M510F47 and M510F49 were measured in the field, but were detected only sporadically and at low levels (average in the top 7.5 cm max. 0.04 mg/kg, after day of last application).

Accumulation studies with boscalid indicated a potential for accumulation in soil with plateau levels ranging between 95% and 167% of the total yearly application rate.

The following endpoints are considered appropriate for use in environmental exposure assessment:

Degradation of boscalid in soil will be described using the DT_{50} values obtained from the field studies. Since the DT_{50} values obtained from US field sites were similar to the European field sites, the US studies provide no new information and exposure assessment for boscalid is performed using the modelling endpoints derived from the European studies.

Degradation of M510F47 will be described based on the laboratory DT_{50} values obtained from the aerobic studies. A formation fraction could not be estimated from laboratory or field data and, therefore, a conservative value will be selected that is derived from the aerobic soil metabolism studies with the parent. The maximum occurrence of M510F47 under aerobic conditions (4.3% TAR, *see CA 7.1.1.1/1, BASF DocID 2002/5002772*) plus the parent occurrence at the end of the study where the metabolite maximum was detected (47.9% TAR) will be used. The resulting value of 52.2% transformed to a formation fraction of 0.522 represents a conservative estimate in the light of the metabolite behaviour in the anaerobic metabolism study where a formation fraction of 0.373 was estimated.

Degradation of M510F49 will be described based on the laboratory DT_{50} values. Regarding the formation fraction, a conservative value will be selected analogous to the procedure described for M510F47. The maximum occurrence of M510F49 under aerobic conditions (14.5% TAR, *see CA 7.1.1.1/1, BASF DocID 2002/5002772*) plus the parent occurrence at the end of the study where the metabolite maximum was detected (47.6% TAR) will be used. The resulting value of 62.1% transformed to a formation fraction of 0.621 represents a conservative estimate in the light of the formation fractions < 0.3 that were estimated from the metabolism study.

CA 7.1.3 Absorption and desorption in soil

CA 7.1.3.1 Adsorption and desorption

For the previous Annex I listing, information on adsorption and desorption behaviour of boscalid was available from one study [see Table 7.1.3.1.1-1]. Substantial adsorption of boscalid to soil was observed with adsorption $K_{F,oc}$ values ranging from 655 to 1110 mL g^{-1}. 1/n values for the adsorption were in the range of 0.839 to 0.887.

New sorption studies were initiated for the metabolites M510F47 and M510F49 due to current trigger values. Low to moderate adsorption of M510F47 to soil was observed with adsorption $K_{F,oc}$ values ranging from 40.9 to 83.9 mL g^{-1}. 1/n values for the adsorption were close to 1 (0.9808 - 1.0126). Substantial adsorption of M510F49 to soil was observed with adsorption $K_{F,oc}$ values ranging from 1101 to 3491 mL g^{-1}. 1/n values for the adsorption were in the range of 0.8976 to 0.9981.

Summary tables of all obtained sorption parameter values for boscalid and the major metabolites can be found at the end of this chapter.

CA 7.1.3.1.1 Adsorption and desorption of the active substance

Table 7.1.3.1.1-1: Studies on soil adsorption/desorption of boscalid

Reference	BASF DocID	Soils (USDA)	Application rate [µg mL^{-1}]	Soil / solution ration	Incubation period [hours]	Remark
Seher A., 1998a	1998/10513	sand/loamy sand sandy loam loamy sand loamy sand loam sandy clay loam	0.02, 0.1, 0.5, 2.5	1 / 5	24	

CA 7.1.3.1.2 Adsorption and desorption of metabolites, breakdown and reaction products

Report: CA 7.1.3.1.2/1
Corden M., 2014a
(14C)-M510F47: Adsorption/desorption on soil
2014/1144358
Guidelines: OECD 106 (2000)
GLP: yes
(certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Executive Summary

The adsorption/desorption characteristics of M510F47 (metabolite of boscalid) in a mixture of [^{14}C]-M510F47 and non-radiolabeled M510F47 were studied in five soils, representing a range of soil types, to provide data for the evaluation of the fate, including the potential for leaching, in the environment.

Preliminary experiments conducted at the highest test concentration (5 µg mL^{-1}) demonstrated that the most suitable solution to soil ratio was 1:1 with an equilibration time of 24 hours. No adsorption was evident on the test vessels used for the experiments.

For the determination of the adsorption isotherm, the soils were treated at five concentrations: 0.045, 0.24, 0.5, 2.5 and 5 µg mL^{-1} in the adsorption solution. The desorption phase was carried out in two steps by incubating the soil residue from the adsorption phase with fresh 0.01 M $CaCl_2$ solution containing no test substance. Concentrations of test substance were determined in the supernatant solutions and calculated in the remaining soil residue at each stage.

After application of [^{14}C]-M510F47 at the highest concentration rate of 5 µg mL^{-1}, the mean recoveries of radioactivity for each of the five soils under study were in the range from 90.6 to 94.6% of the applied radioactivity.

From the measured isotherms the Freundlich adsorption and desorption constants (K_F) were determined, as well as the values corrected for the organic carbon content ($K_{F,OC}$).

Table 7.1.3.1.2-1: Summary of adsorption and desorption isotherms tests of M510F47 on five soils

Soil Name	Soil Type (USDA)	Org. C [%]	Adsorption	Desorption		Organic carbon normalized		
			K_F^{ads} [mL g^{-1}]	K_F^{des1} [mL g^{-1}]	K_F^{des2} [mL g^{-1}]	$K_{F,oc}^{ads}$ [mL g^{-1}]	$K_{F,oc}^{des1}$ [mL g^{-1}]	$K_{F,oc}^{des2}$ [mL g^{-1}]
Bruch West	Sandy loam	1.63	0.6663	0.6504	1.2291	40.88	39.90	75.40
Nierswalde Wildacker	Silt loam	1.85	0.8426	0.6955	1.0802	45.55	37.59	58.39
LUFA 2.1	Sand	0.60	0.5036	0.4652	0.9506	83.93	77.53	158.4
LUFA 2.3	Sandy loam	0.99	0.5802	0.6115	1.0452	58.61	61.77	105.6
La Gironda (Arahal)	Sandy clay loam	1.22	0.5957	0.6107	0.9391	48.83	50.06	76.97

Low to moderate adsorption of M510F47 to soil was observed with adsorption $K_{F,oc}$ values ranging from 40.9 to 83.9 mL g^{-1}. 1/n values for the adsorption were close to 1 (0.9808 - 1.0126).

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

Metabolite code: M510F47
Reg. No.: 107371
Chemical name (IUPAC): 2-chloronicotinic acid
Molecular formula: $C_6H_4ClNO_2$
Molar mass: 157.6 g mol^{-1}

Radiolabeled test item
Label: pyridine-3-^{14}C
Batch No.: 640-2039
Radiochemical purity: > 99% (by Radio-HPLC)
Chemical purity: > 98% (by HPLC)
Specific activity: 11.3 MBq mg^{-1}

Non-radiolabeled test item
Batch No. 01174-232
Purity: 99.8% (±1.0%)

2. Soils

The study was conducted with five different soils originating from Germany (four soils) and Spain (one soil). The physico-chemical properties of the soils are provided in Table 7.1.3.1.2-2.

Table 7.1.3.1.2-2: Characterization of soils used to determine the adsorption behavior of M510F47

Soil designation **Origin**	**Bruch West** **Germany**	**Nierswalde Wildacker** **Germany**	**LUFA 2.1** **Germany**	**LUFA 2.3** **Germany**	**La Gironda (Arahal)** **Spain**
Textural class (DIN 4220)	Loamy sand	Clay silt	Sand	Loamy sand	Sandy clay loam
Soil texture [%], (ISO 11277)					
Sand	61.1	15.1	89.5	66.9	48.0
Silt	27.6	76.2	8.2	24.8	24.3
Clay	11.3	8.8	2.3	8.3	27.7
Textural class (USDA scheme)	Sandy loam	Silt loam	Sand	Sandy loam	Sandy clay loam
Soil texture [%], (USDA)					
Sand	63.7	17.7	90.8	68.6	49.2
Silt	25.1	73.5	6.9	23.1	23.0
Clay	11.3	8.8	2.3	8.3	27.7
Organic carbon [%] (ISO 10694)	1.63	1.85	0.60	0.99	1.22
Cation exchange capacity [$cmol^+ kg^{-1}$]	11.9	3.1	-0.7	7.5	26.3
pH ($CaCl_2$)	7.3	5.7	5.6	6.7	7.4
pH (water)	8.0	6.5	6.5	7.4	8.3
Max. water holding capacity [g $100g^{-1}$ dry soil]	29.2	36.1	23.1	28.2	39.2
Bulk density [g L^{-1}]	1273	1236	1381	1226	1308

B. STUDY DESIGN

1. Experimental conditions

Test solutions were prepared as mixtures of the radiolabeled and the non-radiolabeled test item.

Tier 1: Adsorption Kinetics Preliminary Test

The adsorption of the test substance to the vessel surface was investigated at the highest concentration (5 µg mL^{-1}) in vessels with no soil present (blank).

The solution to soil ratio to be used for the main experiment was investigated using the soils LUFA 2.1 (acidic) and La Gironda (basic), at the highest test substance solution concentration (5 µg mL^{-1}). Adsorption experiments were conducted with duplicate vessels prepared at solution/soil ratios of 1:1, 2:1, 5:1, and 25:1. The vessels were mixed at approximately 20°C for approximately 24 hours at darkness. The solutions were then separated by centrifugation and analyzed for radioactivity by liquid scintillation counting (LSC). The percentage of applied test compound adsorbed to soil was calculated from these measurements with a target percentage adsorbed to the soil above 20% and preferably < 80%. A solution to soil ratio of 1:1 was chosen for the main experiment.

Equilibration time experiments were performed with each soil type at a solution to soil ratio of 1:1 at the highest proposed test substance concentration (5 µg mL^{-1}). Adsorption experiments were set up using the solution/soil ratio determined above using LUFA 2.1 and La Gironda soils. After intervals of 2, 4, 8 and 24 hours, single tubes, containing soil portions of each of the five soils were removed, centrifuged, and measured by LSC. Soils incubated for 24 hours were extracted; extracts and supernatants were analyzed by radio-HPLC to confirm the stability of the test item over the incubation time.

Tier 2 was not required as soils were pre-selected by the Sponsor and a range of concentrations were investigated under Tier 3.

Tier 3: Adsorption and Desorption Isotherms

A solution to soil ratio of 1:1 and equilibration time of 24 hours was chosen for the main experiment for all soils.

Portions of air dried soils (all five) were weighed into centrifuge tubes. The soils were conditioned for use by mixing with 0.01 M $CaCl_2$ overnight prior to the application. Application solutions of the test substance, prepared in acetonitrile, were applied to the test vessels. The achieved test substance concentrations of M510F47 were: 0.045, 0.24, 0.5, 2.5 and 5 µg mL^{-1}. The test was conducted in duplicate at each test substance concentration, except for the highest concentration which was performed in triplicate. Duplicate blank tubes at each concentration excluding soil were also treated to confirm that the test material did not adsorb to the test vessels. Following application the vessels were mixed and stored at approximately 20°C and darkness for the equilibration time of 24 hours. At the end of the equilibration period, each sample tube was centrifuged. Afterwards, the supernatants were decanted and analyzed for radioactivity by LSC.

Following the adsorption phase, fresh 0.01 M $CaCl_2$ solution was added to each vessel to replace the solution removed, including the sample blanks. The soil/aqueous mixtures were then mixed again for the 24 hour equilibration time under the same conditions as described for adsorption. The third replicate at the highest treatment concentration was not continued to the desorption stage. Solution and soil were separated by centrifugation and the desorption process repeated once more. After each centrifugation the supernatant solution was analyzed by LSC.

At the end of the second desorption, material balances were obtained for samples from the highest concentration only. Since the recovery of radioactivity for all soils at the highest concentration was ≥ 90%, the soil residues were not quantified.

2. Description of analytical procedures

As mentioned above, radioactivity in liquid samples was quantified directly by LSC.

Where necessary, soils were extracted with an approximately equal volume of acetonitrile : water (1:1, v/v) by shaking for 30 minutes, followed by centrifugation at 8,500 rpm for 15 minutes. Supernatants were decanted, weighed, and analyzed by LSC. The procedure was repeated two more times. Representative proportions from each extract were taken for pooling. Each pooled extract was reduced down under a stream of nitrogen prior to analysis. Samples were analyzed by radio-HPLC.

II. RESULTS AND DISCUSSION

A. MASS BALANCE

The total mean mass balances of each soil treated at the highest rate of 5 µg mL^{-1} and the mean recovery in the adsorption and desorption phases ranged from 90.6 to 94.6% of the applied radioactivity over the duration of the experiment.

No significant degradation of the test substance was observed in any of the soils, or blank samples throughout the course of the study.

B. FINDINGS

Adsorption Kinetics Preliminary Test

The test substance was shown to have a solubility of > 10 µg mL^{-1} in 0.01 M $CaCl_2$ solution containing 0.1% acetonitrile. The highest test concentration of 5 µg mL^{-1} was confirmed to be suitable for the requirements of the study.

A solution to soil ratio of 1:1 was selected, since the mean % adsorption was 37.4% and 37.7% for LUFA 2.1 and soil La Gironda, respectively. This ratio met the required target of > 20% and < 80% adsorption whilst ensuring good mixing of solution and soil was achieved.

Treated blank samples, comprising test vessels and treated solutions without soil, incubated alongside the test vessels, demonstrated that there was no adsorption of the test substance to the test vessels.

For both, soil LUFA 2.1 and soil La Gironda equilibrium was achieved at 8 to 24 hours. Chromatographic analysis of the 24 hour samples showed that the test substance was stable in the adsorption solution and in soil extracts over this equilibration time. An equilibration time of 24 hours was therefore selected for the main experiment.

Tier 3: Adsorption Isotherms

Adsorption isotherm testing resulted for M510F47 in Freundlich adsorption coefficients (K_F^{ads}) for the five soils in the range from 0.5036 to 0.8426 mL g^{-1}, and in organic carbon normalized values ($K_{F,oc}^{ads}$) from 40.88 to 83.93 mL g^{-1}.

Desorption isotherm testing for M510F47 resulted in Freundlich desorption coefficients for the five soils in the range from 0.4652 to 0.6955 mL g^{-1} (K_F^{des1}) for the first desorption step and in the range from 0.9506 to 1.2291 mL g^{-1} (K_F^{des2}) for the second desorption step. The organic carbon normalized values ($K_{F,oc}^{des1}$ and $K_{F,oc}^{des2}$) ranged from 37.59 to 77.53 mL g^{-1} ($K_{F,oc}^{des1}$) and from 58.39 to 158.4 mL g^{-1} ($K_{F,oc}^{des2}$).

A summary of the experimental results is provided in Table 7.1.3.1.2-3.

Table 7.1.3.1.2-3: Summary of adsorption and desorption isotherms tests of M510F47 on five soils

Soil Name	Soil Type (USDA)	Org. C [%]	pH ($CaCl_2$)	Adsorption		Desorption				Organic carbon normalized		
				K_F^{ads} [mL g^{-1}]	$1/n^{ads}$ [-]	K_F^{des1} [mL g^{-1}]	$1/n^{des1}$ [-]	K_F^{des2} [mL g^{-1}]	$1/n^{des2}$ [-]	$K_{F,oc}^{ads}$ [mL g^{-1}]	$K_{F,oc}^{des1}$ [mL g^{-1}]	$K_{F,oc}^{des2}$ [mL g^{-1}]
Bruch West	Sandy loam	1.63	7.3	0.6663	1.0126	0.6504	1.0274	1.2291	1.036	40.88	39.90	75.40
Nierswalde Wildacker	Silt loam	1.85	5.7	0.8426	0.9677	0.6955	0.9805	1.0802	0.9609	45.55	37.59	58.39
LUFA 2.1	Sand	0.60	5.6	0.5036	0.9808	0.4652	0.9631	0.9506	0.9851	83.93	77.53	158.4
LUFA 2.3	Sandy loam	0.99	6.7	0.5802	0.9945	0.6115	0.9845	1.0452	0.9317	58.61	61.77	105.6
La Gironda (Arahal)	Sandy clay loam	1.22	7.4	0.5957	0.9706	0.6107	0.9067	0.9391	0.8284	48.83	50.06	76.97

Note:
The original GLP report erroneously describes the determination of K_{oc} values (adsorption/desorption coefficients) although actually $K_{F,oc}$ values (Freundlich adsorption/desorption isotherms) were determined. Furthermore, the report incorrectly mentions that these coefficients/isotherms are dimensionless constants. The simplified dimension for adsorption/desorption coefficients/isotherms is ml/g which was introduced for summarizing the results of this study instead of the ultimately correct but rather uncommon unit $\mu g^{1-1/n}(cm^3)^{1/n}g^{-1}$.

III. CONCLUSION

Low to moderate adsorption of M510F47 to soil was observed which indicates potential mobility of this metabolite.

Report: CA 7.1.3.1.2/2
Corden M., 2014b
M510F49 - Adsorption/desorption on soil
2014/1144357
Guidelines: OECD 106 (2000)
GLP: yes
(certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Executive Summary

The adsorption/desorption characteristics of non-radiolabeled M510F49 (metabolite of boscalid) were studied in five soils, representing a range of soil types, to provide data for the evaluation of the fate, including the potential for leaching, in the environment.

Preliminary experiments conducted at the highest test concentration (target 100 ng mL^{-1}) demonstrated that the most suitable solution to soil ratio was 25:1 with an equilibration time of 24 hours. No significant adsorption was evident on the test vessels in the preliminary phase although some adsorption was observed in the main experiment.

For the determination of the adsorption isotherm, the soils were treated at five target concentrations; 1, 5, 10, 50 and 100 ng mL^{-1} in the adsorption solution. The desorption phase was carried out in two steps by incubating the soil residue from the adsorption phase with fresh 0.01 M $CaCl_2$ solution containing no test substance. Concentrations of test substance were determined in the supernatant solutions and calculated in the remaining soil residue at each stage.

After application of M510F49 at the highest concentration rate of 100 ng mL^{-1}, the mean recovery for all soils was 94.3% applied test compound (range 71.9 – 111.0%).

From the measured isotherms the Freundlich adsorption and desorption constants (K_F) were determined, as well as the values corrected for the organic carbon content ($K_{F,OC}$).

Table 7.1.3.1.2-4: Summary of adsorption and desorption isotherms tests of M510F49 on five soils

Soil Name	Soil Type (USDA)	Org. C [%]	Adsorption	Desorption		Organic carbon normalized		
			K_F^{ads} [mL g^{-1}]	K_F^{des1} [mL g^{-1}]	K_F^{des2} [mL g^{-1}]	$K_{F,oc}^{ads}$ [mL g^{-1}]	$K_{F,oc}^{des1}$ [mL g^{-1}]	$K_{F,oc}^{des2}$ [mL g^{-1}]
Bruch West	Sandy loam	1.63	44.5	24.9	83.4	2727	1528	5119
Nierswalde Wildacker	Silt loam	1.85	63.3	72.1	64.2	3423	3896	3468
LUFA 2.1	Sand	0.60	6.6	63.3	14.6 [a]	1101	10557	2433 [a]
LUFA 2.3	Sandy loam	0.99	28.7	61.1	15.7	2899	6167	1581
La Gironda (Arahal)	Sandy clay loam	1.22	42.6	77.8	34.0	3491	6374	2788

[a] K_F^{des2} and $K_{F,oc}^{des2}$ values calculated from highest level sample only.

Substantial adsorption of M510F49 to soil was observed with adsorption $K_{F,oc}$ values ranging from 1101 to 3491 mL g^{-1}. 1/n values for the adsorption were in the range of 0.8976 to 0.9981.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

Metabolite code: M510F49
Reg. No.: 391572
Chemical name (IUPAC): N-(4'-chlorobiphenyl-2-yl)-2-hydroxynicotinamide
Molecular formula: $C_{18}H_{13}ClN_2O_2$
Molar mass: 324.8 g mol^{-1}
Batch No.: L71-12
Purity: 99.7%

2. Soils

The study was conducted with five different soils originating from Germany (four soils) and Spain (one soil). The physico-chemical properties of the soils are provided in Table 7.1.3.1.2-5.

Table 7.1.3.1.2-5: Characterization of soils used to determine the adsorption behavior of M510F49

Soil designation Origin	**Bruch West Germany**	**Nierswalde Wildacker Germany**	**LUFA 2.1 Germany**	**LUFA 2.3 Germany**	**La Gironda (Arahal) Spain**
Textural class (DIN 4220)	Loamy sand	Clay silt	Sand	Loamy sand	Sandy clay loam
Soil texture [%], (ISO 11277)					
Sand	61.1	15.1	89.5	66.9	48.0
Silt	27.6	76.2	8.2	24.8	24.3
Clay	11.3	8.8	2.3	8.3	27.7
Textural class (USDA scheme)	Sandy loam	Silt loam	Sand	Sandy loam	Sandy clay loam
Soil texture [%], (USDA)					
Sand	63.7	17.7	90.8	68.6	49.2
Silt	25.1	73.5	6.9	23.1	23.0
Clay	11.3	8.8	2.3	8.3	27.7
Organic carbon [%] (ISO 10694)	1.63	1.85	0.60	0.99	1.22
Cation exchange capacity [$cmol^+ kg^{-1}$]	11.9	3.1	-0.7	7.5	26.3
pH ($CaCl_2$)	7.3	5.7	5.6	6.7	7.4
pH (water)	8.0	6.5	6.5	7.4	8.3

Table 7.1.3.1.2-5: Characterization of soils used to determine the adsorption behavior of M510F49

Soil designation Origin	Bruch West Germany	Nierswalde Wildacker Germany	LUFA 2.1 Germany	LUFA 2.3 Germany	La Gironda (Arahal) Spain
Max. water holding capacity [g 100g^{-1} dry soil]	29.2	36.1	23.1	28.2	39.2
Bulk density [g L^{-1}]	1273	1236	1381	1226	1308

B. STUDY DESIGN

1. Experimental conditions

Tier 1: Adsorption Preliminary Tests

To confirm the solubility of the test item, a concentration of twice the highest treatment concentration (200 ng mL^{-1}) in 0.01 M $CaCl_2$ (containing 0.1% acetonitrile) was investigated.

The adsorption of the test substance to the vessel surface was investigated at the highest concentration (100 ng mL^{-1}) in duplicate vessels with no soil present (blank).

To demonstrate the extraction efficiency using acetonitrile : water (1:1, v/v), a subsample of Bruch West soil (soil with high organic matter content), was spiked at 2500 ng g^{-1} and extracted as described below in the analytical procedure section.

The solution to soil ratio to be used for the main experiment was investigated using the soils LUFA 2.1 (acidic) and La Gironda (basic), at the highest test substance solution concentration (100 ng mL^{-1}). Adsorption experiments were conducted with duplicate vessels prepared at solution/soil ratios of 1:1, 5:1, 20:1, and 25:1. The vessels were mixed at approximately 20°C for approximately 24 hours in darkness. The solutions were then separated by centrifugation and analyzed by LC-MS/MS. The percentage of applied test compound adsorbed to soil was calculated from these measurements with a target percentage adsorbed to the soil between 20% and 80%. A solution to soil ratio of 25:1 was chosen for the main experiment.

Equilibration time experiments were performed with each soil type at a solution to soil ratio of 25:1 at the highest proposed test substance concentration (100 ng mL^{-1}). Adsorption experiments were set up using the solution/soil ratio determined above using LUFA 2.1 and La Gironda soils. After intervals of 2, 4, 8 and 24 hours, single tubes, containing soil portions of each of the two soils, were removed and centrifuged. Soils incubated for 24 hours were extracted; extracts and supernatants were analyzed by LC-MS/MS.

Tier 2 was not required as soils were pre-selected by the Sponsor and a range of concentrations were investigated under Tier 3.

Tier 3: Adsorption and Desorption Isotherms

A solution to soil ratio of 25:1 and equilibration time of 24 hours was chosen for the main experiment for all soils.

Portions of air dried soils (all five soils; 0.8 g each) were weighed into centrifuge tubes. The soils were conditioned for use by mixing with 0.01 M $CaCl_2$ overnight prior to the application. Application solutions of the test substance, prepared in acetonitrile were applied to the test vessels. The achieved test substance concentrations of M510F49 were: 0.78, 3.3, 5.9, 31 and 86 ng mL^{-1}. The test was conducted in duplicate at each test substance concentration, except for the highest concentration which was performed in triplicate. Duplicate blank tubes at the highest concentration excluding soil were also treated to confirm that the test material did not adsorb to the test vessels. Following application the vessels were mixed and stored at approximately 20°C and in darkness for the equilibration time of 24 hours. At the end of the equilibration period, each sample tube was centrifuged. Afterwards, the supernatants were decanted and analyzed by LC-MS/MS.

Following the adsorption phase, fresh 0.01 M $CaCl_2$ solution was added to each vessel to replace the solution removed, including the sample blanks. The soil/aqueous mixtures were then mixed again for the 24 hour equilibration time under the same conditions as described for adsorption. The third replicate at the highest treatment concentration was not continued to the desorption stage but extracted. Solution and soil were separated by centrifugation and the desorption process repeated once more. After each centrifugation the supernatant solution was analyzed by LC-MS/MS.

2. Description of analytical procedures

As mentioned above, supernatants derived from the adsorption as well as from desorption phases were analyzed by LC-MS/MS.

Following the adsorption phase, the soil residues from the third replicate samples treated at 100 ng mL^{-1} were extracted twice with acetonitrile : water (1:1, v/v) by shaking for 15 minutes, followed by centrifugation at 8,500 rpm for 20 minutes. The extracts were analyzed by LC-MS/MS.

For each soil type, three replicate subsamples of control soil supernatant solution spiked with M510F49 at concentrations of 1 ng mL^{-1} (low) and 100 ng mL^{-1} (high) were analyzed using the procedure described for study samples. Control soil supernatant solutions were obtained by shaking portions of each soil with 0.01 M $CaCl_2$ solution at a ratio of 1:25, followed by centrifugation. The organic content of spiked samples did not exceed 0.1% of the final volume.

In addition, for each soil, three replicate subsamples of control soil extracts, spiked with M510F49 at concentrations of 1 ng mL^{-1} (low) and 100 ng mL^{-1} (high) were analyzed using the procedure described for study samples. Control soil extracts were prepared by extraction of portions of soil with acetonitrile : water (1:1, v/v) followed by centrifugation.

To confirm the efficiency of extraction of M510F49 from soil, duplicate portions of soil Bruch West were spiked at 2.5 µg g^{-1} and extracted three times using acetonitrile : water (1:1, 10 mL). Extracts were pooled and analyzed using the procedure described for study samples.

The limit of quantification (LOQ) was at least 0.5 ng mL^{-1}. The limit of detection (LOD) was 1 ng mL^{-1}.

II. RESULTS AND DISCUSSION

A. MASS BALANCE

Following fortification of M510F49 to soil supernatant solutions, the mean recoveries for all five soils at both concentration levels were in the range of 87 - 106% of the applied M510F49. Following fortification of M510F49 to soil extracts, the mean recoveries for all soils at both concentration levels were in the range of 76 - 94% of the applied M510F49. Hence, M510F49 was shown to be extractable from soil using acetonitrile : water (1:1, v/v).

In untreated control samples of soil, the test compound was below the LOQ.

The mass balances for each soil treated at the highest target rate of 100 ng mL^{-1} in the isotherm experiment were in the range of 91.9 to 111.0%, except soil Nierswalde Wildacker, which gave a slightly lower value of 71.9%. The overall mean recovery for all soils was 94.3%.

B. FINDINGS

Adsorption Preliminary Tests

The test substance was shown to have a solubility of 200 ng mL^{-1} in 0.01 M $CaCl_2$ containing 0.1% organic solvent. The highest test concentration of 100 ng mL^{-1} was therefore considered to be suitable for the requirements of the study.

The solution to soil ratio of 25:1 was selected, since the mean % adsorption was 49.1% and 69.4% for soil LUFA 2.1 and soil La Gironda, respectively.

Treated blank samples, comprising test vessels and treated solutions without soil, incubated alongside the test vessels demonstrated there was no significant adsorption of the test substance to the test vessels.

For both, soil LUFA 2.1 and soil La Gironda equilibrium was achieved at 2 to 24 hours. Extraction and analysis of the 24 hour supernatant solutions and soil extracts showed that the test substance was stable in the adsorption solution and soil extracts over this equilibration time. An equilibration time of 24 hours was therefore selected for the main experiment.

Tier 3: Adsorption Isotherms

Adsorption isotherm testing resulted for M510F49 in Freundlich adsorption coefficients (K_F^{ads}) for the five soils in the range from 6.6 to 63.3, resulting in organic carbon normalized values ($K_{F,oc}^{ads}$) from 1101 to 3491.

Desorption isotherm testing for M510F49 resulted in Freundlich desorption coefficients for the five soils in the range from 24.9 to 77.8 mL g^{-1} (K_F^{des1}) for the first desorption step and in the range from 14.6 to 83.4 mL g^{-1} (K_F^{des2}) for the second desorption step. The organic carbon normalized values ($K_{F,oc}^{des1}$ and $K_{F,oc}^{des2}$) ranged from 1528 to 10557 mL g^{-1} ($K_{F,oc}^{des1}$) and from 1581 to 5119 mL g^{-1} ($K_{F,oc}^{des2}$).

A summary of the experimental results is provided in Table 7.1.3.1.2-6.

Table 7.1.3.1.2-6: Summary of adsorption and desorption isotherms tests of M510F49 on five soils

Soil Name	Soil Type (USDA)	Org. C [%]	pH ($CaCl_2$)	Adsorption		Desorption				Organic carbon normalized		
				K_F^{ads} [mL g^{-1}]	$1/n^{ads}$ [-]	K_F^{des1} [mL g^{-1}]	$1/n^{des1}$ [-]	K_F^{des2} [mL g^{-1}]	$1/n^{des2}$ [-]	$K_{F,oc}^{ads}$ [mL g^{-1}]	$K_{F,oc}^{des1}$ [mL g^{-1}]	$K_{F,oc}^{des2}$ [mL g^{-1}]
Bruch West	Sandy loam	1.63	7.3	44.5	0.9717	24.9	0.8337	83.4	1.0249	2727	1528	5119
Nierswalde Wildacker	Silt loam	1.85	5.7	63.3	0.9981	72.1	0.9527	64.2	0.9573	3423	3896	3468
LUFA 2.1	Sand	0.60	5.6	6.6	0.8976	63.3	1.2861	14.6 [a]	n.c.	1101	10557	2433 [a]
LUFA 2.3	Sandy loam	0.99	6.7	28.7	0.9520	61.1	1.0512	15.7	0.8113	2899	6167	1581
La Gironda (Arahal)	Sandy clay loam	1.22	7.4	42.6	0.9561	77.8	1.0368	34.0	0.8814	3491	6374	2788

n.c. = Not calculated

[a] K_F^{des2} and $K_{F,oc}^{des2}$ values calculated from highest level sample only.

Note:
The original GLP report erroneously describes the determination of K_{oc} values (adsorption/desorption coefficients) although actually $K_{F,oc}$ values (Freundlich adsorption/desorption isotherms) were determined. Furthermore, the report incorrectly mentions that these coefficients/isotherms are dimensionless constants. The simplified dimension for adsorption/desorption coefficients/isotherms is ml/g which was introduced for summarizing the results of this study instead of the ultimately correct but rather uncommon unit $\mu g^{1-1/n}(cm^3)^{1/n}g^{-1}$.

III. CONCLUSION

Substantial adsorption of M510F49 to soil was observed which indicates low mobility of this metabolite.

CA 7.1.3.2 Aged sorption

Information on aged sorption were not considered for the previous Annex I listing, but two new studies are included in the current dossier.

The first study [*BASF DocID 2008/1013108*] that is already included in this dossier under CA 7.1.1.1/2 compared degradation and long-term sorption of freshly applied boscalid to aged residues. Results obtained for freshly applied boscalid were evaluated in order to derive input parameters for use in model simulations and to compare the long-term sorption behavior with measured field data, i.e. measured aged residues of boscalid [*see CA 7.1.3.2/2, BASF DocID 2014/1141729*]. The evaluation showed clear evidence of strong aged sorption of boscalid. Estimated aged-sorption parameters for boscalid are provided for use as model input FOCUS PEARL 4.4.4, PELMO 4.4.3 and PRZM 3.5.2.

Report:	CA 7.1.3.2/1 Beck I.-C., 2008a Boscalid (BAS 510 F): Study on soil degradation and long-term sorption in soil 2008/1013108
Guidelines:	SETAC Procedures for assessing the environmental fate and ecotoxicity for pesticides (March 1995), BBA IV 4-1, OECD 106 (2000), OECD 307 (2002)
GLP:	yes (certified by Umweltministerium Baden-Wuerttemberg, Stuttgart)

This study was already summarized under CA 7.1.1.1/2.

Briefly, freshly applied boscalid showed desorption K_{oc} values increasing over the study duration from about 400 ml g^{-1} at the beginning to about 1000 ml g^{-1} after 182 days. In contrast the desorption K_{oc} of aged residues remained almost constant during this time.
The observed increase of the adsorption of boscalid to the soil over time was attributed to the known effect of time-dependent sorption (non-equilibrium sorption).

Report:	CA 7.1.3.2/2 Hardy I.A.J., 2014a Boscalid (BAS 510 F): Evaluation of aged sorption studies to derive model input parameters 2014/1141729
Guidelines:	CRD - Chemicals Regulation Directorate of the Ministry of Agriculture in the UK, OECD 307, OECD 106, Beulke S. et al. (2010) Proposed guidance on how aged sorption studies for pesticides should be conducted analysed and used in regulatory assessments
GLP:	no

Executive Summary

The aim of this study was to evaluate aged-sorption study data for BAS 510 F - boscalid according to the UK CRD guidance document [*Beulke, S. and van Beinum, W. (2012) - "Guidance on how aged sorption studies for pesticides should be conducted, analysed and used in regulatory assessments", DEFRA projects PS2235 and PS2244*] in order to derive input parameters for use in model simulations and to compare the long-term sorption behavior with measured field data.

The available study reports were firstly evaluated according to the data quality requirements before proceeding to a detailed analysis with the PEARLNeq tool to derive the model input parameters (DT_{50eq}, $K_{om,eq}$, f_{NE} and k_{des}).

The optimized model input parameter values for the virgin soil are summarized in Table 7.1.3.2-1. The evaluations show clear evidence of strong aged-sorption of boscalid based on comparison of equilibrium and non-equilibrium sorption fits.

The results of the standard evaluation, fit of M_{ini}, DT_{50eq}, $K_{om,eq}$, f_{NE} and k_{des}) fully fulfill the fit criteria given in the draft guidance *[Beulke, S. and van Beinum, W. (2012)]* (good visual fit, low χ^2 error, relative standard error < 0.4, similarity of $K_{om,eq}$ and $K_{om,0}$) for the Studernheim virgin soil.

The optimized $K_{om,eq}$ value of 1276 mL g^{-1} for the equilibrium sorption fit of the aged soil reveals a significant increase over that calculated from the virgin soil of 603.4 mL g^{-1} due to the effects of long-term sorption. The calculated apparent K_d for the aged soil of 96.0 mL g^{-1} agrees well with the plateau seen in the non-equilibrium fit for the virgin soil of around 100 mL g^{-1}, thus confirming the long-term sorption behavior.

The reported values are suitable for direct use in the FOCUS PEARL 4.4.4, PELMO 4.4.3 and PRZM 3.5.2 models, but require conversion for use in the FOCUS MACRO 5 model [*FOCUS (2009)*]. The optimized $DegT_{50}$ values have to be normalized to 20°C and pF2 soil moisture according to FOCUS approaches for use as model input.

Table 7.1.3.2-1: Summary of optimized aged-sorption parameters for boscalid for use as model input - standard evaluation

Parameter	Studernheim virgin	
	Value	Relative standard error
$K_{om,0}$ [mL g^{-1}]	657.7	-
M_{ini} [µg]	1.84	0.04
$K_{om,eq}$ [mL g^{-1}]	603.4	0.08
f_{NE} [-]	0.933	0.19
K_{des} [d^{-1}]	0.0136	0.30
$DT_{50,eq}$ [d]	325.9	0.29
$K_{om,eq}$ / $K_{om,0}$ [-]	0.92	-

I. MATERIAL AND METHODS

The long-term sorption behavior of boscalid has been investigated in laboratory studies on one soil. The aim of this study was to evaluate aged-sorption study data for boscalid according to the UK CRD guidance document [*Beulke, S. and van Beinum, W. (2012) - "Guidance on how aged sorption studies for pesticides should be conducted, analysed and used in regulatory assessments", DEFRA projects PS2235 and PS2244*] in order to derive input parameters for use in model simulations.

Long-term sorption in laboratory soil

The long-term sorption behavior of boscalid has been investigated in one soil (Studernheim) under laboratory conditions [*CA 7.1.1.1/2, BASF DocID 2008/1013108*]. Table 7.1.3.2-2 summarizes the soil characterization data.

The soil was collected from a field trial location used for a long-term dissipation study with boscalid on 21st March 2007. 'Virgin' soil was collected from a control plot that had no previous applications of boscalid. 'Aged' soil was collected from the treated plot which had received repeated applications of boscalid over several years, with the last on l7th October 2005 (541 days prior to sampling).

Table 7.1.3.2-2: Soil characteristics - long-term sorption study

Soil	TOC [%]	pH ($CaCl_2$)	Sand [%]	Silt [%]	Clay [%]	Soil texture
Studernheim	2.00	7.5	40.6	38.4	21.0	Loam

TOC = total organic carbon

Important input data for the evaluation as well as the total mass and liquid concentration ($CaCl_2$ extract) data (along with the calculated relative weighting factors) from each of the trials for use in the evaluations are given in the study report.

Batch sorption in laboratory soil

The sorption behavior of boscalid in soil has been investigated in an OECD106 study with six soils [*already peer-reviewed study BASF DocID 1998/10513*]. Table 7.1.3.2-3 summarizes the soil characterization and sorption data. As none of the soils matched the field trial soil, the average l/n value of 0.864 was considered for the evaluations.

Table 7.1.3.2-3: Soil characteristics - batch sorption studies

Soil	TOC [%]	pH ($CaCl_2$)	Sand [%]	Silt [%]	Clay [%]	Soil texture	K_{OC} [L kg^{-1}]	1/n [-]
Lufa Speyer 2.2	2.5	5.8	86	9	5	Sand	1110	0.875
Bruch West	1.5	7.5	73	17	10	Sandy loam	507	0.870
Schlag Li 35 b	1.1	6.5	83	10	7	Loamy sand	594	0.839
USA 538-30-5	0.4	5.8	83	9	8	Loamy sand	987	0.887
USA 538-31-2	0.5	5.2	44	43	13	Loam	655	0.860
Canada 95024 / RCN 95012	3.4	7.5	49	28	23	Sandy clay loam	776	0.851
						Average	**771.5**	**0.864**

TOC = total organic carbon

Data requirements check

The guidance document [*Beulke, S. and van Beinum, W. (2012)*] provides details of a number of data requirements and handling checks to be considered. Details on the data requirement checks are given in the study report [*CA 7.1.3.2/2, BASF DocID 2014/1141729*]. All datasets were considered acceptable for detailed evaluation.

Model description

The guidance document describes the two-site kinetic sorption model as implemented in the latest versions of the FOCUS models [*FOCUS (2009): "Assessing Potential for Movement of Active Substances and their Metabolites to Ground Water in the EU" Report of the FOCUS Ground Water Work Group, EC Document Reference Sanco/13144/2010 version 1, 604 pp*]. Mathematical descriptions of the two-site sorption model can be found in the guidance [*Beulke, S. and van Beinum, W. (2012)*] and FOCUS groundwater [*FOCUS (2009)*] documents. The two-site kinetic model is presented schematically in the study report.

The optimized values of f_{NE} (defined as the ratio of $K_{f,neq}$: K_{feq}) and k_{des} can be used directly in the FOCUS PEARL 4.4.4, PELMO 4.4.3 and PRZM 3.5.2 models, but must be converted for use in the MACRO 5 model [*FOCUS (2009); Beulke, S. and van Beinum, W. (2012)*].

The two-site kinetic sorption model is implemented in PEARLNEQ v4 for the optimization of the parameters required for model input (DT_{50eq}, $K_{om,eq}$, f_{NE} and k_{des}). PEARLNEQ combines the two-site sorption model with the optimization software PEST. The model is simultaneously fitted against data on the total mass of the pesticide in soil (µg) and the concentration in the liquid phase (µg mL^{-1}). PEARLNEQ is run repeatedly by PEST and the parameters are adjusted until the best possible fit to the measured data is achieved based on the least squares method and the Gauss-Marquardt-Levenberg algorithm.

Optimization procedure

Five parameters (M_{ini}, DT_{50eq}, $K_{om,eq}$, f_{NE} and k_{des}) were to be fitted during the optimizations. The Freundlich l/n value was fixed at the value measured during the batch OECD 106 study for the soil and not optimized. Total mass and liquid phase concentration ($CaCl_2$ extract) were used in the optimizations as these are directly measured experimental data. Combinations of starting parameters for f_{NE} and K_{des} and the optimization settings used for PEST are presented in the study report.

Calculated parameters

Apparent K_d (K_{d_app}) values are calculated from the measured data at each time point as well as the simulated concentrations. The calculated $K_{om,eq}$ value on day zero ($K_{om,0}$) is calculated from the measured total mass and concentration in the liquid phase. Equations used for the calculations of K_{d_app} and $K_{om,0}$ are combinations of starting parameters for f_{NE} and K_{des}. Optimization settings are presented in the study report.

Goodness of fit and acceptance criteria

The decision on whether a model fit was acceptable or not was based on:

- An assessment of the visual fit of a model with and without time-dependent sorption
- A χ^2-test to assess the goodness of fit and to compare a model with and without time-dependent sorption
- An assessment of the confidence in the parameter estimates
- The fitted $K_{om,eq}$ value must be within ± 20% of the calculated $K_{om,0}$ value
- $f_{NE} < 10$
- $k_{des} < 0.5$

Details on how these criteria were implemented are given in the study report.

II. RESULTS AND DISCUSSION

Soil Studernheim (virgin)

According to the guidance document [*Beulke, S. and van Beinum, W. (2012)*], standard PEARLNEQ evaluations were conducted with the data for time zero not included in the optimizations. The remaining data started at 7 days. The calculated $K_{om,0}$ value for the virgin soil is summarized in Table 7.1.3.2-4.

Table 7.1.3.2-4: Calculated $K_{om,0}$ values

Soil	Calculated $K_{om,0}$ [mL g^{-1}]		
	Value	-20%	+20%
Studernheim - virgin	657.7	526.1	789.2

The optimized parameter results for the four sets of starting values for f_{NE} and k_{des} as well as the equilibrium sorption fit are summarized in Table 7.1.3.2-5.

Table 7.1.3.2-5: Optimized parameter results from five sets of starting values for soil Studernheim (virgin)

Parameter	Starting parameter combination									
	f_{NE} 0.2 [-] k_{des} 0.004 [d^{-1}]		f_{NE} 0.2 [-] k_{des} 0.05 [d^{-1}]		f_{NE} 1.5 [-] k_{des} 0.004 [d^{-1}]		*f_{NE} 1.5[-] k_{des} 0.05 [d^{-1}]*		f_{NE} 0 [-] k_{des} 0 [d^{-1}]	
	Value	RSE	Value	RSE	Value	RSE	Value	RSE	Value	RSE
M_{ini} [µg]	1.85	0.04	1.84	0.04	1.85	0.04	1.84	0.04	2.07	0.07
$K_{om,eq}$ [mL g^{-1}]	603.3	0.08	605.3	0.08	604.9	0.08	603.4	0.08	1023.3	0.07
f_{NE} [-]	0.930	0.19	0.928	0.19	0.922	0.19	0.933	0.19	0	-
K_{des} [1 d^{-1}]	0.0138	0.30	0.0135	0.28	0.0138	0.29	0.0136	0.30	0	-
$DT_{50,eq}$ [d]	323.1	0.28	328.2	0.30	319.8	0.30	325.9	0.29	246.6	0.20
Phi [-]	0.07206		0.07204		0.07209		0.07200		0.37030	

Values in italics are the final selected values

According to the guidance document [*Beulke, S. and van Beinum, W. (2012)*], the starting parameter combination that results in the lowest objective function value during the PEST optimization should be selected. In this case, f_{NE} = 1.5 and k_{des} = 0.05 d^{-1} resulted in the lowest objective function value - the results for this starting combination were taken forward.

Figure 7.1.3.2-1 shows the optimized model fits obtained for the best-fit starting parameter combination. Comparison of the apparent K_d plots clearly indicates that equilibrium sorption is not able to adequately describe the data and that aged-sorption is occurring.

Figure 7.1.3.2-1: Optimized model fits for equilibrium and non-equilibrium sorption – virgin soil

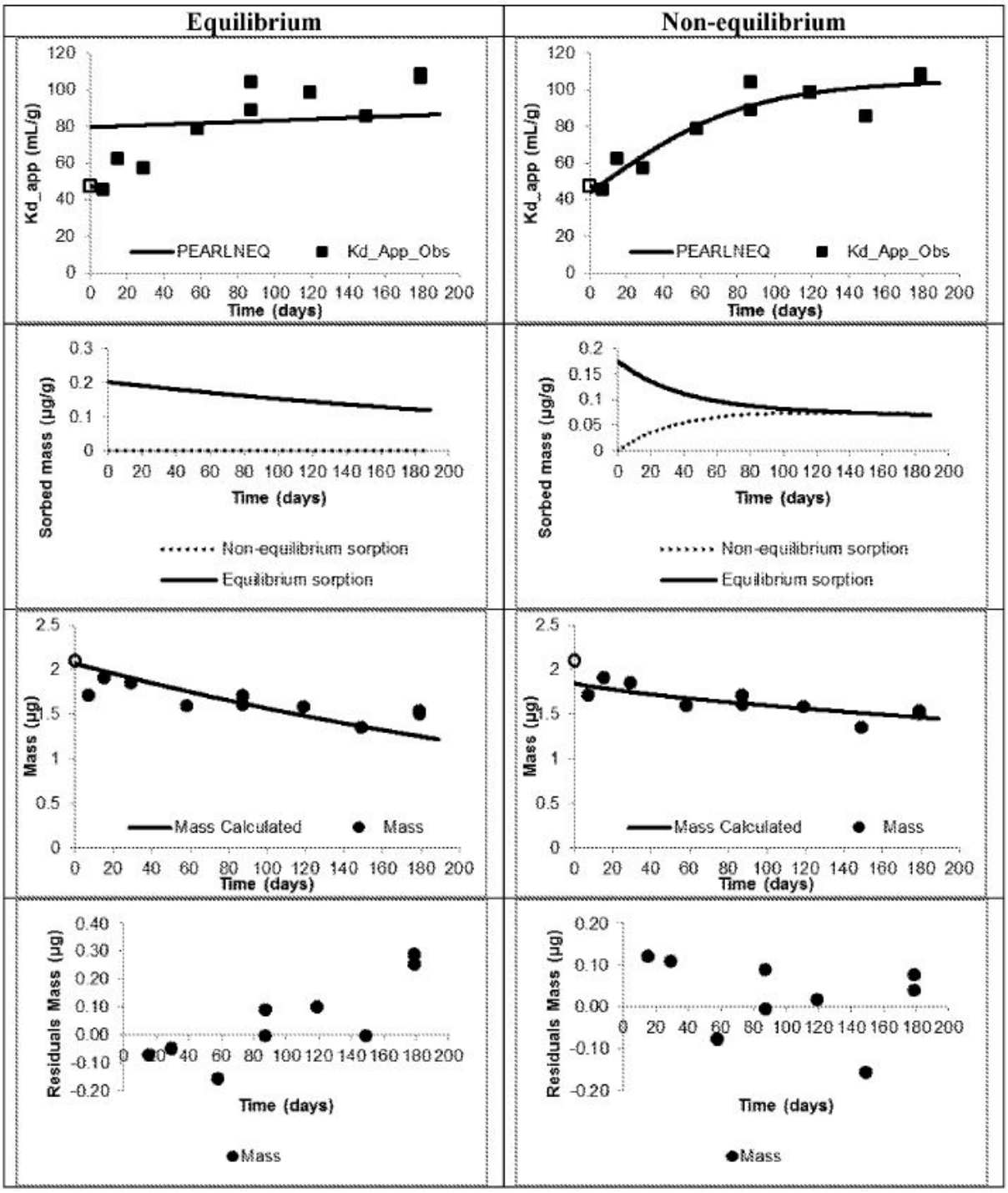
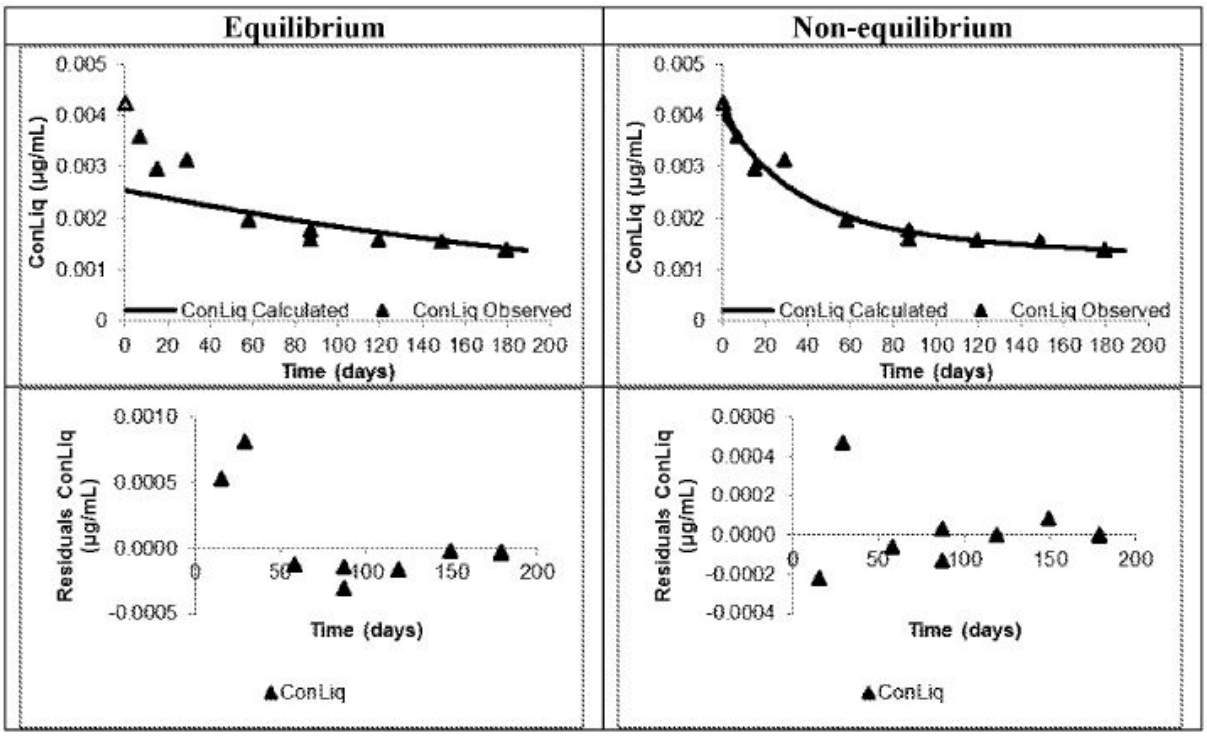


Figure 7.1.3.2-1: Optimized model fits for equilibrium and non-equilibrium sorption – virgin soil (continued)



The optimized $K_{om,eq}$ value of 603.4 mL g^{-1} is within ± 20% of the calculated $K_{om,0}$ value of 657.7 mL g^{-1}.

All acceptability and robustness criteria are met.

Soil Studernheim (aged)

The aged soil data has been evaluated assuming equilibrium sorption (f_{NE} and k_{des} set to zero), as shown in Table 7.1.3.2-6.

Table 7.1.3.2-6: Optimized equilibrium sorption parameter results

Parameter	f_{NE} 0 [-] k_{des} 0 [d^{-1}]	
	Value	RSE
M_{ini} [µg]	3.17	0.04
$K_{om,eq}$ [mL g^{-1}]	1275.8	0.05
f_{NE} [-]	0	-
K_{des} [d^{-1}]	0	-
$DT_{50,eq}$ [d]	486.5	0.25

Figure 7.1.3.2-2 shows the optimized model fits obtained. Evaluation of the apparent K_d plot indicates that equilibrium sorption is able to adequately describe the data, with the increase in apparent K_d being attributed to the Freundlich l/n effect and decreasing soil concentrations.

The calculated $DegT_{50}$ is longer than that from the virgin soil due to the effects of long-term sorption on the aged residues.

Figure 7.1.3.2-2: Optimized model fits for equilibrium sorption - aged soil

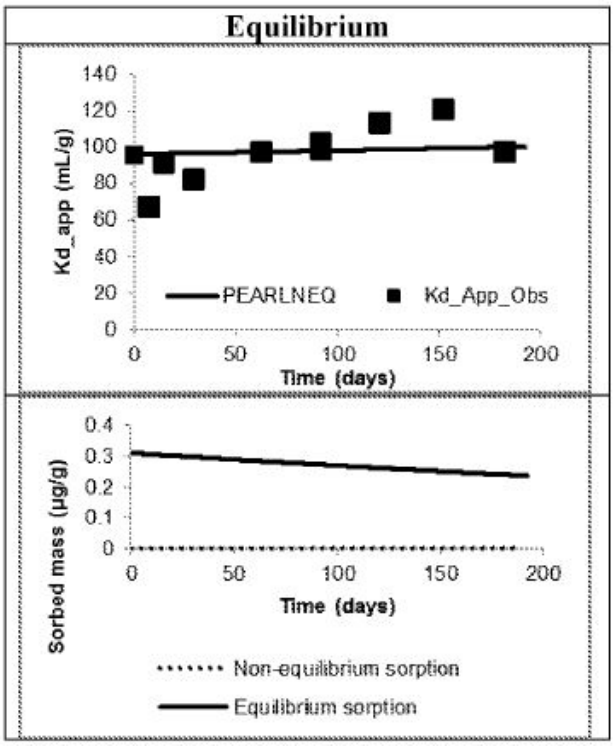
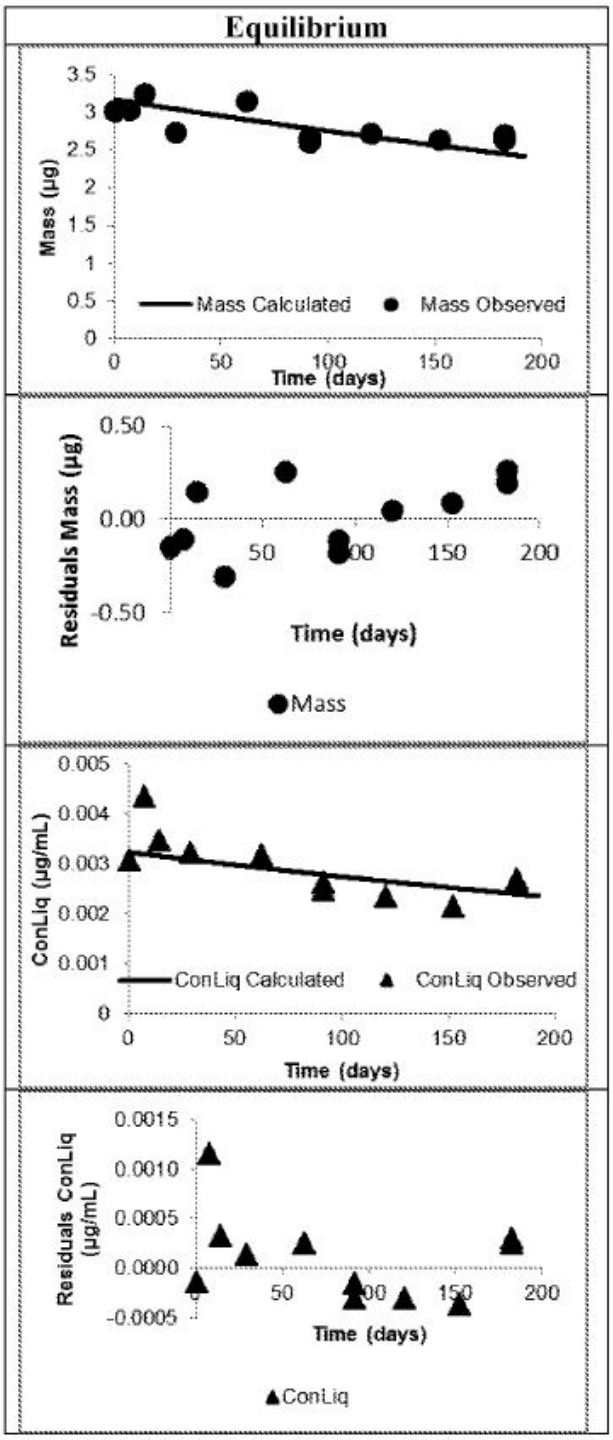


Figure 7.1.3.2-2: Optimized model fits for equilibrium sorption - aged soil (continued)



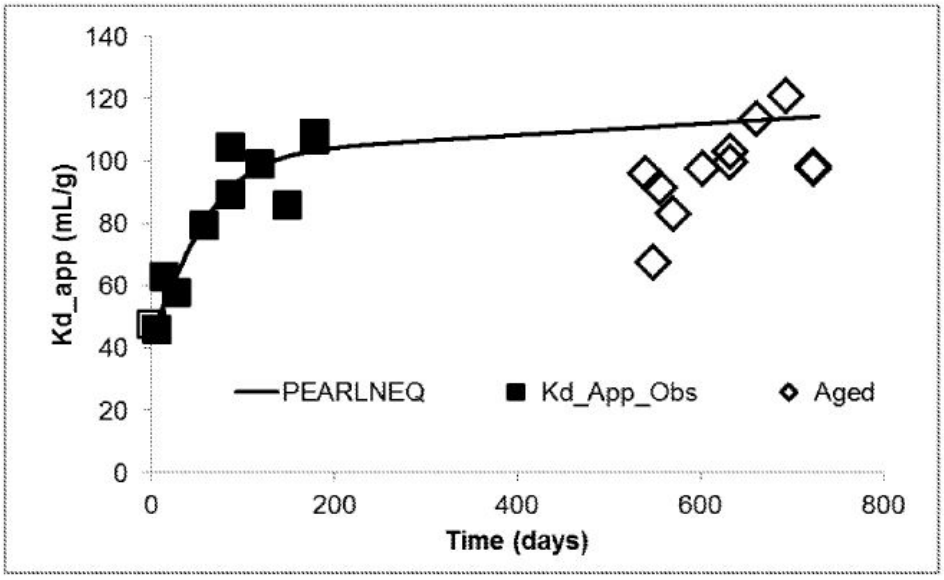
Discussion of results

The evaluations show clear evidence of strong aged-sorption of boscalid based on comparison of equilibrium and non-equilibrium sorption fits for the virgin soil.

The optimized $K_{om,eq}$ value of 1276 mL g^{-1} for the equilibrium sorption fit of the aged soil (Table 7.1.3.2-6) shows a significant increase over that calculated from the virgin soil of 603.4 mL g^{-1} (Table 7.1.3.2-5) due to the effects of long-term sorption. The calculated apparent K_d for the aged soil of 96.0 mL g^{-1} agrees well with the plateau seen in the non-equilibrium fit for the virgin soil of around 100 mL g^{-1}, thus confirming the long-term sorption behavior.

To further compare the long-term sorption behavior of boscalid in the virgin and aged soils, the optimized non-equilibrium sorption parameters for the virgin soil have been used to extrapolate the Apparent K_d up to 730 days (Figure 7.1.3.2-3) - to cover the time period between the last application to the field plot and incubation/extraction in the laboratory (541 days). The increase in apparent K_d beyond 200 days for the virgin soil can be attributed to the effect of decreasing soil concentrations in combination with a Freundlich exponent l/n of 0.864.

Figure 7.1.3.2-3: Comparison of long-term sorption behavior for the virgin and aged soils



The reported values are suitable for direct use in the FOCUS PEARL 4.4.4, PELMO 4.4.3 and PRZM 3.5.2 models, but require conversion for use in the FOCUS MACRO 5 model [*FOCUS (2009)*]. The optimized $DegT_{50}$ values have to be normalized to 20°C and pF2 soil moisture according to FOCUS approaches for use as model input.

III. CONCLUSION

Optimizations using the two-site sorption model provided good visual fits to the data for the virgin soil. The optimized parameters are suitable for use in environmental exposure assessments. Comparison of the long-term sorption behavior determined from the aged soil shows good agreement with that predicted from the non-equilibrium sorption fit for the virgin soil.

Summary of adsorption parameters for boscalid and metabolites obtained for various soil types

Table 7.1.3.2-7: Summary of adsorption parameters for boscalid obtained in various soil types

Soil	Soil type	OC [%]	pH [$CaCl_2$]	K_f [mL g^{-1}]	K_{foc} [mL g^{-1}]	1/n [-]
BASF DocID 1998/10513						
LUFA 2.2	Sand / loamy sand	2.5	5.8	27.8	1110	0.875
Bruch West	Sandy loam	1.5	7.5	7.6	507	0.870
Li 35b	Loamy sand	1.1	6.5	6.5	594	0.839
USA 538-30-5	Loamy sand	0.4	5.8	3.9	987	0.887
USA 538-31-2	Loam	0.5	5.2	3.3	655	0.860
Canada 95024	Sandy clay loam	3.4	7.5	26.4	776	0.851
Geometric mean (n = 6)					**743**	-
Arithmetic mean (n = 6)					-	**0.864**

Table 7.1.3.2-8: Summary of adsorption parameters for boscalid metabolite M510F47 obtained in various soil types

Soil	Soil type	OC [%]	pH [$CaCl_2$]	K_f [mL g^{-1}]	K_{foc} [mL g^{-1}]	1/n [-]
BASF DocID 2014/1144358						
Bruch West	Sandy loam	1.63	7.3	0.6663	40.88	1.0126
Nierswalde Wildacker	Silt loam	1.85	5.7	0.8426	45.55	0.9677
LUFA 2.1	Sand	0.60	5.6	0.5036	83.93	0.9808
LUFA 2.3	Sandy loam	0.99	6.7	0.5802	58.61	0.9945
La Gironda (Arahal)	Sandy clay loam	1.22	7.4	0.5957	48.83	0.9706
Geometric mean (n = 5)					**53.71**	-
Arithmetic mean (n = 5)					-	**0.985**

Table 7.1.3.2-9: Summary of adsorption parameters for boscalid metabolite M510F49 obtained in various soil types

Soil	Soil type	OC [%]	pH [$CaCl_2$]	K_f [mL g^{-1}]	K_{foc} [mL g^{-1}]	1/n [-]
BASF DocID 2014/1144357						
Bruch West	Sandy loam	1.63	7.3	44.5	2727	0.9717
Nierswalde Wildacker	Silt loam	1.85	5.7	63.3	3423	0.9981
LUFA 2.1	Sand	0.60	5.6	6.6	1101	0.8976
LUFA 2.3	Sandy loam	0.99	6.7	28.7	2899	0.9520
La Gironda (Arahal)	Sandy clay loam	1.22	7.4	42.6	3491	0.9561
Geometric mean (n = 5)					**2532**	**-**
Arithmetic mean (n = 5)					**-**	**0.955**

CA 7.1.4 Mobility in soil

CA 7.1.4.1 Column leaching studies

CA 7.1.4.1.1 Column leaching of the active substance

Column leaching studies with boscalid were considered not necessary during the previous Annex I listing, since reliable adsorption coefficient values were obtained from the adsorption/desorption studies. However, a column leaching study was included in the first dossier to compare leaching behavior of freshly applied boscalid to aged residues [see Table 7.1.4.1.1-1]. The study showed that freshly applied boscalid as well as aged residues are not mobile in soil and that there is no risk of transport of boscalid into deeper soil layers.

Table 7.1.4.1.1-1: Studies on column leaching of boscalid

Reference	BASF DocID	Soil type	Application rate [mg kg^{-1}]	MWHC [%]	Incubation temperature [°C]	Incubation period [days]	Remark
Richter T., 2001a	2000/1017037 2001/1000965*	sand	1.5	40	20±2	28	

* Report Addendum
MWHC = Maximum water holding capacity

CA 7.1.4.1.2 Column leaching of metabolites, breakdown and reaction products

No column leaching studies on metabolites, breakdown or reaction products were conducted. Reliable adsorption coefficient values for the soil metabolites M510F47 and M510F49 were obtained from the respective adsorption/desorption studies.

CA 7.1.4.2 Lysimeter studies

The leaching risk of boscalid and its metabolites is addressed by PEC_{gw} calculations using results from degradation rate and adsorption/desorption studies. Neither the active substance nor its metabolites reveal any risk for groundwater contamination.
Lysimeter studies are therefore considered not necessary.

CA 7.1.4.3 Field leaching studies

The leaching risk of boscalid and its metabolites is addressed by PEC_{gw} calculations using results from degradation rate and adsorption/desorption studies. Neither the active substance nor its metabolites reveal any risk for groundwater contamination. Field leaching studies are therefore considered not necessary.

CA 7.2 Fate and behaviour in water and sediment

In the following sections the respective studies that were already peer reviewed for the previous Annex I listing are listed in table form. These studies are considered to be still valid.

CA 7.2.1 Route and rate of degradation in aquatic systems (chemical and photochemical degradation)

CA 7.2.1.1 Hydrolytic degradation

For the previous Annex I listing, information on the hydrolytic degradation behaviour of boscalid was available from one study [see Table 7.2.1.1-1]. Boscalid was hydrolytically stable at pH 4, 5, 7 and 9. No new studies were performed.

Table 7.2.1.1-1: Studies on hydrolytic degradation of boscalid in aquatic systems

Reference	BASF DocID	pH	Application rate [mg L^{-1}]	Incubation temperature [°C]	Incubation period [days]	Remark
von Goetz N., 1999a	1999/11285	4, 7, 9 5, 7, 9	3	50 25	5 30	

CA 7.2.1.2 Direct photochemical degradation

For the previous Annex I listing, information on the direct photochemical degradation behaviour of boscalid was available from one study [see Table 7.2.1.2-1]. Boscalid was practically stable in direct aqueous photolysis. No new studies were performed.

Table 7.2.1.2-1: Studies on photochemical degradation of boscalid in aquatic systems

Reference	BASF DocID	Irradiation [nm]	pH	Application rate [mg L^{-1}]	Incubation temperature [°C]	Incubation period [days]	Remark
von Goetz N., 1999b	1999/11804	290 – 800	5	3	22±1	15	

CA 7.2.1.3 Indirect photochemical degradation

Effectively no degradation of boscalid was observed for direct photochemical degradation [see *CA 7.2.1.2*]. However, as can be deduced from the soil photolysis study, boscalid is more susceptible to degradation under the influence of light [*already peer-reviewed study BASF DocID 2000/1014989*]. An additional laboratory study was conducted to determine whether light influences degradation in the presence of humic or biotic substances found in natural waters [*CA 7.2.1.3/1*]. However no significant degradation of boscalid was observed in this study, with compounds other than boscalid accounting for only 1.3 % of TAR at the end of the study. It is presumed that this study was too short (8 days incubation) for significant degradation to take place. In a higher-tier study with larger scale natural water/sediment systems conducted outdoors for 120 days [*already peer-reviewed study BASF DocID 2000/1017038, see CA 7.2.2.4*] a significant degradation of boscalid was observed and the route and rate of degradation were determined.

Report:	CA 7.2.1.3/1 Goetz N. von, 2002b Photolysis of BAS 510 F in a natural water 2002/1000211
Guidelines:	FAO Revised Guidelines on Environmental Criteria for the Registration of Pesticides Revision 3 (28 August 1993)
GLP:	yes (certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Report:	CA 7.2.1.3/2 Hassink J., 2002b Report amendment No. 1 to final report: Photolysis of BAS 510 F in a natural water 2002/1005320
Guidelines:	FAO Revised Guidelines on Environmental Criteria for the Registration of Pesticides Revision 3 (28 August 1993)
GLP:	yes (certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Executive Summary

The photolytic degradation behavior of boscalid (BAS 510 F) in a natural water was investigated.
After 8 d of continuous irradiation with simulated sunlight, the test substance was practically not degraded. More than 94% TAR were retrieved as boscalid.
Therefore, the first order half-life for photolysis in natural water would exceed twice the study duration by far and is not given.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material Boscalid
Internal code: BAS 510 F
Reg. No.: 300355
Chemical name: 2-chloro-N-(4'-chloro-biphenyl-2-yl)nicotinamide
Molecular formula: $C_{18}H_{12}Cl_2N_2O$
Molecular mass: 343.2 g mol^{-1} (non-labeled)

^{14}C-labeled material:
Position of radiolabel: [pyridine-3-^{14}C]
Lot No.: 640-2037
Radiochemical purity: 99.7%

non-labeled material:
Lot No.: 01183-99
Purity: 99.7%

2. Test system

The test system was a natural pond water. It was sampled from pond "Kleiner Waldsee", Kastenbergheide west of Schifferstadt, Germany and near the motorway A 61, south of the exit Schifferstadt. The physico-chemical properties of the systems are summarized in Table 7.2.1.3-1.

Table 7.2.1.3-1: Characterization of the pond water

Designation **Origin**	**Kleiner Waldsee, Kastenbergheide** **near Schifferstadt, Germany**
sampling date	21.01.1999
pH	8.1
total organic carbon (TOC) [mg L^{-1}]	13.0
NO_3-N [mg L^{-1}]	0.57

B. STUDY DESIGN

1. Experimental conditions

The photolysis test was performed with a concentration of about 2.33 µg mL^{-1}. To achieve this concentration, 155 µL of a non-radioactive stock solution (containing approx. 154 µg unlabeled test item) and 45 µL of a radioactive stock solution (containing approx. 79 µg of labeled test item) were diluted to 100 mL with pond water.
Half of the solution was used for irradiation, the other half served as dark control.

The solution to be irradiated was filled into a thermostated glass vessel, which was airtight covered with quartz glass. The vessel had an air inlet and an air outlet. The incoming air was moistened and CO_2 was removed by 0.5 M NaOH. Three different trapping solutions (ethylenglycole, 0.5 M H_2SO_4, 0.5 M NaOH) were located between vessel and pump to absorb volatile metabolites including CO_2.

With every sampling the trapping solutions were exchanged. The permanently stirred solution was continuously irradiated with a constant intensity over a period of 8 d. The irradiated samples were placed in a SUNTEST CPS+ apparatus and continuously exposed to a Xenon lamp emitting a light spectrum similar to sunlight (> 290 nm) at an intensity of about 3 mW cm^{-2}, simulating a clear summer day.

Dark control samples were stored in Erlenmeyer flasks in a climatic chamber at 22 ± 1°C.

2. Sampling

On each sampling day two samples of both the irradiated test solution and of the dark control were taken at 0, 1, 3, 6 and 8 DAT. One of them was directly analyzed after sampling. The other samples were stored in a freezer. On 0 DAT only samples of the test solutions were taken.

3. Analytical procedures

All samples were measured for radioactivity (LCS) and were analyzed by HPLC to determine the metabolism pattern. Aliquots of all trapping solutions were measured by LSC to determine the amount of produced $^{14}CO_2$ and other volatile degradates at each sampling time.

II. RESULTS

The material balance of the study is shown in Table 7.2.1.3-2. No radioactivity was found in the traps, recovery for the photolysis test ranged from 95.7 to 100% TAR. Recovery for the dark control ranged from 93.7 to 101.9% TAR.
After 8 d of continuous irradiation with simulated sunlight, the test substance was practically not degraded. More than 94% TAR were retrieved as boscalid.

Table 7.2.1.3-2: Recovery (LSC) and results of the HPLC analysis for boscalid

days of treatment [DAT]	photolysis [% TAR]			dark control [% TAR]	
	boscalid	others	recovery	boscalid	recovery
0	99.7	0.3	100.0	99.7	100.0
1	97.5	0.5	98.0	n.a.	94.5
3	95.8	0.7	96.5	n.a.	101.9
6	95.1	1.5	96.6	n.a.	94.6
8	94.4	1.3	95.7	93.2	93.7

n.a. = not analyzed

III. CONCLUSION

After 8 d of continuous irradiation with simulated sunlight, the test substance was practically not degraded. More than 94% TAR were retrieved as boscalid. Therefore, the first order half-live for photolysis in natural water would exceed twice the study duration by far and is not given.

CA 7.2.2 Route and rate of biological degradation in aquatic systems

CA 7.2.2.1 "Ready biodegradability"

For the previous Annex I listing, information on the "ready biodegradability" of boscalid was available from one study [see Table 7.2.2.1-1]. Boscalid is considered "not readily biodegradable". No new studies were performed.

Table 7.2.2.1-1: Studies on "ready biodegradability" of boscalid in aquatic systems

Reference	BASF DocID	Inoculum	Application rate [mg L^{-1}]	Incubation temperature [°C]	Incubation period [days]	Remark
Werner D.I., 1999a	1999/10290	Municipal activated sludge	100	Room temperature	28	

CA 7.2.2.2 Aerobic mineralisation in surface water

The aerobic mineralization study in surface water according to OECD 309 [*CA 7.2.2.2/1*] is a new data requirement introduced since the previous Annex I listing.

Report:	CA 7.2.2.2/1 Yeomans P.,Kelly D., 2015a [14C]-Boscalid (BAS 510 F): Aerobic mineralisation in surface water 2015/1117561
Guidelines:	OECD 309 (April 2004)
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Executive Summary

The purpose of this study was to determine the mineralization and degradation rates of the fungicidal active substance boscalid (BAS 510 F) in an aquatic system under dark conditions. The study was performed according to OECD guideline 309 (Aerobic Mineralization in Surface Water – Simulation Biodegradation Test). The pelagic test system was chosen for this study.

The test was performed at two different boscalid concentrations (10 µg L^{-1} and 100 µg L^{-1}) using pyridine-3-^{14}C-labeled test item. Sterile samples were tested at the higher concentration. The test vessels were attached to a flow-through system for continuous aeration and incubated at a temperature of 20 ± 2°C in the dark. Samples for the experiment were taken at 0, 3, 7, 14, 21, 35 and 59 days after treatment (DAT).

The amount and nature of radioactivity in the water samples was determined by liquid scintillation counting (LSC) and high-performance liquid chromatography (HPLC) coupled with radio detection. Volatiles were trapped in 2 M sodium hydroxide and also analyzed by LSC. Parent substance identification was done by co-chromatography with the corresponding reference items using HPLC and thin-layer chromatography (TLC). TLC also confirmed the quantification of parent substance.

From the results it can be concluded that boscalid is not significantly degraded in the natural water environment provided in this test. After 59 days, at least 97% of the total applied radioactivity (TAR) was recovered as the unchanged active substance. Minor metabolites were observed during the study in small amounts, none of them exceeding a mean of 2.4% TAR. The maximum sum of unknowns amounted to a mean of 3.0% TAR (high concentration, 3 DAT).

Radioactivity in the volatiles traps did not exceed a mean of 1% TAR indicating a low rate of mineralization.

Overall, the compound was considered to be stable in the test systems. Degradation kinetics were not calculated.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

BAS-Code:	BAS 510 F (boscalid)
Reg. No.:	300355
Chemical name:	2-chloro-N-(4'-chlorobiphenyl-2-yl)nicotinamide
Molecular formula:	$C_{18}H_{12}Cl_2N_2O$
Molar mass:	343.2 g mol^{-1} (unlabeled)

Label 1 (pyridine label)

Label:	pyridin-3-^{14}C
Batch No.:	640-2301
Specific activity of a.s.:	5.11 MBq mg^{-1}
Radiochemical purity:	99.5%
Chemical purity:	98.0%

2. Test system

Water and small amounts of sediment were collected from The Lake at Studley Royal (Ripon, United Kingdom). The physico-chemical properties of the system are summarized in Table 7.2.2.2-1.

Prior to use the sediment and water were stored together in the dark at 4 ± 2°C with free access to air. Water was filtered through a 0.1 mm sieve and sediment was passed through a 2 mm sieve prior to use and characterization. The concentration of sediment in the water was adjusted to an approximate concentration of 0.01 g sediment L^{-1}. The test system can still be considered as pelagic.

Table 7.2.2.2-1: Characterization of the water/sediment system

Designation Origin		Fountains Abbey The Lake, Studley Royal, Ripon, UK	
Water			
Temperature [a]	[°C]	5.7	
pH water [b]	-	6.98/7.33	
Oxygen concentration [a]	[mg L^{-1}]	7.19	
Redox potential (Eh) [a]	[mV]	145.2	
Hardness	[mmol L^{-1}]	104	
Total organic carbon	[mg L^{-1}]	8.0	
Total N	[%]	0	
Total P	[mg L^{-1}]	0.09	
Sediment			
Textural class		PSD	USDA
Sand	[%]	61	64
Silt	[%]	30	27
Clay	[%]	9	9
Soil type	-	Sandy loam	Sandy loam
pH (H_2O)	-	6.7	
pH ($CaCl_2$)	-	6.6	
Redox potential (Eh) [a]	[mV]	-106.8	
Organic carbon (OC)	[%]	4.3	

[a] measured at the sampling site

[b] at 0 DAT

B. STUDY DESIGN

1. Experimental conditions

A total of 49 flasks was prepared: 18 flasks for each nominal concentration (10 and 100 µg L^{-1}), 9 flasks for the sterile incubation (100 µg L^{-1}), 2 flasks as system control with radiolabeled sodium benzoate and 2 flasks with sodium benzoate plus treatment solvent.

The vessels were filled with about 100 mL test water. Appropriate amounts of the respective application solutions were pipetted to the water surface to achieve a nominal application rate of 10 µg L^{-1} or 100 µg L^{-1}, respectively.

The systems were incubated at 20 ± 2°C in a metabolism apparatus (incubator) with a gas flow system. Each test vessel was connected to a volatile trapping system of two gas washing bottles containing trapping solutions (2x NaOH) for the ^{14}C-volatiles to be expected. Test vessels containing sterile water were also aerated, however, the air stream was led through sterile filters to avoid contamination of the test system by airborne germs. Vessels were kept in the dark and were agitated by continuous stirring on magnetic stirrers throughout the incubation period.

2. Sampling

Samples, including the sterile groups, were taken at 0, 3, 7, 14, 21, 35 and 59 days after treatment (DAT).

For sampling, the flasks were removed from the rigs and the O_2 content, pH, conductivity and redox potential of the water was measured in the treated samples.

Throughout the test, traps were collected for sampled vessels. Remaining test samples had their traps collected and replenished with fresh solutions at 35 DAT. Reference vessels (incubation groups D and E) had their traps collected and replenished with fresh solutions at 3, 7, 14, 21, 29, 35, 49 and 59 DAT.

3. Description of analytical procedures

The water in the test vessels was transferred into glass jars and weighed. The test vessels were then washed (with sonication) with acetonitrile. Weighed aliquots (*ca.* 200 µL) of the water and acetonitrile were mixed with scintillant for liquid scintillation counting (LSC).

For higher concentration (100 µg L^{-1}) samples, analysis by high-performance liquid chromatography (HPLC) was carried out without further workup. For lower concentration (10 µg L^{-1}) samples, sub-samples of the water (*ca.* 50 mL) were partitioned with dichloromethane (twice with 50 mL). For all except the 59 DAT samples (where procedural recoveries were low), dichloromethane was concentrated to dryness and the samples reconstituted in acetonitrile (5 mL). Procedural recoveries were checked by LSC and were found to be 90% or greater. Where the radioactivity in water after partition was $\geq$ 5% of the total applied radioactivity (TAR) and for the 59 DAT samples, HPLC fraction collection of unextracted water was used.

Volatiles trapped in sodium hydroxide were analyzed by LSC.

4. Calculation of the degradation/dissipation rates

Degradation kinetics were not calculated as no significant degradation was observed.

II. RESULTS AND DISCUSSION

A. MASS BALANCE

The material balance and distribution of radioactivity are shown in Table 7.2.2.2-2.

The applied mass of test item per test vessel containing 100 mL of water was 10.2 µg (high concentration) and 1.0 µg (low concentration).

The material balance ranged from a mean of 97.6 to 106.1% TAR in the viable test vessels, and from 98.9 to 107.5% TAR for the sterilized vessels (with one exception at 84.7% TAR).

The radioactivity recovered from test vessels was predominantly attributed to the water phase, demonstrating that adsorption to the test vessel surface was negligible. Only 0.7 to 3.1% TAR was measured in the vessel wash. At the end of the study (59 DAT), the radioactivity in the water accounted for 97.2 to 104.2% TAR for the viable test vessels (mean of two replicates) and 106.4% TAR for the sterilized vessel (single replicate). For all test samples, the mean total radioactivity in the volatile traps was $\leq$ 0.1% TAR with the exception of one of the low concentration samples (3 DAT) for which a mean value of 1.0% TAR was measured. A low rate of mineralization is therefore indicated.

Table 7.2.2.2-2: Material balance and distribution of radioactivity after application of [^{14}C]-boscalid to lake water (non-sterile and sterile)

Days after treatment (DAT)	Percent of applied radioactivity [% TAR]			
	Water	Vessel wash [a]	Total in NaOH Traps	Material balance
Low concentration (10 µg L^{-1}) [b]				
0	98.5	2.2	NA	100.7
3	99.0	1.7	1.0	101.7
7	94.6	3.1	ND	97.6
14 [c]	101.0	2.3	ND	103.3
21	102.0	1.7	ND	103.6
35	101.5	1.4	ND	102.8
59	104.2	2.0	ND	106.1
High concentration (100 µg L^{-1}) [b]				
0	96.8	1.2	NA	97.9
3	98.3	1.1	0.1	99.4
7	97.0	1.9	0.1	98.9
14	101.5	1.7	ND	103.2
21	100.5	1.8	ND	102.3
35	100.9	1.4	ND	102.2
59	97.2	2.0	ND	99.2
Sterilized lake water (100 µg L^{-1})				
0	97.8	1.1	NA	98.9
3	98.0	1.0	ND	99.0
7	98.1	1.2	ND	99.3
14	101.6	1.2	ND	102.8
21	*83.6*	*1.1*	*ND*	*84.7*
35	101.5	0.7	ND	102.2
59	106.4	1.1	ND	107.5

TAR Total applied radioactivity
NA Not Applicable
ND Not Detected (or < 0.1% TAR)
[a] This is an acetonitrile wash of the incubation vessel
[b] Mean of two replicates
[c] Single replicate
italic sample indicated as outlier

B. TRANSFORMATION OF PARENT COMPOUND

Characterization and identification of residues in water extracts

Water
The results of radio-HPLC analysis are summarized in Table 7.2.2.2-3.

No significant degradation of boscalid was observed during the test. After 59 days, at least 97% TAR could still be recovered as unchanged parent for the different concentrations.

Additional peaks only appeared in small amounts, none of them exceeding a mean of 2.4% TAR at any sampling event. The maximum sum of unknowns amounted to a mean of 3.0% TAR (high concentration, 3 DAT)

The low amount of volatiles, metabolites and other degradation products detected indicate that only negligible microbial degradation took place.

Control samples with sodium benzoate
The control vessels treated with [^{14}C]-sodium benzoate showed that the test system was microbially active both without and with the addition of acetonitrile. The total recoveries of trapped volatile radioactivity after 59 days were 74.6 and 60.9% TAR and the material balances were 76.3 and 66.5% TAR for the samples without and with acetonitrile, respectively.

Sterilized samples
The very limited degradation observed in the viable test vessels resulted in no significant difference in test item concentration between the sterilized incubations and the viable vessels.

Identification of metabolites
Additional peaks generally only appeared in small amounts, none of them exceeding a mean of 2.4% TAR and were therefore not further investigated. Additionally, representative water samples were subjected to thin layer chromatography (TLC) with co-chromatography which confirmed the results obtained by HPLC.

Table 7.2.2.2-3: Metabolite overview for the water phase after application of [^{14}C]-boscalid to lake water

Days After Treatment (DAT)	Percent of applied radioactivity [% TAR]					
	BAS 510 F	Sum of Unknowns	Largest Unknown	Unresolved Background	Aqueous phase [a]	Total
Low concentration (10 µg L^{-1}) [b]						
0	96.8	ND	NA	0.6	1.1	98.5
3	92.8	2.4	1.4	1.2	2.6	99.0
7	93.1	ND	NA	1.1	0.4	94.6
14 [c]	95.4	2.4	2.4	0.1	3.1	101.0
21	99.5	1.3	1.3	0.3	0.8	102.0
35	96.7	2.9	2.2	0.7	1.1	101.5
59	101.2	2.7	1.1	0.3	NA	104.2
High concentration (100 µg L^{-1}) [b]						
0	96.7	ND	ND	0.0	NA	96.8
3	95.1	3.0	2.2	0.2	NA	98.3
7	95.0	1.6	1.6	0.3	NA	97.0
14	101.0	ND	NA	0.4	NA	101.5
21	100.1	ND	ND	0.4	NA	100.5
35	99.8	0.8	0.4	0.3	NA	100.9
59	97.0	ND	NA	0.3	NA	97.2
Sterilized lake water (100 µg L^{-1})						
0	97.6	ND	NA	0.3	NA	97.8
3	97.9	ND	NA	0.1	NA	98.0
7	97.8	ND	NA	0.3	NA	98.1
14	100.7	ND	NA	0.9	NA	101.6
21	*83.4*	*ND*	*NA*	*0.2*	*NA*	*83.6*
35	101.5	ND	NA	0.0	NA	101.5
59	106.3	ND	NA	0.1	NA	106.4

TAR Total applied radioactivity
NA Not Applicable
ND Not Detected (or less than the limit of detection [LOD] of 0.1% TAR)
[a] Activity remaining in the aqueous phase after dichloromethane partition of the water (relevant for low concentration only)
[b] Mean of two replicates
[c] Single replicate
italic indicated as outlier

III. CONCLUSION

It can be concluded that boscalid (BAS 510 F) is not significantly degraded in the natural water environment provided in this test. After 59 days, at least 97% TAR was recovered as the unchanged active substance. Minor metabolites were observed during the study in small amounts, none of them exceeding a mean of 2.4% TAR.

Radioactivity in the volatile traps did not exceed a mean of 1% TAR, indicating a low rate of mineralization. Overall, the compound was stable in the test. Degradation kinetics were not calculated as consistent degradation was not observed.

CA 7.2.2.3 Water/sediment studies

For the previous Annex I listing, information on the degradation behaviour of boscalid in dark water/sediment systems was available from one study with two systems [see Table 7.2.2.3-1]. No new experimental data are provided. However, the kinetic evaluations of the two systems in the dark study were updated according to the newest guidance [*CA 7.2.2.3/1*]. The kinetic evaluations reported in [*CA 7.2.2.3/1*] include additionally the irradiated water/sediment study [*CA 7.2.2.4*].

Table 7.2.2.3-1: List of water/sediment studies performed with boscalid

Reference	BASF DocID	Test system	Application rate [µg L^{-1}]	Incubation temperature [°C]	Incubation period [days]	Remark
Ebert D., 2000a	2000/1000135	river water/ sediment (Rhine). pond water/ sediment	233 (test system)	20±2	100	2 systems considered

Report: CA 7.2.2.3/1
Budde E., 2015a
Kinetic evaluation of two water-sediment studies with BAS 510 F - Boscalid according to FOCUS degradation kinetics
2013/1382371
Guidelines: FOCUS Kinetics (2006) SANCO/10058/2005 version 2.0
GLP: no

Executive Summary

The aim of the study was to evaluate the dissipation and degradation kinetics of boscalid in three aerobic water/sediment systems with two different labels [*already peer-reviewed studies BASF DocID 2000/1000135; BASF DocID 2000/1017038*] and to derive trigger and modeling endpoints according to the recommendations of FOCUS kinetics. The degradation of boscalid under aerobic aquatic conditions was investigated in three aquatic systems in two different studies. In the first study, two different natural systems of water and sediment were incubated in the dark under aerobic conditions. In a higher tier approach, a natural system of water and sediment was incubated under aerobic conditions and natural sunlight for up to 120 days

In the indoor/dark study the aerobic degradation of boscalid was investigated over a period of 100 days in two water/sediment systems called System A and B (a pond and a pond-like side arm of a river located in Southern Germany). In the outdoor study, the aerobic degradation of boscalid was tested over a period of 120 days in a higher tier approach in a natural pond system of water and sediment.

One radiolabel (outdoor study) and two radiolabels (indoor study) of the active substance were used in the studies and were considered independently as replicates in the kinetic evaluation. The experimental data were evaluated using single first order (SFO), first-order multi-compartment (FOMC), double first-order in parallel (DFOP) and hockey stick (HS) kinetic models at the evaluation levels P-I and P-II.

$DegT_{50}$ values (trigger endpoints) for the whole system were 537.7 and 567.5 days in indoor systems A and B, and 69.4 days in the outdoor system. Modeling endpoints ($DegT_{50}$) were 537.7, 567.5, and 376.3 days, respectively. $DisT_{50}$ values in the water compartment ranged from 2.4 to 15.0 days (trigger endpoints) and from 17.3 to 139.2 days (modeling endpoints). For system A and B the modeling endpoints should be interpreted with care, as they are far greater than the study period of 100 days.

Persistence endpoints at level P-II could not be estimated reliably. For modeling endpoints at level P-II, a default approach should be used to set degradation rates in all cases. The degradation rate in water compartment should be set to the overall system half-life, and the degradation rate in the sediment compartment should be set to a default value of 1000 days. The resulting $DegT_{50}$ are 376.3 days for the higher-tier outdoor system, and 537.7 days and 567.5 days for the indoor systems.

In one of the two studies, one metabolite (M510F64) was observed at levels close to 10% of the total applied radioactivity. Kinetic analysis at level M-I was conducted for this metabolite in the total system to derive modeling and trigger endpoints from the respective test system. Evaluation for the metabolite M510F64 resulted in a good visual fit of the metabolite data (SFO kinetics), with a modeling-$DegT_{50}$ value of 10.1 days (temperature-normalized: 14.8 days) and a significant ($p<0.05$) formation fraction of 0.599 (temperature-normalized: 0.492).

I. MATERIAL AND METHODS

The kinetic evaluation of boscalid under aerobic aquatic conditions was investigated in three aquatic systems; two systems in an indoor/dark study and one system in an outdoor study [*already peer-reviewed studies BASF DocID 2000/1999135; BASF DocID 2000/1017038*].

In the first study, two different natural systems of water and sediment were treated with diphenyl-U-[^{14}C] and pyridine-3-[^{14}C] labeled boscalid and incubated in the dark under aerobic conditions for up to 100 days. In a higher tier approach, a natural system of water and sediment was treated with diphenyl-U-[^{14}C] labeled boscalid and incubated under aerobic conditions and natural sunlight for up to 120 days. For all systems, the application rate was equivalent to a field application rate of 700 g a.s. ha^{-1}.

Kinetic evaluation was performed in order to derive persistence and modeling endpoints according to the recommendation of the FOCUS workgroup on degradation kinetics [*FOCUS (2006)*].

Kinetic modeling

The appropriate kinetic model was identified considering the procedures and kinetic models proposed by the FOCUS workgroup on degradation kinetics [*FOCUS (2006)*]. The best-fit model was selected based on visual and statistical assessment, and corresponding DT_{50} and DT_{90} values are reported as *trigger endpoints*. Appropriate DT_{50} values for use in environmental fate models were derived depending on the kinetic model and are reported as *modeling endpoints*.

Kinetic models included in the assessment

The kinetic models employed for this evaluation were described by the FOCUS workgroup on degradation kinetics [*FOCUS (2006)*]. The analysis at P-I level (one-compartment approach) was done for degradation in the whole system as well as the respective dissipations from the water and sediment phases of the test system. At the P-II level (two-compartment approach) the kinetic analysis considered the degradation in water and sediment taking into account the partitioning between the two phases.

Kinetic evaluation at level M-I was performed for one metabolite. In the outdoor test system Kellmetschweiher pond, the metabolite M510F64 occurred with 9.4% total applied radioactivity (TAR) in the water phase 30 days after treatment (DAT). Kinetic analysis at level M-I was conducted for the metabolite in the total system to derive modeling and trigger endpoints from this system.

To obtain endpoints suitable for use in environmental fate models, the data derived from the outdoor study were time-step normalized to derive normalized modeling endpoints (DT_{50}). No correction procedure was necessary for the dark study.

As the purpose of the study was to derive modeling and persistence endpoints, all four kinetic models proposed by FOCUS were used during the evaluation (SFO, FOMC, DFOP, and HS). Details on the models are given in the FOCUS Kinetics guidance [*FOCUS (2006)*].

The appropriateness of the kinetic models to describe degradation was tested with the following checks recommended by *FOCUS (2006)*:

- Visual assessment of goodness-of-fit
- Estimation of the error percentage at which the χ^2 test is passed [*Equation 6-2 in FOCUS (2006)*]
- t-test to evaluate whether estimated degradation parameters differ from zero.

A kinetic model is considered appropriate if the residuals are randomly distributed, the χ^2 - error value is ideally < 15% and the estimated degradation parameters differ from zero as outlined by *FOCUS (2006).*

Data handling

Degradation kinetics were evaluated for the parent substance boscalid. Degradation in the whole system and dissipation from the water compartment (P-I level) were evaluated starting on the day of treatment (i.e. 0 days after treatment, DAT 0). For the P-II level, the initial concentration of the sediment compartment was assumed to be zero. The FOCUS kinetics guidance recommends using the material balance at DAT 0 as the initial value in water for the parent substance. Thus, the total measured occurrence of the radioactive label in water and sediment (parent + metabolites + unknowns) was used as the initial value for the parent. For estimation of degradation at level M-I, the total recovered amount at DAT 0 was considered as the measured initial concentration for the parent compound, while the initial concentration of the metabolite was assumed to be zero.

Values below the quantification (LOQ) or detection limit (LOD) for parent compound and metabolites were treated as recommended by the FOCUS workgroup. This had to be done only for the metabolite in the outdoor system. As no clear indication of the LOQ or LOD was given in the study (‘the LOQ was the 2-fold radioactivity measured in background vials’), the LOD was set to the number of decimals reported, i.e. 0.01% TAR.

Software for kinetic evaluation

The software package KinGUII (version 2.2012.320.1629) was used for parameter fitting [*Schäfer, D., Mikolasch, M., Rainbird, P., Harvey, B. (2007) KinGUII: A new kinetic software tool for evaluations according to FOCUS Degradation Kinetics. In: Del Re, A.A.M. et al. (Eds.): Proceedings of the XIII Symposium on Pesticide Chemistry, Piacenza, 2007, p. 916-923. - BASF DocID 2007/1062781*]. The error tolerance and the number of iterations of the optimization tool were set to 1×10^{-6} and 100, respectively. The method of iteratively reweighted least squares (IRLS) was used.

Experimental data

The kinetic evaluation was based on the findings from two different studies comprising three aquatic systems. The experimental data of the parent substance used as model input values for the kinetic evaluations are given in Table 7.2.2.3-2 and Table 7.2.2.3-3.

Table 7.2.2.3-2: Model input for boscalid – System A (Kellmetschweiher) and System B (Berghäuser Altrhein) of dark study [*BASF DocID 2000/1999135]*

Days after treatment	Concentration [% Total Applied Radioactivity]					
	System A - Kellmetschweiher			System B – Berghäuser Altrhein		
	Water	Sediment	Total system	Water	Sediment	Total system
0	97.4 [a]	0 [b]	97.4	97.5 [a]	0 [b]	97.5
0	96.7 [a]	0 [b]	96.7	96.7 [a]	0 [b]	96.7
1	75.8	20.1	95.9	63.7	32.4	96.1
1	76.0	19.8	95.8	63.0	31.7	94.7
2	69.5	26.5	96.0	54.2	41.4	95.6
2	71.0	24.7	95.7	50.2	44.7	94.9
7	55.2	38.6	93.8	32.3	61.5	93.8
7	51.7	42.2	93.9	33.9	60.4	94.3
14	40.0	53.2	93.2	21.4	69.2	90.6
14	44.5	48.1	92.6	23.0	69.8	92.8
29	30.9	60.4	91.3	12.1	78.4	90.5
29	31.1	60.1	91.2	12.1	79.1	91.2
59	25.3	63.9	89.2	7.4	76.5	83.9
59	21.4	64.5	85.9	8.4	80.1	88.5
100	17.4	68.6	86.0	6.1	80.2	86.3
100	17.3	66.8	84.1	6.2	79.5	85.7

[a] The measured value at DAT 0 was set to the material balance
[b] The measured value at DAT 0 was treated as if the substance was in the water phase

Table 7.2.2.3-3: Model input for boscalid and metabolite M510F64 – Kellmetschweiher Pond of outdoor study [*BASF DocID 2000/1017038]*

Days after treatment	Concentration [% Total Applied Radioactivity]			
	Boscalid			Metabolite M510F64
	Water	Sediment	Total system	Water
0	96.19 [a]	0 [b]	96.19	0.0
1	88.50	8.38	96.88	0.0
2	84.33	11.82	96.15	0.005 [c]
7	63.32	18.79	82.11	7.3
14	51.86	24.04	75.90	9.0
30	31.68	22.06	53.74	9.4
58	25.70	23.56	49.26	2.6
103	19.84	28.21	48.05	- [d]
120	19.17	26.53	45.70	1.9

[a] The measured value at DAT 0 was set to the material balance
[b] The measured value at DAT 0 was treated as if the substance was in the water phase
[c] Set to ½ LOD according to FOCUS (2006)
[d] Should be set to ½ LOD according to FOCUS (2006), but removed as outlier

II. RESULTS AND DISCUSSION

The initial fits for the total system and for the water phase were performed using SFO kinetics. In a further step, it was tested whether the bi-phasic FOMC model was more appropriate, and if so, DFOP and HS kinetics were also tested. Graphical presentations of the tested kinetic models and the results of the χ^2 - test and all other statistical endpoints used in the decision-making process are given in the original study report.

Level P-I

A summary of the trigger and modeling endpoints for boscalid, derived from level P-I kinetic analysis is given in Table 7.2.2.3-4.

Table 7.2.2.3-4: Summary of trigger and modeling endpoints of boscalid (Level P-I)

Test system		Trigger endpoints			Modeling endpoints	
Total system	**Study (DocID)**	**Kinetic model**	**$DegT_{50}$ [d]**	**$DegT_{90}$ [d]**	**Kinetic model**	**$DegT_{50}$ [d]**
Kellmetschweiher (System A)	2000/1000135	SFO	537.7	>1000	SFO	537.7 [a]
Berghäuser Altrhein (System B)		SFO	567.5	>1000	SFO	567.5 [a]
Kellmetschweiher pond (outdoor)	2000/1017038	HS	69.4	n.c.	HS	376.3 [b]
Water compartment	**Study (DocID)**	**Kinetic model**	**$DisT_{50}$ [d]**	**$DisT_{90}$ [d]**	**Kinetic model**	**$DisT_{50}$ [d]**
Kellmetschweiher (System A)	2000/1000135	FOMC	8.5	666.7	DFOP	54.3 [c]
Berghäuser Altrhein (System B)		FOMC	2.4	57.6	FOMC	17.3 [d]
Kellmetschweiher pond (outdoor)	2000/1017038	DFOP	15.0	268.8	DFOP	139.2 [c,e]

n.c. = Not calculated
[a] Much longer than the study period of 100 d; interpret with care
[b] Temperature-normalized value (non-normalized value: 656.1 d)
[c] Derived from slow rate of DFOP (k_2)
[d] DT_{50} back-calculated from FOMC ($DT_{50} = DT_{90}/3.32$)
[e] Temperature-normalized value (non-normalized value: 150.8 d)

Level P-II

Persistence endpoints at level P-II (i.e. sediment $DegT_{50}$) could not be estimated reliably (visual fit not acceptable and/or degradation rate not significant).

For modeling endpoints at level P-II, a default approach was used to set degradation rates in all cases. The degradation rate in the water compartment was set to the overall system half-life, and the degradation rate in the sediment compartment was set to a default value of 1000 days. The resulting $DegT_{50}$ are 537.7 days and 567.5 days for the indoor systems, and 376.3 days for the higher-tier outdoor system.

Level M-I

In the outdoor test system Kellmetschweiher pond, the metabolite M510F64 occurred with a maximum of 9.42% TAR in the water phase. Evaluation for the metabolite M510F64 resulted in a good visual fit of the metabolite data (SFO kinetics), with a modeling-$DegT_{50}$ value of 10.1 days (temperature-normalized: 14.8 days) and a significant ($p < 0.05$) formation fraction of 0.599 (temperature-normalized: 0.492).

III. CONCLUSION

The dissipation and degradation of boscalid in water/sediment systems under dark, as well as under outdoor conditions was evaluated according to the recommendations of the FOCUS workgroup on degradation kinetics [*FOCUS (2006)*] to determine persistence and modeling endpoints.

The experimental data on boscalid in the indoor (Systems A and B) and in the outdoor test systems for both labels (diphenyl- and pyridine-labels) were evaluated at Level P-I and Level P-II. For systems A and B modeling endpoints should be interpreted with care, because they were much longer than the study period of 100 days. Persistence endpoints at level P-II could not be estimated reliably. For modeling endpoints at level P-II the degradation rate in the water compartment should be set to the overall system half-life (376.3 days for the higher-tier outdoor system, and 537.7 days and 567.5 days for the indoor systems), and the degradation rate in the sediment compartment should be set to a default value of 1000 days.

Evaluation for the metabolite M510F64 resulted in a good visual fit of the metabolite data (SFO kinetics), with a modeling-$DegT_{50}$ value of 10.1 days (temperature-normalized: 14.8 days) and a significant ($p<0.05$) formation fraction of 0.599 (temperature-normalized: 0.492).

$DegT_{50}$ values (trigger endpoints) for the whole system were 537.7 and 567.5 days in indoor systems A and B, and 69.3 days in the outdoor system. Modeling endpoints ($DegT_{50}$) were 537.7, 567.5, and 376.3 days, respectively.

$DisT_{50}$ values in the water compartment ranged from 2.4 to 15.0 days (trigger endpoints) and from 17.3 to 139.2 days (modeling endpoints).

CA 7.2.2.4 Irradiated water/sediment study

For the previous Annex I listing, information on the degradation behaviour of boscalid in irradiated water/sediment systems was available from one study [see Table 7.2.2.4-1]. Therefore, no new experimental data are provided. However, the kinetic evaluation of both, the dark and irradiated water/sediment studies was updated according to the newest guidance [*CA 7.2.2.3/1*].

Table 7.2.2.4-1: List of irradiated water/sediment studies performed with boscalid

Reference	BASF DocID	Test system	Application rate [µg L^{-1}]	Incubation temperature [°C]	Incubation period [days]	Remark
Fent G., 2001a; Platz K., 2001d	2000/1017038 2000/1017047	pond water/ sediment	233 (test system)	outdoor conditions	120	

Summary: Route of degradation of boscalid in water/sediment studies

The degradation of boscalid has been investigated in three aerobic water/sediment systems with two different radiolabeled forms of the substance (pyridine-3-^{14}C and diphenyl-U-^{14}C) [*already peer-reviewed studies BASF DocID 2000/1000135; BASF DocID 2000/1017038*].

In the first study [*already peer-reviewed study BASF DocID 2000/1000135*] the degradation was studied in two natural water/sediment systems incubated in the dark. The substance was found to move rapidly from the water to the sediment, where it formed moderate amounts of bound residues. In the water and sediment extracts of both water/sediment systems, the active substance was found to be the only radiolabeled compound. No metabolites were detected.

The degradation of BAS 510 F in natural aqueous systems is insufficiently described by the basic laboratory studies (hydrolysis, aqueous photolysis and water/sediment). BAS 510 F has a low water solubility and a high adsorption coefficient, which leads to a fast movement into the sediment where it is finally transformed into bound residues. Furthermore, as can be deduced from the soil photolysis study, the compound is more susceptible to degradation under the influence of light [*already peer-reviewed study BASF DocID 2000/1014989*].

In a laboratory study in natural water, no significant influence of light on the degradation was observed, presumably due to the short study duration. Thus the second water/sediment study [*already peer-reviewed study BASF DocID 2000/1017038*] was initiated as a higher tier study which integrates all important mechanisms that occur under natural conditions: Photolysis in natural water, biological degradation and sorption to the sediment. One of the water/sediment systems as in the standard study was investigated in large tanks designed to simulate a bigger water body. The tanks were located outdoors in order to have outdoor temperature and light conditions. The results of this study show that BAS 510 F follows two dissipation and degradation pathways in a natural water system. When reaching the water, BAS 510 F undergoes a transformation forming para-Cl-benzoic acid (M510F64) as metabolite, and simultaneously, it adsorbs fast to the sediment where it finally forms bound residues. The metabolite M510F64 reached 9.4% TAR after 30 days in the water phase, however, it degraded thereafter reaching 1.9% TAR at the end of the study. Almost no other degradation and breakdown products could be detected in the water during the study, and also in the sediment, only trace amounts of various metabolites were detected.

Based on the findings of these studies an overall degradation pathway for boscalid in aquatic systems is proposed to be as shown in Figure 7.2.2.4-1.

The proposed trigger and modelling endpoints for the aquatic risk assessment are given in Table 7.2.2.4-2 to Table 7.2.2.4-4

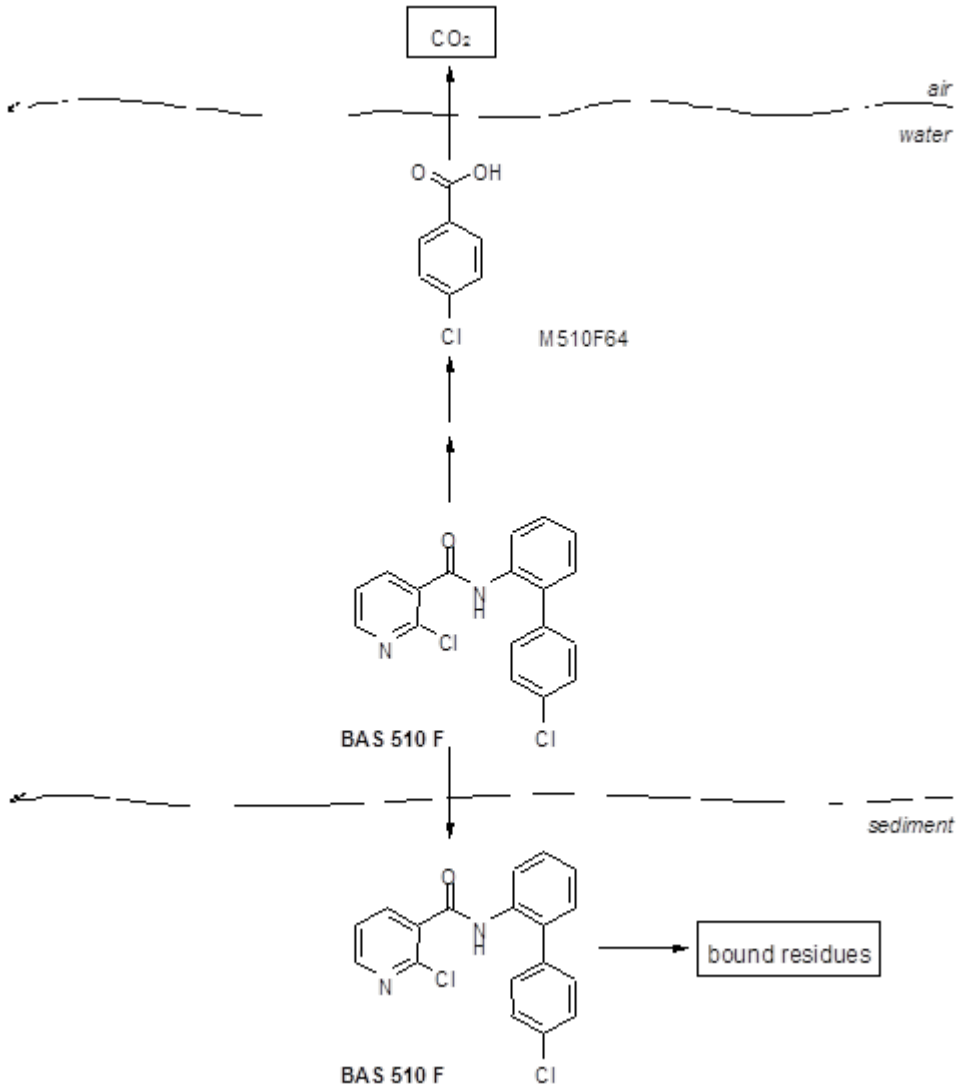


Figure 7.2.2.4-1: Proposed degradation pathway of boscalid in water/sediment systems

Summary of degradation and dissipation endpoints for boscalid and its metabolites in various water/sediment systems

Table 7.2.2.4-2: Summary table of best-fit endpoints of boscalid obtained in water/sediment studies (Level P-I)

Water/ sediment system	pH water	pH sed ($CaCl_2$)	T [°C]	Best-fit $DegT_{50}/DegT_{90}$ whole system [a] [d]	Kinetic model	Best-fit $DisT_{50}/DT_{90}$ water [b] [d]	Kinetic model	Best-fit $DisT_{50}/DT_{90}$ sediment [b] [d]	Kinetic model
BASF DocID 2000/1000135, BASF DocID 2013/1382371									
Kellmetschweiher (p),(d)	8.5	6.8	20	537.7 [c]/ >1000	SFO	8.5 / 666.7	FOMC	Not calc. [d]	-
Berghäuser Altrhein (p),(d)	8.1	7.5	20	567.5 [c] / >1000	SFO	2.4 / 57.6	FOMC	Not calc. [d]	-
BASF DocID 2000/1017038, BASF DocID 2013/1382371									
Kellmetschweiher (outdoor conditions) (d)	8.8	Not given	Mean 18.1 (8.6-28.5)	69.4 / not calc.	HS	15.0 / 268.8	DFOP	Not calc. [d]	-

(p), (d) – pyridine- or diphenyl-labeled test item used
[a] degradation rate
[b] dissipation rate
[c] much longer than the study period of 100 d; interpret with care
[d] no reliable endpoints derived in kinetic evaluation

Table 7.2.2.4-3: Summary table of modeling endpoints of boscalid obtained in water/sediment studies (Level P-I)

Water/ sediment system	pH water	pH sed ($CaCl_2$)	T [°C]	Modeling $DegT_{50}$ whole system [a] [d]	Kinetic model	Modeling $DisT_{50}$ water [b] [d]	Kinetic model	Modeling $DisT_{50}$ sediment [b] [d]	Kinetic model
BASF DocID 2000/1000135, BASF DocID 2013/1382371									
Kellmetschweiher (p),(d)	8.5	6.8	20	537.7 [c]	SFO	54.3 [e]	DFOP	Not calc. [h]	-
Berghäuser Altrhein (p),(d)	8.1	7.5	20	567.5 [c]	SFO	17.3 [f]	FOMC	Not calc. [h]	-
Geometric mean at 20°C				**552.4**		Not calc.		Not calc.	
BASF DocID 2000/1017038, BASF DocID 2013/1382371									
Kellmetschweiher (outdoor conditions) (d)	8.8	Not given	Mean 18.1 (8.6-28.5)	376.3 [d]	HS	139.2 [e, g]	DFOP	Not calc. [h]	-

(p), (d) – pyridine- or diphenyl-labeled test item used
[a] degradation rate
[b] dissipation rate
[c] much longer than the study period of 100 d; interpret with care
[d] temperature-normalized value derived from slow rate of HS (non-normalized value: 656.1 d)
[e] derived from slow rate of DFOP (k_2)
[f] DT_{50} back-calculated from FOMC ($DT_{50}=DT_{90}/3.32$)
[g] temperature-normalized value (non-normalized value: 150.8 d)
[h] no reliable endpoints derived in kinetic evaluation

Table 7.2.2.4-4: Summary table of best-fit endpoints of boscalid metabolite M510F64 obtained in water/sediment studies (Level M-I)

Water/ sediment system	pH water	pH sed ($CaCl_2$)	T [°C]	Best-fit $DegT_{50}/DegT_{90}$ whole system [a] [d]	Kinetic model	Best-fit $DisT_{50}/DT_{90}$ water [b] [d]	Kinetic model	Best-fit $DisT_{50}/DT_{90}$ sediment [b] [d]	Kinetic model
BASF DocID 2000/1017038, BASF DocID 2013/1382371									
Kellmetschweiher (outdoor conditions) (d)	8.8	Not given	Mean 18.1 (8.6-28.5)	10.1 / 33.4	Parent HS / metab. SFO	Not calc. [c]	-	Not calc. [c]	-

(d) – diphenyl-labeled test item used
[a] degradation rate
[b] dissipation rate
[c] no reliable endpoints derived in kinetic evaluation

Table 7.2.2.4-5: Summary table of modeling endpoints of boscalid metabolite M510F64 obtained in water/sediment studies (Level M-I)

Water/ sediment system	pH water	pH sed ($CaCl_2$)	T [°C]	Modeling $DegT_{50}$ whole system [a] [d]	Kinetic model	Modeling $DisT_{50}$ water [b] [d]	Kinetic model	Modeling $DisT_{50}$ sediment [b] [d]	Kinetic model
BASF DocID 2000/1017038, BASF DocID 2013/1382371									
Kellmetschweiher (outdoor conditions) (d)	8.8	Not given	Mean 18.1 (8.6-28.5)	14.8 [c]	Parent HS / metab. SFO	Not calc. [d]	-	Not calc. [d]	-

(d) – diphenyl-labeled test item used
[a] degradation rate
[b] dissipation rate
[c] temperature-normalized value (non-normalized value: 10.1 d)
[d] no reliable endpoints derived in kinetic evaluation

Impact of Water Treatment Procedures

Regulation 1107/2009 mentions in general terms that also substances resulting from water treatment shall have no harmful effects but this provision is not reflected in a data requirement in Regulation 283/2013. No established test methods or agreed guidance exist for assessing the effect of water treatment procedures on residues present in raw water.

Concentrations of boscalid or its metabolites at abstraction points of raw water intended for drinking water production are expected to be very low. In groundwater, concentrations in leachate at 1 m soil depth (represented by PEC_{gw}, see *M-CP 9.2.4.1*) will be reduced by degradation, sorption and dilution, resulting in significantly lower concentrations in aquifers used for drinking water abstraction. In surface water, predicted concentrations in small water bodies at the edge of treated fields (represented by PEC_{sw}, see *M-CP 9.2.5*) will be substantially diluted until they reach abstraction points in rivers or lakes.

Levels in raw water abstracted from ground- or surface water are expected to be reduced by common initial water treatment processes like aeration, flocculation and filtration. Subsequent procedures like oxidation, chlorination or sterilisation are expected to further reduce these levels. For potential degradates produced from these processes, a less than quantitative formation can be reasonably assumed leading to extremely low concentrations of such degradates.

From the chemical structure of boscalid and its metabolites there are no indications that harmful products might be formed by water treatment procedures. However, even if potentially harmful substances were formed, their concentrations are expected to be generally below the threshold of toxicological concern (TTC) of 4.5 µg/L and even below the more strict TTC of 0.075 µg/L covering the risk from geno- and neurotoxicity.

It is concluded that water treatment procedures applied for the production of drinking water are not expected to produce degradates at levels that could cause any risk.

CA 7.2.3 Degradation in the saturated zone

Due to its low leaching potential, boscalid is not expected to reach deeper soil layers or the saturated zone. FOCUS PEARL, PELMO and MACRO calculations [*see CP 9.2.4*] show that the risk of displacement of BAS 510 F into deeper soil layers or into the groundwater is extremely low. Therefore, investigations on the degradation in the saturated zone are considered not to be necessary.

CA 7.3 Fate and behaviour in air

CA 7.3.1 Route and rate of degradation in air

Boscalid is characterized by a low vapor pressure (7.2×10^{-7} Pa at 20 °C) and a low volatilization from soil and plant surfaces (≤ 1% in 24h). Furthermore, in the air it is rapidly degraded by photochemical processes.

Table 7.3.1-1: Studies on degradation of boscalid in air

Reference	BASF DocID	Estimated parameters	Remark
von Goetz N., 2000a	1999/11874	half-live values	
Ohnsorge U., 2000a	2000/1001009	Henry's law constant	
von Goetz N., 2000c	2000/1014979	volatilization rate from soil (sand) and plant (bush bean)	

No new experimental data are available, but a new calculation of the photochemical oxidative degradation in air (Atkinson) according to the newest model is provided below.

Report: CA 7.3.1/1
Hassink J., 2015a
Photochemical oxidative degradation of BAS 510 F (QSAR estimates)
2015/1005043
Guidelines: EC 1107/2009 of the European Parliament
GLP: no

Executive Summary

The degradation rates for reactions of boscalid (BAS 510 F) with OH-radicals and ozone in the atmosphere were calculated using the AOPWIN program based on ATKINSON's increment method.

Based on the resulting degradation rate of $k_{OH} = 9.0426 \times 10^{-12}$ cm^3 $molecule^{-1}$ s^{-1}, the atmospheric degradation half-life of the substance via this reaction route is $t_{1/2} = 1.183$ d (12 h day).

Although O_3 is likely to react with boscalid, the degradation rate resulting from ozone attack could not be estimated.

I. MATERIAL AND METHODS

The rate constant for reaction of boscalid with OH-radicals in the atmosphere was calculated with the AOPWIN Program (Atmospheric Oxidation Program for Microsoft Windows 3.1, Version 1.88, Syracuse Research Corp.) based on ATKINSON's increment method [*Atkinson (1987): A Structure-Activity relationship for the estimation of rate constants for the gas-phase reactions of OH radicals with organic compounds. Int. J. Chem. Kin. 19, 799*]. The degradation rate resulting from attack of ozone was calculated according to an OECD method [*Anonymous (1992): The rate of photochemical transformation of gaseous organic compounds in air under tropospheric conditions. OECD Environment Monographs No. 61, OECD, Paris*].

The rate constant k_{OH} of the active substance was estimated based on the chemical structure. The SMILES notation for boscalid in AOPWIN is:

O=C(c2cccnc2CL)Nc3c(cccc3)c1ccc(cc1)CL

Assuming a constant average OH radical concentration in the troposphere, the degradation of the active substance follows pseudo-first order kinetics with the rate constant k' = k · [OH radicals]:

-d[boscalid]/dt = k' · [boscalid]

The half-life of this process can then be calculated by the following equation:

$t_{1/2}$ = ln2/k' = ln2/k · [OH radicals]

II. RESULTS AND DISCUSSION

The rate constant was estimated to be $k_{OH} = 9.0426 \times 10^{-12}$ cm^3 molecule^{-1} s^{-1}.

Considering a 12 h day and a weighted global average tropospheric hydroxyl-radical concentration of 1.5×10^6 mol cm^{-3}, the half-life for the degradation of boscalid by OH-radicals was calculated as

$t_{1/2}$ = ln2 / ($9.0426\ 10^{-12} \times 1.5\ 10^6$) s
= 14.194 h
= <u>1.183 d (12 h day)</u>

Although boscalid contains reactive sites for an ozone attack, no increments are available and a reasonable approximation by AOPWIN was not possible. Therefore, although O_3 is likely to react with boscalid, no degradation estimation can be given.

Based on this degradation rate, the atmospheric degradation half-life of boscalid via this reaction route is $t_{1/2}$ = 1.183 d (12 h day).

III. CONCLUSION

Based on the results of the Atkinson calculation, boscalid will be rapidly degraded by photochemical processes in the troposphere ($t_{1/2}$ = 1.183 d). Thus, it can be concluded that there is no risk of long-range transport of boscalid via air.

CA 7.3.2 Transport via air

Boscalid has a very low volatilization potential and is degraded fast by photochemical processes. Consequently, there is no risk of long-range transport of boscalid.

CA 7.3.3 Local and global effects

No effects are expected since transport via air is very unlikely (for details see above).

CA 7.4 Definition of the residue

CA 7.4.1 Definition of the residue for risk assessment

According to the results presented in M-CA 7.1 – 7.3 the following compounds have to be considered for the environmental risk assessment:

Soil:
Boscalid and its soil metabolites M510F47 and M510F49

Metabolite M510F47 exceeded the trigger of 5% only after extended periods of anaerobicity which are not expected to occur under real agricultural conditions.
Nevertheless M510F47 was tested besides M510F49 against non-target soil organisms. From the results it can be concluded that the risk of metabolites for soil organisms is very low.

Groundwater:
Boscalid and its soil metabolites M510F47 and M510F49

Predicted environmental concentrations in groundwater (PEC_{gw}) for metabolite M510F47 are <0.1 µg/L (max. 0.057 µg/L). It is concluded that M510F47 poses no risk of leaching to groundwater.

Predicted environmental concentrations in groundwater (PEC_{gw}) for metabolite M510F49 are in some scenarios >0.1µg/L with maximum calculated concentrations of 0.3 µg/L.

Surface Water:
Boscalid and its metabolite M510F64

The aquatic toxicity of M510F64 was tested and very low toxicity was observed. Sufficient margins of safety were reached after FOCUS surface water step 1 calculations and it is concluded that the risk of M510F64 for aquatic organisms is negligible.

Sediment:
Boscalid

No metabolites were detected in the sediment in significant amounts.

Air:
Boscalid

No volatile metabolite was observed.

CA 7.4.2 Definition of the residue for monitoring

According to the results of the risk assessment the following compounds should be considered for environmental monitoring:

Soil: Boscalid (parent only)

Ground Water: Boscalid and metabolite M510F49

Surface Water: Boscalid (parent only)

Sediment: Boscalid (parent only)

Air: Boscalid (parent only)

CA 7.5 Monitoring data

No monitoring data were available for boscalid (BAS 510 F) at the time of the first Annex I review. Since the first Annex I inclusion no monitoring studies have been conducted by the notifier.

In the literature review two relevant articles were found describing monitoring in EU countries for multiple substances including boscalid. These articles are presented in this section. One describes a study monitoring surface water in a Spanish coastal lagoon system [*CA 7.5/1*], the second addresses air monitoring conducted in Strasbourg, France [*CA 7.5/2*]. Neither study indicates findings of boscalid at problematic levels.

Report: CA 7.5/1
Moreno-González R. et al., 2012a
Seasonal input of regulated and emerging organic pollutants through surface watercourses to a Mediterranean coastal lagoon
2015/1226713
Guidelines: none
GLP: no

Executive Summary

Seasonal input of organic compounds (inter alia boscalid) through El Albujón Watercourse to the Mar Menor lagoon was estimated from spring 2009 to winter 2010, including regular periods and two flash flood events. Eighty-two semi-volatile organic compounds (persistent organic compounds, different groups of pesticides and others) were determined by stir bar sorptive extraction and thermal desorption followed by capillary gas chromatography coupled to mass spectrometry from surface waters with quantification limits of a few ng L^{-1} (boscalid 5.8 ng L^{-1}).

Pesticide concentrations varied significantly along the watercourse due to the presence of different sources (ground waters, wastewater effluent, tributary contributions, brackish waters, etc.) and physicochemical/biological processes that take place simultaneously. The most commonly detected analytes were propyzamide, triazine compounds, and chlorpyrifos. A clear seasonal pattern has been detected, with a predominance of insecticides during summer and of herbicides during winter. The input of pesticides through this watercourse is particularly relevant during periods of heavy rain, representing more than 70% of total yearly input for many of them (boscalid 86%).

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material

All analytical standards (unlabeled, including boscalid) were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany).

2. Sampling site

Mar Menor, a hypersaline coastal lagoon located in the south-east of Spain, was selected as sampling site. The site is located close to the Campo de Cartagena area, where an intensive agriculture has been practiced since 1979. Main crops cultivated in this area are lettuce, artichoke, broccoli-cauliflower (winter and spring), as well as pepper and melon (spring and summer). In addition, citrus and almond tree crops are also relevant. Consequently, pesticides are regularly applied in this area and can enter the lagoon in different ways (surface and groundwaters, associated to particulate material or dissolved in air, wastewater effluents, etc.). More than 20 wadis run into the Mar Menor lagoon, El Abujón Watercourse being the main collector with a drainage basin of 441 km².

B. STUDY DESIGN

1. Sampling

The input of organic compounds (inter alia boscalid) into the Mar Menor lagoon via the El Albujón watercourse (main introduction pathway) was investigated from spring 2009 to winter 2010 by taking surface water samples every three months at six different sites along the flow path of the watercourse.

In August 2009 and January 2010, two additional sampling campaigns were conducted to characterize the daily variation of organic compound input at the El Albujón wadi mouth, sampling three times per day for a week. Two additional samples were taken during flood events close to the El Albujón watercourse mouth.

Temperature, pH, conductivity and dissolved oxygen were determined in situ.

2. Analytical procedures

Analyses of the water samples involved a stir bar sorptive extraction (SBSE) step with 200 g L^{-1} NaCl, a subsequent desorption from the stir bar (at 280°C, 12 min), cryofocusing in a PTV injector (at 40°C), and measurement by gas chromatography (GC) coupled with mass spectrometry (MS) in full-scan mode. Deuterated compounds were used as internal standards for the compound quantification.

The limit of quantification (LOQ) for boscalid was 5.8 ng L^{-1}.

II. RESULTS AND DISCUSSION

Findings

The method used was evaluated during the study. It was concluded that the method is sensitive, robust (good reproducibility and repetitively) and reveals a good linearity between 5 and 300 ng L^{-1} for all compounds tested ($r^2 > 0.98$).

The highest concentrations of some herbicides and fungicides (including boscalid) were detected in autumn. In summer 2009, boscalid was detected in 50% of the samples at concentrations ranging from 0 to 28.4 ng L^{-1}. In autumn of the same year, boscalid was detected in 100% of the samples in concentrations between < LOQ and 57.8 ng L^{-1}. In winter 2010, boscalid was detected in 67% of the samples at concentrations ranging from < LOQ to 27.4 ng L^{-1}.

Considering all available data for each season, the seasonal and annual mass inputs of boscalid via the El Albujón watercourse to the Mar Menor lagoon were also estimated < LOQ (spring 2009), 25.5 g $season^{-1}$ (summer 2009), 16.4 g $season^{-1}$ (autumn 2009), and 187.2 $season^{-1}$ (winter 2009). The estimated total input per year was 229.0 g boscalid.

In general, pesticide concentrations (including boscalid) decreased in the downstream direction along the El Albujón watercourse. However, several inputs showed increased concentrations in the main watercourse particularly in the proximity of the watercourse mouth (input from Miranda watercourse through a pipe and groundwater input).

Concentrations of the investigated organic compounds dissolved in water during a flash flood event were several orders of magnitude higher than in regular periods (100 to 10,000 times). During the first flood event (September 15-16, 2009), boscalid was detected in fluxes of 2139.65 mg h^{-1} (spot flux) and 219.96 g (total flux). During the second flood event (September 28, 2009), fluxes were higher, amounting to 68224.84 mg h^{-1} (spot flux) and 1222.52 g (total flux). If the compound concentrations were considered to be constant, the input of boscalid from EI Albujón watercourse to the Mar Menor lagoon during the flood events would represent 86.30% of the total input of boscalid in the period studied (spring 2009 to winter 2010).

III. CONCLUSION

The total input of 70 organic pollutants have been estimated for a year, the most commonly detected being OPPs and triazines. The authors concluded that this contribution is particularly relevant during periods of heavy rain, accounting for more than 70% of the total input of many pesticides through EI Albujón watercourse (in the case of boscalid accounting for 86% of the total yearly input). The authors detected a seasonal pattern, with a predominance of insecticides during winter season.

Report: CA 7.5/2
Schummer C. et al., 2009a
Temporal variations of concentrations of currently used pesticides in the atmosphere of Strasbourg, France
2015/1226715
Guidelines: none
GLP: no

Executive Summary

The aim of this study was to increase the knowledge about the atmospheric behavior of pesticides currently used in Alsace, France. Atmospheric samples (particulate and gas phases) have been collected in Strasbourg between April 18 and May 29, 2007 and were analyzed for 71 current-use pesticides, of which 38 were detected. Average concentrations ranged from 0.09 ng m^{-3} for fenarimol to 110.42 ng m^{-3} for dimethachlor (boscalid 0.53 ng m^{-3}). Overall the concentrations were slightly higher than the concentrations reported from other, comparable agricultural regions.
Significant temporal variations were observed for 30 pesticides, and for most of them it could be shown that these were linked to time (inter alia boscalid), temperature or atmospheric pressure. In several cases, this helped to identify pesticide application just before or at the beginning of the sampling period, or ongoing treatment. Humidity, in contrast to previous reports, could not be linked to these variations.

II. MATERIALS AND METHODS

B. MATERIALS

1. Test material

Unlabeled analytical standards (including boscalid; chemical purity > 98%) were purchased from Promochem (Molsheim, France), Aldrich (I'Isle d'Abeau, France), and Fluka (Buchs, Switzerland).

2. Sampling site

Air samples were collected in Strasbourg between April and May 2007. A high-volume sampler was placed in the botanical garden of Strasbourg University, about 0.5 km away from the city center, about 2 km away from industrial zones, and about 5 km away from the first exploitation of high maize and cereal crops. None of the studied pesticides was used in the Botanical Garden.

B. STUDY DESIGN

1. Sampling

Air sampling was performed using a high-volume sampler on a 48 h basis for the period from April 18 to May 29, 2007. No sampling was done in periods of significant rainfall to avoid influencing the results by wash-out.

Gaseous and particulate samples were collected simultaneously on glass fibre filters (30 cm diameter) and XAD-2 resin (20 g; copolymer of styrene/divinylbenzene and macroporous acrylic ester) for a 48 h-period on average, at a flow rate of 9.96 L min^{-1}. After sampling, filters and resins were stored in the dark at -20°C for a maximum of four days until extraction.

2. Analytical procedures

Prior to sampling, the XAD-2 resin and the glass fibre filters were cleaned by Soxhlet with 50 : 50 (v/v) n-hexane/CH_2Cl_2 and dried. Afterwards, they were individually wrapped in clean plastic bags or aluminum foil, and stored in the dark at -20°C. The efficiency of the cleaning procedure was confirmed by analyzing the extracts of cleaned glass fibre filter and XAD-2 traps for the target pesticides.

The extraction of the pesticides from the filters and the resin was performed separately by Soxhlet extraction with 50:50 (v/v) n-hexane/CH_2Cl_2 (for 20 h). After extraction, the solvents were concentrated to a smaller volume by rotary evaporation (at 40°C) and spiked with Tecnazen, which was used as internal standard. Several samples (filters and resins) were extracted a second time proving the efficiency of the extraction procedure.

Analysis of the 71 target pesticides was performed by gas chromatography (GC) coupled with mass spectrometry with ion trap (MS/MS) or electron capture detection (ECD), depending on the physic-chemical properties of the target pesticides. For the compound quantification, an internal standard (Tecnazen) was used.

The measured pesticide concentrations for both, the particulate and gaseous phases were summed up for each pesticide to obtain the concentrations found in the total atmosphere.

The limit of quantification (LOQ) for boscalid was 27.28 pg m^{-3}.

II. RESULTS AND DISCUSSION

Findings

Boscalid was detected in a frequency of ten out of ten atmospheric samples (combined gaseous and particulate phases). Detected concentrations of boscalid in the total atmosphere ranged from 0.35 to 0.81 ng m^{-3} with an average of 0.53 ng m^{-3} (95% confidence interval: 0.14 ng m^{-3}).

Detected total boscalid concentrations were negatively correlated with time indicating dispersion and degradation of boscalid in the atmosphere (r = -0.9675; p = 0.0317). No correlation of measured total atmospheric boscalid concentrations with air temperature, atmospheric pressure, or humidity could be detected.

In addition, the gas-particle distribution of the detected pesticides (inter alia boscalid) was determined. The distribution may affect the lifetime of a pesticide in the atmosphere as well as its potential for a long-range transport. As a result, detected boscalid concentrations were more related to the particle phase (about 65%) than to the gaseous phase (about 35%).

III. CONCLUSION

From 71 pesticides that were monitored, 38 were detected and quantified in the atmospheric samples (including boscalid). Compared to results from similar studies carried out in Iowa and different regions from Canada, only a limited number of pesticides were in common with the other studies, and concentrations measured for one pesticide sometimes differed considerably in-between the sampling places. The authors concluded that these differences were caused mainly by different sampling periods. Differences were also found when the results from this study were compared to those of a study carried out in similar conditions at the same place in 2002. However, no data for boscalid are presented in this evaluation.
In addition, the measured concentrations were used to study the temporal variations of the target pesticides in Alsace atmosphere and to identify the parameters linked to it. A negative correlation with time was observed for eleven pesticides, and for nine of them a recent application was supposed. Detected total boscalid concentrations were negatively correlated with time indicating dispersion and degradation of boscalid in the atmosphere (r = -0.9675; p = 0.0317). No correlation of measured total atmospheric boscalid concentrations with air temperature, atmospheric pressure, or humidity could be detected.

We create chemistry

Boscalid

Document M-CA, Section 8

ECOTOXICOLOGICAL 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 8 ECOTOXICOLOGICAL STUDIES ON THE ACTIVE SUBSTANCE

Introduction

Boscalid (BAS 510 F), a fungicide for use in vineyards, pulses and rape, is registered in Europe since many years. It was fully reviewed under Directive 91/414/EEC and included in Annex I by Commission Directive No 2008/44/EC. The approval was transferred to the new Regulation (EC) No 1107/2009 in Commission Implementing Regulation (EU) No 540/2011.

All relevant information on the first Annex I review and the endpoints used in ecotoxicological risk assessments can be found in the monographs of boscalid, and in the review report for boscalid (SANCO/3919/2007 - rev.5; Monograph 12945/ECCO/BBA/01, August 2001).

For the current registration renewal under Regulation 1107/2009, a data gap analysis according to new guidelines and new guidance documents was performed and new studies or evaluations were initiated where considered necessary. All new data are provided in this section or in the respective sections of the dossier for the new representative formulation.

Furthermore, a literature search was performed and scientific publications were evaluated for their endpoint relevance and quality. Summaries of relevant and reliable public literature data on boscalid are provided in this section as appropriate. Further information on the literature assessment and respective justifications can be found in M-CA 9.

CA 8.1 Effects on birds and other terrestrial vertebrates

CA 8.1.1 Effect on birds

No new study available.

CA 8.1.1.1 Acute oral toxicity to birds

No new study available

CA 8.1.1.2 Short-term dietary toxicity to birds

No new study available

CA 8.1.1.3 Sub-chronic and reproductive toxicity to birds

No new study available

CA 8.1.2 Effects on terrestrial vertebrates other than birds

No new study available

CA 8.1.2.1 Acute oral toxicity to mammals

No new study available

CA 8.1.2.2 Long-term and reproductive toxicity to mammals

No new study available

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 with the secondary poisoning risk assessments presented in the documents M-CP 10.1.1 and M-CP 10.1.2. All TER values exceed the trigger of 5 set by Commission regulation (EU) 546/2011 for acceptability of effects with considerable margins of safety. Consequently, no additional study is considered to be necessary.

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 either on the type of regulatory testing necessary or how to conduct a risk assessment for amphibian and reptiles.

According to the new aquatic guidance document (EFSA, 2013) amphibians should be included in the aquatic and terrestrial risk assessment. In absence of GLP studies the assessment should be based on any existing relevant information (testing of amphibian is not recommended at first instance due to animal welfare reasons and the absence of standard guidelines for amphibian testing). With regard to the aquatic risk assessment, several data analyses indicate that the risk assessment for aquatic organisms (and fish in particular) covers the risk assessment for aquatic phases of amphibians (Fryday and Thompson, 2012; Weltje et al., 2013). Based on these extensive data reviews it can be concluded that the acute and chronic risk to aquatic life stages of amphibians is covered by the currently requested and conducted risk assessment for aquatic organisms (M-CP 10.2).

Compared to aquatic studies, regulatory ecotoxicological information on amphibians based on dosing studies (LD_{50}) is rather scarce. However, in the few cases where terrestrial stages of amphibians were tested in the same kind of study as birds and mammals, the general pattern is that amphibians are less sensitive than the latter two taxa (see Table 12 and 13 in Fryday and Thompson, 2012).

In the case of reptiles there is even less information available than for amphibians (see the revision by Fryday and Thompson, 2009). The risk from dietary exposure can be assumed to be lower for reptiles than for birds and mammals (Fryday and Thompson 2009). This is because reptiles are poikilotherms (*i.e.* do not maintain a constant body temperature) and as a result feeding activity will peak on warm days and will be zero during hibernation or on cold days. In contrast, birds and mammals will have to maintain a constant body temperature and, hence, will need to feed every day (Fryday & Thompson 2009). Uncertainties remain on the contribution of dermal exposure to the overall exposure to reptiles. However, in contrast to amphibians the skin of reptiles is much less permeable; its function is in general protection and as a barrier, and it is not an organ used for respiration or water/mineral exchange with the environment. Accordingly, reptiles are considered less vulnerable to dermal exposure compared to amphibians. Nevertheless, some uncertainty with respect to the risk to reptiles, i.e. whether they are sufficiently covered by other (more standard) ecotoxicological data will remain and further research is needed.

However, there is no indication from present (eco)toxicological studies, which might raise a specific concern to amphibians or reptiles. Furthermore, boscalid has been used for many years in many countries worldwide. So far, there are no publications indicating a potential risk of this compound to amphibians / reptiles and despite the long term use worldwide, the applicant is not aware of a single finding or (incidence) report that amphibians / reptiles were harmed by applications of this substance.

References:

Commission Regulation (EU) No 283/2013 setting out data requirements for active substances, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market. Official Journal of the European Union: 1st March 2013.

Commission Regulation (EU) No 284/2013 setting out the data requirements for plant protection products, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market. Official Journal of the European Union: 1st March 2013.

Fryday S and Thompson H (2009): Literature reviews on ecotoxicology of chemicals with a special focus on plant protection products. Lot 1. Exposure of reptiles to plant protection products. EFSA (CFT/EFSA/PPR/2008/01).

Fryday S and Thompson, H (2012): Toxicity of pesticides to aquatic and terrestrial life stages of amphibians and occurrence, habitat use and exposure of amphibian species in agricultural; Food and Environment research agency, UK.

Weltje L., Simpson P., Gross M., Crane M., Wheeler J.R. (2013): Comparative acute and chronic sensitivity of fish and amphibians: a critical review of data. Environmental Toxicology and Chemistry, Vol. 32, No. 5, pp. 984-994.

CA 8.1.5 Endocrine disrupting properties

Based on the physical, chemical and structural characteristics of the active substance boscalid, there is no indication of endocrine effects. An assessment of all available data for mammalian toxicology came to the conclusion that there is no indication that boscalid has endocrine disrupting properties (for more detailed information please see M-CA 5.8.4). Furthermore, neither the avian reproduction studies nor any studies conducted with fish give any indication of endocrine disrupting activity (for more detailed information please see M-CP 10.1.1 and M-CP 10.2). Thus, no further studies are required.

CA 8.2 Effects on aquatic organisms

Since Annex I inclusion of boscalid (BAS 510 F), new toxicity studies on the active substance and the metabolites of boscalid have been performed and as a result there are new endpoints which are now used in the aquatic risk assessment. Summaries of these new studies are provided below. For completeness this includes some older studies, which have not been submitted during the previous Annex I inclusion process (*e.g.* because there is no respective data requirement in the EU).

Details on the EU agreed studies which have been already evaluated within the Annex I inclusion of boscalid are provided in the EU Review documents of the active substance (*i.e.* EC Review report SANCO/3919/2007 - rev. 5, 2008; Monograph, Vol. 3, November 2002 and Addendum 2 to the DAR, Vol. 3, Annex B.9, May 2006).

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. Full references used within the following chapters are given at the end of MCA 8.2. Document N3 contains structures and synonyms for the metabolites of boscalid.

Table 8.2-1: Summary of the toxicity values for aquatic organisms obtained in studies with the active substance boscalid (BAS 510 F)

Organism	**Endpoint**	**Value [mg/L]**	**Reference** (BASF DocID)	**EU agreed** (Justification for submission of new data)
Active substance: Boscalid (BAS 510 F)				
Fish				
Oncorhynchus mykiss	96 h LC_{50}	**≅ 2.7**	2001/1001726	yes
Lepomis macrochirus	96 h LC_{50}	> 4.0	2001/1001727	yes
O. mykiss [1)]	96 h LC_{50}	>4.73 < 5.90	1999/10928	no (supplemental data)
L. macrochirus [1)]	96 h LC_{50}	4.34	1999/10933	no (supplemental data)
Cyprinodon variegatus [1), 4)]	96 h LC_{50}	>3.86	2001/5000054	no (new data; generated to address US EPA requirements)
O. mykiss [5)]	28 d NOEC	1.0	1999/10927	yes (but not valid according to current standard) [5)]

Organism	Endpoint	Value [mg/L]	Reference (BASF DocID)	EU agreed (Justification for submission of new data)
O. mykiss	97 d NOEC	**0.125**	1999/11847	yes (but new recalculation of endpoint based on current requirements)
Aquatic invertebrates				
Daphnia magna	48 h EC_{50}	5.33	2000/1018537	yes
Americamysis bahia 1), 4)	48 h LC_{50}	> 3.81 3)	2001/5000086	no (new data; generated to address US EPA requirements)
Crassostrea virginica 1), 4)	96 h EC_{50}	1.66	2001/5000877	no (new data; generated to address US EPA requirements)
D. magna 1)	21 d NOEC	**0.80**	2004/1015006 Amendment: 2004/1015009	no (but data used in previous product registrations)
D. magna	21 d NOEC	1.31	2000/1018539	yes
Sediment dwelling aquatic invertebrates				
Hyalella azteca 1)	10 d LC_{50} (spiked sediment)	> 97.0 mg/kg dry sediment 2)	2001/5000043	no (new data; generated to address US EPA requirements)
Chironomus riparius	28 d NOEC (spiked water)	1.0	2000/1018538	yes
C. riparius	28 d NOEC (spiked sediment)	23.26 mg/kg dry sediment 2)	2005/1022464	yes
Algae 6)				
Pseudokirchneriella subcapitata (Syn. *Selenastrum capricornutum*)	96 h E_rC_{50} 96 h E_yC_{50} 72 h E_rC_{50} 8) 72 h E_yC_{50}	3.75 1.34 **2.61** 7) 1.33 7)	2000/1018524 2009/1044471 (recalculations)	yes (but recalculation of 72 h endpoints)
Navicula pelliculosa 1), 9)	72 h E_bC_{50}	2.0	2001/5000044	no (new data; generated to address US EPA requirements, (but not valid according to current standard))

Organism	Endpoint	Value [mg/L]	Reference (BASF DocID)	EU agreed (Justification for submission of new data)
Anabaena flos-aquae 1), 9)	72 h E_bC_{50}	> 4.2	2001/5000045	no (new data; generated to address US EPA requirements, (but not valid according to current standard))
Skeletonema costatum 1, 4), 9)	72 h E_bC_{50}	> 3.5	2001/5000087	no (new data; generated to address US EPA requirements, (but not valid according to current standard))
Macrophytes 6)				
Lemna gibba 1)	7d EC_{50}	> 3.9 10)	2001/5000046	no (new data; generated to address US EPA requirements)
Bioconcentration				
O.mykiss (35/28 d exposure, 15 d depuration)	BCF_{SS} (TRR) BCF_{SS} (unchanged parent)	70 (whole fish) 125 (whole fish)	2000/1017222	yes

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 macrophytes), the relevant endpoint is used in the TER calculations presented in chapter M-CP 10.2 of this supplementary dossier.

ELS = early life stage

1) Study has not been submitted during the Annex I inclusion process of boscalid; a study summary is provided below.

2) mg/kg dry sediment (spiked sediment study)

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) marine / saltwater species

5) This chronic study on *O. mykiss* is considered to be not valid according to current standard. Therefore the resulting endpoint is provided for completeness, but it is not considered for the aquatic risk assessment.

6) In accordance to the EFSA Aquatic Guidance Document (EFSA 2013) and OECD guidelines 201 (OECD, 2011) and 221 (OECD, 2006), only the EC_{50} values for the more relevant endpoint 'growth rate' (E_rC_{50}) are considered for the risk assessment for aquatic primary producers.

7) In accordance to recent guidelines (EFSA Aquatic GD (2013) and OECD 201 (2011)), the 72 h endpoints of the EU agreed study on the green alga have been (re-)calculated from original data. The re-calculated values are presented here and are used in the aquatic risk assessment; for details on these calculations please refer to the supplement. A summary of this study and the re-calculations is provided below.

8) endpoint extrapolated

9) The study on this alga species is considered to be not valid. For details please see the justifications and study summaries provided below.

10) based on frond no.

Table 8.2-2: List of studies and endpoints for aquatic organisms exposed to the major metabolites of boscalid (BAS 510 F)

Organism	Endpoint	Value [mg/L]	Reference (BASF DocID)	EU agreed (Justification for submission of new data)
Metabolite: M510F64 (Reg. No. 309572, M64)				
Fish				
O. mykiss [1)]	96 h LC_{50}	57.41	2013/1000146	no (new data for the risk assessment of the metabolite)
Aquatic invertebrates				
D. magna [1)]	48 h EC_{50}	102	2013/1005681	no (new data for the risk assessment of the metabolite)
Algae [2)]				
Selenastrum capricornutum (Syn. *Pseudokirchneriella subcapitata*) [1)]	E_rC_{50} (72 h) E_yC_{50} (72 h)	96.2 60.5	2013/1005682 Amendment 2013/1334849	no (new data for the risk assessment of the metabolite)
Metabolite: M510F47 (Reg. No. 107371, M47)				
Fish				
O. mykiss [1)]	96 h LC_{50}	> 100	2015/1001498	no (new data for the risk assessment of the metabolite)
Aquatic invertebrates				
D. magna [1)]	48 h EC_{50}	> 80	2015/1001499	no (new data for the risk assessment of the metabolite)
Algae [2)]				
Selenastrum capricornutum (Syn. *Pseudokirchneriella subcapitata*)	E_rC_{50} (72 h) E_yC_{50} (72 h)	> 100 43.66	2015/1001500	no (new data for the risk assessment of the metabolite)
Metabolite: M510F49 (Reg. No. 391572; M49)				
Fish				
O. mykiss [1)]	96 h LC_{50}	> 0.31	2015/1001501	no (new data for the risk assessment of the metabolite)

Organism	Endpoint	Value [mg/L]	Reference (BASF DocID)	EU agreed (Justification for submission of new data)
Aquatic invertebrates				
D. magna [1)]	48 h EC_{50}	> 0.42	2015/1001502	no (new data for the risk assessment of the metabolite)
Algae [2)]				
Selenastrum capricornutum (Syn. *Pseudokirchneriella subcapitata*) [1)]	E_rC_{50} (72 h) E_yC_{50} (72 h)	> 0.36 > 0.36	2015/1001503	no (new data for the risk assessment of the metabolite)

1) Study has not been submitted during the Annex I inclusion process of boscalid; a study summary is provided below.
2) In accordance to the EFSA Aquatic Guidance Document (EFSA 2013) and OECD guidelines 201 (OECD, 2011) and 221 (OECD, 2006), only the EC_{50} values for the more relevant endpoint 'growth rate' (E_rC_{50}) are considered for the risk assessment for aquatic primary producers.

CA 8.2.1 Acute toxicity to fish

An acute toxicity study with rainbow trout (*Oncorhynchus mykiss*) conducted with boscalid was already evaluated during the previous Annex I inclusion process. The following additional acute toxicity study with rainbow trout performed with the active substance boscalid has not been evaluated previously on EU level. It confirms the toxicity endpoint of the standard study used for the risk assessment (2001/1001726) and is submitted for completeness and as supplemental data.

Report: CA 8.2.1/1
[redacted] 1999a
BAS 510 F - Acute toxicity study on the rainbow trout (Oncorhynchus mykiss WALBAUM 1792) in a static system (96 hours)
1999/10928
Guidelines: EPA 72-1, EEC 84/449, OECD 203
GLP: yes
(certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Executive Summary

In a 96-hour static acute toxicity laboratory study, juvenile rainbow trout were exposed to a dilution water control and to nominal concentrations of 1.0, 2.15, 4.64, 10.0, 21.5 and 46.4 mg boscalid/L (corresponding to mean measured concentrations of 0.83, 1.63, 2.56, 3.20, 3.93, and 4.73 mg a.s./L) in groups of 10 animals in glass aquaria containing 100 L water with 1 replicate per treatment. An additional concentration of 100.0 mg/L (corresponding to a mean measured concentration of 5.90 mg a.s./L) and an untreated control were tested 4 weeks after the start of the test, since no LC_{50} could be achieved with the original concentrations. Fish were observed for survival and symptoms of toxicity after 1, 4, 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 both dilution water controls and at test item concentrations of 0.83, 1.63, 2.56, 3.20 and 4.73 mg a.s./L, whereas 10% and 80% mortality was observed at test item concentrations of 3.93 and 5.90 mg a.s./L, respectively. No sub-lethal effects were found in the control groups and the lowest test item concentration of 0.83 mg a.s./L. At all other test item concentration sub-lethal effects (i.e. apathy, convulsions, narcotic-like states and swimming near the bottom) were found.

In a static acute toxicity study with rainbow trout the LC_{50} (96 h) of boscalid was > 4.73 < 5.90 mg a.s./L based on mean measured concentrations. The NOEC (96 h) based on mortality was determined to be 3.2 mg a.s./L (mean measured).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Boscalid (BAS 510 F; Reg. No. 300355), batch no. N 26, purity: 95.3%.

B. STUDY DESIGN

Test species: Rainbow trout (*Oncorhynchus mykiss* WALBAUM 1792), 3 months old juveniles (4 months for added concentrations); length of fish: 4.2 – 5.1 cm (4.9 – 5.8 cm for added concentration 4 weeks later), wet weight of fish: 0.54 – 0.99 g (1.2 – 1.9 g for added concentration 4 weeks later); supplied by “Trout Breeding Worbis”, Worbis/Thueringen.

Test design: Static system (96 h); 7 test item concentrations plus 2 dilution water controls; 1 replicate per treatment; 10 fish per aquarium; assessment of mortality and sublethal effects directly after 1, 4, 24, 48, 72 and 96 hours after start of exposure.

Endpoints: LC_{50}, NOEC, mortality and sub-lethal effects.

Test concentrations: Control (dilution water), 1.0, 2.15, 4.64, 10.0, 21.5, 46.4 mg/1, corresponding to mean measured concentrations of 0.83, 1.63, 2.56, 3.20, 3.93, 4.73 mg a.s./L; Control 2 (dilution water) and 100 mg/L as additional concentrations 4 weeks after start of the test, corresponding to a mean measured concentration of 5.90 mg a.s./L.

Test conditions:	100 L glass aquaria (80 x 35 x 46 cm), water depth about 40 cm; dilution water: non chlorinated charcoal filtered tap water; temperature: 11.6°C-12.6°C (12.4 – 12.9°C for added concentration 4 weeks later); pH 8.0 - 8.6; acid capacity: approx. 5.5 mmol/L; oxygen content: 8.2 mg/L – 11.0 mg/L (9.1 - 12.5 mg/L for added concentration 4 weeks later); total hardness: 250 mg $CaCO_3$/L; fish loading rate: 0.1 g/L (0.2 g/L for the added concentration 4 weeks later); photoperiod 16 h light: 8 h dark; no food, no aeration.
Analytics:	Analytical verification of test item concentrations was conducted using an HPLC-method with external calibration.
Statistics:	Descriptive statistics, probit analysis for determination EC values

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of boscalid concentrations was conducted in each concentration (filtered samples) at the beginning and at the end of the test. The analyzed contents of boscalid ranged from 5.9% to 79.0% of nominal at test initiation and from 4.9% to 80.0% of nominal at test termination. The test was conducted above the limit of solubility. Undissolved test substance was visible on the bottom and at the water surface of all aquaria with increasing concentrations. The following biological results are based on mean measured concentrations.

Biological results: After 96 hours of exposure, no mortality was observed in both dilution water controls and at test item concentrations of 0.83, 1.63, 2.56, 3.20 and 4.73 mg a.s./L, whereas 10% and 80% mortality was observed at test item concentrations of 3.93 and 5.90 mg a.s./L, respectively. No sub-lethal effects were found in the control groups and the lowest test item concentration of 0.83 mg a.s./L. At all other test item concentration sub-lethal effects (i.e. apathy, convulsions, narcotic-like states and swimming near the bottom) were found. The results are summarized in Table 8.2.1-1.

Table 8.2.1-1: Acute toxicity (96 h) of boscalid to Rainbow trout (*Oncorhynchus mykiss)*

Concentration [mg a.s./L] (nominal)	**Control**	**Control 2 #**	**1.0**	**2.15**	**4.64**	**10.0**	**21.5**	**46.4**	**100 #**
Concentration [mg a.s./L] (mean measured)	**--**	**--**	**0.83**	**1.63**	**2.56**	**3.20**	**3.93**	**4.73**	**5.90**
Mortality [%] (96 h)	0	0	0	0	0	0	10	0	80
Symptoms (after 96 h) *	none	none	none	N; D	K; N	K, N	K, N	K, N	N
Endpoints [mg boscalid/L] (mean measured)									
LC_{50} (96 h)	> 4.73 < 5.90								
NOEC (96 h)	3.20								

* Symptoms after 96 h: K = convulsions, N = narcotic-like state, D = swimming near the bottom
\# Added concentration 4 weeks after start of the test

III. CONCLUSION

In a flow-through acute toxicity study with rainbow trout the LC_{50} (96 h) of boscalid was > 4.73 < 5.90 mg a.s./L based on mean measured concentrations. The NOEC (96 h) based on mortality was determined to be 3.2 mg a.s./L (mean measured).

An acute toxicity study with Bluegill (*Lepomis macrochirus*) conducted with boscalid was already evaluated during the previous Annex I inclusion process. The following additional acute toxicity study with Bluegill performed with the active substance boscalid has not been evaluated previously on EU level and is submitted for completeness and as supplemental data.

Report:	CA 8.2.1/2 [redacted] 1999b BAS 510 F - Acute toxicity study on the bluegill (Lepomis macrochirus RAF.) in a static system (96 hours) 1999/10933
Guidelines:	EPA 72-1, EEC 84/449, OECD 203
GLP:	yes (certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Executive Summary

In a 96-hour static acute toxicity laboratory study, juvenile bluegill were exposed to a dilution water control and to nominal concentrations of 1.0, 2.15, 4.64, 10.0, 21.5, 46.4 and 100 mg boscalid/L (corresponding to mean measured concentrations of 0.84, 1.71, 3.03, 3.93, 4.71, 5.37 and 5.87 mg a.s./L) in groups of 10 animals in glass aquaria containing 100 L water with 1 replicate per treatment. Fish were observed for survival and symptoms of toxicity after 1, 4, 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 and at test item concentrations up to and including 3.03 mg a.s./L, whereas 50%, 50% and 80% mortality was observed at test item concentrations of 3.93, 4.71 and 5.37 mg a.s./L, respectively. At the highest tested concentration, all fish were dead after 96 hours of exposure. No sub-lethal effects were found in the control groups and the two lowest test item concentration of 0.84 and 1.71 mg a.s./L. At all other test item concentration sub-lethal effects (i.e. apathy, narcotic-like states and swimming near the bottom) were found.

In a static acute toxicity study with bluegill the LC_{50} (96 h) of boscalid was 4.34 mg a.s./L based on mean measured concentrations. The NOEC (96 h) based on mortality was determined to be 3.03 mg a.s./L (mean measured).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Boscalid (BAS 510 F; Reg. No. 300355), batch no. N 26, purity: 95.3%.

B. STUDY DESIGN

Test species: Bluegill (*Lepomis macrochirus* RAF.), juveniles; length of fish: 4.8 – 5.7 cm; wet weight of fish: 1.1 – 1.9 g; supplied by "Osage catfisheries INC.", Missouri.

Test design: Static system (96 h); 7 test item concentrations plus a dilution water control; 1 replicate per treatment; 10 fish per aquarium; assessment of mortality and sublethal effects directly after 1, 4, 24, 48, 72 and 96 hours after start of exposure.

Endpoints: LC_{50}, NOEC, mortality and sub-lethal effects.

Test concentrations: Control (dilution water), 1.0, 2.15, 4.64, 10.0, 21.5, 46.4 and 100 mg/1, corresponding to mean measured concentrations of 0.84, 1.71, 3.03, 3.93, 4.71, 5.37 and 5.87 mg a.s./L.

Test conditions: 100 L glass aquaria (80 x 35 x 46 cm), water depth about 40 cm; dilution water: non chlorinated charcoal filtered tap water; temperature: 22°C-24°C; pH 8.0 - 8.6; acid capacity: approx. 5.5 mmol/L; oxygen content: 6.3 mg/L – 9.1 mg/L; total hardness: 250 mg $CaCO_3$/L; fish loading rate: 0.2 g/L; photoperiod 16 h light: 8 h dark; no food, no aeration.

Analytics: Analytical verification of test item concentrations was conducted using an HPLC-method with external calibration.

Statistics: Descriptive statistics, probit analysis for determination EC values

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of boscalid concentrations was conducted in each concentration (filtered samples) at the beginning and at the end of the test. The analyzed contents of boscalid ranged from 5.9% to 74.0% of nominal at test initiation and from 5.6% to 83.3% of nominal at test termination. The test was conducted above the limit of solubility. Undissolved test substance was visible on the bottom and at the water surface of all aquaria with increasing concentrations. 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 and at test item concentrations up to and including 3.03 mg a.s./L, whereas 50%, 50% and 80% mortality was observed at test item concentrations of 3.93, 4.71 and 5.37 mg a.s./L, respectively. At the highest tested concentration, all fish were dead after 96 hours of exposure. No sub-lethal effects were found in the control groups and the two lowest test item concentration of 0.84 and 1.71 mg a.s./L. At all other test item concentration sub-lethal effects (i.e. apathy, narcotic-like states and swimming near the bottom) were found. The results are summarized in Table 8.2.1-2.

Table 8.2.1-2: Acute toxicity (96 h) of boscalid to Bluegill (*Lepomis macrochirus)*

Concentration [mg a.s./L] (nominal)	**Control**	**1.0**	**2.15**	**4.64**	**10.0**	**21.5**	**46.4**	**100**
Concentration [mg a.s./L] (mean measured)	**--**	**0.84**	**1.71**	**3.03**	**3.93**	**4.71**	**5.37**	**5.87**
Mortality [%] (96 h)	0	0	0	0	50	50	80	100
Symptoms (after 96 h) *	none	none	none	D; N	A; D	D, N	N	none
Endpoints [mg boscalid/L] (mean measured)								
LC_{50} (96 h)	4.34							
NOEC (96 h)	3.03							

* Symptoms after 96 h: A = apathy, N = narcotic-like state, D = swimming near the bottom
n.d. = not determined; all fish dead

III. CONCLUSION

In a static acute toxicity study with bluegill the LC_{50} (96 h) of boscalid was 4.34 mg a.s./L based on mean measured concentrations. The NOEC (96 h) based on mortality was determined to be 3.03 mg a.s./L (mean measured).

The following acute toxicity study on sheepshead minnow (*Cyprinodon variegatus*) performed with the active substance boscalid is not required for registration in the EU and it has not been evaluated previously on EU level. The study was conducted due to U.S. data requirements and is submitted for completeness.

Report:	CA 8.2.1/3 [redacted] et al., 2001a Flow-through acute toxicity of BAS 510 F to the sheepshead minnow, Cyprinodon variegatus 2001/5000054
Guidelines:	EPA 72-3(a), EPA 850.1075
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary

In a 96-hour flow-through acute toxicity laboratory study, juvenile sheepshead minnow were exposed to a dilution water control, a solvent control and to nominal concentrations of 0.52, 0.86, 1.4, 2.4 and 4.0 mg boscalid/L (corresponding to mean measured concentrations of 0.490, 0.835, 1.39, 2.33 and 3.86 mg a.s./L) in groups of 10 animals in glass aquaria containing 15 L water with 2 replicates per treatment. 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 test item concentrations of up to and including 0.835 mg a.s./L, whereas 5% mortality was observed at the third highest test item concentration of 1.39 mg a.s./L. At the second highest test item concentration no mortality was observed, while at the highest test item concentration 10% mortality occurred. No sub-lethal effects were found in the control groups and at test item concentrations of up to and including 2.33 mg a.s./L. At the highest test item concentration sub-lethal effects (i.e. loss of equilibrium, lethargy) were found.

In a flow-through acute toxicity study with sheepshead minnow the LC_{50} (96 h) of boscalid was > 3.86 mg a.s./L based on mean measured concentrations. The NOEC (96 h) was determined to be 2.33 mg a.s./L (mean measured).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Boscalid (BAS 510 F; Reg. No. 300355), batch no. N 75, purity: 96.9%.

B. STUDY DESIGN

Test species: Sheepshead minnow (*Cyprinodon variegatus*), juveniles; length of control fish: 27.3 mm; average wet weight of control fish: 0.33 g; supplied by "Aquatic BioSystems", Fort Collins, Colorado, USA.

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; assessment of mortality and sublethal effects directly after start of exposure and 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 (0.5 mL acetone/L), 0.52, 0.86, 1.4, 2.4 and 4.0 mg boscalid/L (nominal), corresponding to mean measured concentrations of 0.490, 0.835, 1.39, 2.33 and 3.86 mg a.s./L.

Test conditions: 20 L glass aquaria, test volume: 15 L; dilution water: carbon filtered natural seawater, salinity: 16 - 17 ‰; flow rate: 6.6 volume additions per 24 hours on average per test vessel; temperature: 21.1°C- 21.9°C; pH 7.7 - 8.0; oxygen content: 7.3 mg/L - 8.4 mg/L; fish loading rate: 0.22 g/L; photoperiod 16 h light : 8 h dark; light intensity: approx. 49 foot candles; no aeration.

Analytics: Analytical verification of test item concentrations was conducted using an HPLC-method with UV-detection.

Statistics: Descriptive statistics.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of boscalid concentrations was conducted in each concentration at the beginning and at the end of the test. The analyzed contents of boscalid ranged from 93.3% to 98.6% of nominal at test initiation and from 93.1% to 99.3% 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 test item concentrations of up to and including 0.835 mg a.s./L, whereas 5% mortality was observed at the third highest test item concentration of 1.39 mg a.s./L. At the second highest test item concentration no mortality was observed, while at the highest test item concentration 10% mortality occurred. No sub-lethal effects were found in the control groups and at test item concentrations of up to and including 2.33 mg a.s./L. At the highest test item concentration sub-lethal effects (i.e. loss of equilibrium, lethargy) were found. The results are summarized in Table 8.2.1-3.

Table 8.2.1-3: Acute toxicity (96 h) of boscalid to sheepshead minnow (*Cyprinodon variegatus*)

Concentration [mg a.s./L] (nominal)	**Control**	**Solvent control**	**0.52**	**0.86**	**1.4**	**2.4**	**4.0**
Concentration [mg a.s./L] (mean measured)	**--**	**--**	**0.490**	**0.835**	**1.39**	**2.33**	**3.86**
Mortality [%] (96 h)	0	0	0	0	5	0	10
Symptoms (after 96 h) *	none	none	none	none	none	none	E, L
Endpoints [mg boscalid/L] (mean measured)							
LC_{50} (96 h)	> 3.86						
NOEC (96 h)	2.33						

* Symptoms after 96 h: E = loss of equilibrium, L = lethargy

III. CONCLUSION

In a flow-through acute toxicity study with sheepshead minnow the LC_{50} (96 h) of boscalid was > 3.86 mg a.s./L based on mean measured concentrations. The NOEC (96 h) was determined to be 2.33 mg a.s./L (mean measured).

The following acute toxicity study on rainbow trout (*Oncorhynchus mykiss*) performed with the metabolite M510F64 (metabolite of boscalid) is provided for the risk assessment of the metabolite. The study has not been evaluated previously on EU level.

Report:	CA 8.2.1/4 [redacted] 2013a Reg.No. 309572 (metabolite of BAS 510 F, Boscalid, M510F64, M64) - Acute toxicity for rainbow trout 2013/1000146
Guidelines:	OECD 203 (1992)
GLP:	yes (certified by Bureau for Chemical Substances and Preparations, Lodz, Poland)

Executive Summary

In a static acute toxicity laboratory study, juvenile rainbow trout (*Oncorhynchus mykiss*) were exposed to M510F64 (metabolite of boscalid) at nominal concentrations of 0 (control), 3.13, 6.25, 12.5, 25, 50 and 100 mg M510F64/L in groups of 10 animals in glass aquaria containing 35 L water. Fish were observed for survival and symptoms of toxicity 3, 6, 24, 48, 72 and 96 hours after start of exposure.

The biological results are based on nominal concentrations. After 96 hours of exposure no mortality was observed in the control and at test item concentrations of up to and including 25 mg M510F64/L. At the two highest tested concentrations of 50 mg M510F64/L and 100 mg M510F64/L, 10% and 100% mortality was observed, respectively. No symptoms of toxicity were observed in any of the test item treatment groups at test end after 96 h of exposure. Statistically significant differences in the mortality rates compared to the control were observed at the highest test item concentration of 100 mg M510F64/L.

In a static acute toxicity study with rainbow trout the LC_{50} (96 h) of M510F64 (metabolite of boscalid) was 57.41 mg/L based on nominal concentrations. The NOEC (96 h) was determined to be 50 mg/L (nominal).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: M510F64 (Reg. No. 309572, synonym: M64, metabolite of boscalid); batch no. AC11643477; purity: 98.4% (± 1.0%).

B. STUDY DESIGN

Test species: Rainbow trout (*Oncorhynchus mykiss* Walb.); juveniles; average body length 5.05 cm ± 0.42 cm; average body weight: 1.81 ± 0.36 g; supplied by "The Culture of Salmonidae", Zawoja', Poland.

Test design: Static system (96 hours); 10 fish per aquarium (loading: 0.52 g fish/L) and per concentration and control, assessments of mortality and symptoms of toxicity 3, 6, 24, 48, 72 and 96 hours after start of exposure.

Endpoints: LC_{50}, NOEC, mortality and sub-lethal effects.

Test concentrations: 0 (control), 3.13, 6.25, 12.5, 25, 50 and 100 mg M510F64/L (nominal).

Test conditions: 35 L glass aquaria; test volume: 35 L; filtered tap water; temperature: 14.7°C - 15.8°C; pH 6.29 - 7.39; oxygen saturation: 88% - 105%; hardness: 0.95 mval/dm^3; photoperiod: 16 h light: 8 h dark, no feeding.

Analytics: Analytical verification of the test item was conducted using an LC-method with UV-VIS detection.

Statistics: Descriptive statistics; Probit analysis for determination of the LC_{50} value; Fisher`s Exact Binomial Test with Bonferroni Correction for determination of the NOEC ($\alpha = 0.05$).

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 mean analyzed contents of M510F64 ranged from 80.2% to 105.1% of nominal at test initiation and from 80.2% to 98.7% of nominal at test termination. As the mean measured concentrations confirmed the correct application of the test item, the following biological results are based on nominal concentrations.

Biological results: After 96 hours of exposure no mortality was observed in the control and at test item concentrations of up to and including 25 mg M510F64/L. At the two highest tested concentrations of 50 mg M510F64/L and 100 mg M510F64/L, 10% and 100% mortality was observed, respectively. No symptoms of toxicity were observed in any of the test item treatment groups at test end after 96 h of exposure. Statistically significant differences in the mortality rates compared to the control were observed at the highest test item concentration of 100 mg M510F64/L (Fisher's Exact Test with Bonferroni Correction, $\alpha = 0.05$). For results see Table 8.2.1-4.

Table 8.2.1-4 Acute toxicity (96 h) of M510F64 (metabolite of boscalid) on rainbow trout (*Oncorhynchus mykiss*)

Concentration [mg/L] (nominal)	**Control**	**3.13**	**6.25**	**12.5**	**25**	**50**	**100**
Mortality [%] (96 h)	0	0	0	0	0	10	100*
Symptoms (96 h)	none	none	none	none	none	none	n.d.
Endpoints [mg M510F64/L] (nominal)							
LC_{50} (96 h)	57.41 (95% confidence limits: -- [#])						
NOEC (96 h)	50						

* Statistically significantly different compared to the control (Fisher's Exact Test with Bonferroni Correction, $\alpha = 0.05$)
n.d. = not determined; all fish dead
[#] not determined due to mathematical reasons

III. CONCLUSION

In a static acute toxicity study with rainbow trout the LC_{50} (96 h) of M510F64 (metabolite of boscalid) was 57.41 mg/L based on nominal concentrations. The NOEC (96 h) was determined to be 50 mg/L (nominal).

The following acute toxicity study on rainbow trout (*Oncorhynchus mykiss*) performed with the metabolite M510F47 (metabolite of boscalid) is provided for the risk assessment of the metabolite. The study has not been evaluated previously on EU level.

Report:	CA 8.2.1/5 [redacted] 2015a Reg.No. 107371 (metabolite of BAS 510 F, Boscalid, M510F47) - Rainbow trout, acute toxicity test 2015/1001498
Guidelines:	OECD 203 (1992), EPA 850.1075
GLP:	yes (certified by Bureau for Chemical Substances and Preparations, Lodz, Poland)

Executive Summary

In a static acute limit test, juvenile rainbow trout (*Oncorhynchus mykiss*) were exposed to M510F47 (metabolite of boscalid) at a single nominal concentration of 100 mg/L and a dilution water control in groups of 15 animals in glass aquaria containing 30 L water. Fish were observed for survival and symptoms of toxicity 3, 6, 24, 48, 72 and 96 hours after start of exposure.

The biological results are based on nominal concentrations. No mortality or other symptoms of toxicity were observed in the control and at the test item treatment after 96 hours of exposure.

In a static acute toxicity study with rainbow trout the LC_{50} (96 h) of M510F47 (metabolite of boscalid) was > 100 mg/L based on nominal concentrations. The NOEC (96 h) was determined to be ≥ 100 mg/L (nominal).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: M510F47 (Reg. No. 107371, metabolite of boscalid), batch no. L80-188, purity: 100.0% (± 1.0%).

B. STUDY DESIGN

Test species: Rainbow trout (*Oncorhynchus mykiss* Walb.); juveniles (approximately 5 months old); average body length 4.6 cm ± 0.3 cm; average body weight: 1.08 ± 0.25 g; supplied by "The Culture of Salmonidae", Zawoja', Poland.

Test design: Static system (96 hours); 15 fish per replicate (loading: 0.54 g fish/L); 2 replicates per concentration and control, assessments of mortality and symptoms of toxicity 3, 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), 100 mg M510F47/L (nominal).

Test conditions: Glass aquaria; test volume: 30 L; reconstituted water (ISO 6341 : 1982); temperature: 13.2°C - 14.1°C; pH 7.12 - 7.51; oxygen saturation: 84% - 99%; conductivity: 648 - 712 µS/cm; hardness: 248 - 266 mg $CaCO_3$/L at exposure initiation; photoperiod: 16 h light: 8 h dark, no feeding.

Analytics: Analytical verification of the test item was conducted using an liquid chromatographic method with DAD detection.

Statistics: Descriptive statistics.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of the test item concentration was conducted in the control and the test item treatment at the beginning and at the end of the test. Measured values of M510F47 in the test item treatment ranged from 81.66% to 82.04% of nominal at test initiation and from 81.24% to 82.88% at test termination. As analytical data confirmed correct application of the test item, the following biological results are based on nominal concentrations.

Biological results: No mortality or other symptoms of toxicity were observed in the control and at the test item treatment after 96 hours of exposure. For results see Table 8.2.1-5

Table 8.2.1-5 Acute toxicity (96 h) of M510F47 (metabolite of boscalid) on rainbow trout (*Oncorhynchus mykiss*)

Concentration [mg/L] (nominal)	**Control**	**100**
Mortality [%] (96 h)	0	0
Symptoms (96 h)	none	none
	Endpoints [mg M510F47/L] (nominal)	
LC_{50} (96 h)	> 100 (95% confidence limits: -- [#])	
NOEC (96 h)	≥ 100	

[#] not determined due to mathematical reasons

III. CONCLUSION

In a static acute toxicity study with rainbow trout the LC_{50} (96 h) of M510F47 (metabolite of boscalid) was > 100 mg/L based on nominal concentrations. The NOEC (96 h) was determined to be ≥ 100 mg/L (nominal).

The following acute toxicity study on rainbow trout (*Oncorhynchus mykiss*) performed with the metabolite M510F49 (metabolite of boscalid) is provided for the risk assessment of the metabolite. The study has not been evaluated previously on EU level.

Report:	CA 8.2.1/6 [redacted] 2015b Reg.No. 391572 (metabolite of BAS 510 F, Boscalid, M510F49) - Rainbow trout, acute toxicity test 2015/1001501
Guidelines:	OECD 203 (1992), EPA 850.1075
GLP:	yes (certified by Bureau for Chemical Substances and Preparations, Lodz, Poland)

Executive Summary

In a static acute limit test, juvenile rainbow trout (*Oncorhynchus mykiss*) were exposed to M510F49 (metabolite of boscalid) at a single nominal concentration of the filtrate of a loading of 100 mg/L (corresponding to a geometric mean measured concentration of 0.31 mg/L) and a dilution water control in groups of 15 animals in glass aquaria containing 30 L water. Fish were observed for survival and symptoms of toxicity 3, 6, 24, 48, 72 and 96 hours after start of exposure.

The biological results are based on geometric mean measured concentrations. No mortality or other symptoms of toxicity were observed in the control and at the test item treatments after 96 hours of exposure.

In a static acute toxicity study with rainbow trout the LC_{50} (96 h) of M510F49 (metabolite of boscalid) was > 0.31 mg/L based on geometric mean measured concentrations. The NOEC (96 h) was determined to be ≥ 0.31 mg/L (geometric mean measured).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: M510F49 (Reg. No. 391572; metabolite of boscalid), batch no. L86-2, purity: 99.9% (± 1.0%).

B. STUDY DESIGN

Test species: Rainbow trout (*Oncorhynchus mykiss* Walb.); juveniles (approximately 5 months old); average body length 4.7 cm ± 0.3 cm; average body weight: 1.12 ± 0.26 g; supplied by "The Culture of Salmonidae", Zawoja', Poland.

Test design: Static system (96 hours); 15 fish per replicate (loading: 0.56 g fish/L); 2 replicates per concentration and control, assessments of mortality and symptoms of toxicity 3, 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), filtrate of a loading of 100 mg/L (corresponding to a geometric mean measured concentration of 0.31 mg M510F49/L)

Test conditions: Glass aquaria; test volume: 30 L; reconstituted water (ISO 6341 : 1982); temperature: 13.1°C - 13.7°C; pH 7.12 - 7.57; oxygen saturation: 83% - 99%; conductivity: 646 - 678 µS/cm; hardness: 244 - 260 mg $CaCO_3$/L at exposure initiation; photoperiod: 16 h light: 8 h dark, no feeding.

Analytics: Analytical verification of the test item was conducted using an LC-method with UV-VIS detection.

Statistics: Descriptive statistics.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of the test item concentration was conducted in the control and the test item treatment at the beginning and at the end of the test. The mean measured concentration of the test item determined in samples collected at exposure initiation was 0.31 mg/L. Mean measured values for M510F49 at test termination were 95.16% of initial concentration. The following biological results are based on geometric mean measured concentrations.

Biological results: No mortality or other symptoms of toxicity were observed in the control and at the test item treatment after 96 hours of exposure. For results see Table 8.2.1-6.

Table 8.2.1-6 Acute toxicity (96 h) of M510F49 (metabolite of boscalid) on rainbow trout (*Oncorhynchus mykiss*)

Concentration [filtrate of the loading of 100 mg/L] (initial mean measured)	**Control**	**0.31**
Concentration [mg/L] (geometric mean measured)	**--**	**0.31**
Mortality [%] (96 h)	0	0
Symptoms (96 h)	none	none
	Endpoints [mg M510F49/L] (geometric mean measured)	
LC_{50} (96 h)	> 0.31 (95% confidence limits: -- [#])	
NOEC (96 h)	≥ 0.31	

[#] not determined due to mathematical reasons

III. CONCLUSION

In a static acute toxicity study with rainbow trout the LC_{50} (96 h) of M510F49 (metabolite of boscalid) was > 0.31 mg/L based on geometric mean measured concentrations. The NOEC (96 h) was determined to be ≥ 0.31 mg/L (geometric mean measured).

CA 8.2.2 Long-term and chronic toxicity to fish

Due to the reasons given below, the chronic toxicity study on *O.mykiss* (DocID 1999/10927) 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 boscalid and is provided below.

Executive Summary

Fish, *Oncorhynchus mykiss*: The chronic toxicity of boscalid to the freshwater fish *Oncorhynchus mykiss* was tested in the laboratory according to OECD guideline 210 and EPA E-72-4.
In a flow-through chronic toxicity laboratory study, juvenile rainbow trout (*Oncorhynchus mykiss*) were exposed to the active substance boscalid (BAS 510 F) at nominal concentrations of 0 (control), 0.10, 0.464, 1.0 and 2.15 mg a.s./L in groups of 20 animals in glass aquaria with a flow rate of 10 L/h test solution/aquarium. The temperature was about 14 - 16°C, pH ranged from 8.0 – 8.4 and the oxygen content was between 8.0 and 10.9 mg/L.
The mean analytically detected concentrations of the test compound were in the range of 92.0% to 94.7% of the nominal concentrations during the exposure period. Hence the biological results are based on the nominal concentrations. Mortality occurred only in the highest test item concentration of 2.15 mg a.s./L. It started on day 6 (5%) and increased to 30% on day 28 of exposure (end of study). The "No Observed Adverse Effect Concentration (NOAEC)" for mortality was 1.0 mg a.s./L. Compound-related toxic signs were observed only in the highest concentration (2.15 mg a.s./L) starting on day 2 of exposure. The following effects were observed: mucous excretion from the anus, narcotic like state, tumbling, lying and swimming near the bottom, apathy, convulsion, attempts to escape and reduced or no food uptake. The "No Observed Adverse Effect Concentration (NOAEC)" for toxic signs was 1.0 mg a.s./L. The results are summarized in Table 8.2.2-1.

Table 8.2.2-1: Sub-lethal toxicity (28 d) of BAS 510 F on rainbow trout (*Oncorhynchus mykiss*)

Concentration(nominal) [mg a.s./L]	**Control**	**0.10**	**0.464**	**1.0**	**2.15**
Concentration(mean measured) [% of nominal]	**n. d.**	**92.6**	**94.7**	**93.3**	**92.0**
Mortality [%]	0	0	0	0	60
Symptoms	none	none	none	none	A, Bo, F, J, K, N, T, Y, Z
Mean weight [g] (28 d)	7.27	7.63	7.51	7.47	3.73
Mean length [cm] (28 d)	8.45				
	Endpoints [mg boscalid/L]				
NOAEC (28 d)	1.0				
LOEC (28 d)	2.15				
Threshold level of lethal effects (28 d)	> 1.0 < 2.15				

Symptoms: A = apathy, Bo = swimming near the bottom, F = attempts to escape, J = lying on the bottom, K = spasms, convulsions, N = narcosis like state, T = tumbling, Y = mucous excretion from anus, Z = reduced or no feed consumption.
n. d. = not detected

This study on *Oncorhynchus mykiss* shows some apparent deficiencies / deviations from current guideline OECD 204. Body length of test animals varied between 4.0 and 7.0 cm, with a mean of 5.6 cm (guideline: 5 cm max.). In addition, due to technical problems fish were exposed to only 10% of the nominal concentration of the test compound during the first week. Therefore the test was prolonged for an additional period of 1 week. However, nominal concentrations were not achieved until day 4 in the 1.0 mg/L-group. Only 4 concentration levels have been tested (guideline: 5). The water volume in the test aquaria was only exchanged twice a day due to the reduction of the dilution water flow rates in order to achieve nominal concentration levels (guideline recommends 4 times). Therefore, already during the Annex I inclusion process it was stated by the RMS that the study was not valid (see also Monograph of Boscalid, Vol. 3, November 2002, p. 559). Thus, the results of the EU-agreed 97 d ELS study with *Oncorhynchus mykiss* are considered as relevant endpoints for the risk assessment.

CA 8.2.2.1 Fish early life stage toxicity test

A fish early life stage toxicity test performed with rainbow trout has already been evaluated during the previous Annex I inclusion process of boscalid. No additional fish early life stage toxicity studies are required and no (new) study has been conducted.

CA 8.2.2.2 Fish full life cycle test

The chronic toxicity to fish is fully addressed by the other available chronic studies (see above). No additional fish full life cycle study is required and no (new) study has been conducted.

CA 8.2.2.3 Bioconcentration in fish

A bioconcentration study performed with rainbow trout has already been evaluated during the previous Annex I inclusion process of boscalid. No additional bioconcentration studies are required and no (new) study has been conducted.

CA 8.2.3 Endocrine disrupting properties

Based on the physical, chemical and structural characteristics of the active substance boscalid as well as the results of available long-term fish studies and long-term reproduction studies with terrestrial vertebrates (see chapter M-CA 8.1.5) there is no indication of endocrine disrupting properties of this active substance. This is supported by several impact assessments of different organizations (see M-CA 5.8.3 and M-CA 5.8.4). Thus, 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 the metabolite M510F64 is provided for the risk assessment of the metabolite. The study has not been evaluated previously on EU level.

Report:	CA 8.2.4.1/1 Handlos F.,Wydra V., 2013a Acute toxicity of Reg.No. 309572 (metabolite of BAS 510 F, Boscalid) to Daphnia magna in a 48-hour immobilisation test 2013/1005681
Guidelines:	OECD 202 (2004), (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part C.2
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

Executive Summary

In a static acute toxicity laboratory study, water flea neonates were exposed to M510F64 (metabolite of boscalid) at nominal concentrations of 10.0, 18.0, 32.0, 56.0 and 100 mg M510F64/L in 4 replicates per concentration containing 5 daphnids each. Additionally, a dilution water control and a solvent control were set up. Daphnids were observed for immobility 24 hours and 48 hours after start of exposure.

The biological results are based on nominal concentrations. After 48 h of exposure, no immobility of daphnids was observed in the control groups and at test item concentrations of up to and including 56.0 mg/L, whereas 45% of the daphnids were immobile at the highest test item concentration of 100 mg/L.

In a 48-hour static acute toxicity study with *Daphnia magna* the EC_{50} of M510F64 (metabolite of boscalid) was determined to be 102 mg/L based on nominal concentrations. The NOEC was 56.0 mg/L (nominal).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: M510F64 (Reg. No. 309572, synonym: M64, metabolite of boscalid); batch no AC11643477; purity: 98.4% (± 1.0%).

B. STUDY DESIGN

Test species: Water flea (*Daphnia magna*), neonates from in-house culture, < 24 hours old at test initiation and not first brood progeny.

Test design: Static system (48 hours), 5 test concentrations plus dilution water control and solvent control, 4 replicates with 5 daphnids in each; assessment of immobility after 24 and 48 hours.

Endpoints: EC_{50} and NOEC based on immobility of daphnids.

Test concentrations: Control (dilution water), solvent control (0.050 mL Tween 80/L); 10.0, 18.0, 32.0, 56.0 and 100 mg M510F64/L (nominal).

Test conditions: 100 mL glass beakers, test volume 60 mL, dilution water: "M4" (Elendt medium); temperature: 20°C - 21°C; pH 6.2 - 7.8; oxygen content: 7.4 mg/L - 9.0 mg/L; photoperiod: 16 hours light : 8 hours dark; light intensity: 980 - 1220 lux; no feeding; no aeration.

Analytics: Analytical verification of test item concentrations was conducted using an HPLC-method with UV-Vis detection.

Statistics: Descriptive statistics; probit analysis for determination of the EC_{50}.

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 analyzed contents of M510F64 ranged from 102% to 106% of nominal at test initiation and from 103% to 106% 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 groups and at test item concentrations of up to and including 56.0 mg/L, whereas 45% of the daphnids were immobile at the highest test item concentration of 100 mg/L. For results see Table 8.2.4.1-1.

Table 8.2.4.1-1: Effects of M510F64 on *Daphnia magna* mobility

Concentration [mg/L] (nominal)	**Control**	**Solvent control**	**10.0**	**18.0**	**32.0**	**56.0**	**100**
Immobility (24 h) [%]	0	0	0	0	0	0	0
Immobility (48 h) [%]	0	0	0	0	0	0	45
Endpoints [mg M510F64/L] (nominal)							
EC_{50} (48 h)	102 (95% confidence limits: n.d.)						
NOEC (48 h)	56.0						

n.d.= not determined

III. CONCLUSION

In a 48-hour static acute toxicity study with *Daphnia magna* the EC_{50} of M510F64 (metabolite of boscalid) was determined to be 102 mg/L based on nominal concentrations. The NOEC was 56.0 mg/L (nominal).

The following acute toxicity study on *Daphnia magna* performed with the metabolite M510F47 is provided for the risk assessment of the metabolite. The study has not been evaluated previously on EU level.

Report:	CA 8.2.4.1/2 Brzozowska-Wojoczek K., 2015a Reg.No. 107371 (metabolite of BAS 510 F, Boscalid, M510F47) - Daphnia magna, acute immobilization test 2015/1001499
Guidelines:	OECD 202 (2004), EPA 850.1010
GLP:	yes (certified by Bureau for Chemical Substances and Preparations, Lodz, Poland)

Executive Summary

In a static acute toxicity laboratory study, water flea neonates were exposed to M510F47 (metabolite of boscalid) at nominal concentrations of 0 (control), 1.25, 2.5, 5.0, 10.0, 20.0, 40.0 and 80.0 mg/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 biological results are based on nominal concentrations of the test item. After 48 h of exposure, no immobility of daphnids was observed in the control and at test item concentrations of 1.25, 2.5, 5.0, 10.0 and 40.0 mg/L, whereas 5% and 10% of the daphnids were immobile at test item concentration of 20.0 and 80.0 mg/L, respectively. No statistically significant effects on mobility of daphnids after 48 h of exposure were observed.

In a 48-hour static acute toxicity study with *Daphnia magna* the EC_{50} of M510F47 (metabolite of boscalid) was determined to be > 80.0 mg/L based on nominal concentrations. The NOEC was ≥ 80.0 mg/L (nominal).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: M510F47 (Reg. No. 107371, metabolite of boscalid,), batch no. L80-188, purity: 100.0% (± 1.0%).

B. STUDY DESIGN

Test species: Water flea (*Daphnia magna* STRAUS), neonates from in-house culture, < 24 hours old at test initiation and not first brood progeny.

Test design: Static system (48 hours), 7 test concentrations plus dilution water control, 4 replicates with 5 daphnids in each; assessment of immobility after 24 and 48 hours.

Endpoints: EC_{50} and NOEC based on immobility of daphnids.

Test concentrations: Control (dilution water), 1.25, 2.5, 5.0, 10.0, 20.0, 40.0 and 80.0 mg M510F47/L (nominal).

Test conditions: 150 mL glass beakers covered with transparent lids, test volume 100 mL, dilution water: Elendt M7 medium; temperature: 18.8°C - 20.2°C; pH 6.50 - 7.39; oxygen saturation: 93% - 99%; photoperiod: 16 hours light : 8 hours dark; fluorescent light source; no feeding; no aeration.

Analytics: Analytical verification of test item concentrations was conducted using a HPLC-method with DAD.

Statistics: Descriptive statistics. Fisher`s Exact Binomial Test with Bonferroni Correction for determination of the NOEC; probit analysis for determination of the EC_{50} value.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentration was conducted in each concentration at the beginning and at the end of the test. Mean measured values for M510F47 ranged from 81.7 to 84.3% of nominal concentrations at test initiation and from 82.0 to 89.5% 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 1.25, 2.5, 5.0, 10.0 and 40.0 mg/L, whereas 5% and 10% of the daphnids were immobile at test item concentration of 20.0 and 80.0 mg/L, respectively. No statistically significant effects on mobility of daphnids after 48 h of exposure were observed. For results see Table 8.2.4.1-2.

Table 8.2.4.1-2: Effect of M510F47 (metabolite of boscalid) on *Daphnia magna* immobility

Concentration [mg/L] (nominal)	Control	1.25	2.5	5.0	10.0	20.0	40.0	80.0
Immobility (24 h) [%]	0	0	0	0	0	0	0	0
Immobility (48 h) [%]	0	0	0	0	0	5	0	10
	Endpoints [mg M510F47/L] (nominal)							
EC_{50} (48 h)	> 80.0							
NOEC (48 h)	≥ 80.0							

III. CONCLUSION

In a 48-hour static acute toxicity study with *Daphnia magna* the EC_{50} of M510F47 (metabolite of boscalid) was determined to be > 80.0 mg/L based on nominal concentrations. The NOEC was ≥ 80.0 mg/L (nominal).

The following acute toxicity study on *Daphnia magna* performed with the metabolite M510F49 is provided for the risk assessment of the metabolite. The study has not been evaluated previously on EU level.

Report: CA 8.2.4.1/3
Turek T., 2015a
Reg.No. 391572 (metabolite of BAS 510 F, Boscalid, M510F49) - Daphnia magna, acute immobilization test
2015/1001502
Guidelines: OECD 202 (2004), EPA 850.1010
GLP: yes
(certified by Bureau for Chemical Substances and Preparations, Lodz, Poland)

Executive Summary

In a static acute limit test, water flea neonates were exposed to M510F49 (metabolite of boscalid) at a single nominal concentration of the filtrate of a loading of 100 mg/L (corresponding to geometric mean measured concentration of 0.42 mg/L) and a dilution water control in 8 replicates per treatment containing 5 daphnids each. Daphnids were observed for immobility 24 hours and 48 hours after start of exposure.

The biological results are based on geometric mean measured concentrations of the test item. No mortality or other symptoms of toxicity were observed in the control and the test item treatments after 48 hours of exposure.

In a 48-hour static acute limit test with *Daphnia magna* the EC_{50} of M510F49 (metabolite of boscalid) was determined to be > 0.42 mg/L based on geometric mean measured. The NOEC was ≥ 0.42 mg/L (geometric mean measured).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: M510F49 (Reg. No. 391572; metabolite of boscalid), batch no. L86-2, purity: 99.9% (± 1.0%).

B. STUDY DESIGN

Test species: Water flea (*Daphnia magna* STRAUS), neonates from in-house culture, < 24 hours old at test initiation and not first brood progeny.

Test design: Static system (48 hours), limit test: 1 test concentration plus a control; 8 replicates with 5 daphnids in each; assessment of immobility after 24 and 48 hours.

Endpoints: EC_{50} and NOEC based on immobility of daphnids.

Test concentrations: Control (dilution water), filtrate of a loading of 100 mg/L (corresponding to geometric mean measured concentration of 0.42 mg M510F49/L)

Test conditions: 150 mL glass beakers covered with transparent lids, test volume 100 mL, dilution water: Elendt M7 medium; temperature: 18.9°C - 20.2°C; pH 7.27 – 7.31; oxygen saturation: 91 % - 83 %; photoperiod: 16 hours light : 8 hours dark; fluorescent light source; no feeding and no aeration.

Analytics: Analytical verification of test item concentrations was conducted using an HPLC-method with UV-VIS-detection.

Statistics: Descriptive statistics.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of the test item concentration was conducted in the control and the test item treatment at the beginning and at the end of the test. The concentration of the test item determined in samples collected at exposure initiation was 0.43 mg/L. Mean measured values for M510F49 at test termination were 93.02% of initial concentration. The following biological results are based on geometric mean measured concentrations.

Biological results: No mortality or other symptoms of toxicity were observed in the control, and at the test item treatments after 48 hours of exposure. For results see Table 8.2.4.1-3.

Table 8.2.4.1-3: Effect of M510F49 (metabolite of boscalid) on *Daphnia magna* mobility

Concentration [filtrate of the loading of 100 mg a.s./L] (initial mean measured)	**Control**	**0.43**
Concentration [mg a.s./L] (geometric mean measured)	**--**	**0.42**
Immobility (24 h) [%]	0	0
Immobility (48 h) [%]	0	0
	Endpoints [mg M510F49/L] (nominal)	
EC_{50} (48 h)	> 0.42	
NOEC (48 h)	≥ 0.42	

III. CONCLUSION

In a 48-hour static acute limit test with *Daphnia magna* the EC_{50} of M510F49 (metabolite of boscalid) was determined to be > 0.42 mg/L based on geometric mean measured. The NOEC was ≥ 0.42 mg/L (geometric mean measured).

CA 8.2.4.2 Acute toxicity to an additional aquatic invertebrate species

The following acute flow-through toxicity study with the saltwater mysid *Americamysis bahia* performed with the active substance boscalid was conducted for registrations outside the EU. The study is provided for completeness and has not been evaluated previously on EU level.
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 the 48 h results are presented below additionally to the 96 h results.

Report:	CA 8.2.4.2/1 Boeri R.L.et al., 2001b Flow-through acute toxicity of BAS 510F to the mysid, Americamysis bahia 2001/5000086
Guidelines:	EPA 72-3(c), EPA 850.1035
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary

In a flow-through acute toxicity laboratory study, saltwater mysids were exposed to a dilution water control, a solvent control and to nominal concentrations of 0.52, 0.88, 1.4, 2.4 and 4.0 mg boscalid/L (corresponding to mean measured concentrations of 0.419, 0.827, 1.38, 2.31 and 3.81 mg a.s./L) in two replicates per treatment containing 10 mysids each. Saltwater mysids 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 48 hours of exposure no mortality was observed in the control and the solvent control, whereas 5%, 15%, 10%, 5% and 10% mortality were observed at the test item concentrations of 0.419, 0.827, 1.38, 2.31 and 3.81 mg a.s./L, respectively. No other sub-lethal toxic effects occurred in the control groups and at test item concentrations of up to and including the highest concentration tested.

In a flow-through acute toxicity study with saltwater mysids (*Americamysis bahia*) the LC_{50} (48 h) for boscalid was determined to be > 3.81 mg a.s./L based on mean measured concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Boscalid (BAS 510 F; Reg. No. 300355), batch no. N 75, purity: 96.9%.

B. STUDY DESIGN

Test species: Saltwater mysid (*Americamysis bahia*), juveniles, age: less than 24 hours old; average wet weight of control mysids: 0.31 mg; source: in-house cultures; originally obtained from "Aquatic BioSystems", Fort Collins, Colorado, USA.

Test design: Flow-through system (96 hours); 5 test item concentrations plus a control and a solvent control, 2 replicates per treatment; 10 mysids per replicate (loading 0.00021 g mysid/L); assessment of mortality and symptoms of toxicity directly after start of exposure and 24, 48, 72 and 96 hours after start of exposure.

Endpoints: LC_{50} (48 h), mortality and sub-lethal effects.

Test concentrations: Control (dilution water), solvent control (0.5 mL acetone/L) and 0.52, 0.88, 1.4, 2.4 and 4.0 mg boscalid/L (nominal), corresponding to mean measured concentrations of 0.419, 0.827, 1.38, 2.31 and 3.81 mg a.s./L.

Test conditions: Glass aquaria (20 L), test volume 15 L; exposure chambers: glass cylinders (8 cm in height and 8 cm in diameter) with mesh screen attached to the bottom; dilution water: carbon filtered, sterilized natural seawater; flow rate: 6.8 volume additions per 24 hours on average; salinity: 16 - 17‰; temperature: 21.5°C - 22.3°C; pH 7.7 - 8.1; oxygen content: 7.0 - 8.2 mg/L; photoperiod 16 h light : 8 h dark; light intensity: 32 foot-candles; feeding: live *Artemia salina* nauplii at least once daily; no aeration.

Analytics: Analytical verification of test item concentrations was conducted using an HPLC-method with UV-detection.

Statistics: Descriptive statistics.

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 analytically determined concentrations of boscalid ranged from 80.0% to 100.0% of nominal concentrations at test initiation and from 79.2% to 99.3% of nominal at test termination. The following biological results are based on mean measured concentrations.

Biological results: After 48 hours of exposure no mortality was observed in the control and the solvent control, whereas 5%, 15%, 10%, 5% and 10% mortality were observed at the test item concentrations of 0.419, 0.827, 1.38, 2.31 and 3.81 mg a.s./L, respectively. No other sub-lethal toxic effects occurred in the control groups and at test item concentrations of up to and including the highest concentration tested. The results are summarized in Table 8.2.4.2-1.

Table 8.2.4.2-1: Acute toxicity of boscalid to saltwater mysids (*Americamysis bahia*)

Concentration [mg a.s./L] (nominal)	**Control**	**Solvent control**	**0.52**	**0.88**	**1.4**	**2.4**	**4.0**
Concentration [mg a.s./L] (mean measured)	**--**	**--**	**0.419**	**0.827**	**1.38**	**2.31**	**3.81**
Mortality [%] (48 h)	0	0	5	15	10	5	10
Symptoms after 48 h	none	none	none	none	none	none	none
Endpoints [mg boscalid/L] (mean measured)							
LC_{50} (48 h)	> 3.81						

III. CONCLUSION

In a flow-through acute toxicity study with saltwater mysids (*Americamysis bahia*) the LC_{50} (48 h) for boscalid was determined to be > 3.81 mg a.s./L based on mean measured concentrations.

The following acute toxicity study on the eastern oyster (*Crassostrea virginica*) performed with the active substance boscalid is not required for registration in the EU and it has not been evaluated previously on EU level. The study was conducted due to U.S. data requirements and it is provided for completeness.

Report:	CA 8.2.4.2/2 Boeri R.L.et al., 2001c Flow-through mollusc shell deposition test with BAS 510 F 2001/5000877
Guidelines:	EPA 72-3(c)
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary

In a 96-hour acute toxicity laboratory study the effect of boscalid 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 0.52, 0.88, 1.4, 2.4 and 4.0 mg boscalid/L (corresponding to mean measured concentrations of 0.421, 0.777, 1.26, 2.20, 3.58 mg a.s./L) in groups of 10 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. After 96 hours of exposure, no mortality of oysters occurred in the control groups and test item concentrations of up to and including the highest concentration tested. No sublethal effects were noted during the exposure period in the controls and the test item treatments. Control and solvent control oysters deposited an average of 2.0 and 2.3 mm of new shell during the test, respectively. Statistically significant inhibition of shell growth compared to the pooled control was observed at all tested concentrations.

In a flow-through acute toxicity study with eastern oysters (*Crassostrea virginica*), the EC_{50} (96 h) for boscalid was 1.66 mg a.s./L based on mean measured concentrations. The NOEC (96 h) was determined to be < 0.421 mg a.s./L (mean measured).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Boscalid (BAS 510 F; Reg. No. 300355), batch no. N 75, purity: 96.9%.

B. STUDY DESIGN

Test species: Eastern oyster (*Crassostrea virginica*), juveniles, height: 36 - 49 mm; source: "Middle Peninsula Aquaculture", Virginia, USA.

Test design: Flow-through system (96 hours); 5 test item concentrations plus a control and a solvent control, 2 replicates for each test item concentration and the controls with 10 oysters per replicate (20 animals per treatment); initially and 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, mortality and symptoms of toxicity.

Test concentrations: Control (dilution water), solvent control (0.5 mL acetone/L), 0.52, 0.88, 1.4, 2.4, 4.0 mg boscalid/L (nominal), corresponding to mean measured concentrations of 0.421, 0.777, 1.26, 2.20, 3.58 mg a.s./L.

Test conditions: 20 L glass aquaria, test volume 15 L, unfiltered, natural seawater, flow rate: average of 9.1 volume additions per 24 hours in each test vessel (0.57 L per oyster per hour); salinity: 34 - 35‰; temperature: 20.2°C - 21.9°C; pH 7.8 - 8.1; oxygen content: 6.0 mg/L - 8.0 mg/L; photoperiod 16 h light : 8 h dark; light intensity: approx. 34 foot candles; no aeration; live marine phytoplankton as supplement to existing food in unfiltered seawater used as dilution water.

Analytics: Analytical verification of test item concentrations was conducted using an HPLC-method with UV-detection.

Statistics: Descriptive statistics; t-test ($\alpha = 0.05$) for comparison of shell deposition data in the control groups; binomial/nonlinear interpolation method for calculation of EC_{50} based on shell deposition data; ANOVA followed by Bonferroni's test for determination of the NOEC value based on shell deposition data ($\alpha = 0.05$).

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 boscalid ranged from 80.4% to 93.3% of nominal concentrations at test initiation and from 78.7% to 91.3% of nominal at test termination. The following biological results are based on mean measured concentrations.

Biological results: After 96 hours of exposure, no mortality of oysters occurred in the control groups and test item concentrations of up to and including the highest concentration tested.
No sublethal effects were noted during the exposure period in the controls and the test item treatments. Control and solvent control oysters deposited an average of 2.0 and 2.3 mm of new shell during the test, respectively. No statistically significant difference in shell deposition was observed between the control groups (t-test, $\alpha = 0.05$). 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 all tested concentrations (ANOVA followed by Bonferroni's test, $\alpha = 0.05$). The results are summarized in Table 8.2.4.2-2.

Table 8.2.4.2-2: Acute toxicity (96 h) of boscalid to eastern oysters (*Crassostrea virginica*)

Concentration [mg a.s./L] (nominal)	**Control**	**Solvent control**	**0.52**	**0.88**	**1.4**	**2.4**	**4.0**
Concentration [mg a.s./L] (mean measured)	**--**	**--**	**0.421**	**0.777**	**1.26**	**2.20**	**3.58**
Mortality after 96 h [%]	0	0	0	0	0	0	0
Mean shell deposition after 96 h [% of control]	--	115	75 *	70 *	65 *	35 *	0 *
Endpoints [mg boscalid/L] (mean measured)							
EC_{50} (96 h)	1.66 (95% confidence limits: 1.26 - 2.20)						
NOEC (96 h)	< 0.421						

* Statistically significant difference compared to the pooled control (ANOVA followed by Bonferroni's test, $\alpha = 0.05$).

III. CONCLUSION

In a flow-through acute toxicity study with eastern oysters (*Crassostrea virginica*), the EC_{50} (96 h) for boscalid was 1.66 mg a.s./L based on mean measured concentrations. The NOEC (96 h) was determined to be < 0.421 mg a.s./L (mean measured).

CA 8.2.5 Long-term and chronic toxicity to aquatic invertebrates

CA 8.2.5.1 Reproductive and development toxicity to Daphnia magna

The following 21-day semi-static toxicity study on *Daphnia magna* performed with the active substance boscalid is provided in support of the aquatic risk assessment and has not been evaluated previously on EU level.

Report:	CA 8.2.5.1/1 Jatzek J., 2004a BAS 510 F - Determination of the chronic effect on the reproduction of the water flea Daphnia magna STRAUS 2004/1015006
Guidelines:	EPA 850.1300, OECD 211
GLP:	yes (certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Report:	CA 8.2.5.1/2 Jatzek J., 2004b Amendment No. 1 to the report: BAS 510 F - Determination of the chronic effect on the reproduction of the water flea Daphnia magna STRAUS 2004/1015009
Guidelines:	OECD 211, EPA 850.1300
GLP:	yes (certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Executive Summary

In a 21-day semi-static toxicity test, effects of boscalid to water fleas (*Daphnia magna*) were examined. Neonates less than 24 hours old were exposed to nominal concentrations of 0.1, 0.2, 0.4, 0.8, 1.6 and 3.2 mg a.s./L and a water control. All treatment groups and the control consisted of 10 replicates with one parent daphnid in each. Adult 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 were based on nominal concentrations. After 21 days of exposure no parent mortality was observed in the control and at concentrations up to and including 0.8 mg a.s./L, whereas 10% mortality was observed at the two highest concentrations of 1.6 and 3.2 mg a.s./L. The number of offspring ranged between 63.9 and 121.4 in the test item treatments, compared to 122.2 in the control. At test end the body length of the adult daphnids ranged from 4.40 to 4.99 mm and the body weight of the adult daphnids ranged from 0.4 to 1.2 mg. Statistically significant effects on reproduction were observed at the two highest tested concentrations of 1.6 and 3.2 mg a.s./L. Body length and weight of the parent daphnids was statistically significantly reduced at the highest test concentration of 3.2 mg a.s./L.

In a 21-day semi-static toxicity study with *Daphnia magna* the NOEC of boscalid was determined to be 0.8 mg a.s./L based on nominal concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Boscalid (BAS 510 F; Reg. No. 300355), batch no. N 46, purity: 94.3%.

B. STUDY DESIGN

Test species: Water flea (*Daphnia magna* STRAUS), neonates from in-house culture (originally obtained from Institut National de Recherche Chimique Appliquée, France); > 2 < 24 hours old at test initiation.

Test design: Semi-static system (21 days), 6 test concentrations plus control, ten replicates per treatment with one parent daphnid per in each; 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 mortality, reproduction, parent length and dry weight.

Test concentrations: Control, 0.1, 0.2, 0.4, 0.8, 1.6 and 3.2 mg a.s./L (nominal).

Test conditions: Glass vessels, test volume 50 mL, dilution water “M4” (Elendt medium); temperature: 19.5 °C - 20.3 °C; pH 7.8 - 8.2; oxygen content: 7.8 mg/L - 9.9 mg/L; total hardness: 2.2 - 3.2 mmol/L, conductivity: 550 µS/cm - 650 µS/cm; light intensity: about 1 -8 µE/(m^2 x s) at 400 - 700 nm; photoperiod 16 hours light : 8 hours dark; daily feeding with algae (*Desmodesmus subspicatus*), no aeration.

Analytics: The test item concentrations were analyzed using a HPLC-method with UV detection.

Statistics: Descriptive statistics; Dunnett`s multiple range test (one-sided, $p \leq 0.01$) for determination of the NOEC.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of the test item concentrations was conducted in all treatments at day 0, 2, 11, 14, 16 and 18. Mean recoveries of boscalid were in the range of 90.1% - 103.9% of nominal concentrations during the course of the study. The following biological results are based on nominal test item concentrations.

Biological results: After 21 days of exposure no parent mortality was observed in the control and at concentrations up to and including 0.8 mg a.s./L, whereas 10% mortality was observed at the two highest concentrations of 1.6 and 3.2 mg a.s./L. The number of offspring varied between 63.9 and 121.4 in the test item treatments compared to 122.2 in the control. At test end the body length of the adult daphnids ranged from 4.40 to 4.99 mm and the dry weight of the adult daphnids ranged from 0.4 to 1.2 mg. Statistically significant effects on reproduction were observed at the two highest tested concentrations of 1.6 and 3.2 mg a.s./L (Dunnett`s multiple range test, $p \leq 0.01$). Body length and weight of the parent daphnids was statistically significantly reduced at the highest test concentration of 3.2 mg a.s./L (Dunnett`s multiple range test, $p \leq 0.01$). The results are summarized in Table 8.2.5.1-1.

Table 8.2.5.1-1: Effects of boscalid (21 d) on *Daphnia magna* reproduction, growth and parent mortality

Concentration [mg/L] nominal	**Control**	**0.1**	**0.2**	**0.4**	**0.8**	**1.6**	**3.2**
Parent mortality [%]	0	0	0	0	0	10	10
Av. living offspring/parent	122.2	110.9	106.4	116.5	121.4	94.6 *	63.9 *
Av. body weight [mg]	1.0	1.0	1.2	1.2	1.0	1.0	0.4 *
Av. body length [mm]	4.95	4.83	4.94	4.99	4.83	4.91	4.40 *
Endpoints [mg boscalid/L] (nominal)							
NOEC (21 d)	0.8						

* Statistically significant effects compared to the control (Dunnett`s multiple range test (one-sided, $p \leq 0.01$).

III. CONCLUSION

In a 21-day semi-static toxicity study with *Daphnia magna* the NOEC of boscalid was determined to be 0.8 mg a.s./L based on nominal concentrations.

CA 8.2.5.2 Reproductive and development toxicity to an additional aquatic invertebrate species

No study required; thus, this point is not addressed *via* (new) toxicity studies.

CA 8.2.5.3 Development and emergence in Chironomus riparius

A spiked water toxicity study on *Chironomus riparius* performed with boscalid 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

The following acute spiked sediment toxicity study on the amphipod *Hyalella azteca* was performed with the active substance boscalid and is not required for registration in the EU. It has not been evaluated previously on EU level. The study was conducted due to U.S. data requirements and is provided for completeness.

Report:	CA 8.2.5.4/1 Holmes C. et al., 2001a Acute toxicity of BAS 510 F in whole sediment to the amphipod, Hyalella azteca 2001/5000043
Guidelines:	EPA 850.1735
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary

In a 10-day static acute spiked sediment study, amphipods (*Hyalella azteca*) were exposed to boscalid at nominal concentrations of 6.25, 12.5, 25.0, 50.0 and 100 mg a.s./kg dry sediment (corresponding to mean measured concentrations of 6.37, 12.5, 25.8, 49.5, and 97.0 mg a.s./kg dry sediment). Additionally, a solvent control and a dilution water control were set up. All test item concentrations and the control groups had 8 replicates consisting of 10 amphipods per replicate. Assessment of survival and growth of the amphipods was performed at test end.

The biological results are based on mean measured sediment concentrations. After 10 days of exposure, the mean survival was 95% and 94% in the dilution water control and the solvent control, respectively. The pooled control survival was 94%. Survival rates at the test item concentrations of 6.37, 12.5, 25.8, 49.5 and 97.0 mg a.s./kg dry sediment were 91%, 99%, 91%, 79% and 94%, respectively. Statistically significant differences compared to the pooled control were observed at the tested concentration of 49.5 mg a.s./kg dry sediment. However, this does not appear to be a dose dependent response to the animals' exposure to boscalid, since there were no other statistically significant reductions in animal survival for the other treatment levels.
Regarding the endpoint growth (dry weight), the mean control and solvent control individual dry weights measured at test termination were 0.159 and 0.230 mg per animal, respectively. The individual dry biomass of the control animals was determined to be statistically reduced as compared to the average dry biomass of the solvent control animals. Therefore, the statistical analyses of the treatment values were compared to the solvent control data. The average individual dry weights ranged from 0.134 to 0.245 mg per animal in the treated sediments. Statistically significantly differences compared to the solvent control were observed in the control and the test item concentrations of 6.37, 12.5, and 25.8 mg a.s./kg dry sediment.

In a 10-day static acute sediment test with *Hyalella azteca*, the LC_{50} of boscalid was determined to be >97.0 mg a.s./kg dry sediment based on mean measured concentrations. The NOEC was 97.0 mg a.s./kg dry sediment (mean measured).

I. MATERIAL AND METHODS

Test item:	BAS 510 F (Reg. No. 300355), batch no. N 75, purity: 96.9%
Test species:	Amphipods (*Hyalella azteca*), juveniles (approximately 7 days old), mean individual dry weight at test initiation: 0.06 mg/individual; in-house culture maintained at ABC Laboratories.
Test design:	Static system (10 days); 5 test concentrations plus a control and a solvent control (acetone), 8 replicates per test item concentration and per control group, 10 amphipods per replicate; assessment of survival and growth after 10 days.
Endpoints:	Mortality, growth (dry weight).
Test concentrations:	Control (dilution water), solvent control, 6.25, 12.5, 25.0, 50.0 and 100 mg a.s./kg dry sediment (nominal); corresponding to mean measured TRR concentrations (^{14}C-labeled boscalid equivalents) of 6.37, 12.5, 25.8, 49.5 and 97 mg a.s./kg dry sediment.

Test conditions: 1 L glass jars with lids filled with ~190 g treated sediment (70% fine industrial sand, 20% kaolinite clay, 10% sphagnum peat), 600 mL dilution water (prepared by blending naturally hard well water with de-mineralized well water and passed through UV sterilizer and polypropylene cartridge filters); pH 6.37 - 8.47; oxygen content: 4.71 mg/L - 8.24 mg/L; water temperature: 22.1 - 23.8°C; conductivity: 304 - 311 µS at test initiation and 320 - 339 µS at test termination; alkalinity: 146 - 154 mg $CaCO_3$/L at test initiation and 140 - 152 mg $CaCO_3$/L at test termination; total hardness: 148 - 152 mg $CaCO_3$/L at test initiation and 142 - 152 mg $CaCO_3$/L at test termination; ammonia: 0.0011 - 0.015 ppm at test initiation and 0.0033 - 0.53 ppm at test termination; light intensity: 411.4 - 475.5 lux; photoperiod: 16 h light : 8 h dark; continuous aeration; food: invertebrate food suspension daily.

Analytics: Overlaying water, interstitial (pore) water and sediment were analyzed for total radioactive residues (TRR) using a liquid scintillation counting (LSC) method. The concentration of boscalid at the highest treatment was also measured in the overlying test water using an HPLC-method with UV-detection.

Statistics: Descriptive statistics, contingency table method and Fisher's exact test for determination of the NOEC value based on survival data (α =0.05) and a one-way ANOVA followed by Dunnett's test for determination of the NOEC value based on growth data (α = 0.05).

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of ^{14}C-labeled boscalid concentrations in the sediment and the overlying water and the interstitial water was conducted in the control and in each concentration at the beginning and the end of the test via LSC analysis. Mean measured concentrations of ^{14}C-labeled boscalid TRR in the sediment were in a range between 97.1 and 107.6% of nominal concentrations at test initiation and between 95.2 and 100.3% of nominal at test termination. The mean measured concentrations of ^{14}C-labeled boscalid in the overlaying water samples were 0.0267, 0.0586, 0.1348, 0.286 and 0.519 mg TRR/L for the 6.25, 12.5, 25.8, 49.5 and 97.0 mg a.s./kg dry sediment samples, respectively. The respective mean measured concentrations of ^{14}C-labeled boscalid in the interstitial water samples were 0.0652, 0.136, 0.298, 0.664 and 1.066 mg TRR/L.
Additionally, the concentration of boscalid in the controls and at the highest treatment level was measured in the overlying water at test initiation and test termination via HPLC/UV analysis. Measured concentrations in overlaying water samples of the 97.0 mg a.s./kg dry sediment treatment were 0.204 mg a.s./L at test initiation and 0.486 mg a.s./L at test termination.
The following biological results are based on the mean measured ^{14}C-labeled boscalid sediment concentrations. The TRR concentrations were corrected for dry weight of sediment.

Biological results: After 10 days of exposure, the mean survival was 95% and 94% in the dilution water control and the solvent control, respectively. The pooled control survival was 94%. Survival rates at the test item concentrations of 6.37, 12.5, 25.8, 49.5 and 97.0 mg a.s./kg dry sediment were 91%, 99%, 91%, 79% and 94%, respectively. Statistically significant differences compared to the pooled control were observed at the tested concentration of 49.5 mg a.s./kg dry sediment (Fisher's exact test, $\alpha = 0.05$). However, this does not appear to be a dose dependent response to the animals' exposure to boscalid, since there were no other statistically significant reductions in animal survival for the other treatment levels.
Regarding the endpoint growth (dry weight), the mean control and solvent control individual dry weights measured at test termination were 0.159 and 0.230 mg per animal, respectively. The individual dry biomass of the control animals was determined by Dunnett's test ($\alpha = 0.05$) to be statistically reduced as compared to the average dry biomass of the solvent control animals. Therefore, the statistical analyses of the treatment values were compared to the solvent control data. The average individual dry weights ranged from 0.134 to 0.245 mg per animal in the treated sediments. Statistically significantly differences compared to the solvent control were observed in the control and the test item concentrations of 6.37, 12.5, and 25.8 mg a.s./kg dry sediment (Dunnett's test; $\alpha = 0.05$). The results are summarized in Table 8.2.5.4-1.

Table 8.2.5.4-1: Effect of boscalid on survival of *Hyalella azteca*

Concentration [mg a.s./kg dry sediment] (nominal)	**Control**	**Solvent control**	**6.25**	**12.5**	**25.0**	**50.0**	**100**
Concentration [mg a.s/kg dry sediment] (mean measured)	**--**	**--**	**6.37**	**12.5**	**25.8**	**49.5**	**97.0**
Survival (10 d) [%]	95	94	91	99	91	79 *	94
Dry weight [mg/individual]	0.159 #	0.230	0.134 #	0.134 #	0.135 #	0.209	0.245
Endpoints [mg a.s./kg dry sediment] (mean measured)							
LC_{50} (10 d)	> 97.0						
NOEC $_{\text{mortality; dry biomass}}$ (10 d)	97.0						

* Statistically significantly difference compared to the pooled control (Fisher's exact test, $\alpha = 0.05$).
\# Statistically significantly difference compared to the solvent control (Dunnett's test; $\alpha = 0.05$).

III. CONCLUSION

In a 10-day static acute sediment test with *Hyalella azteca*, the LC_{50} of boscalid was determined to be >97.0 mg a.s./kg dry sediment based on mean measured concentrations. The NOEC was 97.0 mg a.s./kg dry sediment (mean measured).

CA 8.2.6 Effects on algal growth

CA 8.2.6.1 Effects on growth of green algae

Report:	CA 8.2.6.1/1 Kubitza J., 2001a Effect of BAS 510 F on the growth of the green alga Pseudokirchneriella subcapitata 2000/1018524
Guidelines:	OECD 201
GLP:	yes (certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

The following 96-hour algae study on the freshwater green algae *Pseudokirchneriella subcapitata* has already been submitted and accepted during the previous Annex I inclusion process. Meanwhile the 72 h endpoints have been calculated from the original data for use in the aquatic risk assessment. The calculations and for completeness also the already submitted study are summarised below.

Executive Summary

In a 96-hour static toxicity laboratory study, the effect of boscalid on the growth of the green alga *Pseudokirchneriella subcapitata* was investigated. The following nominal concentrations were applied: 0.1, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg boscalid/L (corresponding to mean measured concentrations of 0.09, 0.49, 0.97, 1.44, 1.71, 2.24 and 2.45 mg boscalid/L).
Additionally, a dilution water control was set up. Assessment of growth was conducted daily.

The biological results are based on mean measured concentrations of the test item. No morphological effects on algae were observed in the control group and all test item concentrations.

In a 96-hour algae test with *Pseudokirchneriella subcapitata,* the E_rC_{50} (96 h) of boscalid was determined to be 3.75 mg/L based on nominal concentrations

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 510 F (Reg. No. 300355), batch no. N 37, purity: 94.4%

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 (96 hours); 7 test 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 biomass after exposure over 96 hours.

Test concentrations: Control, 0.1, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg boscalid/L (nominal), corresponding to mean measured concentrations of 0.09, 0.49, 0.97, 1.44, 1.71, 2.24 and 2.45 mg boscalid/L.

Test conditions: 100 mL Erlenmeyer dimple flasks, test volume: 60 mL; nutrient solution according to OECD 201; pH 7.64 – 7.94; temperature: 22 ±1°C; initial cell densities: 3×10^3 cells/mL; continuous light: about 8000 lux, continuous shaking.

Analytics: Analytical verification of test item concentrations was conducted using a HPLC-method with UV-detection.

Statistics: Descriptive statistics, probit analysis for determination of EC_x values for growth rate and biomass.

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 boscalid ranged from 82.5 to 96.7% of nominal concentrations at test initiation and from 80.8 to 108.9% of nominal at test termination. As the initially measured concentrations confirmed the correct application of the test item, the following biological results are based on mean measured concentrations.

Biological results: No morphological effects on algae were observed in the control group and all test item concentrations. The effects on algal growth are summarized in Table 8.2.6.1-1.

Table 8.2.6.1-1: Effect of boscalid on the growth of the green algae *Pseudokirchneriella subcapitata*

Concentration [mg/L] (nominal)	**Control**	**0.1**	**0.5**	**1.0**	**1.5**	**2.0**	**2.5**	**3.0**
Concentration [mg/L] (mean measured)	**--**	**0.09**	**0.49**	**0.97**	**1.44**	**1.71**	**2.24**	**2.45**
Inhibition in 96 h (growth rate) [%]	--	0.5	1.7	4.7	7.1	11.2	20.3	48.3
Inhibition in 96 h (biomass) [%]	--	3.0	12.7	28.5	40.3	53.2	73.0	94.8
Endpoints [mg boscalid/L] (nominal)								
E_rC_{50} (96 h)	3.75 (95% confidence limits: 3.48 – 4.04)							
E_rC_{10} (96 h)	1.28 (95% confidence limits: 1.22 – 1.34)							
E_bC_{50} (96 h)	1.34 (95% confidence limits: 1.30 – 1.38)							
E_bC_{10} (96 h)	0.41 (95% confidence limits: 0.38 – 0.44)							

III. CONCLUSION

In a 96-hour algae test with *Pseudokirchneriella subcapitata,* the E_rC_{50} (96 h) of boscalid was determined to be 3.75 mg/L based on nominal concentrations

Report: CA 8.2.6.1/2
Hoffmann F., 2009a
Effect of BAS 510 F on the growth of the green alga Pseudokirchneriella subcapitata - Additional calculation of the inhibition values for growth rate and yield data after a test period of 72 h
2009/1044471
Guidelines: OECD 201
GLP: no

Executive Summary (recalculations; BASF Doc ID 2009/1044471)

The study BASF DocID 2000/1018524 with the unicellular fresh water green alga *P. subcapitata* was conducted according to Good Laboratory Practice (GLP) following OECD Guideline 201, however, over a longer test duration than recommended (*i.e.* 96 h instead of 72 h). In the original study report only the 96 hour endpoints related to growth rate and biomass are reported.
Additional endpoints related to growth rate (r) and yield (y) after 72 hours of exposure were recalculated according to current recommendations (OECD 201, March, 2011). The following 72-hour endpoints were obtained based on mean measured concentrations of the test item:

E_rC_{50} (72 h) = 2.61 mg/L (95% confidence limits: 2.53 - 2.70 mg/L)
E_rC_{10} (72 h) = 1.19 mg/L (95% confidence limits: 1.14 - 1.25 mg/L)
E_yC_{50} (72 h) = 1.33 mg/L (95% confidence limits: 1.30 - 1.36 mg/L)
E_yC_{10} (72 h) = 0.42 mg/L (95% confidence limits: 0.39 - 0.46 mg/L)

The following acute toxicity study on the freshwater green algae *Pseudokirchneriella subcapitata* performed with the metabolite M510F64 is provided for the risk assessment of the metabolite. The study has not been evaluated previously on EU level.

Report:	CA 8.2.6.1/3 Handlos F.,Wydra V., 2013b Toxicity of Reg.No. 309572 (metabolite of BAS 510 F, Boscalid) to Pseudokirchneriella subcapitata in an algal growth inhibition test 2013/1005682
Guidelines:	OECD 201 (2011), (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)

Report:	CA 8.2.6.1/4 Handlos F.,Wydra V., 2013c 1st final report amendment - Toxicity of Reg.No. 309572 (metabolite of BAS 510 F, Boscalid) to Pseudokirchneriella subcapitata in an algal growth inhibition test 2013/1334849
Guidelines:	OECD 201 (2011), (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 toxicity laboratory study, the effect of M510F64 (metabolite of boscalid) 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, 25.0, 50.0 and 100 mg M510 F64/L. Assessment of growth was conducted 24 h, 48 h and 72 h after test initiation.

The biological results are based on nominal concentrations of the test item. No morphological effects on algae were observed in the control group and at up to and including the test item concentration of 50.0 mg/L. Algal cells of the highest test item concentration of 100 mg/L were smaller and showed some deformation signs compared to the control. Statistically significant differences for growth rate and yield compared to the control were observed at the two highest test item concentrations of 50.0 and 100 mg/L.

In a 72-hour algae test with *Pseudokirchneriella subcapitata,* the E_rC_{50} and the E_yC_{50} values of M510 F64 (metabolite of boscalid) were determined to be 96.2 mg/L and 60.5 mg/L, respectively, based on nominal concentrations. The 72 h NOE_rC and NOE_yC values for M510 F64 were both determined to be 25.0 mg/L (nominal).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: M510F64 (Reg. No. 309572, synonym: M64, metabolite of boscalid); batch no AC11643477; purity: 98.4% (± 1.0%)

B. STUDY DESIGN

Test species: Unicellular fresh water green alga, *Pseudokirchneriella subcapitata*; . Strain No. SAG 61.81; stock obtained from "Sammlung von Algenkulturen, Universität Göttingen", Göttingen, Germany.

Test design: Static system (72 hours); 7 test concentrations with 3 replicates for each plus a control 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, 1.56, 3.13, 6.25, 12.5, 25.0, 50.0 and 100 mg M510 F64/L (nominal).

Test conditions: 50 mL Erlenmeyer flasks, test volume: 50 mL; nutrient solution according to OECD 201; pH 7.8 - 8.0 at test initiation and pH 8.4 - 9.5 at test termination; temperature: 22 - 23°C; initial cell densities: 5 x 10^3 cells/mL; continuous light at 6600 - 7960 lux.

Analytics: Analytical verification of test item concentrations was conducted using an HPLC-method with UV detection.

Statistics: Descriptive statistics, probit analysis for determination of EC_x values for growth rate and yield, one-sided William´s t-test for determination of the NOEC values ($\alpha = 0.05$).

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 analyzed contents of M510 F64 ranged from 89% to 101% of nominal at test initiation and from 88% to 105% 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: No morphological effects on algae were observed in the control group and at up to and including the test item concentration of 50.0 mg/L. Algal cells of the highest test item concentration of 100 mg/L were smaller and showed some deformation signs compared to the control. Statistically significant differences for growth rate and yield compared to the control were observed at the two highest test item concentrations of 50.0 and 100 mg/L (one-sided William´s t-test, $\alpha = 0.05$). The effects on algal growth are summarized in Table 8.2.6.1-2.

Table 8.2.6.1-2: Effect of M510F64 (metabolite of boscalid) on the growth of the green alga *Pseudokirchneriella subcapitata*

Concentration [mg/L] (nominal)	**1.56**	**3.13**	**6.25**	**12.5**	**25.0**	**50.0**	**100**
Inhibition in 72 h (growth rate) [%]	1.3	2.0	1.3	1.4	1.7	6.7*	53.5*
Inhibition in 72 h (yield) [%]	6.4	9.8	6.4	6.1	7.9	28.3*	93.5*
Endpoints [mg M510F64/L] (nominal)							
E_rC_{50} (72 h)	96.2 (95% confidence limits: 94.1 – 98.4)						
E_rC_{10} (72 h)	54.8 (95% confidence limits: 51.3 – 57.9)						
E_yC_{50} (72 h)	60.5 (95% confidence limits: 55.4 – 68.0)						
E_yC_{10} (72 h)	38.9 (95% confidence limits: 31.0 – 43.9)						
NOE_rC (72 h) / NOE_yC (72 h)	25.0						

* Statistically significantly different compared to the control (one-sided William´s t-test, $\alpha = 0.05$)

III. CONCLUSION

In a 72-hour algae test with *Pseudokirchneriella subcapitata,* the E_rC_{50} and the E_yC_{50} values of M510F64 (metabolite of boscalid) were determined to be 96.2 mg/L and 60.5 mg/L, respectively, based on nominal concentrations. The 72 h NOE_rC and NOE_yC values for M510F64 were both determined to be 25.0 mg/L (nominal).

The following toxicity study on the freshwater green algae *Pseudokirchneriella subcapitata* performed with the metabolite M510F47 is provided for the risk assessment of the metabolite. The study has not been evaluated previously on EU level.

Report:	CA 8.2.6.1/5 Brzozowska-Wojoczek K., 2015b Reg.No. 107371 (metabolite of BAS 510 F, Boscalid, M510F47) - Pseudokirchneriella subcapitata SAG 61.81 - Growth inhibition test 2015/1001500
Guidelines:	OECD 201 (2006), EPA 850.4500
GLP:	yes (certified by Bureau for Chemical Substances and Preparations, Lodz, Poland)

Executive Summary

In a 96-hour static toxicity laboratory study, the effect of M510F47 (metabolite of boscalid) on the growth of the green algae *Pseudokirchneriella subcapitata* was investigated. The following nominal concentrations were applied: 2.6, 6.4, 16.0, 40.0 and 100.0 mg M510F47/L. Additionally, a dilution water control was set up. Assessment of growth was conducted 24, 48, 72 and 96 h after test initiation.

The biological results are based on nominal concentrations of the test item. No morphological effects on algae were observed in the control group and at test item concentrations of up to and including 40 mg/L. In the highest test item concentration of 100 mg/L algae cells were opalescent after 72 and 96 hours of exposure. Statistically significant differences for growth rate and yield compared to the control were observed at the two highest test item concentrations of 40.0 and 100.0 mg/L after 72 h of exposure and in all test item concentrations, except the lowest of 2.6 mg/L after 96 h of exposure.

In a 96-hour algae test with *Pseudokirchneriella subcapitata,* the E_rC_{50} (96 h) of M510F47 (metabolite of boscalid) was determined to be > 100 mg/L based on nominal concentrations After 72 hours of exposure, the respective E_rC_{50} value was equally determined to be > 100 mg/L.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: M510F47 (Reg. No. 107371, metabolite of boscalid,), batch no. L80-188, purity: 100.0% (± 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 (96 hours); 5 test concentrations with 8 replicates for each plus a control with 4 replicates; daily assessment of growth.

Endpoints: EC_{10} and EC_{50} with respect to growth rate and yield after exposure over 72 and 96 hours.

Test concentrations: Control, 2.6, 6.4, 16.0, 40.0 and 100.0 mg M510F47/L (nominal).

Test conditions: 250 mL Erlenmeyer flasks, test volume: 100 mL; nutrient solution according to OECD 201 (AAP medium); pH 7.00 – 7.56 at test initiation and pH 7.84 - 8.34 at test termination; temperature: 24.9 – 25.4°C; initial cell densities: 1×10^4 cells/mL; continuous light: 3700 - 3860 lux, continuous shaking.

Analytics: Analytical verification of test item concentrations was conducted using a HPLC-method with DAD.

Statistics: Descriptive statistics, probit analysis for determination of EC_x values for growth rate and yield. Williams' Multiple Sequential t-test procedure for determination of the NOEC for growth rate and yield ($\alpha = 0.05$).

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. Mean measured values for M510F47 ranged from 84.4 to 87.5% of nominal concentrations at test initiation and from 82.8 to 85.9% of nominal at test termination. As the initially measured concentrations confirmed the correct application of the test item, the following biological results are based on nominal concentrations.

Biological results: No morphological effects on algae were observed in the control group and at test item concentrations of up to and including 40 mg/L. In the highest test item concentration of 100 mg/L algae cells were opalescent after 72 and 96 hours of exposure. Statistically significant differences for growth rate and yield compared to the control were observed at the two highest test item concentrations of 40.0 and 100.0 mg/L after 72 h of exposure and in all test item concentrations, except the lowest of 2.6 mg/L after 96 h of exposure (Williams t-test, $\alpha = 0.05$). The effects on algal growth are summarized in Table 8.2.6.1-3

Table 8.2.6.1-3: Effect of M510F47 (metabolite of boscalid) on the growth of the green algae *Pseudokirchneriella subcapitata*

Concentration [mg/L] (nominal)	**Control**	**2.6**	**6.4**	**16.0**	**40.0**	**100.0**
Inhibition in 72 h (growth rate) [%] *	0.0	-2.95	-2.98	2.39	14.77 #	33.77 #
Inhibition in 72 h (yield) [%] *	0.0	-14.09	-13.93	10.39	49.31 #	79.12 #
Inhibition in 96 h (growth rate) [%] *	0.0	-1.12	1.54 #	2.07 #	5.54 #	16.40 #
Inhibition in 96 h (yield) [%] *	0.0	-5.68	8.44 #	10.99 #	26.17 #	58.98 #
Endpoints [mg M510F47/L] (nominal)						
E_rC_{50} (72 h)	> 100					
E_rC_{10} (72 h)	31.01 (95% confidence limits: 27.95 – 33.86)					
E_yC_{50} (72 h)	43.66 (95% confidence limits: 40.04 – 47.65)					
E_rC_{50} (96 h)	> 100					
E_rC_{10} (96 h)	61.65 (95% confidence limits: 57.82 – 65.28)					
E_yC_{50} (96 h)	79.61 (95% confidence limits: 71.58 – 90.01)					

* Negative values indicate stimulated growth compared to the control.
# Statistically significant difference compared to the control (Williams Multiple t-test, $\alpha = 0.05$).

III. CONCLUSION

In a 96-hour algae test with *Pseudokirchneriella subcapitata*, the E_rC_{50} (96 h) of M510F47 (metabolite of boscalid) was determined to be > 100 mg/L based on nominal concentrations After 72 hours of exposure, the respective E_rC_{50} value was equally determined to be > 100 mg/L.

The following toxicity study on the freshwater green algae *Pseudokirchneriella subcapitata* performed with the metabolite M510F49 is provided for the risk assessment of the metabolite. The study has not been evaluated previously on EU level.

Report:	CA 8.2.6.1/6 Turek T., 2015b Reg.No. 391572 (metabolite of BAS 510 F, Boscalid, M510F49) - Pseudokirchneriella subcapitata SAG 61.81 growth inhibition test 2015/1001503
Guidelines:	OECD 201 (2006), EPA 850.4500
GLP:	yes (certified by Bureau for Chemical Substances and Preparations, Lodz, Poland)

Executive Summary

In a 96-hour static limit test, the effect of M510F49 (metabolite of boscalid) on the growth of the green alga *Pseudokirchneriella subcapitata* was investigated at a single nominal concentration of the filtrate of a loading of 100 mg/L (corresponding to a geometric mean measured concentration of 0.36 mg/L) and a dilution water control. Assessment of growth was conducted 24, 48, 72 and 96 h after test initiation.

The biological results are based on geometric mean measured concentrations of the test item. No morphological effects on algae were observed in the control group and the test item concentration.

In a 96-hour algae test with *Pseudokirchneriella subcapitata,* the E_rC_{50} (96 h) of M510F49 (metabolite of boscalid) was determined to be > 0.36 mg/L based on geometric mean measured concentrations After 72 hours of exposure, the respective E_rC_{50} value was equally determined to be > 0.36 mg M510F49/L (geometric mean measured).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: M510F49 (Reg. No. 391572, metabolite of boscalid), batch no. L86-2, purity: 99.9% (± 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 (96 hours); limit test with one test item concentration and control; six replicates per treatment; daily assessment of growth.

Endpoints: EC_{10} and EC_{50} with respect to growth rate and yield after exposure over 72 and 96 hours.

Test concentrations: Control (dilution water), filtrate of a loading of 100 mg/L (corresponding to a geometric mean measured concentration of 0.36 mg M510F49/L).

Test conditions: 250 mL Erlenmeyer flasks, test volume: 100 mL; nutrient solution according to OECD 201 (AAP medium); pH 7.43 - 7.46 at test initiation and pH 8.16 - 8.73 at test termination; temperature: 24.6 - 25.1°C; initial cell densities: 1×10^4 cells/mL; continuous light: 3773 - 3850 lux, continuous shaking.

Analytics: Analytical verification of test item concentrations was conducted using a LC-method with UV-VIS detection.

Statistics: Descriptive statistics

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of the test item concentration was conducted in the control and the test item treatment at the beginning and at the end of the test. The mean measured concentration of the test item determined in samples collected at exposure initiation was 0.37 mg/L. Mean measured values for M510F49 at test termination were 91.89% of initial concentration. The following biological results are based on geometric mean measured concentrations.

Biological results: No morphological effects on algae were observed in the control group and at the test item concentration. The effects on algal growth are summarized in Table 8.2.6.1-4.

Table 8.2.6.1-4: Effect of M510F49 (metabolite of boscalid) on the growth of the green algae *Pseudokirchneriella subcapitata*

Concentration [filtrate of the loading of 100 mg/L] (initial mean measured)	**Control**	**0.37**
Concentration [mg/L] (geometric mean measured)	**--**	**0.36**
Inhibition in 72 h (growth rate) [%] *	0.0	-1.18
Inhibition in 72 h (yield) [%] *	0.0	-6.00
Inhibition in 96 h (growth rate) [%] *	0.0	-0.10
Inhibition in 96 h (yield) [%] *	0.0	-0.37
	Endpoints [mg M510F49/L] (geometric mean measured)	
E_rC_{50} (72 h)	> 0.36	
E_rC_{10} (72 h)	> 0.36	
E_yC_{50} (72 h)	> 0.36	
E_rC_{50} (96 h)	> 0.36	
E_rC_{10} (96 h)	> 0.36	
E_yC_{50} (96 h)	> 0.36	

* Negative values indicate stimulated growth compared to the control.

III. CONCLUSION

In a 96-hour algae test with *Pseudokirchneriella subcapitata,* the E_rC_{50} (96 h) of M510F49 (metabolite of boscalid) was determined to be > 0.36 mg/L based on geometric mean measured concentrations After 72 hours of exposure, the respective E_rC_{50} value was equally determined to be > 0.36 mg M510F49/L (geometric mean measured).

CA 8.2.6.2 Effects on growth of an additional algal species

The following 96-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 was significantly > 35% (*i.e.* 60%).

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 8.2.6.2/1 Palmer S.J. et al., 2001a BAS 510 F: A 96-hour toxicity test with the freshwater diatom (Navicula pelliculosa) 2001/5000044
Guidelines:	EPA 850.5400
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary

The effect of boscalid on the growth of the freshwater diatom *Navicula pelliculosa* was investigated in a 96-hour static laboratory study. The following nominal concentrations were applied: 0.078, 0.16, 0.31, 0.63, 1.3 and 2.5 mg boscalid//L (corresponding to mean measured concentrations of 0.069, 0.14, 0.28, 0.60, 1.2 and 2.4 mg a.s./L). Additionally, a solvent control (DMF) and a dilution water control were set up. Assessment of growth was conducted 24, 48, 72 and 96 h after test initiation.

The biological results are based on mean measured concentrations of the test item. After 96 h of exposure, no morphological effects on algae were observed at any of the test item treatments compared to the control replicates. Statistically significant differences in cell density compared to the pooled control were observed at the three highest and at the four highest test item concentrations after exposure over 72 h and 96 h.

In a 96 hour algae toxicity test with *Navicula pelliculosa,* the EC_{50} (96 h) for boscalid was determined to be 1.8 mg a.s./L. After 72 hours of exposure, the respective EC_{50} was determined to be 2.0 mg a.s./L.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Boscalid (BAS 510 F; Reg. No. 300355), batch no. N 75; purity: 96.9%.

B. STUDY DESIGN

Test species: Freshwater diatom, *Navicula pelliculosa*, stock originally obtained from the "UTEX - Culture Collection of Algae", University of Texas, Austin, USA.

Test design: Static system; test duration 96 hours; 6 test concentrations plus a dilution water control and a solvent control with 3 replicates per treatment; daily assessment of growth.

Endpoints: EC_{10} and EC_{50} with respect to cell density after exposure over 72 and 96 hours.

Test concentrations: Control (dilution water), solvent control (0.1 mL DMF/L), 0.078, 0.16, 0.31, 0.63, 1.3 and 2.5 mg boscalid/L (nominal), corresponding to mean measured concentrations of 0.069, 0.14, 0.28, 0.60, 1.2 and 2.4 mg a.s./L.

Test conditions: 250 mL Erlenmeyer flasks; test volume 100 mL; sterile freshwater algal medium (ASTM 1218-90E) with silica and selenium constituents; pH 7.2 at test initiation and pH 7.5 - 7.6 at test termination; temperature: 23.3°C - 24.0°C; initial cell densities 1 x 10^4 cells/mL; continuous light at 3890 - 4470 lux; constant shaking at 100 rpm.

Analytics: Analytical verification of test item concentrations was conducted using an HPLC-method with variable wavelength detection.

Statistics: Descriptive statistics; Student's t-test ($\alpha = 0.05$) for comparison of control and the solvent control data; determination of EC_x values using linear interpolation; ANOVA followed by Dunnett's test for determination of the NOEC values ($\alpha = 0.05$).

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 analyzed contents of boscalid ranged from 88.5% to 100% of nominal concentrations at test initiation and from 86.7% to 92.4% of nominal at test termination. The following biological results are based on mean measured concentrations.

Biological results: After 96 h of exposure, no morphological effects on algae were observed at any of the test item treatments compared to the control replicates. No statistically significant differences were determined between the control and the solvent control data (Student's 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. Statistically significant differences in cell density compared to the pooled control were observed at the three highest and at the four highest test item concentrations after exposure over 72 h and 96 h, respectively (ANOVA followed by Dunnett's test; $\alpha = 0.05$). The effects on algal growth are summarized in Table 8.2.6.2-1.

Table 8.2.6.2-1: Effect of boscalid on growth of the freshwater diatom *Navicula pelliculosa*

Concentration [mg a.s./L] (nominal)	**Control**	**Solvent control**	**0.078**	**0.16**	**0.31**	**0.63**	**1.3**	**2.5**
Concentration [mg a.s./L] (mean measured)	**--**	**--**	**0.069**	**0.14**	**0.28**	**0.60**	**1.2**	**2.4**
Inhibition in 72 h (cell density) [%] #	--	--	2.4	-5.5	-15	15 *	18 *	58 *
Inhibition in 96 h (cell density) [%] #	--	--	13	10	19 *	28 *	44 *	56 *
Endpoints [mg boscalid/L] (mean measured)								
EC_{50} (72 h)	2.0 (95% confidence limits: 1.8 - 2.4)							
EC_{10} (72 h)	0.76 (95% confidence limits: 0.49 - 1.2)							
EC_{50} (96 h)	1.8 (95% confidence limits: 1.3 - 2.6)							
EC_{10} (96 h)	0.12 (95% confidence limits: 0.046 - 0.33)							

Negative values indicate stimulated growth compared to the pooled control.

* Statistically significant difference compared to the pooled control (Dunnett´s test, $\alpha = 0.05$)

III. CONCLUSION

In a 96 hour algae toxicity test with *Navicula pelliculosa,* the EC_{50} (96 h) for boscalid was determined to be 1.8 mg a.s./L. After 72 hours of exposure, the respective EC_{50} was determined to be 2.0 mg a.s./L.

The following 96-hour algae study on the freshwater algae *Anabaena flos-aquae* 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 was significantly > 35% (*i.e.* 55%).

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 8.2.6.2/2 Palmer S.J. et al., 2001b BAS 510 F: A 96-hour toxicity test with the freshwater alga (Anabaena flos-aquae) 2001/5000045
Guidelines:	EPA 850.5400
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary

In a 96-hour static toxicity laboratory study, the effect of boscalid on growth of the blue-green alga *Anabaena flos-aquae* was investigated. The following nominal concentrations were applied: 0.25, 0.50, 1.0, 2.0 and 4.0 mg boscalid/L (corresponding to mean measured concentrations of 0.25, 0.53, 0.99, 2.0 and 4.2 mg a.s./L). Additionally, a solvent control (dimethylformamide (DMF)) and a dilution water control were set up. Assessment of growth was conducted 24 h, 48 h, 72 h and 96 h after test initiation.

The biological results are based on mean measured concentrations of the test item. After 96 h of exposure, no morphological effects on algae were observed at any of the test item treatments compared to the control replicates. No statistically significant differences in cell density compared to the pooled control were observed at test item concentrations of up to and including the highest concentration tested.

In a 96-hour algae toxicity test with *Anabaena flos-aquae*, both the EC_{50} (72 h) and the EC_{50} (96 h) for boscalid were determined to be > 4.2 mg a.s./L based on mean measured concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Boscalid (BAS 510 F; Reg. No. 300355), batch no. N 75; purity: 96.9%.

B. STUDY DESIGN

Test species: Freshwater blue-green alga, *Anabaena flos-aquae*; stock originally obtained from the "UTEX - Culture Collection of Algae", University of Texas, Austin, USA.

Test design: Static system; test duration 96 hours; 5 test concentrations plus a dilution water control and a solvent control with 3 replicates per treatment; daily assessment of growth.

Endpoints: EC_{10} and EC_{50} with respect to cell density after exposure over 72 hours and 96 hours.

Test concentrations: Control (dilution water), solvent control (0.1 mL DMF/L), 0.25, 0.50, 1.0, 2.0 and 4.0 mg boscalid/L (nominal), corresponding to mean measured concentrations of 0.25, 0.53, 0.99, 2.0 and 4.2 mg a.s./L.

Test conditions: 250 mL Erlenmeyer flasks; test volume 100 mL; sterile freshwater algal medium (ASTM 1218-90E); pH 7.3 at test initiation and pH 7.3 - 7.4 at test termination; temperature: 23.2°C - 23.8°C; initial cell densities: 1 x 10^4 cells/mL; continuous light at 2240 - 2460 lux, continuous shaking at 100 rpm.

Analytics: Analytical verification of test item concentrations was conducted using an HPLC-method with variable wavelength detection.

Statistics: Descriptive statistics; Student's t-test ($\alpha = 0.05$) for comparison of control and the solvent control data; determination of EC_x values using linear interpolation; ANOVA followed by Dunnett's test for determination of the NOEC values ($\alpha = 0.05$).

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each test item concentration at the beginning and at the end of the test. Measured concentrations of boscalid ranged from 100% to 108% of nominal concentrations at test initiation and from 97% to 105% of nominal at test termination. The following biological results are based on mean measured concentrations.

Biological results: After 96 h of exposure, no morphological effects on algae were observed at any of the test item treatments compared to the control replicates. No statistically significant differences were determined between the control and the solvent control data (Student's 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. No statistically significant differences in cell density compared to the pooled control were observed at test item concentrations of up to and including the highest concentration tested (ANOVA followed by Dunnett's test; $\alpha = 0.05$). The effects on algal growth are summarized in Table 8.2.6.2-2.

Table 8.2.6.2-2: Effect of boscalid on growth of the blue-green alga *Anabaena flos-aquae*

Concentration [mg a.s./L] (nominal)	**Control**	**Solvent control**	**0.25**	**0.50**	**1.0**	**2.0**	**4.0**
Concentration [mg a.s./L] (mean measured)	**--**	**--**	**0.25**	**0.53**	**0.99**	**2.0**	**4.2**
Inhibition in 72 h (cell density) [%] #	--	--	-26	12	14	18	-4.9
Inhibition in 96 h (cell density) [%] #	--	--	25	-12	11	-1.1	-9.6

	Endpoints [mg boscalid/L] (mean measured)
EC_{50} (72 h & 96 h)	> 4.2
EC_{10} (72 h)	0.41 (95% confidence limits: 0.32 - 1.4)
EC_{10} (96 h)	> 4.2

Negative values indicate stimulated growth compared to the pooled control.

III. CONCLUSION

In a 96-hour algae toxicity test with *Anabaena flos-aquae*, both the EC_{50} (72 h) and the EC_{50} (96 h) for boscalid were determined to be > 4.2 mg a.s./L based on mean measured concentrations.

The following 96-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 valid based on 72 h data, however based on 96 h data 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 was significantly > 35% (*i.e.* 50%).

- The mean coefficients of variation (CV) for section by section average specific growth rate was > 7% (*i.e.* 7.5%).

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 8.2.6.2/3 Palmer S.J. et al., 2001a BAS 510 F: A 96-hour toxicity test with the marine diatom (Skeletonema costatum) 2001/5000087
Guidelines:	EPA 850.5400
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary

The effect of boscalid on the growth of the marine diatom *Skeletonema costatum* was investigated in a 96-hour static laboratory study. The following nominal concentrations were applied: 0.25, 0.50, 1.0, 2.0 and 4.0 mg boscalid/L (corresponding to mean measured concentrations of 0.23, 0.46, 0.99, 1.9 and 3.5 mg a.s./L). Additionally, a solvent control (dimethylformamide (DMF)) and a dilution water control were set up. Assessment of growth was conducted 24, 48, 72 and 96 h after test initiation.

The biological results are based on mean measured concentrations of the test item. After 96 h of exposure, no morphological effects on algae were observed at any of the test item treatments compared to the control replicates. No statistically significant differences in cell density compared to the pooled control were observed at test item concentrations of up to and including the highest concentration tested.

In a 96 hour algae toxicity test with *Skeletonema costatum*, both the EC_{50} (72 h) and the EC_{50} (96 h) for boscalid were determined to be > 3.5 mg a.s./L based on mean measured concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Boscalid (BAS 510 F; Reg. No. 300355), batch no. N 75; purity: 96.9%.

B. STUDY DESIGN

Test species: Marine diatom, *Skeletonema costatum*, stock originally obtained from the "UTEX - Culture Collection of Algae", University of Texas, Austin, USA.

Test design: Static system; test duration 96 hours; 5 test concentrations plus a dilution water control and a solvent control with 3 replicates per treatment; daily assessment of growth.

Endpoints: EC_{10} and EC_{50} with respect to cell density after exposure over 72 and 96 hours.

Test concentrations: Control (dilution water), solvent control (0.1 mL DMF/L), 0.25, 0.50, 1.0, 2.0 and 4.0 mg boscalid/L (nominal), corresponding to mean measured concentrations of 0.23, 0.46, 0.99, 1.9 and 3.5 mg a.s./L.

Test conditions: 250 mL Erlenmeyer flasks; test volume 100 mL; saltwater algal medium; pH 7.9 at test initiation and pH 8.4 - 8.9 at test termination; temperature: 20.2°C - 21.0°C; initial cell densities 7.7 x 10^4 cells/mL; fluorescent lighting 16 hours per day at 3710 - 4140 lux; constant shaking at 100 rpm.

Analytics: Analytical verification of test item concentrations was conducted using an HPLC-method with variable wavelength detection.

Statistics: Descriptive statistics; Student's t-test ($\alpha = 0.05$) for comparison of control and the solvent control data; determination of EC_x values using linear interpolation; ANOVA followed by Dunnett's test for determination of the NOEC values ($\alpha = 0.05$).

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 analyzed contents of boscalid ranged from 96.0% to 107% of nominal concentrations at test initiation and from 67.5% to 94.8% of nominal at test termination. The following biological results are based on mean measured concentrations.

Biological results: After 96 h of exposure, no morphological effects on algae were observed at any of the test item treatments compared to the control replicates. No statistically significant differences were determined between the control and the solvent control data (Student's 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. No statistically significant differences in cell density compared to the pooled control were observed at test item concentrations of up to and including the highest concentration tested (ANOVA followed by Dunnett's test; $\alpha = 0.05$). The effects on algal growth are summarized in Table 8.2.6.2-3.

Table 8.2.6.2-3: Effect of boscalid on the growth of the marine diatom *Skeletonema costatum*

Concentration [mg a.s./L] (nominal)	**Control**	**Solvent control**	**0.25**	**0.50**	**1.0**	**2.0**	**4.0**
Concentration [mg a.s./L] (mean measured)	**--**	**--**	**0.23**	**0.46**	**0.99**	**1.9**	**3.5**
Inhibition in 72 h (cell density) [%] #	--	--	-12	-21	-11	-9.7	-12
Inhibition in 96 h (cell density) [%] #	--	--	-14	16	-12	15	-10
Endpoints [mg boscalid/L] (mean measured)							
EC_{50} (72 h & 96 h)	> 3.5						
EC_{10} (72 h & 96 h)	> 3.5						

Negative values indicate stimulated growth compared to the pooled control.

III. CONCLUSION

In a 96 hour algae toxicity test with *Skeletonema costatum*, both the EC_{50} (72 h) and the EC_{50} (96 h) for boscalid were determined to be > 3.5 mg a.s./L based on mean measured concentrations.

CA 8.2.7 Effects on aquatic macrophytes

The following aquatic plant toxicity study performed with the active substance boscalid was conducted for registrations outside the EU. The study is provided for completeness and has not been evaluated previously on EU level.

Report:	CA 8.2.7/1 Palmer S.J. et al., 2001c BAS 510 F: A 7-day toxicity test with duckweed (Lemna gibba G3) 2001/5000046
Guidelines:	EPA 850.4400
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary

In a 7-day static toxicity laboratory study, the effect of boscalid on the growth of the duckweed *Lemna gibba* was investigated. The following nominal concentrations were applied: 0.25, 0.50, 1.0, 2.0 and 4.0 mg boscalid/L (corresponding to mean measured concentrations of 0.27, 0.50, 0.99, 2.0 and 3.9 mg a.s./L). Additionally, a solvent control (dimethylformamide (DMF)) and a dilution water control were set up. Assessment of plant growth and other effects was conducted at test initiation and on days 3, 5 and 7. Percent growth inhibition relative to the pooled control was calculated for each test concentration based upon frond number.

The biological results are based on mean measured concentrations of the test item. The duckweed population in the control and the solvent control vessels showed sufficient growth, increasing from an average of 5 fronds per vessel to an average of 127 and 128 fronds per vessel, corresponding to an 25 x and 26 x multiplication respectively. After 7 days of exposure, chlorotic fronds were observed in the solvent control and at the test item concentrations of 0.50 and 2.0 mg a.s./L. Necrotic fronds occurred at three highest test item concentrations. Statistically significant effects on frond number compared to the pooled control were observed at the second highest tested concentration of 2.0 mg a.s./L, however the reduction was not considered treatment-related.

In a 7-day aquatic-plant test with *Lemna gibba,* the EC_{50} value of boscalid based on frond number was determined to be > 3.9 mg a.s./L (mean measured).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Boscalid (BAS 510 F; Reg. No. 300355), batch no. N 75; purity: 96.9%.

B. STUDY DESIGN

Test species: Duckweed (*Lemna gibba* G3); cultures maintained in-house; stock obtained from "United States Department of Agriculture".

Test design: Static system; test duration 7 days; 5 test item concentrations plus a control and a solvent control, 3 replicates for each test item concentration, the control and the solvent control; 5 plants with a total of 15 fronds per replicate; assessment of growth and other effects at test initiation and on days 3, 5 and 7.

Endpoints: EC_{50} and NOEC with respect to biomass development based on frond number after exposure over 7 days.

Test concentrations: Control, solvent control (0.1 mL DMF/L), 0.25, 0.50, 1.0, 2.0 and 4.0 mg boscalid/L (nominal), corresponding to mean measured concentrations of 0.27, 0.50, 0.99, 2.0 and 3.9 mg a.s./L.

Test conditions: 250 mL glass beakers, test volume: 100 mL, sterile M-Hoagland's media without EDTA or sucrose; pH 4.8 at test initiation and pH 5.7 - 5.8 at test termination; temperature: 24.7°C - 25.5°C, continuous light, light intensity: 4660 - 5140 lux

Analytics: Analytical verification of the test item was conducted using an HPLC-method with variable wavelength-detection.

Statistics: Descriptive statistics, Student's t-test ($\alpha = 0.05$) for comparison of control and the solvent control data; determination of EC_{50} values using linear interpolation; ANOVA followed by Bonferroni's test for determination of the NOEC value ($\alpha = 0.05$).

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each concentration at the beginning and at the end of the test. The analyzed contents of boscalid ranged from 93.8% to 105% of nominal at test initiation and from 99.7% to 109% of nominal at test termination. The following biological results are based on mean measured concentrations.

Biological results: No statistically significant differences in frond numbers were determined between the control and the solvent control groups (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. The duckweed population in the control and the solvent control vessels showed sufficient growth, increasing from an average of 5 fronds per vessel to an average of 127 and 128 fronds per vessel, corresponding to an 25 x and 26 x multiplication respectively. After 7 days of exposure, chlorotic fronds were observed in the solvent control and at the test item concentrations of 0.50 and 2.0 mg a.s./L. Necrotic fronds occurred at three highest test item concentrations. Statistically significant effects on frond number compared to the pooled control were observed at the second highest tested concentration of 2.0 mg a.s./L (Bonferroni's test; $\alpha = 0.05$), however the reduction was not considered treatment-related. Effects on the growth of *Lemna gibba* are summarized in Table 8.2.7-1.

Table 8.2.7-1: Effects of boscalid on the growth of duckweed *Lemna gibba*

Concentration [mg a.s./L] (nominal)	**Control**	**Solvent control**	**0.25**	**0.50**	**1.0**	**2.0**	**4.0**
Concentration [mg a.s./L] (mean measured)	**--**	**--**	**0.27**	**0.50**	**0.99**	**2.0**	**3.9**
Mean plant number at day 7	22	22	23	21	22	18	18
Mean frond no.	127	128	119	115	115	113	114
Inhibition after 7 d [%] (based on frond no.)	--	--	6.7	9.5	9.5	11 *	11
Endpoints [mg boscalid/L] (mean measured)							
EC_{50} (7 d) based on frond no	> 3.9						
NOEC (7 d)	0.99						

* Statistically significantly different compared to the pooled control (Bonferroni's test, $\alpha = 0.05$)

III. CONCLUSION

In a 7-day aquatic-plant test with *Lemna gibba,* the EC_{50} value of boscalid based on frond number was determined to be > 3.9 mg a.s./L (mean measured).

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.

OECD (2006) OECD Guidelines for the Testing of Chemicals, Guideline 221, *Lemna* sp. Growth Inhibition Test. OECD Publishing. Adopted: 23 March 2006, pp. 22.

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 boscalid (BAS 510 F), new studies on honeybees have been performed with the active substance. As a result there are new endpoints, which are considered in the honey bee risk assessment. Summaries of these new studies are provided below and an overview on studies and endpoints is given in Table 8.3.1-1.

Table 8.3.1-1: List of studies and endpoints with honeybees and the active substance boscalid (BAS 510 F)

Substance	Test species	Endpoint	Value	Reference (BASF DocID)	EU agreed
Boscalid	Honeybee	48 h acute oral LD_{50}	> 166 µg a.s./bee	1999/10823	yes
		48 h acute contact LD_{50}	> 200 µg a.s./bee		
Boscalid	Honeybee larva	72 h LD_{50}	> 30 µg a.s./larva	2013/1275399	no, new study
		72 h LC_{50}	> 914.6 mg a.s./kg food		
Boscalid [1)]	Residue study in bee relevant matrixes. Applied rate on sunflower: 0.5 L BAS 540 01 F/ha, equivalent to 100 g boscalid/ha	highest residue: 3.88 mg boscalid/kg (90^{th} percentile, found in pollen; hand sampling)		2014/100181	no, new study
Boscalid [1)]	Residue study in bee relevant matrixes. Applied rate on winter oilseed rape: 0.5 L BAS 540 01 F/ha, equivalent to 100 g boscalid/ha	highest residue: 5.45 mg boscalid/kg (90^{th} percentile, found in pollen; collected by forager bees)		2015/1000383	no, new study

[1)] Studies have been submitted also for renewal of approval of the active substance dimoxystrobin (BAS 505 F). The results presented here refer to the active substance boscalid (BAS 510 F) only.

CA 8.3.1.1 Acute toxicity to bees

No new studies are available.

CA 8.3.1.1.1 Acute oral toxicity

No new studies are available.

CA 8.3.1.1.2 Acute contact toxicity

No new studies are available.

CA 8.3.1.2 Chronic toxicity to bees

No new studies are available.

CA 8.3.1.3 Effects on honeybee development and other honeybee life stages

Report:	CA 8.3.1.3/1 Royer S., 2014a Effect of Reg.No. 300355 (BAS 510 F) on survival and development of honey bee brood (Apis mellifera), using an in vitro rearing method 2013/1275399
Guidelines:	OECD 237 (2013) Honey bee (Apis mellifera) larval toxicity test single exposure
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

In a single feeding toxicity test, four day old (D4) honeybee larvae (*Apis mellifera carnica* P.) were exposed to one application of BAS 510 F diluted in the larvae food. The toxicity of the test item was determined at doses of 1.875, 3.75, 7.5, 15.0 and 30.0 µg a.s./larva. The concentrations of test item in the diet were 57.2, 114.3, 228.6, 457.3 and 914.6 mg a.s./kg food. Additionally, honeybee larvae were treated with dimethoate tech. as a reference item at a dose of 8.8 µg dimethoate/larva. Untreated diet served as control, in addition a solvent control with acetone (2% v/v) was used.

The untreated control and solvent control group showed a mortality of 5.56% and 0.0% after 72 hours (D7). In the test item group, the larvae fed with 1.875, 3.75, 7.5, 15.0 and 30.0 µg a.s. revealed mortality, which was not statistically significant in comparison to the solvent control after 72 hours (D7).

In an acute larval toxicity test with BAS 510 F, the NOED was ≥ 30.0 µg a.s./larva and the corresponding NOEC was ≥ 914.6 mg a.s./kg food. No LD_{50}/LC_{50} (72 h) could be determined.

I. MATERIAL AND METHODS

A. MATERIALS

Test item:	BAS 510 F; batch no. COD-001035; content of a.s.: boscalid (Reg. No. 300355): 99.4 % analyzed purity (tolerance ± 1.0%).

B. STUDY DESIGN

Test species:	*Apis mellifera carnica* P. (honeybee), synchronized first instar larvae; collected from three healthy and queen-right colonies; source: BASF-owned colonies.
Test design:	One day old honeybee larvae (D1) of *Apis mellifera carnica* P. were transferred from brood combs to plastic queen cups in 48-well cell culture plates 3 days before start of the treatment. After this, in a 72 hour (D7) acute test, the 4 day old (D4) larvae were exposed to a single application of BAS 510 F diluted in the larvae food (aqueous sugar solution mixed with royal jelly). In total, 8 treatment groups were set up: 5 doses of the test item, 2 controls: 1 untreated control and 1 acetone solvent control, and 1 dose of the reference item, all with 3 replicates per dose and 12 larvae per replicate. Assessments of larval mortality were done 24 hours prior to (D3) and 24, 48, 72 and 96 hours (respectively D4, D5, D6, D7) after dosing. Additionally, body condition of the larvae was noted daily from D3 to D7. The presence of uneaten food was documented after 72 hours (D7).
Endpoints:	Mortality
Reference item:	Dimethoate (99.8% purity analyzed, tolerance ± 1.0%).
Test doses/concentrations:	Control (50% aqueous sugar solution with 50% royal jelly); solvent control (control solution with 2% acetone); BAS 510 F: 1.875, 3.75, 7.5, 15.0 and 30.0 µg a.s./larva, the concentrations of test item in the diet were 57.2, 114.3, 228.6, 457.3 and 914.6 mg a.s./kg food; reference item: 8.8 µg dimethoate/larva.
Test conditions:	Temperature: 33.1 °C – 34.9 °C (mean 34.7 °C), relative humidity: 51.9% - 97.6% (mean: 96.4%), food: 50% aqueous sugar solution and 50% royal jelly.
Statistics:	Descriptive statistics; Fisher's Exact Binomial Test with Bonferroni Correction (one-sided greater, $\alpha = 0.05$), Probit analysis.

II. RESULTS AND DISCUSSION

The untreated control (AC) showed a mortality of 5.56% after 72 hours (D7). The solvent control showed no mortality after 72 hours (D7). In the test item group, larvae fed with 1.875, 3.75, 7.5, 15.0 and 30.0 µg a.s./larva revealed mortality, which was not statistically significant in comparison to the solvent control group after 72 hours (D7) (Fisher's Exact Binomial Test with Bonferroni Correction, one-sided greater, a=0.05). The results are summarized in Table 8.3.1.3-1.

Table 8.3.1.3-1: Toxicity of BAS 510 F to *Apis mellifera carnica* P. (honeybee) in an acute larval toxicity test

Treatment		Mortality after 72 hours (D7)	
dosage [µg a.s./larva]	concentration [g a.s./kg food]	mean mortality [%] absolute	corrected [1)]
Control	Control	5.56	--
Solvent control	Solvent control	0.0	--
1.875	57.2	8.33	8.33
3.75	114.3	2.78	2.78
7.5	228.6	5.56	5.56
15.0	457.3	2.78	2.78
30.0	914.6	0.0	0.0
Endpoints		72 hours (D7)	
Test item doses	LD_{50}	> 30.0 µg a.s./larva	
	NOED	≥ 30.0 µg a.s./larva	
Test item concentrations	LC_{50}	> 914.6 mg a.s./kg food	
	NOEC	≥ 914.6 mg a.s./kg food	

1) Test item corrected for solvent control mortality, reference item corrected for control mortality (according to Schneider-Orelli 1947).

III. CONCLUSION

In an acute larval toxicity test with BAS 510 F, the NOED was ≥ 30.0 µg a.s./larva and the corresponding NOEC was ≥ 914.6 mg a.s./kg food. No LD_{50}/LC_{50} (72 h) could be determined.

CA 8.3.1.4 Sub-lethal effects

Report:	CA 8.3.1.4/1 Barth M., 2015a Determination of residues of BAS 540 01 F in sunflower inflorescences and their respective honeybee food items 2014/1000181
Guidelines:	SANCO/3029/99 rev. 4, EU Regulation 1107/2009 with Regulation 283/2013, EEC 91/414 (1607/IV/97 Rev. 2), EU Regulation 1107/2009 with Regulation 284/2013
GLP:	yes (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

Executive Summary

The purpose of the study was to determine residues of BAS 510 F (Reg. No. 300355) and BAS 505 F (Reg. No. 285028) in/on nectar, pollen and inflorescences from sunflower (*Helianthus annuus* L.) treated once with BAS 540 01 F under field conditions. During the 2013 growing season, five separate field trials were conducted in different representative growing areas located in Germany (n = 3) and Spain (n = 2).
Two treatment groups were set up per trial, one untreated control and one test item treatment. BAS 540 01 F was applied once at a rate of 0.5 L/ha, equivalent to 100 g BAS 510 F/ha and 100 g BAS 505 F/ha. The spray volume used was 400 L/ha and the applications were done at BBCH 63-65. Two samplings were performed per treatment group, one on day of application and one seven days after application. Pollen and inflorescences specimens were collected directly from sunflower plants. For the nectar specimens, sunflower inflorescences were collected and the nectar was extracted with capillary tubes at the field.

No residues of boscalid and dimoxystrobin were detected (below limit of quantification, LOQ; <0.01 mg/kg) in untreated pollen, nectar and inflorescences specimens of the two samplings (days after treatment, DAT 0, DAT 7) from all trials. The highest boscalid and dimoxystrobin residues analyzed over all trials were found in pollen samples. Here residues ranged from 2.05 mg/kg to 4.37 mg/kg (boscalid) and 2.38 mg/kg to 5.15 mg/kg (dimoxystrobin) at the first sampling (DAT 0) and from 0.06 mg/kg to 0.65 mg/kg (boscalid) and 0.06 mg/kg to 0.68 mg/kg (dimoxystrobin) at the second sampling (DAT 7).

Based on the highest boscalid residues, found in pollen, the 90th percentile was determined to be 3.88 mg/kg at the first sampling with an average value of 2.97 mg/kg. The reduction of boscalid residues at DAT 7 compared to DAT 0 was 95.0%, 92.6%, and 93.9% in nectar, pollen and inflorescences, respectively.
Based on the highest dimoxystrobin residues, found in pollen, the 90th percentile was determined to be 4.57 mg/kg at the first sampling with an average value of 3.42 mg/kg. The reduction of dimoxystrobin residues at DAT 7 compared to DAT 0 was 95.0%, 93.9%, and 94.9% in nectar, pollen and inflorescences, respectively.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 540 01 F, SC (suspension concentrate) formulation; batch no.: 0003354823, content of a.s.: BAS 510 F (boscalid; Reg. No. 30055), 200 g/L (197.2 g/L analyzed); BAS 505 F (dimoxystrobin; Reg. No. 285028), 200 g/L (198.1 g/L analyzed).

B. STUDY DESIGN

Test system: Sunflower – *Helianthus annuus* (Asteraceae).

Test plots: The test fields were located in Spain (n = 2) and Germany (n = 3).

Trial no.	Field location
1	Maribanez, Andalusia, Spain
2	Guadalema de los Quinteros, Andalusia, Spain
3	Priesteblich, Saxony, Germany
4	Bad Dürrenberg, Saxony-Anhalt, Germany
5	Kleinkrausnik, Brandenburg, Germany

Test design: Nectar, pollen and inflorescence samples collected from sunflower were analyzed for residues of boscalid (BAS 510 F) and dimoxystrobin (BAS 505 F). The study comprised 5 trials, covering different geographical regions in Europe (2 in Spain and 3 in Germany), each trial comprising a test item treatment and an untreated control. The minimum distance between test item treatment and control was at least 10 m.

Sampling method: In total two samplings were performed per trial, one on day of application of the test item and one 7 days after application. All samples were taken from different locations across the plot (pooled samples). For determination of residues in sunflower the inflorescences and the respective bee food items (pollen and nectar) were sampled by hand in the test item treatment and the respective control of each trial. For the nectar specimens, sunflower inflorescences were collected and the nectar was extracted with capillary tubes at the field. For pollen and nectar samples, a pooled sample of approx. 2 g was collected on each sampling day and divided into two subsamples samples of at least 1 g for analysis. For flower samples a pooled sample of at least 10 g of sunflower inflorescences were collected on each sampling day and divided into two subsamples samples of at least 5 g, each. The sampling on DAT 0 was performed within 24 hours after application of the test item. The second sampling took place on DAT 7.

Specimen storage: After collection of the specimens, the respective sample container was sealed and deep-frozen immediately on dry ice. The specimens were transported and stored under deep-frozen conditions (≤ 18 °C).

Analytics: Specimens were analyzed for BAS 510 F and BAS 505 F (BASF method No. 535/1 (L0076/01); DocID 2010/1018946). Limit of quantification (LOQ) and limit of detection (LOD) were set to 0.01 mg/kg and 0.001 mg/kg, respectively.

Endpoints: Residues of BAS 510 F (boscalid) and BAS 505 F (dimoxystrobin) in nectar, pollen and inflorescences.

Test rates: Control, 0.5 L BAS 540 01 F (corresponding to 100 g boscalid/ha and 100 g dimoxystrobin/ha).

Trial No.	Date / Growth stage [1)]	Application rate actually applied			Spray volume actually applied
		[mL product /ha]	[g a.s./ha [2)]]		[L/ha]
			BAS 510 F	BAS 505 F	
1	01.07.2013 BBCH 65	492	97.1	97.6	394
2	01.07.2013 BBCH 65	498	98.3	98.8	399
3	23.07.2013 BBCH 63-65	506	99.8	100.3	404
4	26.07.2013 BBCH 63-65	492	97.0	97.4	393
4	25.07.2013 BBCH 63-65	518	102.1	102.6	414

[1)] BBCH code, Meier (2001).
[2)] Based on analyzed content of a.s.

Test conditions: Natural field conditions. Weather conditions were well during application. Environmental conditions (air temperature, air humidity and rainfall) were recorded under non-GLP conditions as close as possible to each the trial location. The weather data was recorded during the entire study period.
Trial 1: During application (01.07.2013; 11:55-12:00), warm and sunny conditions (27.8°-28.1°C; 0% cloud cover), no wind and no precipitation. For the remaining sampling phase the weather was good with warm temperatures and no precipitation occurring.
Trial 2: During application (01.07.2013; 10:55-11:00), warm and sunny conditions (24.7°-24.8°C; 0% cloud cover), no wind and no precipitation. For the remaining sampling phase the weather was good with warm temperatures and no precipitation occurring.
Trial 3: During application (23.07.2013; 05:47-05:53), warm and sunny conditions (15.1°-15.2°C; 0% cloud cover), no wind and no precipitation. For the remaining sampling phase the weather was generally good with warm temperatures and only slight rain occurring on DAT 1 (2.5 mm), DAT 3 (11.2 mm), DAT 4 (1.4 mm); DAT 5 (6.5 mm), DAT 6 (6.8 mm) and DAT 7 (0.1 mm).
Trial 4: During application (26.07.2013; 07:57-08:08), warm and sunny conditions (20.9°-21.2°C; 0 % cloud cover), light wind (0.1 m/s) and no precipitation. For the remaining sampling phase the weather was generally good with warm temperatures and only slight rain occurring on DAT 1 (1.4 mm), DAT 2 (6.5 mm), DAT 3 (6.8 mm) and DAT 4 (0.1 mm).
Trial 5: During application (25.07.2013; 14:36-14:44), warm and mostly sunny conditions (25.7°-25.9°C; 30% cloud cover), light wind (0.2 m/s) and no precipitation. For the remaining sampling phase the weather was good with warm temperatures and only slight rain occurring on DAT 1 (3.5 mm), DAT 3 (3.7 mm), DAT 4 (16.2 mm) and DAT 6 (1.2 mm).

II. RESULTS AND DISCUSSION

During the 2013 growing season, five separate field trials were conducted in flowering sunflower (*Helianthus annuus* L.) treated once with BAS 540 01 F to determine residues of BAS 510 F (boscalid) and BAS 505 F (dimoxystrobin) in/on nectar, pollen and inflorescences.

No residues of boscalid and dimoxystrobin were detected (below LOQ; <0.01 mg/kg) in untreated pollen, nectar and inflorescences samples of the two samplings (DAT 0, DAT 7) from all trials.
The highest boscalid and dimoxystrobin residues analyzed over all trials were found in pollen samples. Here residues ranged from 2.05 mg/kg to 4.37 mg/kg (boscalid) and 2.38 mg/kg to 5.15 mg/kg (dimoxystrobin) at the first sampling (DAT 0) and from 0.06 mg/kg to 0.65 mg/kg (boscalid) and 0.06 mg/kg to 0.68 mg/kg (dimoxystrobin) at the second sampling (DAT 7).
In inflorescences samples residues of all trials ranged from 0.15 mg/kg to 0.52 mg/kg (boscalid) and 0.16 mg/kg to 0.62 mg/kg (dimoxystrobin) at the first sampling (DAT 0). At the second sampling (DAT 7) residues were between <LOQ to 0.02 mg/kg (boscalid) and <LOD and 0.03 mg/kg (dimoxystrobin).

In nectar samples lowest residues of all matrices were analyzed and ranged from <LOD to 0.03 mg/kg (boscalid) and <LOD to 0.03 mg/kg (dimoxystrobin) at the first sampling (DAT 0). At the second sampling residues of in average <LOD were found for both analytes. The results are summarized in Table 8.3.1.4-1 and Table 8.3.1.4-2.

Table 8.3.1.4-1: Summarized results of residues of BAS 510 F (boscalid) and BAS 505 F (dimoxystrobin) in nectar, pollen and inflorescences of sunflower

Matrix	Treatment	Sampling day	Average [mg/kg]	Range of residues [mg/kg]	90th percentile[1)] [mg/kg]	Average [mg/kg]	Range of residues [mg/kg]	90th percentile[1)] [mg/kg]
			BAS 510 F (Boscalid)			BAS 505 F (Dimoxystrobin)		
Nectar	Control	DAT 0	<LOD	<LOD	-	<LOD	<LOD	-
		DAT 7	<LOD	<LOD	-	<LOD	<LOD	-
	Test item	DAT 0	0.02	<LOD-0.03	**0.03**	0.02	<LOD-0.03	**0.03**
		DAT 7	<LOD	<LOD	-	<LOD	<LOD	-
Pollen	Control	DAT 0	<LOQ	<LOQ	-	<LOQ	<LOQ	-
		DAT 7	0.01	<LOQ-0.01	-	<LOQ	<LOQ	-
	Test item	DAT 0	2.97	2.05-4.37	**3.88**	3.42	2.38-5.15	**4.57**
		DAT 7	0.22	0.06-0.65	**0.45**	0.21	0.06-0.68	**0.46**
Inflorescences	Control	DAT 0	<LOD	<LOD	-	<LOD	<LOD	-
		DAT 7	<LOD	<LOD	-	<LOD	<LOD	-
	Test item	DAT 0	0.33	0.15-0.52	**0.50**	0.39	0.16-0.62	**0.60**
		DAT 7	0.02	<LOQ-0.02	**0.02**	0.02	<LOD-0.03	**0.03**

1) 90th percentile: over all trials; DAT: days after application.

The bee-relevant matrix with the highest residues analyzed was pollen. Due to the morphology of sunflower inflorescences, the pollen is more exposed during application compared to nectar. This fact results in higher residue values in pollen compared to nectar. Based on the highest boscalid and dimoxystrobin residues, found in pollen (manual sampling), the 90th percentile was determined to be 3.88 mg/kg and 4.57 mg/kg, respectively at the first sampling with an average value of 2.97 mg/kg for boscalid and 3.42 mg/kg for dimoxystrobin. For nectar, the 90th percentile was determined to be 0.03 mg/kg boscalid and 0.03 mg/kg dimoxystrobin, respectively.

For all matrices, a reduction of the residues between the first and second sampling was shown. The table below shows the residue reduction from the 1st to the 2nd sampling in percent, calculated from the average residue values from all 5 trials.

Table 8.3.1.4-2: Residue reduction

Matrix	Sampling day	BAS 510 F (Boscalid)		BAS 505 F (Dimoxystrobin)	
		Average [mg/kg]	**Reduction compared to DAT 0 [%]**	Average [mg/kg]	**Reduction compared to DAT 0 [%]**
Nectar	DAT 0	0.02	-	0.02	-
	DAT 7	<LOD	**95.0**	<LOD	**95.0**
Pollen	DAT 0	2.97	-	3.42	-
	DAT 7	0.22	**92.6**	0.21	**93.9**
Inflorescences	DAT 0	0.33	-	0.39	-
	DAT 7	0.02	**93.9**	0.02	**94.9**

Values below limit of detection (LOD) were set to 0.001 mg/kg.

III. CONCLUSION

Based on the highest boscalid residues, found in pollen, the 90th percentile was determined to be 3.88 mg/kg at the first sampling with an average value of 2.97 mg/kg. The reduction of boscalid residues at DAT 7 compared to DAT 0 was 95.0%, 92.6%, and 93.9% in nectar, pollen and inflorescences, respectively.
Based on the highest dimoxystrobin residues, found in pollen, the 90th percentile was determined to be 4.57 mg/kg at the first sampling with an average value of 3.42 mg/kg. The reduction of dimoxystrobin residues at DAT 7 compared to DAT 0 was 95.0%, 93.9%, and 94.9% in nectar, pollen and inflorescences, respectively.

Report:	CA 8.3.1.4/2 Schnurr A., 2015a Residues of BAS 510 F (Boscalid) and BAS 505 F (Dimoxystrobin) in winter oilseed rape inflorescences and their respective honeybee food items after application of BAS 540 01 F under semi-field conditions on five different trials in Germany 2015/1000383
Guidelines:	EEC 91/414 (1607/IV/97 Rev. 2), EU Regulation 1107/2009 with Regulation 283/2013, EU Regulation 1107/2009 as set out in Regulation (EU) 284/2013, SANCO/3029/99 rev. 4
GLP:	yes (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

Executive Summary

The objective of the study was to determine residues of boscalid (BAS 510 F) and dimoxystrobin (BAS 505 F), in/on nectar, pollen and flowers from winter oilseed rape *Brassica napus* L. treated once with BAS 540 01 F under field conditions. During the 2014 growing season five separate field trials were conducted in two different representative growing areas in Germany.

Two treatment groups (two tunnels with one bee hive) were set up per trial, one untreated control tunnel and one test item treatment tunnel. BAS 540 01 F was applied once at a rate equivalent to 100 g/ha of dimoxystrobin and 100 g/ha of boscalid (0.5 L of product/ha). The spray volume used was 400 L/ha and the applications were carried out at full flowering winter oilseed rape (BBCH 63-65).
Five samplings were performed per treatment group, one before application and four samplings during till seven days after application. Pollen and nectar specimens were collected by forager bees during the field phase of the study. Nectar was harvested from honey sacs of forager bees. For the flower specimens, winter oilseed rape petals were collected directly in the tunnels from different locations across the crop area. All specimens were collected in triplicates, *i.e.* three samples per sample timing and matrix. One triplicate (three samples) was pooled to one specimen and afterwards divided into two specimens (one for each analytical phase: determination of residues and protein content).

No residues of boscalid (BAS 510 F) and dimoxystrobin (BAS 505 F) were detected (above LOD) in untreated nectar, pollen and inflorescences from all trials.
The boscalid and dimoxystrobin residues in pollen ranged from 2.79 mg/kg to 6.86 mg/kg and 3.58 mg/kg and 8.21 mg/kg, respectively at the first sampling after the application (DAA 0). At the last sampling (DAA 7) residues decreased. A reduction of 97.2% (BAS 510 F) and 97.0% (BAS 505 F) was visible.
Residues of nectar samples ranged between 0.028 mg/kg and 0.425 mg/kg for boscalid and 0.047 mg/kg and 0.533 mg/kg for dimoxystrobin at the first sampling (DAA 0). Seven days later (DAA 7), at the last sampling, no residues above < LOQ (-96.1% and -93.3%, respectively, compared to DAA 0) were detected in nectar specimen.

For inflorescences (petals), residues between 3.12 mg/kg and 33.5 mg/kg for boscalid and 2.90 mg/kg and 36.7 mg/kg for dimoxystrobin were measured at the first sampling after application (DAA 0). A reduction of 92.9% and 93.2% was determined for boscalid and dimoxystrobin until the last sampling (DAA 7), respectively.

Based on the highest boscalid and dimoxystrobin residues, found in pollen, the 90th percentile was determined to be 5.45 mg/kg and 6.51 mg/kg, respectively, at the first sampling after application. For nectar the 90th percentile was determined to be 0.414 mg/kg boscalid and 0.517 mg/kg dimoxystrobin, respectively, at the first sampling after application.
For all matrices a reduction of the residues between the first and last sampling after application is measureable.

Based on the highest boscalid residues, found in the bee-relevant matrix pollen, the 90th percentile was determined to be 5.45 mg/kg at the first sampling after application (bee sampling) with an average value of 3.79 mg/kg.
Based on the highest dimoxystrobin residues, found in the bee-relevant matrix pollen, the 90th percentile was determined to be 6.51 mg/kg at the first sampling after application (bee sampling) with an average value of 4.68 mg/kg.

For all matrices a reduction of the residues between the first and last sampling after application is visible.
The reduction of boscalid residues at DAT 7 compared to DAT 0 was 97.2%, 96.1%, and 92.9% in pollen, nectar, and inflorescences, respectively.
The reduction of dimoxystrobin residues at DAT 7 compared to DAT 0 was 97.0%, 93.3%, and 93.2% in pollen, nectar, and inflorescences, respectively.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 540 01 F, SC (suspension concentrate) formulation, Batch No.: FRE-000949, content of a.s.: BAS 510 F (boscalid; Reg. No. 300355), 200 g/L (202.3 g/L analyzed); BAS 505 F (dimoxystrobin; Reg. No. 285028), 200 g/L (202.5 g/L analyzed).

B. STUDY DESIGN

Test system: Winter oilseed rape – *Brassica napus* (Brassicaceae).

Test plots: The study comprised five separate field trials (trials 1 to 5) which covered different regions in Germany.

Trial no.	Field location
1	47574 Goch-Nierswalde, North Rhine-Westphalia, Germany
2	47652 Weeze, North Rhine-Westphalia, Germany
3	04668 Motterwitz, Saxony, Germany
4	04451 Cunnersdorf, Saxony, Germany
5	04828 Bennewitz, Saxony, Germany

Test design: Petals collected by hand from winter oilseed rape, nectar prepared from forager bees and pollen collected by forager bees were analyzed for residues of the test item active ingredients boscalid and dimoxystrobin. Each trial comprised two tunnels, each with one bee colony, an untreated control tunnel and a test item treatment tunnel. The minimum distance between both tunnels was at least 10 m. Five samplings were performed per trial, one before application of the test item treatment and the other four within 7 days after application (DAA 0, DAA 2, DAA 4/5 and DAA 6/7).
For determination of residues in winter oilseed rape, the petals and the respective bee food items (pollen and nectar) were sampled either by hand or by forager bees in the test item treatment tunnel and the respective control tunnel of each trial. All specimens were collected in triplicates. Thus, three samples per sample timing and matrix. One triplicate (three specimens) was pooled to one specimen and afterwards divided into three specimens (one for each analytical phase). The rest of the pooled specimen was kept as retained specimen. For pollen and nectar, each triplicate sample was approx. 1 g. For petals a target amount of 5 g per each triplicate specimen was collected.

Specimen storage: After collection of the specimens, the respective sample container was sealed and immediately deep-frozen on dry ice. The specimens were transported and stored at ≤ 18 °C.

Analytics: Specimens were analyzed for boscalid (BAS 510 F) and dimoxystrobin (BAS 505 F) based on the BASF method No. 535/1 (L0076/01; DocID 2010/1018946). The limit of detection (LOD) for each analyte was set as 0.001 mg/kg fresh weight. The limit of quantification (LOQ) for each analyte was set as 0.01 mg/kg fresh weight.

Endpoints: Residues of boscalid and dimoxystrobin in pollen, nectar and flowers.

Test rates: Control, 0.5 L BAS 540 01 F (corresponding to 100 g boscalid/ha and 100 g dimoxystrobin/ha).

Trial No.	Date / Growth stage [1)]	Application rate actually applied			Spray volume actually applied
		[mL product /ha]	[g a.s./ha [2)]]		[L/ha]
			boscalid	dimoxystrobin	
1	09.04.2014 BBCH 65[1)]	515	104.1	104.2	412
2	09.04.2014 BBCH 65[1)]	493	99.8	99.9	395
3	24.04.2014 BBCH 65[1)]	516	104.4	104.5	413
4	22.04.2014 BBCH 65[1)]	501	101.4	101.5	401
5	22.04.2014 BBCH 65[1)]	491	99.3	99.4	393

[1)] According to Meier (2001).
[2)] Based on analyzed content of a.s.

II. RESULTS AND DISCUSSION

No residues of boscalid (BAS 510 F) and dimoxystrobin (BAS 505 F) were detected (above LOD) in untreated nectar, pollen and inflorescences from all trials.
The boscalid and dimoxystrobin residues in pollen ranged from 2.79 mg/kg to 6.86 mg/kg and 3.58 mg/kg and 8.21 mg/kg, respectively at the first sampling after the application (DAA 0). At the last sampling (DAA 7) residues decreased. A reduction of 97.2% (BAS 510 F) and 97.0% (BAS 505 F) was visible.
Residues of nectar samples ranged between 0.028 mg/kg and 0.425 mg/kg for boscalid and 0.047 mg/kg and 0.533 mg/kg for dimoxystrobin at the first sampling (DAA 0). Seven days later (DAA 7), at the last sampling, no residues above < LOQ (-96.1% and -93.3%, respectively, compared to DAA 0) were detected in nectar specimen.
For inflorescences (petals), residues between 3.12 mg/kg and 33.5 mg/kg for boscalid and 2.90 mg/kg and 36.7 mg/kg for dimoxystrobin were measured at the first sampling after application (DAA 0). A reduction of 92.9% and 93.2% was determined for boscalid and dimoxystrobin until the last sampling (DAA 7), respectively.
Based on the highest boscalid and dimoxystrobin residues, found in pollen, the 90th percentile was determined to be 5.45 mg/kg and 6.51 mg/kg, respectively, at the first sampling after application. For nectar the 90th percentile was determined to be 0.414 mg/kg boscalid and 0.517 mg/kg dimoxystrobin, respectively, at the first sampling after application.
For all matrices a reduction of the residues between the first and last sampling after application is visible.

The results are summarized presented in Table 8.3.1.4-3 and Table 8.3.1.4-4 below.

Table 8.3.1.4-3: Analyzed residues of boscalid and dimoxystrobin as contained in BAS 540 01 F in samples of pollen, nectar and inflorescences

Matrix	Treatment	Sampling day	Range of residues [mg/kg]	90th percentile[1)] [mg/kg]	Range of residues [mg/kg]	90th percentile[1)] [mg/kg]
			BAS 510 F (Boscalid)		BAS 505 F (Dimoxystrobin)	
Pollen	C	DAA -1	<LOD	-	<LOD	-
		DAA 0	<LOD-<LOQ	-	<LOD-<LOQ	-
		DAA 2	<LOD-<LOQ	-	<LOD-<LOQ	-
		DAA 5	<LOD-<LOQ	-	<LOD-<LOQ	-
		DAA 7	<LOD-<LOQ	-	<LOD	-
	T	DAA -1	<LOD	-	<LOD	-
		DAA 0	2.79-6.86	**5.45**	3.58-8.21	**6.51**
		DAA 2	0.105-1.29	**1.06**	0.099-1.73	**1.52**
		DAA 5	0.083-0.647	**0.566**	0.076-1.07	**0.980**
		DAA 7	0.056-0.188	**0.167**	0.059-0.248	**0.243**
Nectar	C	DAA -1	<LOD	-	<LOD	-
		DAA 0	<LOD-<LOQ	-	<LOD-<LOQ	-
		DAA 2	<LOD	-	<LOD	-
		DAA 5	<LOD	-	<LOD	-
		DAA 7	<LOD	-	<LOD-<LOQ	-
	T	DAA -1	<LOD-<LOQ	-	<LOD-<LOQ	-
		DAA 0	0.028-0.425	**0.414**	0.047-0.533	**0.517**
		DAA 2	<LOQ-0.089	**0.088**	0.020-0.148	**0.142**
		DAA 5	<LOD-0.011	**0.011**	<LOQ-0.022	**0.019**
		DAA 7	<LOD-0.028	**0.021**	<LOD-0.056	**0.041**
Inflorescences	C	DAA -1	<LOD-<LOQ	-	<LOD-<LOQ	-
		DAA 0	<LOQ	-	<LOQ	-
		DAA 2	<LOQ	-	<LOQ	-
		DAA 5	<LOD-<LOQ	-	<LOQ	-
		DAA 7	<LOQ	-	<LOQ	-
	T	DAA -1	<LOD-<LOQ	-	<LOQ	-
		DAA 0	3.12-33.5	**25.7**	2.90-36.7	**28.1**
		DAA 2	5.65-25.4	**24.4**	5.50-27.5	**25.5**
		DAA 5	0.342-38.3	**31.3**	0.230-39.3	**32.2**
		DAA 7	0.066-2.79	**2.48**	0.042-2.94	**2.58**

DAA: days after application; C: Control; T: Test item.
LOQ = 0.01mg/kg, LOD = 0.001 mg/kg.
1) 90th percentile over all trials: values below LOQ were set as LOQ (0.01 mg/kg), values below LOD were set ad LOD (0.001 mg/kg).

Table 8.3.1.4-4: Residue decline of BAS 510 F (boscalid) and BAS 505 F (dimoxystrobin) in pollen, nectar and inflorescences of winter oilseed rape during the 7 day sampling period from first to last sampling after application

Matrix	Sampling day	Boscalid		Dimoxystrobin	
		Average * [mg/kg]	**Reduction compared to DAA 0 [%]**	Average * [mg/kg]	**Reduction compared to DAA 0 [%]**
Pollen	DAA 0	3.79	**-**	4.68	**-**
	DAA 2	0.557	**-85.3**	0.815	**-82.6**
	DAA 5	0.273	**-92.8**	0.434	**-90.7**
	DAA 7	0.108	**-97.2**	0.140	**-97.0**
Nectar	DAA 0	0.206	**-**	0.268	**-**
	DAA 2	0.049	**-76.2**	0.076	**-71.6**
	DAA 5	0.010	**-95.1**	0.013	**-95.1**
	DAA 7	<LOQ	**-96.1**	0.018	**-93.3**
Inflorescences	DAA 0	14.2	**-**	15.3	**-**
	DAA 2	15.2	**+7**	15.6	**+2**
	DAA 5	12.1	**-14.8**	12.4	**-19.0**
	DAA 7	1.01	**-92.9**	1.04	**-93.2**

* Values below limit of quantification (LOQ) were set as LOQ (0.01 mg/kg); values below limit of detection (LOD) were set ad LOD (0.001 mg/kg).

III. CONCLUSION

Based on the highest boscalid residues, found in the bee-relevant matrix pollen, the 90th percentile was determined to be 5.45 mg/kg at the first sampling after application (bee sampling) with an average value of 3.79 mg/kg.
Based on the highest dimoxystrobin residues, found in the bee-relevant matrix pollen, the 90th percentile was determined to be 6.51 mg/kg at the first sampling after application (bee sampling) with an average value of 4.68 mg/kg.

For all matrices a reduction of the residues between the first and last sampling after application is visible.
The reduction of boscalid residues at DAT 7 compared to DAT 0 was 97.2%, 96.1%, and 92.9% in pollen, nectar, and inflorescences, respectively. The reduction of dimoxystrobin residues at DAT 7 compared to DAT 0 was 97.0%, 93.3%, and 93.2% in pollen, nectar, and inflorescences, respectively.

CA 8.3.2 Effects on non-target arthropods other than bees

No new studies are available.

CA 8.3.2.1 Effects on Aphidius rhopalosiphi

No new studies are available.

CA 8.3.2.2 Effects on Typhlodromus pyri

No new studies are available.

CA 8.4 Effects on non-target soil meso- and macrofauna

Since Annex I inclusion of the active substance boscalid (BAS 510 F), new studies on soil macro-organisms have been performed with the active substance. As a result there are new endpoints, which are considered in the respective risk assessment. Summaries of these new studies are provided below and an overview on studies and endpoints is given in Table 8.4-1.

Table 8.4-1 Toxicity to non-target soil meso- and macrofauna of boscalid

Substance (Reg. No, synonyms)	Species	Endpoint	Value [mg/kg dry soil]	Reference (BASF DocID)	EU agreed
Boscalid	*Eisenia fetida*	$LC_{50\ CORR}$	> 500 *	1999/10816	yes
Boscalid		$NOEC_{CORR}$	12.5 *	2014/1083454	no, new study
M510F47 (Reg. No. 107371)		NOEC	250	2014/1111126	no, new study
M510F49 (Reg. No. 391572)		$NOEC_{CORR}$	31.25 *	2015/1000864	no, new study
Boscalid	*Folsomia candida*	NOEC	≥ 1000	2014/1083456	no, new study
M510F49 (Reg. No. 391572)		NOEC	≥ 1000	2015/1000868	no, new study
M510F49 (Reg. No. 391572)	*Hypoaspis aculeifer*	NOEC	≥ 500	2015/1000986	no, new study

* Toxicity endpoint is 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., 2014a Sublethal toxicity of BAS 510 F (Boscalid) to the earthworm Eisenia fetida in artificial soil 2014/1083454
Guidelines:	OECD 222 (2004)
GLP:	yes (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

Executive Summary

The effects of BAS 510 F (boscalid) on *Eisenia fetida* (Annelida: Oligochaeta) mortality, biomass development and reproduction were investigated in a chronic laboratory study over 56 days. Five test item concentrations (6.25, 12.5, 25, 50 and 100 mg BAS 510 F/kg dry soil) were incorporated into the soil (10% peat) with 4 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, biomass, and feeding activity was carried out after 28 days; assessment of reproduction (number of juveniles) was carried out after 56 days.

BAS 510 F did not show any statistically significant effects on mortality and biomass. The mortality of adult worms was between 0.0% and 2.5% in the test item treatments and 1.3% in the control group. The weight change of adult worms was between 26.7% and 29.2% in the test item treatments and 27.9% in the control group.
In the control, 135.6 juveniles were counted after 56 days. In the test item treatment groups, the number of juveniles ranged between 90.0 and 143.0. The reproduction rate was statistically significantly different compared to the control at 50 and 100 mg a.s./kg dry soil, the highest two treatment rates tested. No behavioral abnormalities were observed in any of the treatment groups. The feeding activity in all test item treated groups was comparable to the control.

In a 56-day reproduction study with BAS 510 F (Reg. No. 300355), no adverse effects on survival and biomass development could be determined at concentrations up to and including 100 mg a.s./kg dry soil. Statistically significant effects on the number of *Eisenia fetida* juveniles were determined at 50 and 100 mg a.s./kg dry soil. Therefore, the NOEC for mortality and biomass was ≥ 100 mg a.s./kg dry soil, and the NOEC for reproduction was determined to be 25 mg a.s./kg dry soil.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 510 F (boscalid, Reg. No. 300355), batch no. COD-001035, analyzed purity: 99.4% (± 1.0%).

B. STUDY DESIGN

Test species: *Eisenia fetida*; adult worms with clitellum and weight of 300 – 499 mg, approximately 3 months old; source: W. Neudorff GmbH KG followed by in-house culture.

Test design: In a 56-day test, adults of *Eisenia fetida* were exposed to 5 concentrations of BAS 510 F in treated artificial soil according to OECD 222 (10% peat). In total, 6 treatment groups were set up (5 concentrations of the test item and 1 untreated control group) with 4 replicates for the test item treatments and 8 replicates for the control, 10 adult worms per replicate. The artificial soil was treated and filled into vessels, before the earthworms were introduced on the top of the soil.
Assessment of worm mortality, behavioral effects and weight change was done after 28 days of exposure, after an additional 28 days (56 days after application) reproduction (number of juveniles) was assessed.

Endpoints: Mortality, weight change, feeding activity, reproduction rate.

Reference item: Nutdazim 50 Flow (carbendazim, SC 500).

Test concentrations: Control, 6.25, 12.5, 25, 50 and 100 mg BAS 510 F/kg dry soil.

Test conditions: Artificial soil according to OECD 222 with 10% peat; pH 6.00 - 6.02 at test initiation, pH 5.77 – 5.81 at test termination; water content 54.9% - 55.2% of its maximum water holding capacity (WHC) at test initiation and 54.4% - 55.0% of WHC at test termination, temperature: 18.0°C – 21.7°C; photoperiod: 16 hours light : 8 hours dark, light intensity: 570 lux, feeding with horse manure.

Statistics: Descriptive statistics; Fisher's Exact Binomial test for mortality ($\alpha = 0.05$, one-sided greater).Williams-t-test for weight change and reproduction data ($\alpha = 0.05$, one-sided smaller), Probit analysis (Finney 1971).

II. RESULTS AND DISCUSSION

BAS 510 F did not show any statistically significant effects on mortality and biomass (Fisher's Exact Binomial test for mortality, $\alpha = 0.05$, one-sided greater; Williams-t-test for biomass, $\alpha = 0.05$, one-sided smaller). The mortality of adult worms was between 0.0% and 2.5% in the test item treatments and 1.3% in the control group. The weight change of adult worms was between 26.7% and 29.2% in the test item treatments and 27.9% in the control group.
In the control, 135.6 juveniles were counted after 56 days. In the test item treatment groups, the number of juveniles ranged between 90.0 and 143.0. The reproduction rate was statistically significantly different compared to the control at 50 and 100 mg a.s./kg dry soil, the highest two treatment rates tested (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 test item treated groups was comparable to the control.

The results are summarized in Table 8.4.1-1.

Table 8.4.1-1: Effects of BAS 510 F on *Eisenia fetida* in a 56-day reproduction study

BAS 510 F [mg a.s./kg dry soil]	**Control**	**6.25**	**12.5**	**25**	**50**	**100**
Mortality (day 28) [%]	1.3	0.0	2.5	0.0	2.5	0.0
Weight change (day 28) [%]	27.9	26.8	28.3	29.2	26.7	27.7
Number of juveniles (day 56)	135.6	143.0	139.3	129.8	113.5 *	90.0 *
Reproduction (day 56) [% of control]	--	105.4	102.7	95.7	83.7	6.4
Endpoints [mg BAS 510 F/kg dry soil]						
NOEC (day 28)	≥ 100					
NOEC (day 56)	25					
EC_{50} (day 56)	> 100					

* Statistically significantly different compared to the control (Williams-t-test, $\alpha = 0.05$, one-sided smaller).

In a separate study with the reference item Nutdazim 50 Flow (carbendazim, SC 500), the number of juveniles was reduced by 39 and 100% at concentrations of 5 and 10 mg product/kg dry soil (mean number of juveniles = 77 and 0) after 8 weeks of test duration when compared to control (mean number of juveniles = 127).

III. CONCLUSION

In a 56-day reproduction study with BAS 510 F (Reg. No. 300 355), no adverse effects on survival and biomass development could be determined at concentrations up to and including 100 mg a.s./kg dry soil. Statistically significant effects on the number of *Eisenia fetida* juveniles were determined at 50 and 100 mg a.s./kg dry soil. Therefore, the NOEC for mortality and biomass was ≥ 100 mg a.s./kg dry soil, and the NOEC for reproduction was determined to be 25 mg a.s./kg dry soil.

Report:	CA 8.4.1/2 Ganssmann M., 2014a Effects of Reg.No. 107371 (metabolite of BAS 510 F, Boscalid) on reproduction and growth of earthworms Eisenia fetida in artificial soil with 10% peat 2014/1111126
Guidelines:	OECD 222 - Earthworm reproduction Test (2004)
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

Executive Summary

The effects of Reg. No. 107371 (metabolite of BAS 510 F, boscalid) on *Eisenia fetida* (Annelida: Oligochaeta) mortality, biomass development and reproduction were investigated in a chronic laboratory study over 56 days. Five test item concentrations (31.25, 62.5, 125, 250 and 500 mg Reg. No. 107 371/kg dry soil) were incorporated into the soil (10% peat) with 4 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, biomass and feeding activity was carried out after 28 days; assessment of reproduction (number of juveniles) was carried out after 56 days.

Reg. No. 107371 did not show any statistically significant effects on mortality and biomass. No mortality was observed in any test item treatment or in the control group. The biomass development of adult worms was between 35.1% and 43.7% in the test item treatments and 37.9% in the control group.
In the control, 201 juveniles were counted after 56 days. In the test item treatment groups, the number of juveniles ranged between 157 and 230. The reproduction rate was statistically significantly different compared to the control at 500 mg Reg. No. 107 371/kg dry soil, the highest treatment rate tested. No behavioral abnormalities were observed in any of the treatment groups. The feeding activity in all test item treated groups was comparable to the control.

In a 56-day reproduction study with Reg. No. 107371 (metabolite of BAS 510 F, boscalid), no adverse effects on survival and biomass development could be determined at concentrations up to and including 500 mg/kg dry soil. Statistically significant effects on the number of *Eisenia fetida* juveniles were determined at 500 mg Reg. No. 107371/kg dry soil. Therefore, the NOEC for mortality and biomass was ≥ 500 mg Reg. No. 107371/kg dry soil, and the NOEC for reproduction was determined to be 250 mg Reg. No. 107371/kg dry soil.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Reg. No. 107371 (metabolite of BAS 510 F, boscalid), batch no. 01174-232, analyzed purity: 99.8% (± 1.0%).

B. STUDY DESIGN

Test species: *Eisenia fetida*; adult worms with clitellum and weight of 301 – 600 mg, approximately 10 to 11 months old; source: in-house culture.

Test design: In a 56-day test, adults of *Eisenia fetida* were exposed to 5 concentrations of Reg. No. 107 371 in treated artificial soil according to OECD 222 (10% peat). In total, 6 treatment groups were set up (5 concentrations of the test item and an untreated control group) with 4 replicates for the test item treatments and 8 replicates for the control, 10 adult worms per replicate. The artificial soil was treated and filled into vessels, before the earthworms were introduced on the top of the soil.
Assessment of worm mortality, behavioral effects and biomass development was done after 28 days of exposure, after an additional 28 days (56 days after application) reproduction (number of juveniles) was assessed.

Endpoints: Mortality, weight change, feeding activity, reproduction rate.

Reference item: Carbendazim (499 g/kg nominal).

Test concentrations: Control, 31.25, 62.5, 125, 250 and 500 mg Reg. No. 107 371/kg dry soil.

Test conditions: Artificial soil according to OECD 222 with 10% peat; pH 5.5 - 5.7 at test initiation, pH 5.7 – 6.0 at test termination; water content 52.5% - 54.5% of its maximum water holding capacity (WHC) at test initiation and 56.0% - 59.2% of WHC at test termination, temperature: 18.0°C – 22.0°C; photoperiod: 16 hours light : 8 hours dark, light intensity: 400 - 800 lux, feeding with cattle manure.

Statistics: Descriptive statistics; Williams-t-test for biomass development ($\alpha = 0.05$, two-sided) and reproduction data ($\alpha = 0.05$, one-sided smaller).

II. RESULTS AND DISCUSSION

Reg. No. 107371 (metabolite of BAS 510 F, boscalid) did not show any statistically significant effects on mortality and biomass (Williams t-test, $\alpha = 0.05$, two-sided). No mortality was observed in any test item treatment or in the control group. The biomass development of adult worms was between 35.1% and 43.7% in the test item treatments and 37.9% in the control group.
In the control, 201 juveniles were counted after 56 days. In the test item treatment groups, the number of juveniles counted ranged between 157 and 230. The reproduction rate was statistically significantly different compared to the control at 500 mg Reg. No. 107 371/kg dry soil, the highest treatment rate tested (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 test item 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. 107371 (metabolite of BAS 510 F, boscalid) on *Eisenia fetida* in a 56-day reproduction study

Reg. No. 107371 [mg/kg dry soil]	**Control**	**31.25**	**36.5**	**125**	**250**	**500**
Mortality (day 28) [%]	0.0	0.0	0.0	0.0	0.0	0.0
biomass development (day 28) [%]	37.9	40.3	43.7	35.8	35.1	35.8
Number of juveniles (day 56)	201	230	225	202	188	157 *
Reproduction (day 56) [% of control]	--	114.0	111.8	100.1	93.4	78.1
Endpoints [mg Reg. No. 107371/kg dry soil]						
NOEC (day 28)	≥ 500					
NOEC (day 56)	250					

* Statistically significantly different compared to the control (Williams-t-test, $\alpha = 0.05$, one-sided smaller).

In a separate study the reference item carbendazim caused statistically significant effects on reproduction at a concentration of 1.95 mg carbendazim/kg dry soil and higher. The EC_{50} for reproduction was calculated as 1.87 mg carbendazim/kg dry soil.

III. CONCLUSION

In a 56-day reproduction study with Reg. No. 107371 (metabolite of BAS 510 F, boscalid), no adverse effects on survival and biomass development could be determined at concentrations up to and including 500 mg/kg dry soil. Statistically significant effects on the number of *Eisenia fetida* juveniles were determined at 500 mg Reg. No. 107371/kg dry soil. Therefore, the NOEC for mortality and biomass was ≥ 500 mg Reg. No. 107371/kg dry soil, and the NOEC for reproduction was determined to be 250 mg Reg. No. 107371/kg dry soil.

Report:	CA 8.4.1/3 Friedrich S., 2015a Sublethal toxicity of Reg.No. 391572 (metabolite of BAS 510 F, M510F49) to the earthworm Eisenia fetida in artificial soil 2015/1000864
Guidelines:	OECD 222 (2004)
GLP:	yes (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

Executive Summary

In a chronic toxicity study, adults of *Eisenia fetida* (Annelida: Oligochaeta), were exposed to Reg. No. 391572 (M510F49, metabolite of BAS 510 F, boscalid). The test item was mixed into artificial soil (10% peat) at concentrations of 15.63, 31.25, 62.5, 125 and 250 mg Reg. No. 391572/kg dry soil with 4 replicates per treatment (each containing 10 worms). An untreated control with 8 replicates was included. The reference item was tested in a separate study. Assessments of mortality of the adults, behavioral effects and biomass development were carried out after 28 days and reproduction (number of juveniles) was assessed after 56 days.

Reg. No. 391572 neither showed any effects on mortality nor statistically significant effects on body weight. The mortality of adult worms was between 0.0% in the test item treatments and 0.0% in the control group. The weight change of adult worms was between 31.3% and 39.6% in the test item treatments and 37.7% in the control group. In the control, a mean of 186.6 juveniles was counted. In the test item treatment groups, mean numbers of juveniles between 130.8 and 187.3 were counted. The reproduction rate was significantly different compared to the control at 125 and 250 mg Reg. No. 391572/kg dry soil, the two highest treatment rates tested. No behavioral abnormalities were observed in any of the treatment groups. The feeding activity in all test item treated groups was comparable to the control.

In a 56-day reproduction study with Reg. No. 391572 (M510F49, metabolite of BAS 510 F, boscalid), no adverse effects on survival and biomass development could be determined at concentrations up to and including 250 mg Reg. No. 391572/kg dry soil. Statistically significant effects on the number of juveniles of *Eisenia fetida* were determined at 125 and 250 mg Reg. No. 391572/kg dry soil. Therefore, the NOEC for mortality and biomass was ≥ 250 mg Reg. No. 391572/kg dry soil. The NOEC for reproduction was 62.5 mg Reg. No. 391572/kg dry soil.

I. MATERIAL AND METHODS

Test item:	Reg. No. 391572 (M510F49, metabolite of BAS 510 F, boscalid), batch no. L86-2, analyzed purity: 99.9% (± 1.0%).
Test species:	*Eisenia fetida*; adult worms with clitellum and weight of 250 - 450 mg, approximately 3 months old; source: W. Neudorff GmbH KG followed by in-house culture.
Test design:	In a 56-day test, adults of *Eisenia fetida* were exposed to five concentrations of Reg. No. 391 572 in treated artificial soil according to OECD 222 (10% peat). In total, 6 treatment groups were set up (5 concentrations of the test item and 1 untreated control) with 4 replicates for the test item treatments and 8 replicates for the control, 10 adult worms per replicate. The artificial soil was treated and filled into vessels, before the earthworms were introduced on the top of the soil. Assessment of worm mortality, behavioral effects and biomass development was done after 28 days of exposure, after additional 28 days (56 days after application) reproduction (number of juveniles) was assessed.
Endpoints:	Mortality, biomass development, feeding activity, reproduction rate.
Reference item:	Nutdazim 50 Flow (Carbendazim SC 500).
Test concentrations:	Control, 15.63, 31.25, 62.5, 125 and 250 mg Reg. No. 391572/kg dry soil.
Test conditions:	Artificial soil according to OECD 222 with 10% peat; pH 6.01 - pH 6.06 at test initiation, pH 5.74 – 5.79 at test termination; water content 56.0% - 56.2% of its maximum water holding capacity (WHC) at test initiation and 55.1% - 56.0% of WHC at test termination, temperature: 18.4°C – 22.0°C; photoperiod: 16 hours light : 8 hours dark, light intensity: 570 lux, feeding with horse manure.
Statistics:	Descriptive statistics; Dunnett-t-test for weight change and Williams-t-test for reproduction data (α = 0.05, one-sided smaller), Probit analysis.

II. RESULTS AND DISCUSSION

Reg. No. 391572 neither showed any effects on mortality nor statistically significant effects on body weight (Dunnett-t-test, $\alpha = 0.05$, one-sided smaller). The mortality of adult worms was between 0.0% in the test item treatments and 0.0% in the control group. The weight change of adult worms was between 31.3% and 39.6% in the test item treatments and 37.7% in the control group. In the control, a mean of 186.6 juveniles was counted. In the test item treatment groups, mean numbers of juveniles between 130.8 and 187.3 were counted. The reproduction rate was significantly different compared to the control at 125 and 250 mg Reg. No. 391572/kg dry soil, the two highest treatment rates tested (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 test item treated groups was comparable to the control.

The results are summarized in Table 8.4.1-3.

Table 8.4.1-3: Effects of Reg. No. 391572 (metabolite of BAS 510 F, boscalid) on *Eisenia fetida* in a 56-day reproduction study

Reg. No. 391572 [mg/kg dry soil]	**Control**	**15.63**	**31.25**	**62.5**	**125**	**250**
Mortality (day 28) [%]	0.0	0.0	0.0	0.0	0.0	0.0
Weight change (day 28) [%]	37.7	36.1	39.6	38.0	37.3	31.3
Number of juveniles (day 56)	186.6	183.5	187.3	176.8	154.0 *	130.8 *
Reproduction (day 56) [% of control]	100.0	98.3	100.3	94.7	82.5	70.1
Endpoints [mg Reg. No. 391572/kg dry soil]						
NOEC (day 28)	≥ 250					
NOEC (day 56)	62.5					
EC_{50} (day 56)	> 250					

* Statistically significantly different compared to the control (Williams-t-test, $\alpha = 0.05$, one-sided smaller).

In a separate study the reference item Nutdazim 50 Flow (Carbendazim SC 500) caused a statistically significant lower biomass increase and reproduction of *Eisenia fetida*. The reproduction rate was clearly inhibited by 46% and 100% compared to the control at the test concentrations of 5 and 10 mg product/kg dry soil.

III. CONCLUSION

In a 56-day reproduction study with Reg. No. 391572 (M510F49, metabolite of BAS 510 F, boscalid), no adverse effects on survival and biomass development could be determined at concentrations up to and including 250 mg Reg. No. 391572/kg dry soil. Statistically significant effects on the number of juveniles of *Eisenia fetida* were determined at 125 and 250 mg Reg. No. 391572/kg dry soil. Therefore, the NOEC for mortality and biomass was ≥ 250 mg Reg. No. 391572/kg dry soil. The NOEC for reproduction was 62.5 mg Reg. No. 391572/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., 2014b Effects of BAS 510 F (Boscalid) on the reproduction of the collembolan Folsomia candida 2014/1083456
Guidelines:	OECD 232 (2009)
GLP:	yes (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

Executive Summary

The effects of BAS 510 F (boscalid) on mortality and reproduction of the collembolan *Folsomia candida* were investigated in a chronic laboratory experiment over a time period of 28 days. The test item was mixed into artificial soil at rates of 62.5, 125, 250, 500 and 1000 mg BAS 510 F/kg dry soil. For the control, the soil was left untreated. 4 replicates were prepared for the treatment groups and 8 replicates were prepared for the control, each containing 10 collembolans. Assessment of mortality, reproduction and behavior was made 28 days after treatment.

No statistically significant effect on parental mortality was found for any concentration tested. Mortality rates of 2.5% to 5.0% were recorded in the test item treatment groups. In the control, the mortality rate was 5.0%.
No statistically significant effects on the number of juveniles compared to the control were recorded at any concentration tested. The mean reproduction in the control reached 734 juveniles. Reproduction rates in 62.5, 125, 250, 500 and 1000 mg BAS 510 F/kg dry soil were 713, 728, 737, 715 and 735 juveniles, respectively.

In a 28-day collembolan reproduction study with BAS 510 F (boscalid), the LC_{50} was determined to be > 1000 mg BAS 510 F kg dry soil. The NOEC based on mortality and reproduction was determined to be ≥ 1000 mg BAS 510 F/kg dry soil.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 510 F (boscalid, Reg. No. 300355), batch no.: COD-001035, analyzed purity: 99.4% (± 1.0%).

B. STUDY DESIGN

Test species: Collembola (*Folsomia candida*), age: 9 - 12 days; source: in-house culture.

Test design: 28-day test in treated artificial soil (with 5% peat); different concentrations of the test item were mixed homogeneously into the soil which was filled in glass vessels before collembolans were introduced on top of the soil. 6 treatment groups (5 test item concentrations, 1 control) were set up with 4 replicates for the test item treatments and 8 replicates for the control, each containing 10 juvenile collembolans. Feeding of collembola occurred with about 2 mg dry yeast at the beginning of the test for each test vessel and additional feeding on day 14. Assessment of adult collembolans mortality, reproduction rate (number of juveniles) and behavioral effects was carried out after 28 days.

Endpoints: Mortality and 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, 62.5, 125, 250, 500 and 1000 mg BAS 510 F/kg dry soil.

Test conditions: Artificial soil according to OECD 232 with a peat content of 5%; water content: 58.3% - 58.8% of the maximum water holding capacity (WHC) at test initiation and 57.1% - 58.3% of the maximum WHC at test termination; pH 6.02 - 6.08 at test initiation, pH 5.77 - 5.82 at test termination; temperature 18.1°C - 19.3°C; photoperiod: 16 h light : 8 h dark; light intensity 450 lux.

Statistics: Descriptive statistics. Fisher's Exact Binomial test with Bonferroni Correction for mortality ($\alpha = 0.05$, one-sided greater), Williams-t-test for reproduction ($\alpha = 0.05$, one-sided smaller).

II. RESULTS AND DISCUSSION

No statistically significant effect on parental mortality was found for any concentration tested (Fisher`s Exact Binomial Test with Bonferroni Correction, $\alpha = 0.05$, one-sided greater).
Mortality rates of 2.5% to 5.0% were recorded in the test item treatment groups. In the control, the mortality rate was 5.0%.
No statistically significant effects on the number of juveniles compared to the control were recorded at any concentration tested (Williams-t-test, $\alpha = 0.05$, one-sided smaller). The mean reproduction in the control reached 734 juveniles. Reproduction in 62.5, 125, 250, 500 and 1000 mg BAS 510 F/kg dry soil reached 713, 728, 737, 715 and 735 juveniles, respectively.

The results are summarized in Table 8.4.2.1-1.

Table 8.4.2.1-1: Effects of BAS 510 F (boscalid) on collembola (*Folsomia candida*) in a 28-day reproduction study

BAS 510 F [mg a.s./kg dry soil]	**Control**	**62.5**	**125**	**250**	**500**	**1000**
Mortality (day 28) [%]	5.0	5.0	2.5	5.0	2.5	2.5
No. of juveniles (day 28)	734	713	728	737	715	735
Reproduction (day 28) [% of control])	100	97	99	100	97	100
Endpoints [mg BAS 510 F/kg dry soil]						
$NOEC_{mortality,\ reproduction}$	≥ 1000					
LC_{50}	> 1000					

III. CONCLUSION

In a 28-day collembolan reproduction study with BAS 510 F (boscalid) the LC_{50} based was determined to be > 1000 mg BAS 510 F/kg dry soil. The NOEC based on mortality and reproduction was determined to be ≥ 1000 mg BAS 510 F/kg dry soil.

Report:	CA 8.4.2.1/2 Ganssmann M., 2015a Effects of Reg.No. 391572 (metabolite of BAS 510 F, Boscalid) on reproduction of the collembolan Folsomia candida in artificial soil with 5% peat 2015/1000868
Guidelines:	OECD 232 (2009)
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

Executive Summary

The effects of Reg. No. 391572 (metabolite of BAS 510 F, boscalid) on mortality and reproduction of the collembolan *Folsomia candida* were investigated in a chronic laboratory experiment over a time period of 28 days. The test item was mixed into artificial soil at rates of 62.5, 125, 250, 500 and 1000 mg Reg. No. 391572/kg dry soil. For the control, the soil was left untreated. 4 replicates were prepared for the treatment groups and 8 replicates were prepared for the control, each containing 10 collembolans. Assessment of mortality, reproduction and behavior was made 28 days after treatment.

No statistically significant effect on parental mortality was found for any concentration tested.
Mortality rates ranged from 3.0% to 13.0% in the test item treatment groups. In the control, the mortality rate was 5.0%.
No statistically significant effects on the number of juveniles compared to the control were recorded at any concentration tested. The mean reproduction in the control reached 715 juveniles. Reproduction in 62.5, 125, 250, 500 and 1000 mg Reg. No. 391572/kg dry soil reached 633, 761, 753, 622 and 670 juveniles, respectively. No behavioral abnormalities were observed in any of the treatment groups.

In a 28-day collembolan reproduction study with Reg. No. 391572 (metabolite of BAS 510 F, boscalid) the LC_{50} based on mortality was estimated to be > 1000 mg/kg dry soil. The NOEC based on mortality and reproduction was determined to be ≥ 1000 mg Reg. No. 391572/kg dry soil.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Reg. No. 391572 (metabolite of BAS 510 F, boscalid), batch no.: L86-2, analyzed purity: 99.9% (± 1.0%).

B. STUDY DESIGN

Test species: Collembola (*Folsomia candida*), age: 10 - 12 days; source: in-house culture.

Test design: 28-day test in treated artificial soil (with 5% peat); different concentrations of the test item were mixed homogeneously into the soil which was filled in glass vessels before collembolans were introduced on top of the soil. 6 treatment groups (5 test item concentrations, 1 control) were set up with 4 replicates for the test item treatments and 8 replicates for the control, each containing 10 juvenile collembolans. Feeding of collembola occurred with about 2 mg dry yeast at the beginning of the test for each test vessel and additional feeding on day 14. Assessment of adult collembolans mortality, reproduction rate (number of juveniles) and behavioral effects was carried out after 28 days.

Endpoints: Mortality, behavioral effects and reproduction rate after 28 days.

Reference item: Boric acid (analyzed purity: 100.3%). The effects of the reference item were investigated in a separate study.

Test concentrations: Control, 62.5, 125, 250, 500 and 1000 mg Reg. No. 391572/kg dry soil.

Test conditions: Artificial soil according to OECD 232 with a peat content of 5%; water content: 52.0% - 53.1% of the maximum water holding capacity (WHC) at test initiation and 45.7% - 51.3% of the maximum WHC at test termination; pH 6.0 - 6.2 at test initiation, pH 6.0 at test termination; temperature 18.0°C – 22.0°C; photoperiod: 16 h light : 8 h dark; light intensity 400 - 800 lux.

Statistics: Descriptive statistics. Fisher's Exact Binomial Test with Bonferroni Correction for mortality ($\alpha = 0.05$, one-sided greater), Dunnett’s-t-test for reproduction ($\alpha = 0.05$, one-sided smaller).

II. RESULTS AND DISCUSSION

No statistically significant effect on parental mortality was found for any concentration tested (Fisher's Exact Binomial Test with Bonferroni Correction, $\alpha = 0.05$, one-sided greater). Mortality rates ranged from 3.0% to 13.0% in the test item treatment groups. In the control, the mortality rate was 5.0%.
No statistically significant effects on the number of juveniles compared to the control were recorded at any concentration tested (Dunnett's-t-test for reproduction, $\alpha = 0.05$, one-sided smaller).
The mean reproduction in the control reached 715 juveniles. Reproduction in 62.5, 125, 250, 500 and 1000 mg Reg. No. 391572/kg dry soil reached 633, 761, 753, 622 and 670 juveniles, respectively. No behavioral abnormalities were observed in any of the treatment groups.

The results are summarized in Table 8.4.2.1-2.

Table 8.4.2.1-2: Effects of Reg. No. 391572 (metabolite of BAS 510 F, boscalid) on collembola (*Folsomia candida*) in a 28-day reproduction study

Reg. No. 391572 [mg/kg dry soil]	**Control**	**62.5**	**125**	**250**	**500**	**1000**
Mortality (day 28) [%]	5	13	10	3	10	8
No. of juveniles (day 28)	715	633	761	753	622	670
Reproduction (day 28)[% of control]	--	89	106	105	87	94
Endpoints [mg Reg. No. 391572/kg dry soil]						
LC_{50}	> 1000					
$NOEC_{mortality,\ reproduction}$	≥ 1000					
$EC_{50\ reproduction}$	> 1000					

III. CONCLUSION

In a 28-day collembolan reproduction study with Reg. No. 391572 (metabolite of BAS 510 F, boscalid) the LC_{50} based on mortality was estimated to be > 1000 mg/kg dry soil. The NOEC based on mortality and reproduction was determined to be ≥ 1000 mg Reg. No. 391572/kg dry soil.

Report:	CA 8.4.2.1/3 Schulz L., 2015b Effects of Reg.No. 391572 (metabolite of BAS 510 F, Boscalid) on the reproduction of the predatory mite Hypoaspis aculeifer 2015/1000986
Guidelines:	OECD 226 (2008)
GLP:	yes (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

Executive Summary

The effects of Reg. No. 391572 (metabolite of BAS 510 F, boscalid) on mortality and reproduction of the soil mite *Hypoaspis aculeifer* were investigated in a chronic laboratory study over 14 days. The test item was mixed into artificial soil at rates of 31.25, 62.5, 125, 250 and 500 mg Reg. No. 391572/kg dry soil. Test item treatments were replicated 4 times each. As a control treatment, untreated soil with 8 replicates was included. Each treatment contained 10 adult female soil mites. Assessments of mortality and reproduction were carried out after 14 days of exposure.

Test item treatment groups had mortality rates of between 0.0% - 5.0%. In the untreated control the mortality rate was 0.0%, respectively. The observed mortality rates for adult mites in the test item treatment groups compared to the control were not statistically significant.

In the untreated control group, mean numbers of 327.3 juveniles were counted, respectively. In the test item treatment groups the mean number of juveniles was between 310.3 and 323.8. Reg. No. 391 572 (metabolite of BAS 510 F, boscalid) showed no statistically significantly adverse effects on reproduction at all test concentrations. No differences in behavior and morphology between mites in the control and the test item treatments could be observed.

In a 14-day reproduction study with Reg. No. 391572 (metabolite of BAS 510 F, boscalid) on predatory soil mites (*Hypoaspis aculeifer*), the LC_{50} and EC_{50} values were determined to be > 500 mg Reg. No. 391572/kg dry soil. The NOEC for mortality and reproduction was determined to be ≥ 500 mg Reg. No. 391572/kg dry soil, the highest concentration tested.

I. MATERIAL AND METHODS

Test item:	Reg. No. 391572 (metabolite of BAS 510 F, boscalid), batch no. L86-2, analyzed purity: 99.9% (± 1.0%).
Test species:	*Hypoaspis aculeifer* (CANESTRINI), adult female predatory mites (age difference: 3 days); source: in-house culture.
Test design:	14-day chronic laboratory test (according to OECD 226) on effects of Reg. No. 391572 on mortality and reproduction of soil mites. Different concentrations of the test item were homogenously mixed into artificial soil (5% peat) which was then filled in glass vessels before the soil mites were introduced on top of the soil; 6 treatment groups (1 control, 5 test item concentrations); 8 replicates for the control treatment and 4 replicates for test item treatments, each with 10 soil mites; assessment of adult mortality and reproduction effects (number of juveniles) after 14 days.
Endpoints:	Mortality and reproduction rate after 14 days.
Reference item:	Dimethoate (analyzed purity: 99.8%, ±1.0%). The effects of the reference item were investigated in a separate study.
Test rates:	Control, 31.25, 62.5, 125, 250 and 500 mg Reg. No. 391572/kg dry soil.
Test conditions:	Artificial soil according to OECD 226; pH 5.7 at test initiation, pH 5.5 – pH 5.8 at test termination; water content at test initiation 45.29% – 48.91% of maximum water holding capacity (WHC) and 43.73% – 47.48% of maximum WHC at test termination; temperature: 19.7°C – 20.7°C; photoperiod: 16 h light : 8 h dark; light intensity: 523 lux; food: cheese mites (*Tyrophagus putrescentiae*) at beginning and *ad libitum* during the test.
Statistics:	Descriptive statistics; Fisher's Exact Binomial Test with Bonferroni Correction for mortality ($\alpha = 0.05$, one-sided greater), Dunnett-t-test for reproduction ($\alpha = 0.05$, one-sided smaller).

II. RESULTS AND DISCUSSION

Test item treatment groups had mortality rates of between 0.0% - 5.0%. In the untreated control the mortality rate was 0.0%, respectively. The observed mortality rates for adult mites in the test item treatment groups compared to the control were not statistically significant (Fisher's Exact Binomial Test with Bonferroni Correction, $\alpha = 0.05$, one-sided greater).

In the untreated control group, mean numbers of 327.3 juveniles were counted, respectively. In the test item treatment groups the mean number of juveniles was between 310.3 and 323.8. Reg. No. 391572 (metabolite of BAS 510 F, boscalid) showed no statistically significantly adverse effects on reproduction at all test concentrations (Dunnett-t-test, $\alpha = 0.05$, one-sided smaller). No differences in behavior and morphology between mites in the control and the test item treatments could be observed.

The results are summarized in Table 8.4.2.1-3.

Table 8.4.2.1-3: Effects of Reg. No. 391572 (metabolite of BAS 510 F, boscalid) on predatory mite (*Hypoaspis aculeifer*) mortality and reproduction (day 14)

Reg. No. 391572 [mg/kg dry soil]	**Control**	**31.25**	**62.5**	**125**	**250**	**500**
Mortality [%]	0.0	0.0	0.0	2.5	0.0	5.0
No. of juveniles (day 14)	327.3	323.8	323.0	318.8	310.3	310.5
Reproduction (day 14) [% of control]	100	99	99	97	95	95
Endpoints [mg Reg. No. 391572/kg dry soil]						
$NOEC_{mortality,\ reproduction}$	≥ 500					
LC_{50}	> 500					
EC_{50}	> 500					

The reference item was tested in a separate study. The EC_{50} (reproduction) was calculated to be 6.2 mg dimethoate/kg dry soil. The results of the reference test demonstrate the sensitivity of the test system.

III. CONCLUSION

In a 14-day reproduction study with Reg. No. 391572 (metabolite of BAS 510 F, boscalid) on predatory soil mites (*Hypoaspis aculeifer*), the LC_{50} and EC_{50} values were determined to be > 500 mg Reg. No. 391572/kg dry soil. The NOEC for mortality and reproduction was determined to be ≥ 500 mg Reg. No. 391572/kg dry soil, the highest concentration tested.

CA 8.5 Effects on nitrogen transformation

Since Annex I inclusion of the active substance boscalid (BAS 510 F), new studies with metabolites of the active substance on nitrogen transformation have been performed. As a result, there are new endpoints which are considered in the risk assessment. Summaries of these new studies are provided under CA 8.5/1 below. Endpoints are listed in Table 8.5-1 .

Table 8.5-1 Toxicity to nitrogen transformation of boscalid and relevant metabolites

Test substance	Endpoint	Endpoint (< 25% effect) [mg/kg dry soil]	Reference (BASF DocID)	Study EU agreed?
Boscalid (tested as BAS 510 01 F)	Effects on nitrogen transformation	8.0	2000/1018517 + amendment 2001/1014651	yes
510F47 (Reg.No. 107371)	Effects on nitrogen transformation	2.0	2014/1111052	no, new study
510F49 (Reg.No. 391572)	Effects on nitrogen transformation	10.0	2015/1000863	no, new study

Report:	CA 8.5/1 Stojanowitsch M., 2014a Reg.No. 107371 (metabolite of BAS 510 F, M510F47): Effects on the activity of the soil microflora under laboratory conditions (nitrogen transformation) 2014/1111052
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. 107371 (= M510F47, metabolite of BAS 510 F) on the nitrogen transformation were investigated in a medium loamy sand soil. Reg. No. 107371 was applied to samples of the soil at nominal test concentrations of 0.2 mg Reg. No. 107371/kg and 2.0 mg Reg. No. 107371/kg dry soil. Reg. No. 107371 treated soils and controls were incubated at approx. 20.0 °C in the dark. Triplicate samples of each treatment were removed for analysis of mineral nitrogen 0, 7, 14 and 28 days after application.

No adverse effects of Reg. No. 107371 (= M510F47, metabolite of BAS 510 F) on nitrogen transformation in soil could be observed at both test concentrations, 0.2 and 2.0 mg Reg. No. 107371/kg dry soil, after 28 days. Only negligible deviations from the control of -3.34% (0.2 mg Reg. No. 107371/kg dry soil) and +2.34% (2.0 mg Reg. No. 107371/kg dry soil) were measured at the end of the 28-day incubation period.

Based on the results of this study, in accordance with OECD guideline 216, Reg. No. 107371 (= M510F47, metabolite of BAS 510 F) caused no adverse effects (< 25.0% deviation from control) on the soil nitrogen transformation (measured as NO_3-N production) in a field soil tested up to a concentration of 2.0 mg Reg. No. 107371/kg dry soil after a 28-day incubation period.

I. MATERIALS AND METHODS

A. MATERIALS

Test item: Reg. No. 107371 (= M510F47 metabolite of BAS 510 F), batch no. 01174-232; 99.8% purity (analyzed).

B. STUDY DESIGN

Test species: Biologically active agricultural soil: medium silty sand (DIN)/sandy loam (USDA), pH 5.9, 0.72% C_{org}, WHC_{max} 33.19%.

Test design: Determination of the N-transformation (NO_3-N-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-N formed from the nitrification process was determined using an ion selective electrode. Sampling scheme: 0, 7, 14 and 28 days after treatment. Sub-samples (3 replicates per treatment) were withdrawn from the bulk batches and subjected to the measurement.

Endpoints: Effects on the NO_3-N production 0, 7, 14 and 28 days after application..

Test rates: Control, solvent control, 0.2 mg and 2.0 mg Reg. No. 107371 (= M510F47, metabolite of BAS 510 F)/kg dry soil.

Reference item: Sodium chloride; 99.9% purity (analyzed). The reference item was tested in a separate study at a rate of 20.0 g a.s./kg dry soil.

Test conditions: Soil moisture: 42.0% of its maximum water holding capacity; pH: 5.4 - 6.2. Soil samples were incubated at 19.5 °C – 22.1 °C while stored in glass jars in the dark.

Statistics: Descriptive statistics.

II. RESULTS AND DISCUSSION

No adverse effects of Reg. No. 107371 (= M510F47, metabolite of BAS 510 F) on nitrogen transformation in soil could be observed at both test concentrations, 0.2 and 2.0 mg Reg. No. 107371/kg dry soil, after 28 days. Only negligible deviations from the control of-3.34% (0.2 mg Reg. No. 107371/kg dry soil) and +2.34% (2.0 mg Reg. No. 107371/kg dry soil) were measured at the end of the 28-day incubation period.

The results are summarized in Table 8.5-2.

Table 8.5-2: Effects of Reg. No. 107371 (= M510F47, metabolite of BAS 510 F) on soil micro-organisms (nitrogen transformation) on days 7, 14, and 28 of incubation

Soil (days)	Control	Solvent control	0.2 mg Reg. No. 107371/kg dry soil		2.0 mg Reg. No. 107371/kg dry soil	
	NO_3-N [mg/kg dry soil]	NO_3-N [mg/kg dry soil]	NO_3-N [mg/kg dry soil]	% Deviation from control [1)]	NO_3-N [mg/kg dry soil]	% Deviation from control [1)]
7	-1.09	-1.12	-1.18	-5.14	n.c. [2)]	n.c. [2)]
14	1.17	1.26	1.22	-3.00	1.16	-7.84
28	1.50	1.68	1.62	-3.34	1.72	+2.34

[1)] Based on NO_3-N production; - = inhibition, + = stimulation.

[2)] not calculable, measured nitrate content was below LOQ.

The reference item (sodium chloride), tested in a separate study (see appendix) had significant effects on the soil nitrogen turnover (increase of the nitrogen level of 264.0%) and the short-term respiration (90.5% inhibition) in a field soil tested at a concentration of 20.0 g/kg dry soil.

III. CONCLUSION

Based on the results of this study, in accordance with OECD guideline 216, Reg. No. 107371 (= M510F47, metabolite of BAS 510 F) caused no adverse effects (< 25.0% deviation from control) on the soil nitrogen transformation (measured as NO_3-N production) in a field soil tested up to a concentration of 2.0 mg Reg. No. 107371/kg dry soil, after a 28-day incubation period.

Report:	CA 8.5/2 Schulz L., 2015a Effects of Reg.No. 391572 (metabolite of BAS 510 F, M510F49) on the activity of soil microflora (Nitrogen transformation test) 2015/1000863
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. 391572 (= M510F49, metabolite of BAS 510 F) on the nitrogen transformation were investigated in a loamy sand soil. Reg. No. 391572 was applied to samples of the soil at nominal test concentrations of 1.0 mg Reg. No. 391572/kg and 10.0 mg Reg. No. 391572/kg dry soil. Reg. No. 391572 treated soils and controls were incubated at approx. 20.0 °C in the dark. Triplicate samples of each treatment were removed for analysis of mineral nitrogen 0, 7, 14 and 28 days after application.

No adverse effects of Reg. No. 391572 (= M510F49, metabolite of BAS 510 F) on nitrogen transformation in soil could be observed at both test concentrations, 1.0 and 10.0 mg Reg. No. 391572/kg dry soil, after 28 days. Only negligible deviations from the control of -0.9% (1.0 mg Reg. No. 391572/kg dry soil) and -0.5% (10.0 mg Reg. No. 391572/kg dry soil) were measured at the end of the 28-day incubation period.

Based on the results of this study, in accordance with OECD guideline 216, Reg. No. 391572 (= M510F49, metabolite of BAS 510 F) caused no adverse effects (< 25% deviation from control) on the soil nitrogen transformation (measured as NO_3-N production) in a field soil tested up to a concentration of 10.0 mg Reg. No. 391572/kg dry soil, after a 28-day incubation period.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Reg. No. 391572 (metabolite of BAS 510 F), batch no. L86-2; 99.9% purity (analyzed, ± 1.0%).

B. STUDY DESIGN

Test species: Biologically active agricultural soil: loamy sand (DIN 4220)/sandy loam (USDA), pH 6.2, 1.34% C_{org}, WHC 39.02 g/100.0 g dry soil.

Test design: Determination of the N-transformation (NO_3-N-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-N formed from organically bound nitrogen and NO_3-N formed from the nitrification process was determined using an Autoanalyzer (BRAN and LUEBBE). Sampling scheme: 0, 7, 14 and 28 days after treatment. Sub-samples (3 replicates per treatment) were withdrawn from the bulk batches and subjected to the measurement.

Endpoints: Effects on the NO_3-N production after 28 days.

Test rates: Control, 1.0 mg and 10.0 mg Reg. No. 391572 (= M510F49, metabolite of BAS 510 F)/kg dry soil.

Reference item: Dinoterb; 98.0% purity (analyzed, ± 0.5%). The reference item was tested in a separate study at rates of 6.80, 16.00 and 27.00 mg a.s./kg dry soil.

Test conditions: Soil moisture: 45.0% of its maximum water holding capacity; pH 5.9 - 6.0. Soil samples were incubated at 19.2 °C – 20.8 °C while stored in glass flasks in the dark.

Statistics: Descriptive statistics.

II. RESULTS AND DISCUSSION

No adverse effects of Reg. No. 391572 (= M510F49, metabolite of BAS 510 F) on nitrogen transformation in soil could be observed at both test concentrations, 1.0 and 10.0 mg Reg. No. 391572/kg dry soil, after 28 days. Only negligible deviations from the control of -0.9% (1.0 mg Reg. No. 391572/kg dry soil) and -0.5% (10.0 mg Reg. No. 391572/kg dry soil) were measured at the end of the 28-day incubation period.

The results are summarized in Table 8.5-3.

Table 8.5-3: Effects of Reg. No. 391572 (= M510F49, metabolite of BAS 510 F) on soil micro-organisms (nitrogen transformation) on days 7, 14, and 28 of incubation

Soil (days)	Control	1.0 mg Reg. No. 391572/kg dry soil		10.0 mg Reg. No. 391572/kg dry soil	
	NO_3-N [mg/kg dry soil]	NO_3-N [mg/kg dry soil]	% Deviation from control [1)]	NO_3-N [mg/kg dry soil]	% Deviation from control [1)]
7	30.13	34.33	+13.9	34.33	+13.9
14	57.43	57.43	0.0	59.13	+3.0
28	86.17	85.37	-0.9	85.77	-0.5

1) Based on NO_3-N production; - = inhibition, + = stimulation.

The reference item dinoterb caused an effect of +33.2% and +46.9% on the nitrogen transformation in a field soil at the tested concentrations of 16.00 mg and 27.00 mg dinoterb/kg dry soil, respectively, 28 days after application.

III. CONCLUSION

Based on the results of this study, in accordance with OECD guideline 216, Reg. No. 391572 (= M510F49, metabolite of BAS 510 F) caused no adverse effects (< 25% deviation from control) on the soil nitrogen transformation (measured as NO_3-N production) in a field soil tested up to a concentration of 10.0 mg Reg. No. 391572/kg dry soil, after a 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.

CA 8.7 Effects on other terrestrial organisms (flora and fauna)

In the literature search performed in the course of the renewal of approval, it was concluded that the following extended laboratory study on the effects of several fungicides, including boscalid, on a species of predatory mites is reliable with restrictions (reliability index (RI) 2). The study was not performed according to an accepted testing guideline, no information regarding physio-chemical properties were given, the tested concentrations were not verified and no positive control was tested. Aside from that, the study is well documented and was conducted to a certain extent comparable with current guidelines.
As low risk for non-target arthropods was concluded in the environmental risk assessment and no further studies are required, the study is presented as additional information.

Report:	CA 8.7/1 Bostanian N.J. et al., 2008a Toxicity of six novel fungicides and sulphur to Galendromus occidentalis (Acari: Phytoseiidae) 2009/1132343
Guidelines:	none
GLP:	no

Executive Summary

An extended laboratory study with the predatory mite *Galendromus occidentalis* collected from apple and cherry orchards in the Okanagan Valley, British Columbia, Canada was carried out. Six novel fungicides and sulphur were tested in the study, in the present summary only Lance 70 WDG (boscalid 70%) is considered. The mites were exposed to Lance 70 WDG at recommended label rate (0.26 kg a.s./ha) on leaf disks from apple plants. Mortality of adults, larvae and eggs, and fecundity were recorded. Assessment of mortality was made after 144 h post treatment for eggs and larvae. The mortality of adults was evaluated 72 h after treatment. Fecundity assessments were done every 24 h until 72 h after treatment.

After 144 h a corrected mortality of 1.1% was observed in eggs, 11.9% for larvae and after 72 h 1.7% for adults. The results for fecundity were 1.83 eggs/female /day after 24 h, 1.97 eggs/female/day after 48 h and 2.10 eggs/female/day after 72 h. Regarding the results, boscalid had caused no significant mortality or fecundity reduction in *Galendromus occidentalis* in a worst case extended laboratory scenario.

The results reported for Lance 70 WDG (boscalid 70%) indicate that there were no adverse effects on mortality of eggs, larvae and adults of *G. occidentalis*. Furthermore, fecundity was also not adversely affected. The results of the laboratory study indicate that Lance 70 WDG (boscalid 70%), can immediately be integrated into IPM programs in British Columbia orchards.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Lance 70 WDG, boscalid (70%)

B. STUDY DESIGN

Test species: Predatory mite *Galendromus occidentalis*, collected from IPM (integrated pest management program) apple and cherry orchards in the Okanagan Valley, British Columbia, Canada.

Test design: The fungicide was applied with a thin layer chromatography sprayer set at 10.34 kPa (1.5 PSI) and held perpendicular to the Petri dishes at about 30 cm. Each Petri dish was treated for 5 s and it received 0.0044 ± 0.0002 ml/cm²/s of the fungicide. To discover effects on eggs, two to three adult female *Galendromus occidentalis* were released for 48 h on the lower side of 10 apple leaf discs (20 mm diameter) placed with the upper side on wet cotton wool in a 14.5 cm diameter Petri dish. After the egg laying period the eggs were counted and treated with the test item. The treated eggs were air-dried and the number of live eggs and larvae were recorded at 72, 96, 120 and 144 h after treatment. Three replicates were done.
For the adult treatment 108 twenty-four hour old females were placed on apple leafs in petri dishes (1 female/leaf, 12 leafs/petri dish and3 petri dishes /replicate). The petri dishes were treated as described above and the mortality was estimated at 24, 48, and 72 h post treatment.
The test of fecundity was done with 24 h old females, released on individual pre infested leaf discs. The mortality and laid eggs were recorded at 24, 48 and 72 h post treatment.

Endpoints: Mortality of eggs and larvae after exposure over 144 h; mortality of adults after 72 h exposure; Fecundity (No. of laid eggs) over 72 h

Reference item: No reference item was used.

Test rates: Control, tap water. The label rate of 0.26 kg/ha were calculated to g a.s. /L based on the application of 600 L of sprayable material/ha.

Test conditions: Exposure period: mortality: 72 h adults, 144 h larvae and eggs, fecundity: 72 h; temperature: 27.0 °C; relative humidity: 65.0%; photoperiod: 16 h light: 8 h dark; food: two-spotted spider mite.

Statistics: Statistics were done using Henderson and Tilton (1955) formula to correct natural mortality of G. *occidentalis*. Logarithmic transformation was used to transform fecundity data before ANOVA and Tukey-Kramer tests were carried out with JMP statistics and graphics guide (Institute SAS 2002).

II. RESULTS AND DISCUSSION

After 144 h mortality of 1.1% was observed in eggs, 11.9% for larvae and 1.7% for adults after 72 h. The results for fecundity were 1.83 eggs/female after 24 h, 1.97 eggs/female after 48 h and 2.10 eggs/female after 72 h. regarding the results, boscalid had caused no significant mortality or fecundity reduction to *Galendromus occidentalis* in a worst case extended laboratory scenario (ANOVA and Tukey-Kramer tests $\alpha = 0.05$).

Table 8.7-1: Effects on predatory mites (*Galendromus occidentales*) exposed to Lance 70 WDG in a laboratory trial

a.s.	Trade Name	Application rate (kg a.s./ha)	% Mortality[1)]		
			144 h Eggs $\alpha = 0.792$[2)]	144 h Larvae $\alpha = 0.001$[3)]	72 h Adults $\alpha = 0.585$[2)]
Boscalid	Lance 70 WDG	0.26	1.1	11.9[2)]	1.7
a.s.	**Trade Name**	**Application rate (kg a.s./ha)**	**Number of eggs laid at 24 h intervals**		
			24 h $\alpha = 0.792$[4)]	**48 h $\alpha = 0.792$[4)]**	**72 h $\alpha = 0.792$[4)]**
--	Control	--	2.18	2.24	2.14
Boscalid	Lance 70 WDG	0.26	1.83	1.97	2.10

1) Cumulative percent mortality was calculated according to Henderson and Tilton (1955)

2) No significant differences between treatments, based on ANOVA performed on arcsine-transformed data

3) Means within columns followed by the same letter are not significantly different, based on Tukey-Kramer test performed on arcsine-transformed data ($\alpha = 0.05$)

4) No significant differences between treatments, based on ANOVA performed on log (eggs laid/ female)

No reference item was used in this study.

III. CONCLUSION

The results reported for Lance 70 WDG (boscalid 70%) indicate that there were no adverse effects on mortality of eggs, larvae and adults of *G. occidentalis*. Furthermore, fecundity was also not adversely affected. The results of the laboratory study indicate that Lance 70 WDG (boscalid 70%), can immediately be integrated into IPM programs in BC orchards.

In the literature search performed in the course of the renewal of approval, it was concluded that the following extended laboratory study on the effects of several fungicides, including boscalid, on a species of predatory mites is reliable with restrictions (reliability index (RI) 2). The study was not performed according to an accepted testing guideline, no information regarding physio-chemical properties were given and no positive control was tested. Aside from that, the study is well documented and was conducted to a certain extent comparable with current guidelines.
As low risk for non-target arthropods was concluded in the environmental risk assessment and no further studies are required, the study is presented as additional information.

Report: CA 8.7/2
Laurin M.-C., Bostanian N.J., 2007a
Short-Term contact Toxicity of seven fungicides on Anystis baccarum
2007/1071225
Guidelines: none
GLP: no

Executive Summary

A short-term laboratory study with the predatory mite *Anystis baccarum*, collected from an experimental block of apple cv. "Liberty", located at Frelingsburgh, Quebec, Canada, was carried out. Seven fungicides were tested in the study, in the present summary only Lance 70 WDG (boscalid) is discussed. The mites were exposed to Lance 70 WDG at the recommended label rate for vineyards, regarding that Lance 70 WDG was not registered for apples in Canada. In addition, five different multiples of the recommended rate were tested. In total six rates with four replicates and 15 mites per replicate were tested. The mites were released to the test cages (50 x 9 mm petri dishes) after the fungicide was applied by using a thin-layer chromatography and let them dry for 2 h. No food was offered during the 48 h test period. During the test, the mites were held in growth chambers under controlled climatic conditions.

The six test rates(X = 0.55 g a.s/L [recommended dose], 2X, 4X, 8X, 16X and 32X), elicited a mortality of 6.8% at 2X, 5.1% at 4X, 3.3% at 8X, 10.0% at 16X and 33.3% at 32X. The recommended concentration "X" caused no adverse effects compared to the control.

The results reported for Lance 70 WDG (boscalid) indicate that there were no adverse effects on adult *Anystis baccarum* 48 h after treatment. Therefore Lance 70 WDG (boscalid 70%) can be considered to be harmless to *Anystis baccarum* at the recommended label concentration.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Lance 70 WDG (boscalid, 70%).

B. STUDY DESIGN

Test species: Predatory mite *Anystis baccarum*, collected from an experimental block of apple cv. “Liberty”, located at Frelingsbourgh, Quebec, Canada.

Test design: Plastic petri dishes (50 x 9 mm) were used as cages for mite treatment. The test fungicide was applied with a thin-layer chromatography (2 mg/cm²) sprayer set at 10.3 kPa. The treated cages were stored in a growth chamber at 22 °C and 70% relative humidity to dry for at least 2 h. A pre-test was carried out with 30 mites to detect the response of the mites on the fungicide, above and below the recommended label rates. For Lance 70 WDG the recommended label rates for vineyards were used, as Lance 70 WDG was not registered for apples in Canada. Six concentrations with four replicates and 15 mites per replicate were tested. No food was offered during the 48 h test period. To avoid cannibalism only one *Anystis baccarum* was introduced in each compartment. The test cages were stored in growth chambers. Mortality was counted after 48 h, specimens were considered dead when they were unable to move a distance equivalent to their own circumference when the cage was shaken.

Endpoints: Mortality of adult mites after 48 h exposure.

Reference item: No reference item was used.

Test rates: Control, X (0.55 g a.s./L), 2 X, 4 X, 8 X, 16 X and 32 X.

Test conditions: Exposure period: 48 h, Temperature: 21.0 °C, relative humidity: 80.0%; photoperiod: 16 h light: 8 h dark. Food: no food was offered.

Statistics: Statistics were carried out using Probit analyses, mortality was calculated according to Abbott (1925).

II. RESULTS AND DISCUSSION

The short term toxicity test of Lance 70 WDG (boscalid) with the predatory mite *Anystis baccarum* showed no statistically significant effects on mortality after 48 h (Abbott).

The six tested concentrations (X = 0.55 g a.s./L [recommended dose], 2X, 4X, 8X, 16X and 32X), elicited a corrected mortality of 6.8% at 2X, 5.1% at 4X, 3.3% at 8X, 10.0% at 16X and 33.3% at 32X of the recommended concentration. The recommended concentration "X" showed no adverse effects compared to the control (Abbott).

Table 8.7-2: Effects on predatory mites (*Anystis baccarum*) exposed to Lance 70 WDG in a laboratory trial

Fungicide	% mortality[2)] by pesticide concentration[3)]							
	32X	16X	8X	4X	2X	X	X/2	Control
Lance 70 WDG (boscalid)[1)]	33.3	10.0	3.3	5.1	6.8	0	-	0

1) n = 60 mites treated in each concentration

2) Mortality of each concentration calculated according to Abbott.

3) "X" represents the recommended concentration for a use in apples (0.55 g a.s./L)

No reference item was used in this study.

III. CONCLUSION

The results reported for Lance 70 WDG (boscalid) indicate that there were no adverse effects on adult *Anystis baccarum* 48 h after treatment. Therefore, Lance 70 WDG (boscalid) can be considered to be harmless to *Anystis baccarum* at recommended label concentration.

In the literature search performed in the course of the renewal of approval, it was concluded that the following peer-reviewed study on the effects of boscalid on soil micro-organism activity (including carbon transformation) is not reliable (Reliability Index (RI) 3). The study was not performed according to an accepted testing guideline, the tested concentrations were not verified and no positive control was tested. Furthermore, no definite endpoint was determined in the study and exposure to boscalid was unrealistic high. Aside from that, the study is well documented and conducted.
Testing of effects on carbon transformation is not required according to EU Commission Regulation No. 283/2013. Nonetheless, for the sake of completeness, the study is presented as additional information.

Report: CA 8.7/3
Xiong D. et al., 2013a
Influence of boscalid on the activities of soil enzymes and soil respiration
2014/1327532
Guidelines: none
GLP: no

Executive Summary

The effect of boscalid on soil respiration was tested beside of soil enzyme activity, which is not part of this summary. Boscalid was applied on a natural soil sample, taken from the campus of the Agricultural University, Beijing, China, at test concentrations of 10, 100 and 200 mg boscalid/kg dry soil. The treated soils and untreated control were incubated at 28 ± 1 °C in the dark for 60 days (application of the test item on day 7). Triplicate samples of each treatment were removed for analysis of carbon transformation (oxygen consumption) 7, 21, 35 and 60 days after application.

No difference in the respiration between soil samples treated with 200 mg/kg boscalid and without boscalid were detected in the whole stage of incubation. Inhibitory effects occurred at concentrations of 10 and 100 mg boscalid/kg dry soil during the treatment period, but differences were statistically not significant.

No pre-sampling was included. The differences at 10 and 100 mg/kg might be due to experimental variation. The tested rates are well above the PECs calculated for BAS 510 01 F use pattern.

Based on the results of this study, boscalid caused no adverse effects at the highest tested concentration of 200 mg/kg dry on the soil carbon transformation (measured as respiration) in biologically active soil after 60 days of incubation. No significant differences were observed at the lower concentrations of 10 and 100 mg/kg dry soil over the treatment period of 60 days. The differences might be due to a lack of a pre-sampling and experimental variation which is not accounted for.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: boscalid, purity: 95% (w/w).

B. STUDY DESIGN

Test soil: Biologically active soil from the upper 20 cm of campus land in China Agricultural University, Beijing China. The soil was air-dried at room temperature, ground and sieved through a 2 mm sieve. Physicochemical characteristics: pH 7.5, 1.24% C_{org}, water content is not given.

Test design: Determination of carbon transformation in soil was performed as follows. The soil (50 g dry weight) treated with 1 g glucose, placed into a 250 ml serum vial, was adjusted to 60% of the maximum WHC (water holding capacity) and incubated for 7 days under test conditions. These serum vials were removed to 2500 ml sealable tanks with another serum vial contained with 20 ml 1 mol/L1 NaOH in every sealable tank. Afterwards, the soil was artificially contaminated by stock standard solution (boscalid 10 000mg/L + acetone) at the doses of 10, 100 and 200 mg/kg dry soil. The soil samples were incubated in the dark for 53 more days at 28 ± 1 °C. The measurements were done at day 7, 21, 35 and 60 after the treatment, regarding CO2 formed in the headspace of the serum vials, absorbed by NaOH. The respiration rate was determined by using the titration method and expressed as respiration of soil in ml CO_2/100 g soil/d. During the test, distilled water was added to keep the soil WHC. An untreated soil sample was used as control.

Endpoints: Effects on O_2 consumption 7, 21, 35, and 60 days after test start.

Test concentrations: Control, 10, 100, 200, mg boscalid°/kg dry soil; test concentrations related to a soil depth of 20 cm, cation exchange capacity of 24.5 cmol/kg and a physical clay content of (< 0.01 mm)V% 19.52. .

Reference item: No reference item was used in the study.

Test conditions: Soil moisture: approx. 60% of maximum water holding capacity; pH 7.50. Soil samples were incubated at 28 ± 1 °C while stored in serum vials in the dark.

Statistics: Descriptive statistics. Duncan’s new multiple range test ($\alpha = 0.05$).

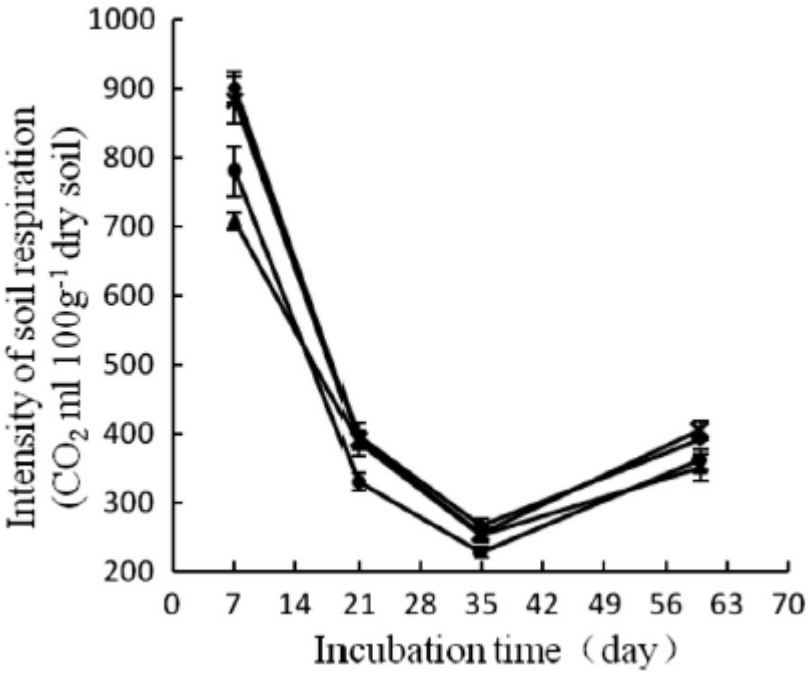
II. RESULTS AND DISCUSSION

No difference in the respiration between soil samples treated with 200 mg/kg and without boscalid was detected during the whole incubation period. In the initial 7 days, respiration was reduced to 78.5% and 86.5% of the control. The treatments with 10 and 100 mg/kg dry soil showed inhibitory effects on respiration during the whole incubation period, but not significantly different to the control (Duncan's new multiple range test).
From day 7 to 35 respiration of all treatments and the control declined sharply.

BASF evaluation

No pre-sampling was included. The differences at 10 and 100 mg/kg might be due to experimental variation. The tested rates are well above the PECs calculated for BAS 510 01 F use pattern.

Figure 8.7-1: Effects of boscalid on soil micro-organisms (carbon transformation) on days 7, 21, 35 and 60 of incubation



[1)] Effects of boscalid on soil respiration. Symbols: ♦: without boscalid, ▲: 10 mg/kg boscalid, ●: 100 mg/kg boscalid, x: 200 mg/kg boscalid

No reference item for positive control was used in this study.

III. CONCLUSION

Based on the results of this study, boscalid caused no adverse effects at the highest tested concentration of 200 mg/kg dry soil on the carbon transformation (measured as respiration) in biologically active soil after 53 days of incubation. No significant differences were observed at the lower concentrations of 10 and 100 mg/kg dry soil over the treatment period of 60 days. The differences might be due to a lack of a pre-sampling and experimental variation which is not accounted for.

CA 8.8 Effects on biological methods for sewage treatment

The results of the already peer-reviewed and accepted study are still valid and are summarized in Table 8.8-1. No new study has been performed.

Table 8.8-1: Effects on biological methods for sewage treatment

Test item	Study type	Endpoint [mg a.s./L]	Reference (BASF DocID)	EU agreed
BAS 510 F (Boscalid)	Respiration inhibition test (inhibition of oxygen consumption activated sludge from wastewater plant)	EC_{50} (0.5 h) >1000	1999/10289	yes

CA 8.9 Monitoring data

According to the knowledge of the applicant, there are currently no monitoring studies available, which are assessing ecotoxicological effects of boscalid (BAS 510 F).

We create chemistry

Boscalid

Document M-CA, Section 9

LITERATURE DATA

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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 9 LITERATURE DATA

A literature search on boscalid was performed by the BASF Group Information Center. The Literature Search Report on boscalid 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 2015/1249174).

The first step of the search result processing based on summary records was done by the Information Center and involved the separation into "hits" and "ballast" (obviously irrelevant records). The “ballast” was not further processed.

The “hits” were further evaluated by the scientific experts and categorized into “not relevant”, “not reliable”, and “used for dossier”. This is documented in EXCEL files which are attached to the search report in K-CA 9 with the file names as listed below (alphabetical order):

Analytics:	Boscalid Literature Analytics
Ecotoxicology:	Boscalid Literature Ecotox Aquatic
	Boscalid Literature Ecotox General
	Boscalid Literature Ecotox Terrestric
	Boscalid Literature Ecotox Wildlife
Environmental Fate:	Boscalid Literature Environmental Fate
Consumer Safety:	Boscalid Literature Metabolism and Residues in Animals
	Boscalid Literature Metabolism and Residues in Plants
Product Chemistry:	Boscalid Literature Product Chemistry
Toxicology:	Boscalid Literature Toxicology

The hits in Ecotox General, Ecotox Aquatic, Ecotox Wildlife, Metabolism and Residues in Animals and Plants as well as Product Chemistry did not contribute to the risk assessment and were therefore not further discussed in the dossier.

We create chemistry

Boscalid

Document M-CA, Section 10

CLASSIFICATION AND LABELLING OF THE ACTIVE SUBSTANCE

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CA 10 CLASSIFICATION AND LABELLING OF THE ACTIVE SUBSTANCE

There is no harmonized classification for boscalid.

BASF proposes the following self-classification and labelling in accordance with Regulation (EC) No 1272/2008, based on already peer reviewed data and on new studies:

Table 10-1: Proposed Classification and Labelling for Boscalid

Legislation	Classification	Labelling
Regulation (EC) No 1272/2008	Aquatic Chronic 2	 Hazard statement: H411 Toxic to aquatic life with long lasting effects. Precautionary Statements (Prevention): P273 Avoid release to the environment. Precautionary Statements (Response): P391 Collect spillage. Precautionary Statements (Disposal): P501 Dispose of contents/container to hazardous or special waste collection point.

Physico - chemical properties

Table 10-2: Physico-chemical data relevant for classification of boscalid

Study Type	Results (triggered classification and labelling)	Reference
Explosivity	**Not explosive**	[see 2013/1164740 Achhammer G. 2013 a]
Oxidizing properties	Not oxidizing	[see 2013/1164740 Achhammer G. 2013 a]
Flammability	Not flammable	[see 2013/1164740 Achhammer G. 2013 a]
Content of hydrocarbon	Due to the molecular structure boscalid is not regarded as a hydrocarbon.	See chapter MCA 1
Viscosity (kinematic)	Not applicable as boscalid is a solid	SANCO/3919/2007-rev. 5, Appendix I, 21 January 2008

Toxicological properties

The available toxicological data on boscalid do not meet the criteria for classification according to Regulation (EC) 1272/2008.

Ecotoxicology/Environment

Table 10-3: Ecotoxicology/Environment data relevant for classification of boscalid [1]

Study Type (duration)	Results (triggered risk phrase)	Reference
Oncorhynchus mykiss (96 h)	96 h $LC_{50} \cong$ 2.7 mg/L	DocID 2001/1001726
O. mykiss **(ELS, 97 d)**	**97 d NOEC = 0.125 mg/L (Chronic aquatic hazard Cat. 2; H411, Toxic to aquatic life with long lasting effects)**	**DocID 1999/11847**
Daphnia magna (48 h)	48 h EC_{50} = 5.33 mg/L	DocID 2000/1018537
D. magna (21 d) [2]	21 d NOEC = 0.80 mg/L	DocID 2004/1015006 Amendment: DocID 2004/1015009
Pseudokirchneriella subcapitata **(Syn. *Selenastrum capricornutum*)** [2]	**72 h E_rC_{50} = 2.61 mg/L (No Acute aquatic hazard Cat.)** [3]	**Doc ID 2000/1018524 Recalculations: Doc ID 2009/1044471**
	72 h E_rC_{10} = 1.19 mg/L	
Lemna gibba (7 d) [2]	7 d E_rC_{50} > 3.9 mg/L	DocID 2001/5000046
	7 d NOEC = 0.99 mg/L	
Biodegradation	Boscalid is not readily biodegradable	DocID 1999/10290

1 The lowest acute and chronic endpoint (basis for classification) is marked in **bold**.

2 Study was not submitted during Annex I inclusion process of the active substance (for details see chapter CA 8.2).

3 According to Regulation EC) No 1272/2008 no Acute aquatic hazard Category is triggered, as the lowest $L(E)C_{50}$ > 1 mg/L.