

FINAL REGISTRATION REPORT

Part B

Section 9

Ecotoxicology

Detailed summary of the risk assessment

Product code: BAS 758 00 F

Product name(s): Revyflex Plus

Chemical active substance(s):

Mefentrifluconazole, 66.6 g/L

Metrafenone, 100 g/L

Pyraclostrobin, 80 g/L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(authorization)

Applicant: BASF

Submission date: June 2022

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Version history

When	What
03/2022	Initial dRR – BASF DocID 2022/2012030
06/2022	Updated version - BASF DocID 2022/2035488
10/2022	zRMS finalized dRR evaluation
January 2023	Final version prepared by zRMS after Commenting period

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9 Ecotoxicology (KCP 10)

Review Comments:

This document describes the acceptable use conditions required for registration of BAS 758 00 F, an emulsifiable concentrate containing 66.7 g/L mefentrifluconazole, 80.0 g/L pyraclostrobin and 100.0 g/L metrafenone, for use as a fungicide in cereals.

This Part B document only reviews data and additional information that has not previously been considered within the EU review process.

Since this document is based on the information provided by the applicant, all review comments, additions and corrections have been made using commenting boxes or highlighted in grey.

9.1 Critical GAP and overall conclusions

Table 9.1-1: Table of critical GAPs

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Use- No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, Fpn G, Gn, Gpn or I **	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	Application				Application rate			PHI (days)	Remarks: e.g. g saf- ener/ synergist per ha	Conclusion						
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target	Soil organisms	Non-target plants
Zonal uses (field or outdoor uses, certain types of protected crops)																				
1	AT, BE, DE, IE, NL, PL	wheat TRZAW, TRZAS TRZDU, TRZSP	F	<i>Oculimacula spp.</i> - PSDCHE <i>Blumeria graminis</i> - ERYSGR <i>Zymoseptoria tritici</i> - SEPTTR <i>Puccinia triticina</i> - PUCCRT <i>Puccinia striiformis</i> - PUCCST <i>P. tritici-repentis</i> - PYRNTR	Spraying (SP)	30 - 59	a) 2 b) 2	14	a) 1.50 b) 3.00	a) 0.100 ¹⁾ / 0.150 ²⁾ / 0.120 ³⁾ b) 0.200 ¹⁾ / 0.300 ²⁾ / 0.240 ³⁾	100 - 300	56	For eyespot control, only one application at BBCH 30-32							
2	AT, BE, DE, IE, NL, PL	barley HORVW HORVS	F	<i>B. graminis</i> - ERYSGR <i>Pyrenophora teres</i> - PYRNTE <i>R. secalis</i> - RHYNSE <i>R. collo-cygni</i> - RAMUCC <i>Puccinia hordei</i> - PUCCHD	Spraying (SP)	30 - 59	a) 2 b) 2	14	a) 1.50 b) 3.00	a) 0.100 ¹⁾ / 0.150 ²⁾ / 0.120 ³⁾ b) 0.200 ¹⁾ / 0.300 ²⁾ / 0.240 ³⁾	100 - 300	56								
3	AT, BE, DE, IE, NL, PL	rye SECCW SECCS SECCE	F	<i>R. secalis</i> - RHYNSE <i>Puccinia recondita</i> - PUCCRE	Spraying (SP)	30 - 59	a) 2 b) 2	14	a) 1.50 b) 3.00	a) 0.100 ¹⁾ / 0.150 ²⁾ / 0.120 ³⁾ b) 0.200 ¹⁾ /	100 - 300	56								

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
										0.300 ²⁾ / 0.240 ³⁾										
4	AT, BE, DE, IE, NL, PL	triticale TTLWI TTLSO	F	<i>B. graminis</i> - ERYSGR <i>Septoria spp.</i> - SEPTSP <i>Puccinia recondita</i> - PUCCRE <i>Puccinia striiformis</i> - PUCCST	Spraying (SP)	30 - 59	a) 2 b) 2	14	a) 1.50 b) 3.00	a) 0.100 ¹⁾ / 0.150 ²⁾ / 0.120 ³⁾ b) 0.200 ¹⁾ / 0.300 ²⁾ / 0.240 ³⁾	100 - 300	56								
5	AT, DE, BE, NL, IE	oat AVESA	F	<i>B. graminis</i> - ERYSGR <i>Puccinia coronata</i> - PUCCCA	Spraying (SP)	30 - 59	a) 2 b) 2	14	a) 1.50 b) 3.00	a) 0.100 ¹⁾ / 0.150 ²⁾ / 0.120 ³⁾ b) 0.200 ¹⁾ / 0.300 ²⁾ / 0.240 ³⁾	100 - 300	56								
6	CZ	wheat TRZAW, TRZAS TRZDU, TRZSP	F	<i>Oculimacula spp.</i> - PSDCHE <i>Blumeria graminis</i> - ERYSGR <i>Zymoseptoria tritici</i> - SEPTTR <i>Puccinia triticina</i> - PUCCRT <i>Puccinia striiformis</i> - PUCCST <i>P. tritici-repentis</i> - PYRNTR	Spraying (SP)	30 - 59	a) 1 b) 1		a) 1.00 - 1.50 b) 1.00 - 1.50	a) 0.100 ¹⁾ / 0.150 ²⁾ / 0.120 ³⁾ b) 0.100 ¹⁾ / 0.150 ²⁾ / 0.120 ³⁾	100 - 300	56	For eyespot control, only one application at BBCH 30-32							
7	CZ	barley HORVW HORVS	F	<i>B. graminis</i> - ERYSGR <i>Pyrenophora teres</i> - PYRNTE <i>R. secalis</i> - RHYNSE <i>R. collo-cygni</i> - RAMUCC <i>Puccinia hordei</i> - PUCCHD	Spraying (SP)	30 - 59	a) 1 b) 1		a) 1.00 - 1.50 b) 1.00 - 1.50	a) 0.100 ¹⁾ / 0.150 ²⁾ / 0.120 ³⁾ b) 0.100 ¹⁾ / 0.150 ²⁾ / 0.120 ³⁾	100 - 300	56								
8	CZ	rye SECCW SECCS SECCE	F	<i>R. secalis</i> - RHYNSE <i>Puccinia recondita</i> - PUCCRE	Spraying (SP)	30 - 59	a) 1 b) 1		a) 1.00 - 1.50 b) 1.00 - 1.50	a) 0.100 ¹⁾ / 0.150 ²⁾ / 0.120 ³⁾ b) 0.100 ¹⁾ / 0.150 ²⁾ / 0.120 ³⁾	100 - 300	56								

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
9	CZ	triticale TTLWI TTLWO	F	<i>B. graminis</i> - ERYSGR <i>Septoria spp.</i> - SEPTSP <i>Puccinia recondita</i> - PUCCRE <i>Puccinia striiformis</i> – PUC CST	Spraying (SP)	30 - 59	a) 1 b) 1		a) 1.00 - 1.50 b) 1.00 - 1.50	a) 0.100 ¹⁾ / 0.150 ²⁾ / 0.120 ³⁾ b) 0.100 ¹⁾ / 0.150 ²⁾ / 0.120 ³⁾	100 - 300	56								
10	CZ	oat AVESA	F	<i>B. graminis</i> - ERYSGR <i>Puccinia coronata</i> – PUC CCA	Spraying (SP)	30 - 59	a) 1 b) 1		a) 1.00 - 1.50 b) 1.00 - 1.50	a) 0.100 ¹⁾ / 0.150 ²⁾ / 0.120 ³⁾ b) 0.100 ¹⁾ / 0.150 ²⁾ / 0.120 ³⁾	100 - 300	56								
11	HU, RO, SK	wheat TRZAW, TRZAS TRZDU, TRZSP	F	<i>Oculimacula spp.</i> - PSDCHE <i>Blumeria graminis</i> - ERYSGR <i>Zymoseptoria tritici</i> - SEPTTR <i>Puccinia triticina</i> - PUC CRT <i>Puccinia striiformis</i> - PUC CST <i>P. tritici-repentis</i> – PYRNTR	Spraying (SP)	30 - 59	a) 2 b) 2	14	a) 0.50 - 1.00 b) 0.50 - 2.00	a) 0.067 ¹⁾ / 0.100 ²⁾ / 0.080 ³⁾ b) 0.133 ¹⁾ / 0.200 ²⁾ / 0.160 ³⁾	100 - 300	56	For eyespot control, only one application at BBCH 30-32							
12	HU, RO, SK	barley HORVW HORVS	F	<i>B. graminis</i> - ERYSGR <i>Pyrenophora teres</i> - PYRNTE <i>Puccinia hordei</i> – PUC HD	Spraying (SP)	30 - 59	a) 2 b) 2	14	a) 0.50 - 1.00 b) 0.50 - 2.00	a) 0.067 ¹⁾ / 0.100 ²⁾ / 0.080 ³⁾ b) 0.133 ¹⁾ / 0.200 ²⁾ / 0.160 ³⁾	100 - 300	56								
13	HU, RO, SK	rye SECCW SECCS SECCE	F	<i>R. secalis</i> - RHYNSE <i>Puccinia recondita</i> - PUC CRE	Spraying (SP)	30 - 59	a) 2 b) 2	14	a) 0.50 - 1.00 b) 0.50 - 2.00	a) 0.067 ¹⁾ / 0.100 ²⁾ / 0.080 ³⁾ b) 0.133 ¹⁾ / 0.200 ²⁾ / 0.160 ³⁾	100 - 300	56								

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
14	HU, RO, SK	triticale TTLWI TTLSO	F	<i>B. graminis</i> - ERYSGR <i>Septoria spp.</i> - SEPTSP <i>Puccinia recondita</i> - PUCCRE <i>Puccinia striiformis</i> – PUC CST	Spraying (SP)	30 - 59	a) 2 b) 2	14	a) 0.50 - 1.00 b) 0.50 - 2.00	a) 0.067 ¹⁾ / 0.100 ²⁾ / 0.080 ³⁾ b) 0.133 ¹⁾ / 0.200 ²⁾ / 0.160 ³⁾	100 - 300	56								
15	HU, RO, SK	oat AVESA	F	<i>B. graminis</i> - ERYSGR <i>Puccinia coronata</i> - PUCCCA	Spraying (SP)	30 - 59	a) 2 b) 2	14	a) 0.50 - 1.00 b) 0.50 - 2.00	a) 0.067 ¹⁾ / 0.100 ²⁾ / 0.080 ³⁾ b) 0.133 ¹⁾ / 0.200 ²⁾ / 0.160 ³⁾	100 - 300	56								

* Use number(s) in accordance with the list of all intended GAPs in Part B, Section 0 should be given in column 1

** F: professional field use, Fn: non-professional field use, Fpn: professional and non-professional field use, G: professional greenhouse use, Gn: non-professional greenhouse use, Gpn: professional and non-professional greenhouse use, I: indoor application

1) Mefentrifluconazole

2) Metrafenone

3) Pyraclostrobin

Explanation for column 15 – 21 “Conclusion”

A	Acceptable, Safe use
R	Further refinement and/or risk mitigation measures required
C	To be confirmed by cMS
N	No safe use

Remarks table:

- (1) Numeration necessary to allow references
- (2) Use official codes/nomenclatures of EU
- (3) For crops, the EU and Codex classifications (both) should be used; where relevant, the use situation should be described (e.g. fumigation of a structure)
- (4) F: professional field use, Fn: non-professional field use, Fpn: professional and non-professional field use, G: professional greenhouse use, Gn: non-professional greenhouse use, Gpn: professional and non-professional greenhouse use, I: indoor application
- (5) Scientific names and EPPO-Codes of target pests/diseases/ weeds or when relevant the common names of the pest groups (e.g. biting and sucking insects, soil born insects, foliar fungi, weeds) and the developmental stages of the pests and pest groups at the moment of application must be named
- (6) Method, e.g. high volume spraying, low volume spraying, spreading, dusting, drench
Kind, e.g. overall, broadcast, aerial spraying, row, individual plant, between the plants - type of equipment used must be indicated
- (7) Growth stage at first and last treatment (BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4), including where relevant, information on season at time of application
- (8) The maximum number of application possible under practical conditions of use must be provided
- (9) Minimum interval (in days) between applications of the same product.
- (10) For specific uses other specifications might be possible, e.g.: g/m³ in case of fumigation of empty rooms. See also EPPO-Guideline PP 1/239 Dose expression for plant protection products
- (11) The dimension (g, kg) must be clearly specified. (Maximum) dose of a.s. per treatment (usually g, kg or L product / ha).
- (12) If water volume range depends on application equipments (e.g. ULVA or LVA) it should be mentioned under “application: method/kind”.
- (13) PHI - minimum pre-harvest interval
- (14) Remarks may include: Extent of use/economic importance/restrictions

9.1.1 Overall conclusions

9.1.1.1 Effects on birds (KCP 10.1.1), Effects on terrestrial vertebrates other than birds (KCP 10.1.2), Effects on other terrestrial vertebrate wildlife (reptiles and amphibians) (KCP 10.1.3)

9.1.1.1.1 Effects on birds (KCP 10.1.1)

Dietary risk assessment

Exposure to active substances separately

In the screening step, all TER_A and TER_{LT} values for mefentrifluconazole, metrafenone and pyraclostrobin exceed the triggers set by Commission Regulation (EU) 546/2011 for acceptability of effects.

Exposure to combined active substances and to the formulation

The two acute risk assessment approaches carried out (combined toxicity of the active substances as virtual compound and formulation toxicity) resulted in TER values at the screening or tier 1 level above the trigger of 10 for acceptability of effects. The combined reproductive risk assessment using the concentration addition model resulted in tier 1 TER_{LT combi} values above the trigger of 5 for acceptability of effects.

Therefore, the acute and reproductive dietary risk to birds from BAS 758 00 F according to the proposed use pattern is acceptable.

Drinking water risk assessment

Following EFSA/2009/1438, the puddle scenario is considered relevant for application of BAS 758 00 F according to the proposed use pattern. Since the ratios of the effective application rate to the relevant endpoints are below the value of 3000 for mefentrifluconazole, metrafenone and pyraclostrobin, a quantitative risk assessment for the proposed use pattern of BAS 758 00 F is not necessary.

Secondary poisoning and biomagnification

The log P_{ow} of the active substances mefentrifluconazole, metrafenone and pyraclostrobin are > 3, which triggers an assessment of the potential risk from secondary poisoning for all active substances. According to the tier 1 risk assessments for earthworm- and fish-eating birds, the TER values for mefentrifluconazole, metrafenone and pyraclostrobin are above the trigger value of 5, indicating an acceptable risk for the intended use of BAS 758 00 F.

Low potential for accumulation of mefentrifluconazole, metrafenone and pyraclostrobin in animal tissue was concluded in the respective EU reviews and therefore further evaluation of biomagnification is not necessary.

Overall conclusion

It can be concluded that the risk to birds from the application of BAS 758 00 F according to good agricultural practice is acceptable.

9.1.1.1.2 Effects on terrestrial vertebrates other than birds (KCP 10.1.2)

Dietary risk assessment

Exposure to active substances separately

In the screening step and/or tier 1 risk assessment, all TER_A values for mefentrifluconazole, metrafenone and pyraclostrobin and all TER_{LT} values for mefentrifluconazole and metrafenone exceed the triggers set by Commission Regulation (EU) 546/2011 for acceptability of effects.

For pyraclostrobin, the TER_{LT} values in the tier 1 risk assessment are above the relevant trigger of 5 for acceptability of effects for all scenarios, except for the small herbivorous mammal “vole” scenario at BBCH ≥ 40 . Using field foliage residue dissipation data ~~and applying refined ecological data on the diet of the common vole~~ as well as refinement of the deposition factor, the refined TER_{LT} value for the “vole” scenario is above the trigger of 5.

In conclusion, quantitative risk assessments ~~as well as additional evidence from field effect studies for pyraclostrobin~~ indicate low and acceptable acute and reproductive risks for mammals from the intended uses of BAS 758 00 F according to the proposed use pattern.

Exposure to combined active substances and to formulation

The two acute risk assessment approaches carried out (combined toxicity of the active substances as virtual compound and formulation toxicity) resulted in TER values at the screening or tier 1 level above the trigger of 10 for acceptability of effects. The combined reproductive risk assessment approach resulted in TER_{LT combi} values above the trigger of 5 with the exception of the small herbivorous mammal “vole” scenario at BBCH ≥ 40 . Using field foliage residue dissipation data for pyraclostrobin ~~and mefentrifluconazole and applying refined ecological data on the diet of the common vole~~ as well as refinement of the deposition factor ~~for all three active substances in the formulation~~, the refined TER_{LT combi} value for the small herbivorous mammal “vole” scenario at BBCH ≥ 40 is above the trigger of 5.

Therefore, the acute and reproductive dietary risk to mammals from BAS 758 00 F according to the proposed use pattern is acceptable.

Drinking water risk assessment

Following EFSA/2009/1438, the puddle scenario is the one relevant for mammals. Since the ratios of the effective application rate to the relevant endpoints are below the value of 3000 for mefentrifluconazole, metrafenone and pyraclostrobin, a quantitative risk assessment for the proposed use pattern of BAS 758 00 F is not necessary.

Secondary poisoning and biomagnification

The log P_{ow} of the active substances mefentrifluconazole, metrafenone and pyraclostrobin are > 3 , which triggers an assessment of the potential risk from secondary poisoning for all three active substances. According to the tier 1 risk assessment for earthworm- and fish-eating mammals, the TER values for mefentrifluconazole, metrafenone and pyraclostrobin are above the trigger value of 5, indicating an acceptable risk for the intended use of BAS 758 00 F.

Low potential for accumulation of mefentrifluconazole, metrafenone and pyraclostrobin in animal tissue was concluded in the respective EU reviews and therefore further evaluation of biomagnification is not necessary.

Overall conclusion

It can be concluded that the risk to mammals from the application of BAS 758 00 F according to good agricultural practice is acceptable.

9.1.1.1.3 Effects on other terrestrial vertebrate wildlife (reptiles and amphibians) (KCP 10.1.3)

In the EU, there is no requirement to test terrestrial amphibians or reptiles and there is also no guidance available on how to conduct risk assessments for these groups. There are no studies in the open literature on amphibians and reptiles for the active substances mefentrifluconazole (BAS 750 F) and metrafenone (BAS 560 F) or the product BAS 758 00 F, or other information from the field related to potential negative impacts from the use of this product on amphibians or reptiles. There is some information in the literature concerning formulations containing the active substance pyraclostrobin (BAS 500 F). However, BASF is not aware of any incidents where amphibians or reptiles were affected by pyraclostrobin applications following the label instructions. This lack of effects at field rates was confirmed by BASF-conducted semi-field studies in cereals showing no effects on small juvenile common frogs and toads (see the AIR3 dossier for more information).

In conclusion, the risk to terrestrial life stages of amphibians from applications of BAS 758 00 F according to good agricultural practice under realistic field conditions will be low.

9.1.1.2 Effects on aquatic organisms (KCP 10.2)

The standard risk assessment for the active substances mefentrifluconazole and metrafenone indicates an acceptable risk for all groups of aquatic organisms following the intended uses of BAS 758 00 F ‘in spring and winter cereals’ with no need for additional mitigation measures. Regarding the active substance pyraclostrobin, acute and chronic PEC/RAC ratios for fish and aquatic invertebrates did not meet the required trigger value of 1 for application of BAS 758 00 F in ‘spring and winter cereals’, based on standard worst-case assumptions. However, a range of higher-tier studies and approaches (such as a mesocosm study, an SSD for acute fish and a WoE approach for chronic fish) allow a refined risk assessment that indicates an acceptable risk if non-sprayed buffer zones of 5 m or the use of 50% drift reducing nozzles are considered.

Single and twofold application (1x and 2 x 1 L/ha) of BAS 758 00 F

A non-sprayed buffer zone of 5 m or 50% drift reducing nozzles are considered for the intended use of BAS 758 00 F in ‘spring cereals’ for scenarios D4 and D5. A non-sprayed buffer zone of 5 m or 75% drift reducing nozzles are considered for the intended use of BAS 758 00 F in ‘spring cereals’ for scenarios D3. For scenario R4 non-sprayed, vegetated buffer zone of 10 m is required.

Single and twofold application (1x and 2 x 1.5 L/ha) of BAS 758 00 F

A non-sprayed buffer zone of 5 m or 75% drift reducing nozzles are considered for the intended use of BAS 758 00 F in ‘spring cereals’ for scenarios D3, D4 and D5. For scenario R4 non-sprayed, vegetated buffer zone of 10 m is required.

Single and twofold application (1x and 2 x 1 L/ha) of BAS 758 00 F

A non-sprayed buffer zone of 5 m or 50% drift reducing nozzles are considered for the intended use of BAS 758 00 F in ‘winter cereals’ for scenarios D4, D5, R1 and R3. A non-sprayed buffer zone of 5 m or 75% drift reducing nozzles are considered for the intended use of BAS 758 00 F in ‘winter cereals’ for scenarios D3. For scenario R4 non-sprayed, vegetated buffer zone of 10 m is required.

Single and twofold application (1x and 2 x 1.5 L/ha) of BAS 758 00 F

A non-sprayed buffer zone of 5 m or 75 % drift reducing nozzles are considered for the intended use of BAS 758 00 F in 'winter cereals' for scenarios D3, D4 and D5. For scenarios R1, R3 and R4 non-sprayed, vegetated buffer zone of 10 m is required.

The PEC/RAC ratios for the relevant metabolites of mefentrifluconazole, metrafenone and pyraclostrobin are significantly below the trigger of 1 based on standard worst-case assumptions or of negligible relevance in aquatic systems for all proposed uses; they are thus considered not to be of ecotoxicological relevance.

Studies performed with the formulated product BAS 758 00 F indicate no significantly higher (or unexpected) toxicity than predicted based on the results of the active substance for fish, aquatic invertebrates and algae. Toxic unit calculations for fish and aquatic invertebrates indicated that pyraclostrobin is driving the toxicity of the formulated product. The formulation risk assessment revealed an acceptable risk to algae and aquatic plants following the intended uses of BAS 758 00 F in 'spring and winter cereals' with no need for additional mitigation measures.

The standard and refined risk assessment for the fungicidal product BAS 758 00 F, the active substances mefentrifluconazole, metrafenone and pyraclostrobin as well as their major metabolites demonstrates that the application of BAS 758 00 F in 'spring and winter cereals' according to good agricultural practice is of low risk to aquatic ecosystems if a non-sprayed buffer zone of 5 m or 50% drift-reducing nozzles are employed.

9.1.1.3 Effects on bees (KCP 10.3.1)

The risk to honey bees from the use of mefentrifluconazole, metrafenone, pyraclostrobin and BAS 758 00 F was assessed using the maximum single application rate and the LD₅₀ values to calculate hazard quotients (HQ) for oral exposure (Q_{HO}) and contact exposure (Q_{HC}) [OEPP/EPPO, 2010: *Environmental risk assessment scheme for plant protection products, Chapter 10: Honeybees* (PP 3/10 (3), *Bulletin OEPP/EPPO Bulletin 40*, 323–331)]. Furthermore, under Regulation (EC) No 1107/2009, no risk assessment scheme exists currently for chronic honey bee or honey bee larvae studies. In the absence of clear guidance (noted and agreed by member states) a preliminary risk assessment according to the current legal requirements (SANCO/10329/2002 and EPPO 2010) has been conducted.

The hazard quotients for BAS 758 00 F and the active substances mefentrifluconazole, metrafenone and pyraclostrobin for acute oral and acute contact exposure of honey bees are considerably below the Commission Regulation (EU) 546/2011 trigger value of 50. Additionally, the chronic TER for larvae and adult bees exceed the suggested trigger. Considering the very protective assumptions the risk can be considered acceptable.

Based on these results it can be concluded that low risk to honey bees is expected from applications of BAS 758 00 F according to the proposed uses. This is confirmed by a worst case assessment following EPPO (2010) for chronic adult and honey bee larvae as well as a honey bee semi-field study with BAS 758 00 F.

9.1.1.4 Effects on arthropods other than bees (KCP 10.3.2)

The testing and risk assessment strategy used here follow the approach recommended in the ESCORT 2 guidance document, ESCORT 3, and the EC Guidance Document on Terrestrial Ecotoxicology (SANCO/10329, 17 October 2002). The risk assessment for BAS 758 00 F is based on Tier I tests with the standard test species *Typhlodromus pyri* and *Aphidius rhopalosiphi* and Tier II tests on *A. rhopalosiphi* and *Chrysoperla carnea* as well as an aged residue study on *C. carnea*. The risk assessment is based on the worst-case application rate according to the proposed use pattern.

Based on the results of the conducted first and higher tier risk assessments it can be concluded that low risk for non-target arthropods is expected from the use of BAS 758 00 F according to the proposed use pattern. No unacceptable effects on non-target arthropods are expected in in-field and off-field habitats.

9.1.1.5 Effects on non-target soil meso- and macrofauna (KCP 10.4), Effects on soil microbial activity (KCP 10.5)

The evaluation of the risk for earthworms and other non-target soil organisms (meso- and macrofauna), as well as for soil microorganisms was performed in accordance with the recommendations of the “Guidance Document on Terrestrial Ecotoxicology”, as provided by the Commission Services (SANCO/10329/2002 rev 2 (final), October 17, 2002).

Effects on non-target soil meso- and macrofauna

The potential risk of BAS 758 00 F, mefentrifluconazole, metrafenone, pyraclostrobin and the relevant metabolites to earthworms and other non-target soil macro-organisms was assessed by comparing the maximum PEC_{soil} values with NOEC or EC_{10} values, to generate long-term TER values (TER_{lt}).

All TER values for BAS 758 00 F, mefentrifluconazole, metrafenone, pyraclostrobin and the relevant metabolites for chronic exposure of earthworms and other non-target soil organisms (meso- and macrofauna) are considerably higher than the Commission Regulation (EU) 546/2011 trigger value of 5. This indicates that BAS 758 00 F poses no unacceptable risk to earthworms and other non-target soil organisms (meso- and macrofauna) when applied according to the proposed use rate.

Effects on soil microbial activity

The potential risk of BAS 758 00 F, mefentrifluconazole, metrafenone, pyraclostrobin and the relevant metabolites to soil micro-organisms was assessed by comparing the maximum PEC_{soil} values with the maximum concentration with effects $\leq 25\%$.

For the formulation BAS 758 00 F, the active substances mefentrifluconazole, metrafenone and pyraclostrobin as well as their relevant metabolites, the maximum concentration with effects $< 25\%$ (SANCO/10329/2002 trigger) are all above the maximum PEC_{soil} values. Therefore, it is concluded that the use of BAS 758 00 F will not pose an unacceptable risk to non-target soil micro-organisms, if applied according to good agricultural practice.

9.1.1.6 Effects on non-target terrestrial plants (KCP 10.6)

The toxicity of BAS 758 00 F to non-target terrestrial plants has been investigated by carrying out vegetative vigour and seedling emergence studies with up to six dicotyledonous and four monocotyledonous non-target plant species. Plants showed similar sensitivity to pre- emergence exposure than to post-emergence exposure. The risk assessment is thus carried out with the respective most sensitive endpoints obtained from the vegetative vigour and seedling emergence tests.

The risk assessment is based on the “Guidance Document on Terrestrial Ecotoxicology”, (SANCO/10329/2002 rev.2 final, 2002). It is restricted to off-field areas, as non-target plants are non-crop plants located outside the treated area. The amount of spray drift reaching off-crop habitats is calculated using the 90th percentile estimates in Appendix IV of ESCORT 2. For a single application to field crops and vegetables < 50 cm, 2.77% of the application rate was assumed to reach areas at 1 m from the edge of the crop (worst-case scenario). The highest single application rate of BAS 758 00 F is used to calculate the maximum off-field predicted environmental rate ($PER_{off-field}$). The potential risk of BAS 758 00 F to non-

target plants was assessed by comparing the calculated PER value to the ER_{50} values in order to generate TER values (TER).

Based on the results of the greenhouse trials, all the TER values were above the standard trigger of 5.

Based on the risk assessment it can be concluded that BAS 758 00 F poses no unacceptable risk to non-target plants, if applied according to the recommended use pattern. Particular precautions to reduce the environmental concentrations resulting from BAS 758 00 F applications are not required for the protection of terrestrial non-target plants.

9.1.1.7 Effects on other terrestrial organisms (flora and fauna) (KCP 10.7)

Not relevant.

9.1.2 Grouping of intended uses for risk assessment

The following table documents the grouping of the intended uses to support application of the risk envelope approach (according to SANCO/11244/2011).

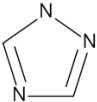
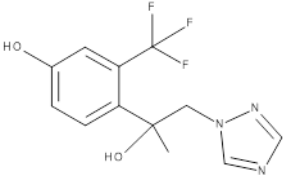
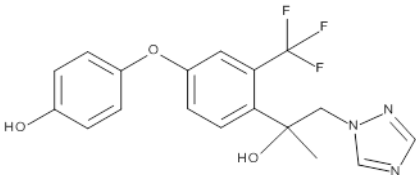
Table 9.1-2: Critical use pattern of BAS 758 00 F grouped according to applications

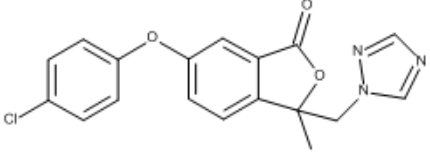
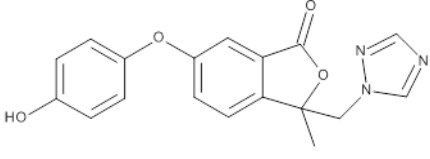
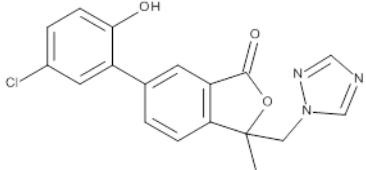
Grouping according to worst-case application				
Area	Group	Intended uses	Relevant use parameters for grouping	Relevant parameter or value for sorting
Birds and mammals	Application rate and number of applications	All intended uses	EFSA crop group: Cereals	Maximum worst-case application rate = 2 x 1.5 L/ha (corresponding to 2 x 0.100 kg mefentrifluconazole/ha, 2 x 0.150 kg metrafenone/ha and 2x 0.120 kg pyraclostrobin/ha)
Aquatic organisms	Grouping according to Section 8 – Environmental Fate			
Bees, non-target plants	Application rate	All intended uses	Risk assessments are based on the maximum single application rate of 1 x 1.5 L/ha (corresponding to 1 x 0.100 kg mefentrifluconazole, 1 x 0.150 kg metrafenone and 1 x 0.120 kg pyraclostrobin/ha), which covers all other intended uses.	Maximum application rate = 1.5 L/ha
Non-target arthropods	Application rate	All intended uses	Risk assessments are based on the maximum application rate of 2 x 1.5 L/ha (corresponding to 2 x 0.100 kg mefentrifluconazole, 2 x 0.150 kg metrafenone and 2 x 0.120 kg pyraclostrobin/ha), which covers all other intended uses	Maximum application rate = 2 x 1.5 L/ha
Soil macro- and micro-organisms	Worst case PEC _{soil} value	All intended uses	Risk assessment is based on the worst case PEC _{soil} value derived from maximum application rate of 2 x 0.100 kg mefentrifluconazole, 2 x 0.150 kg metrafenone and 2 x 0.120 kg pyraclostrobin/ha), which covers all other intended uses	Maximum application rate = 2 x 1.5 L /ha

9.1.3 Consideration of metabolites

A list of metabolites found in environmental compartments is provided below. The need for conducting a metabolite-specific risk assessment in the context of the evaluation of BAS 758 00 F is indicated in the table. Metabolites relevant in other areas than soil and aquatics will be dealt in the respective parts of this dossier.

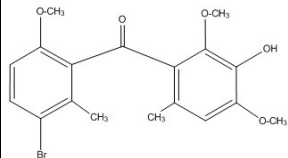
Table 9.1-3 Metabolites of mefentrifluconazole

Metabolite	Chemical structure	Molar mass [g mol ⁻¹]	Maximum occurrence in compartments [%]	Risk assessment required?
M750F001 (1,2,4- triazole) Reg. No. 87084		69.1	Soil: 5.1 ^a Water: 10.2 Sediment: 4.9 Total w/s system: 15.1	Terrestrial Metabolite relevant for RA: yes RA conducted: yes Aquatic Metabolite relevant for RA: yes RA conducted: yes
M750F003 Reg. No. 5924326		287.2	Soil: 1.8 Water: 3.8 Sediment: 5.4 Total w/s system: 8.5	Terrestrial Metabolite relevant for RA: no RA conducted: no Aquatic Metabolite relevant for RA: yes RA conducted: yes
M750F005 Reg. No. 6003433		379.3	Soil: not detected in soil Water: 32.2 (max. in aqueous photolysis study) Sediment: not detected in sediment Total w/s system: not detected in w/s study	Terrestrial Metabolite relevant for RA: no RA conducted: no Aquatic Metabolite relevant for RA: yes RA conducted: yes

Metabolite	Chemical structure	Molar mass [g mol ⁻¹]	Maximum occurrence in compartments [%]	Risk assessment required?
M750F006 Reg. No. 5863469		355.8	Soil: not detected in soil Water: 30.7 (max. in aqueous photolysis study) Sediment: not detected in sediment Total w/s system: not detected in w/s study	Terrestrial Metabolite relevant for RA: no RA conducted: no Aquatic Metabolite relevant for RA: yes RA conducted: yes
M750F007 Reg. No. 6003432		337.3	Soil: not detected in soil Water: 43.9 (max. in aqueous photolysis study) Sediment: not detected in sediment Total w/s system: not detected in w/s study	Terrestrial Metabolite relevant for RA: no RA conducted: no Aquatic Metabolite relevant for RA: yes RA conducted: yes
M750F008 Reg. No. 6010286		355.8	Soil: not detected in soil Water: 7.3 (max. in aqueous photolysis study) Sediment: not detected in sediment Total w/s system: not detected in w/s study	Terrestrial Metabolite relevant for RA: no RA conducted: no Aquatic Metabolite relevant for RA: yes RA conducted: yes

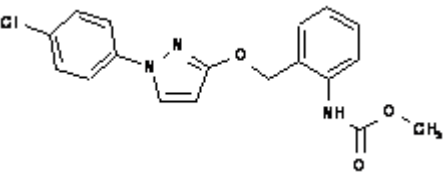
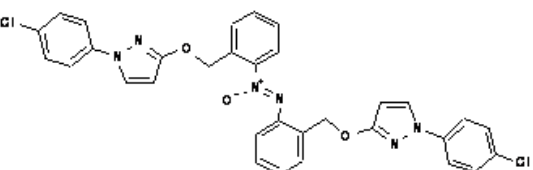
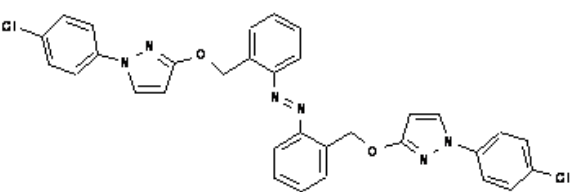
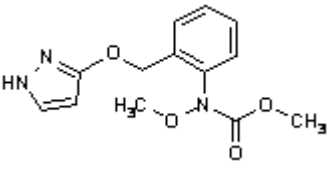
^a The metabolite was observed at a single time point above 5% in one soil (max. 5.1% at 90 d with subsequent decline – average of two replicates). For precautionary reasons, it was included in the exposure assessment for soil and groundwater

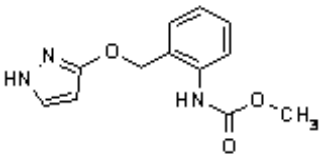
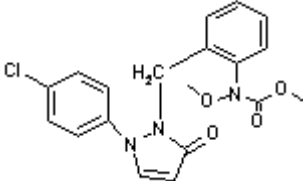
Table 9.1-4 Metabolites of metrafenone

Metabolite	Chemical structure	Molar mass	Maximum occurrence in compartments	Risk assessment required?
CL 377160		395.3	18.9% in soil	Terrestrial Metabolite relevant for RA: yes RA conducted: yes

Note: To support the approval renewal of metrafenone, additional studies have been performed to update the supporting data package to meet the requirements as set out in Regulations (EU) Nos 283/2013 and 284/2013. Therefore, data on additional metabolites of metrafenone which are not ecologically relevant according to Commission Regulation (EU) 544/2011, are available and can be submitted on request.

Table 9.1-5 Metabolites of pyraclostrobin

Metabolite	Chemical structure	Molar mass	Maximum occurrence in compartments	Risk assessment required?
BF 500-3 "des-methoxy" 500M07		357	Soil: max. 95.8% after 7 d (anaerobic degradation study, not found in field studies) Water: max. 2.3% after 61 d Sediment: max. 65.7% after 14 d (river system), max. 12% after 100 d (pond system)	Terrestrial not relevant Aquatic Metabolite relevant for RA: yes RA conducted: yes
BF 500-6 "azoxy" 500M01	 cis-trans isomerization possible	611	Soil: max. 30.9% after 120 d (aerobic laboratory degradation study, only found sporadically in minor quantities in field studies) Sediment: max. 6.5% after 61 d (only in pond system)	Terrestrial Metabolite relevant for RA: yes RA conducted: yes Aquatic Metabolite relevant for RA: yes RA conducted: yes
BF 500-7 "azo" 500M02	 cis-trans isomerization possible	596	Soil: max. 12.5% after 62 d (aerobic laboratory degradation study, not found in field studies) Sediment: max. 6.3% after 61 d (only in pond system)	Terrestrial Metabolite relevant for RA: yes RA conducted: yes Aquatic Metabolite relevant for RA: yes RA conducted: yes
BF 500-11 "M277" 500M60		277	<i>Photolysis study</i> Water: 44.5% after 21 d (tolyl label) <i>Irradiated water/sediment study:</i> Water: max. 11.4% after 21 d Sediment: max. 0.6% after 62 d	Terrestrial Metabolite relevant for RA: no RA conducted: no Aquatic Metabolite relevant for RA: yes RA conducted: yes

Metabolite	Chemical structure	Molar mass	Maximum occurrence in compartments	Risk assessment required?
BF 500-13 "M247" 500M62		247	<i>Photolysis study</i> Water: 16.8% after 6 d (tolyl label) <i>Irradiated water/sediment study:</i> Water: max. 15.7% after 62 d Sediment: max. 2.1% after 45 d	Terrestrial Metabolite relevant for RA: no RA conducted: no Aquatic Metabolite relevant for RA: yes RA conducted: yes
BF 500-14 "M387TypeA" 500M76		387	<i>Photolysis study</i> Water: 14.8 % after 6 h (tolyl label) <i>Irradiated water/sediment study:</i> Water: max. 11.4% after 14 d Sediment: max. 0.7% after 7 d	Terrestrial Metabolite relevant for RA: no RA conducted: no Aquatic Metabolite relevant for RA: yes RA conducted: yes

9.2 Effects on birds (KCP 10.1.1)

The risk assessment for birds is carried out following the latest guidance document by EFSA (*Anonymous 2009: Guidance Document on risk assessment for Birds & Mammals on request from EFSA. EFSA Journal 2009; 7(12):1438. European Food Safety Authority*), hereafter cited as EFSA/2009/1438.

9.2.1 Toxicity data

Avian toxicity studies have been carried out with mefentrifluconazole, metrafenone and pyraclostrobin. Full details of these studies are provided in the respective EU DAR and related documents.

Active substances

An overview of the EU agreed endpoints is given in Table 9.2-1 for mefentrifluconazole and in Table 9.2-2 for metrafenone. In case the selection of studies and endpoints for the risk assessment deviates from the results of the EU review process, justifications are provided below.

Two new bird toxicity studies have been submitted in the course of the ongoing renewal process for metrafenone. Therefore, the EU renewal process for metrafenone and the latest version (October 2018) of the Renewal Assessment Report (RAR) of metrafenone presented by RMS Latvia have been considered for the two newly submitted studies.

An overview of the EU agreed endpoints for pyraclostrobin is given in Table 9.2-3. The selection of studies and endpoints for the risk assessment of pyraclostrobin reflects the current status of the ongoing EU renewal process for pyraclostrobin and the latest version (January 2020) of the Renewal Assessment Report (RAR) of pyraclostrobin presented by RMS Germany. In some cases these endpoints deviate from the results of the previous EU review process (SANCO/1420/2001-Final. 2004, 1-24). Justifications are provided below. A new study is available for pyraclostrobin (BASF DocID 2013/1400375) and being reviewed in the ongoing EU renewal process for pyraclostrobin (RAR, 2020). This study is listed in Appendix 1 and summarized in Appendix 2.

Review Comments:

The zRMS is of the opinion that the endpoints given in the current LoEP of metrafenone and pyraclostrobin should be considered for the risk assessment.

For birds, the new endpoint of both active substances will not be used in the evaluation.

Table 9.2-1: Mefentrifluconazole (BAS 750 F): Endpoints relevant for the risk assessment for birds

Species	Substance	Exposure System	Results	Reference [BASF DocID]
<i>Colinus virginianus</i>	Mefentrifluconazole	Oral, 1 d Acute	LD ₅₀ = 816 mg/kg bw	EFSA Journal 2018;16(7):5379 [2014/1095701]
<i>Anas platyrhynchos</i>	Mefentrifluconazole	Oral, 1 d Acute	LDD ₅₀ > 2000 mg/kg bw	EFSA Journal 2018;16(7):5379 [2014/1095700]
<i>Serinus canaria</i>	Mefentrifluconazole	Oral, 1 d Acute	LD ₅₀ > 2860 mg/kg bw	EFSA Journal 2018;16(7):5379 [2015/1085493]
<i>Colinus virginianus</i>	Mefentrifluconazole	Dietary, 8d Short-term	LC ₅₀ = 6377 mg/kg diet LDD ₅₀ = 858 mg/kg bw/d	DAR (2017) [2014/1127963, amendment 2015/1223324]
<i>Anas platyrhynchos</i>	Mefentrifluconazole	Dietary, 8d Short-term	LC ₅₀ = 8347 mg/kg diet LDD ₅₀ = 1213 mg/kg bw/d	DAR (2017) [2014/1117035]
<i>Colinus virginianus</i>	Mefentrifluconazole	Dietary Reproductive toxicity	NOEL = 25.3 mg/kg bw/d	EFSA Journal 2018;16(7):5379 [2013/1281276]
<i>Anas platyrhynchos</i>	Mefentrifluconazole	Dietary Reproductive toxicity	NOEL = 80.5 mg/kg bw/d	EFSA Journal 2018;16(7):5379 [2015/7005819]
Endpoint used for acute risk assessment	Mefentrifluconazole	Oral, 1d Acute	LD₅₀ = 816 mg/kg bw	EFSA Journal 2018;16(7):5379 [2014/1095701]
Endpoint used for reproductive risk assessment	Mefentrifluconazole	Dietary Reproductive toxicity	NOEL = 25.3 mg/kg bw/d	EFSA Journal 2018;16(7):5379 [2013/1281276]

Table 9.2-2: Metrafenone (BAS 560 F): Endpoints relevant for the risk assessment for birds

Species	Substance	Exposure System	Results	Reference [BASF DocID]
<i>Colinus virginianus</i>	Metrafenone	Oral, 1 d Acute	LD ₅₀ > 2025 mg a.s./kg bw	EFSA Scientific Report (2006) 58, 1-72; [2000/7000117]
<i>Anas platyrhynchos</i>	Metrafenone	Oral, 1 d Acute	LD ₅₀ > 2025 mg a.s./kg bw	EFSA Scientific Report (2006) 58, 1-72; [2000/7000115]
<i>Taeniopygia guttata</i>	Metrafenone	Oral, 1 d Acute	LD ₅₀ > 2000 mg a.s./kg bw	RAR, October 2018; [2011/1263863]
<i>Colinus virginianus</i>	Metrafenone	Dietary Reproductive toxicity	NOEC = 1 350 mg a.s./kg diet NOEL = 125.4 mg a.s./kg bw/day	EFSA Scientific Report (2006) 58, 1-72; [2002/7005090]
<i>Anas platyrhynchos</i>	Metrafenone	Dietary Reproductive toxicity	NOEC = 900 mg a.s./kg diet NOEL = 114.7 mg a.s./kg bw/day	RAR, October 2018; [2006/1018046]
Endpoint used for acute assessment	Metrafenone	Oral, 1 d Acute	LD₅₀ (extrapolated, geometric mean) = 3807 mg/kg bw > 2025 mg a.s./kg bw	Extrapolation and geometric mean of quail, mallard and zebra finch LD₅₀ [2000/7000117, 2000/7000115, 2011/1263863]
Endpoint used for reproductive assessment	Metrafenone	Dietary Reproductive toxicity	NOEL = 114.7 mg a.s./kg bw/day 125.4 mg a.s./kg bw/day	RAR, October 2018; [2006/1018046]

Table 9.2-3: Pyraclostrobin (BAS 500 F): Endpoints relevant for the risk assessment for birds

Species	Substance	Exposure System	Results	Reference [BASF DocID]
<i>Colinus virginianus</i>	Pyraclostrobin	Oral, 1 d Acute	LD ₅₀ > 2000 mg a.s./kg bw	RAR; Rev.1; 10. Jan. 2020 [1997/11136] SANCO/1420/2001-Final. 2004, 1-24
<i>Serinus canaria</i>	Pyraclostrobin	Oral, 1 d Acute	LD₅₀ > 1446 mg a.s./kg bw	RAR; Rev.1; 10. Jan. 2020 [2013/1400375]¹⁾
<i>Colinus virginianus</i>	Pyraclostrobin	Dietary Reproductive toxicity	NOEL = 105 mg a.s./kg bw/d	RAR; Rev.1; 10. Jan. 2020 [1999/11207] SANCO/1420/2001-Final. 2004, 1-24
<i>Anas platyrhynchos</i>	Pyraclostrobin	Dietary Reproductive toxicity	NOEL = 128 mg a.s./kg bw/d	RAR; Rev.1; 10. Jan. 2020 [1999/11206] SANCO/1420/2001-Final. 2004, 1-24
Endpoint used for acute assessment	Pyraclostrobin	Oral, 1 d Acute	LD₅₀ (geometric mean) = 1701 mg/kg bw > 2000 mg a.s./kg bw	RAR; Rev.1; 10. Jan. 2020 [1997/11136 and 2013/1400375]¹⁾ SANCO/1420/2001-Final. 2004, 1-24
Endpoint used for reproductive assessment	Pyraclostrobin	Dietary Reproductive toxicity – Tier 1	NOEL = 105 mg a.s./kg bw/d	RAR; Rev.1; 10. Jan. 2020 [1999/11207] SANCO/1420/2001-Final. 2004, 1-24

¹⁾ This study was not evaluated in the previous EU review process (SANCO/1420/2001-Final. 2004, 1-24), but is now included and evaluated in the Renewal Assessment Report (RAR); Rev.1; 10. Jan. 2020 presented by RMS Germany.

Metabolites

Metabolites of mefentrifluconazole

According to the EFSA conclusion regarding the peer review of mefentrifluconazole (EFSA Journal 2018; 16(7): 5379), it was concluded that no specific risk assessment for birds and mammals for any of the mefentrifluconazole metabolites is necessary. Therefore, no risk assessment for metabolites is presented in this dossier.

Metabolites of metrafenone

No relevant metabolites of metrafenone occur in plants. In conclusion, the risk to birds from metabolites of metrafenone is covered by the risk as evaluated for the parent metrafenone.

Metabolites of pyraclostrobin

The metabolism of pyraclostrobin in potential food items of wild living birds or mammals (i.e., green plant matter, fruits, or seeds) was investigated in plant metabolism studies in grapes (BASF DocIDs 1998/10988 and 2000/1000201), potatoes (BASF DocIDs 1999/11419 and 2000/1000048) and wheat (BASF DocID 1999/11137). Most metabolites occurred only at trace amounts far below 10% TRR in the potential food items. The only metabolite that occurred at higher levels in potential food items was the desmethyl metabolite (BF 500-3, synonym: 500M07). It was found at levels of up to 15.3% TRR in grapes, 21.4% TRR in green matter of potatoes, 13.1% TRR in wheat forage, and 10.5% TRR in wheat grain. However, this desmethyl metabolite was also detectable in rats (faeces) at levels of up to 5.77% TRR (BASF DocID 1999/11781), at 21.7% TRR in goats (BASF DocID 2000/1000004) and at 38.9% TRR in hens (BASF DocID 1999/11480). Hence, it can be concluded that the mammalian and avian toxicity studies with pyraclostrobin cover this metabolite, and that the dietary risk assessment for pyraclostrobin provided for birds and mammals covers the potential risk from this metabolite.

Water metabolites are of minor importance for the risk assessment for wild living birds and mammals, considering the predominant route of exposure being via food items like plants, seeds, or arthropods. Water uptake itself or exposure via the aquatic compartment, however, can play a role in the drinking water and the secondary poisoning risk assessment for fish-eaters. Hence in the following part the risk from relevant surface water metabolites to birds and mammals is considered. The metabolites BF 500-5, BF 500-11, BF 500-13, BF 500-14 were found at levels slightly above 10% TAR in surface water (for details see section 8). However, the risk from these metabolites to birds and mammals is considered to be covered by the presented risk assessments for the parent compound pyraclostrobin due to the following reasons:

- i) PEC values of the metabolites are far below the PEC values of the parent (for details see section 8).
- ii) There are no specific toxicity studies with the metabolites BF 500-5, BF 500-11, BF 500-13, BF 500-14 available in birds or mammals. However, the low toxicity of the metabolites was confirmed in standard acute fish toxicity studies with *O. mykiss* (for details see chapter 9.5).
- iii) An assessment of the risk of secondary poisoning from these metabolites is not triggered because all have a $\log P_{ow} < 3$ (1.8 for BF 500-5, 1.87 for BF 500-11, 1.71 for BF 500-13, 2.54 for BF 500-14).

Soil metabolites can contribute to secondary poisoning via consumption of earthworms, so the risk to birds and mammals from relevant soil metabolites is considered. In the aerobic soil study, the metabolites BF 500-6 and BF 500-7 occurred at >10% TAR (for details see section 8). However, the risk from these metabolites to birds and mammals is considered to be covered by the presented risk assessments for the parent compound pyraclostrobin due to the following reasons:

- i) PEC values of the metabolites are far below the PEC values of the parent (for details see section 8).
- ii) There are no specific toxicity studies with the metabolites BF 500-6 and BF 500-7 available in birds or mammals, but the low toxicity of these two metabolites was confirmed in standard acute and chronic earthworm studies with *Eisenia fetida* (for details see chapter 9.8).

Formulation toxicity

An acute oral toxicity study with BAS 758 00 F in northern bobwhite quails (*Colinus virginianus*) was carried out according to OECD 223 (BASF DocID 2021/2037989, see Appendix A 2.1.1.1), as required by EU Commission Regulation No. 284/2013 due to the acute toxicity of the formulation to rats. No mortality occurred in the highest dose tested and therefore the LD₅₀ after oral administration of BAS 758 00 F was >2000 mg formulation/kg bw. This indicates low toxicity of the formulation and no increased toxicity of the formulation compared to the active substances. Consequently, the acute risk from the formulation is covered by the acute risk assessment for the active substances.

9.2.1.1 Justification for new endpoints

Mefentrifluconazole

Acute – Not applicable. Endpoint is EU agreed.

Reproductive – Not applicable. Endpoint is EU agreed.

Metrafenone

~~Acute – Because no treatment-related mortality occurred in the acute oral toxicity studies in the bobwhite quail (BASF DocID 2000/7000117), mallard duck (BASF DocID 2000/7000115) and zebra finch (BASF DocID 2011/1263863), the endpoints from each study (> 2025 mg a.s./kg bw and > 2000 mg a.s./kg bw) were extrapolated using a factor of 1.888 to LD_{50 (extrapolated)} = 3823 mg a.s./kg bw for both the bobwhite quail and mallard duck, and to LD_{50 (extrapolated)} = 3776 mg a.s./kg bw for the zebra finch, in accordance with EFSA/2009/1438. The geometric mean out of three extrapolated LD₅₀ values resulted in an LD_{50 (extrapolated, geometric mean)} = 3807 mg a.s./kg bw.~~

~~Reproductive – For the long term risk assessment, either the lowest NOAEL from reproductive toxicity studies or the LD₅₀/10 (> 200 mg a.s./kg bw) is used, as specified in EFSA/2009/1438. In the case of metrafenone, the endpoint derived from the mallard duck reproductive toxicity study (114.7 mg a.s./kg bw/d) is the lowest endpoint.~~

Acute – Not applicable. Endpoint is EU agreed. At the screening step LD₅₀ value of >2000 mg a.s./kg bw will be used in the risk assessment.

Reproductive – Not applicable. Endpoint is EU agreed.

Pyraclostrobin

~~Acute – In line with the latest evaluation in the ongoing EU renewal process for pyraclostrobin (RAR, 2020) the LD₅₀ (geometric mean) = 1701 mg a.s./kg bw of the acute oral gavage study in the quail (BASF DocID 1997/11136) and the canary (BASF DocID 2013/1400375) is the relevant endpoint for the acute risk assessment.~~

Acute – Not applicable. Endpoint is EU agreed.

Reproductive – Not applicable. The endpoint is EU-agreed in the previous EU process of pyraclostrobin (SANCO/1420/2001-Final. 2004, 1-24) and no deviation is proposed in the ongoing EU renewal process.

9.2.2 Risk assessment for spray applications

Proposed use pattern for the risk assessments

The proposed use pattern for the use of BAS 758 00 F is summarized in Table 9.2-4. The detailed use pattern table is presented at the beginning of the ecotoxicology chapter (section 9.1).

Table 9.2-4: Proposed use pattern

Crop	Crop group according to EFSA/2009/1438	Application time (BBCH growth stage)	Number of applications	Interval between applications [d]	Application rate per application			
					Mefentrifluconazole [kg/ha]	Metrafenone [kg/ha]	Pyraclostrobin [kg/ha]	BAS 758 00 F [L/ha]
Cereals (winter, spring)	Cereals	30-59	2	14	0.100	0.150	0.120	1.5
		30-59	1	--	0.100	0.150	0.120	1.5
		30-59	2	14	0.067	0.100	0.080	1.0

The application scenario with the highest number of applications and the highest use rate shown **in bold** is used for the risk assessment and covers all possible application scenarios according to the GAP in section 9.1

9.2.2.1 First-tier assessment (screening/generic focal species)

The dietary TER acute (TER_A) and reproductive (TER_{LT}) values for the screening step and tier 1 risk assessment were calculated with the EFSA calculator tool (version of 9 July 2010, <http://www.efsa.europa.eu/de/efsajournal/pub/1438.htm>) according to EFSA/2009/1438.

Dietary risk assessment for the active substances

Acute risk assessment

The dietary TER acute values for the screening step are presented in Table 9.2-5 (mefentrifluconazole), Table 9.2-6 (metrafenone), and Table 9.2-7 (pyraclostrobin). All TER_A values for mefentrifluconazole, metrafenone and pyraclostrobin are above the relevant trigger of 10 for acceptability of acute effects at the screening step.

Table 9.2-5: Mefentrifluconazole: Screening step calculations of the acute risk for birds due to the use of BAS 758 00 F for the crop group “cereals”

Data from Data_Entry worksheet	Crop	Application rate (kg a.s./ha)	Number of applications	Application Interval	DT ₅₀	LD ₅₀	
	Cereals	0.1	2	14	10.0	816.0	
Screening step:							
Acute risk assessment screening step	Indicator species	Shortcut value	Daily Dietary Dose (single)	MAF (90)	Daily Dietary Dose (Multiple)	TER	No refinement step required
	Small omnivorous bird	158.8	15.88	1.2	19.06	42.8	

Table 9.2-6: Metrafenone: Screening step calculations of the acute risk for birds due to the use of BAS 758 00 F for the crop group “cereals”

Data from Data_Entry worksheet	Crop	Application rate (kg a.s./ha)	Number of applications	Application Interval	DT ₅₀	LD ₅₀	
	Cereals	0.15	2	14	10.0	3807.0 ≥2025	
Screening step:							
Acute risk assessment screening step	Indicator species	Shortcut value	Daily Dietary Dose (single)	MAF (90)	Daily Dietary Dose (Multiple)	TER	No refinement step required
	Small omnivorous bird	158.8	23.82	1.2	28.58	133.2 70.85	

Table 9.2-7: Pyraclostrobin: Screening step calculations of the acute risk for birds due to the use of BAS 758 00 F for the crop group “cereals”

Data from Data_Entry worksheet	Crop	Application rate (kg a.s./ha)	Number of applications	Application Interval	DT ₅₀	LD ₅₀	
	Cereals	0.12	2	14	10.0	1701 ≥2000	
Screening step:							
Acute risk assessment screening step	Indicator species	Shortcut value	Daily Dietary Dose (single)	MAF (90)	Daily Dietary Dose (Multiple)	TER	No refinement step required
	Small omnivorous bird	158.8	19.06	1.2	22.87	74.4 26.9 87.5	

Reproductive risk assessment

The dietary TER reproductive values for the screening step and tier 1 risk assessments are presented in Table 9.2-8 (mefentrifluconazole), Table 9.2-9 (metrafenone) and Table 9.2-10 (pyraclostrobin). All TER_{LT} values for mefentrifluconazole, metrafenone and pyraclostrobin are above the relevant trigger of 5 for acceptability of reproductive effects at the screening step.

Table 9.2-8: Mefentrifluconazole: Screening step and tier 1 calculations of the long-term/reproductive risk for birds due to the use of BAS 758 00 F for the crop group “cereals”

Data from Data_Entry worksheet	Crop	Application rate (kg a.s./ha)	Number of applications	Application Interval	DT ₅₀	Reproductive End Point (mg/kg b.w./d)	Time weighted average (TWA)
	Cereals	0.1	2	14	10	25.3	0.53
Screening step:							
Reproductive risk assessment screening step	Indicator species	Shortcut value	Daily Dietary Dose (single)	MAF mean	Daily Dietary Dose (Multiple)	TER	No refinement step required
	Small omnivorous bird	64.8	6.48	1.4	4.81	5.3	
First Tier Risk Assessment: ^b							
Calculate TER for each generic focal species	Crop	Generic focal species			Shortcut value	TER	No refinement required
	Cereals BBCH 30 -39	Small omnivorous bird “lark” Combination (invertebrates with interception) 25% crop leaves 25% weed seeds 50% ground arthropods			5.4	63.1	
	Cereals BBCH ≥ 40	Small omnivorous bird “lark” Combination (invertebrates with interception) 25% crop leaves 25% weed seeds 50% ground arthropods			3.3	103.3	

¹⁾ The screening step resulted in a TER value above the relevant trigger of 5. Hence, a first-tier risk assessment is not necessary. However, first-tier TER calculations are presented as they are required for calculation of combined reproductive toxicity.

Table 9.2-9: Metrafenone: Screening step and tier 1 calculations of the long-term/reproductive risk for birds due to the use of BAS 758 00 F for the crop group “cereals”

Data from Data_Entry worksheet	Crop	Application rate (kg a.s./ha)	Number of applications	Application Interval	DT ₅₀	Reproductive End Point (mg/kg b.w./d)	Time weighted average (TWA)
	Cereals	0.15	2	14	10	114.7 125.4	0.53
Screening step:							
Reproductive risk assessment screening step	Indicator species	Shortcut value	Daily Dietary Dose (single)	MAF mean	Daily Dietary Dose (Multiple)	TER	Please perform first tier risk assessment (see below)
	Small omnivorous bird	64.8	9.72	1.4	7.21	15.9 17.4	
First Tier Risk Assessment: ¹⁾							
Calculate TER for each generic focal species	Crop	Generic focal species			Shortcut value	TER	No refinement required
	Cereals BBCH 30 -39	Small omnivorous bird “lark” Combination (invertebrates with interception) 25% crop leaves 25% weed seeds 50% ground arthropods			5.4	190.8 209.0	
	Cereals BBCH ≥ 40	Small omnivorous bird “lark” Combination (invertebrates with interception) 25% crop leaves 25% weed seeds 50% ground arthropods			3.3	312.3 330.0	

¹⁾ The screening step resulted in a TER value above the relevant trigger of 5. Hence, a first-tier risk assessment is not necessary. However, first-tier TER calculations are presented as they are required for calculation of combined reproductive toxicity.

Table 9.2-10: Pyraclostrobin: Screening step and tier 1 calculations of the long-term/reproductive risk for birds due to the use of BAS 758 00 F for the crop group “cereals”

Data from Data_Entry worksheet	Crop	Application rate (kg a.s./ha)	Number of applications	Application Interval	DT ₅₀	Reproductive End Point (mg/kg b.w./d)	Time weighted average (TWA)
	Cereals	0.12	2	14	10	105.0	0.53
Screening step:							
Reproductive risk assessment screening step	Indicator species	Shortcut value	Daily Dietary Dose (single)	MAF mean	Daily Dietary Dose (Multiple)	TER	Please perform first tier risk assessment (see below)
	Small omnivorous bird	64.8	7.78	1.4	5.77	18.2	
First Tier Risk Assessment: ¹⁾							
Calculate TER for each generic focal species	Crop	Generic focal species			Shortcut value	TER	No refinement required
	Cereals BBCH 30 -39	Small omnivorous bird “lark” Combination (invertebrates with interception) 25% crop leaves 25% weed seeds 50% ground arthropods			5.4	218.4	
	Cereals BBCH ≥ 40	Small omnivorous bird “lark” Combination (invertebrates with interception) 25% crop leaves 25% weed seeds 50% ground arthropods			3.3	357.3	

¹⁾ The screening step resulted in a TER value above the relevant trigger of 5. Hence, a first-tier risk assessment is not necessary. However, first-tier TER calculations are presented as they are required for calculation of combined reproductive toxicity.

The conclusions for the dietary risk assessments for each of the active substances are as follows: acceptable acute and reproductive risks for birds were shown at the screening levels for mefentrifluconazole, metrafenone and pyraclostrobin. No higher-tier dietary risk assessments are necessary.

Dietary risk assessment for combined effects of simultaneous exposure to several active substances

Combined acute toxicity

According to EFSA/2009/1438 section 2.5, this assessment is relevant for BAS 758 00 F because this formulation contains more than one active substance.

Following Appendix B (step 1) in EFSA/2009/1438 a surrogate LD₅₀ = 1591.8 mg/kg bw is calculated based on the assumption of dose additivity (Table 9.2-11). A combined acute risk assessment is not required if for one active substance the deviation between ‘tox per fraction (a.s.)’ and ‘tox per fraction (mix)’ is ≤ 10% as in that case the risk is covered by the assessment for that active substance. For BAS 758 00 F this does not apply because the deviation for all active substances is more than 10% (Table 9.2-11).

Table 9.2-11: Calculation of surrogate LD₅₀ for the mixture of active substances

Active substance	Concentration a.s. in mixture [g/L]	Fraction a.s. in mixture	LD ₅₀ a.s. [mg/kg bw]	Fraction a.s./ LD ₅₀ a.s.	Surrogate LD ₅₀ [mg/kg b.w.]	Tox per fraction (a.s.)	Deviation tox per fraction (a.s.) and tox per fraction (mix) [%]
Mefentrifluconazole	66.6	0.27	816	0.00033	1591.8 1449.3	3021.4	99 108
Metrafenone	100	0.41	3807 > 2025	0.00011 0.00020		9388.1 4939.0	490 241
Pyraclostrobin	80	0.32	1701 > 2000	0.00019 0.00016		5243.3 6250.0	229 331

A laboratory study on the acute toxicity of the formulation BAS 758 00 F to birds (BASF DocID 2021/2037989) has been conducted and resulted in an acute LD₅₀ > 2000 mg/kg bw (see 9.2.1).

Appendix B of EFSA/2009/1438 recommends comparing the surrogate LD₅₀ with the experimental LD₅₀ from formulation testing and running the risk assessment with the lowest of the two values. However, Appendix B does not provide clear recommendations if, for the comparison of the two LD₅₀ values and for the calculation of the exposure scenarios, only the content of the active substances should be considered as the surrogate LD₅₀ is based on toxicity and concentration of active substances, while the experimental LD₅₀ is based on all components of the formulation. Due to this lack of guidance in Appendix B the most comprehensive approach is adopted by the notifier by presenting the two possible risk assessments, one for the virtual compound and another for the formulation.

Exposure and risk assessment for the combined active substances (virtual compound approach)

The potential exposure to the combined substances follows step 4 of Appendix B of EFSA/2009/1438. The maximum application rate of formulation BAS 758 00 F is 1.5 L product/ha (corresponding to 0.1 kg/ha mefentrifluconazole, 0.15 kg/ha metrafenone and 0.12 kg/ha pyraclostrobin) for the use in cereals; applying the concept for dose additivity to the exposure calculations results in a combined application rate of 0.370 kg virtual compound/ha.

The dietary TER acute value for the screening step presented in Table 9.2-12 is above the trigger of 10. Therefore, the acute risk to birds from combined effects of the three active substances in BAS 758 00 F is acceptable.

Table 9.2-12: Screening step calculation of the acute risk for birds due to the use of BAS 758 00 F in the crop group “cereals”- exposure to the combined active substances (virtual compound approach)

Data from Data_Entry worksheet	Crop	Application rate (kg/ha)	Number of applications	Application Interval	DT ₅₀	LD ₅₀	
	Cereals	0.370	2	14	10.0	1591.8 1449.3	
Screening step:							
Acute risk assessment screening step	Indicator species	Shortcut value	Daily Dietary Dose (single)	MAF (90)	Daily Dietary Dose (Multiple)	TER	No refinement step required
	Small omnivorous bird	158.8	58.76	1.2	70.51	22.6 20.6	

Note that this virtual compound acute TER was calculated according to the concentration addition approach and thus gives the same value as if calculated using equation $TER_{Acombi} = trigger / ((trigger / TER_{substance\ 1}) + (trigger / TER_{substance\ 2}) + (trigger / TER_{substance\ 3}))$.

Exposure and acute risk assessment for combined active substances (formulation approach)

BAS 758 00 F is intended to be used in the crop groups “cereals” with a maximum single application rate of 1.5 L product/ha. Taking into account the density of the formulation of 1.092 g/cm³, this will result in an application rate of 1.638 kg BAS 758 00 F/ha.

The acute dietary risk assessment for birds is presented in Table 9.2-13. The dietary TER acute values in the tier 1 risk assessment are above the trigger of 10, therefore the acute risk to birds from exposure to BAS 758 00 F is acceptable.

Table 9.2-13: Screening step and tier 1 calculations of the acute risk for birds due to the use of BAS 758 00 F in the crop group “cereals” – formulation approach

Data from Data_Entry worksheet	Crop	Application rate (kg formulation/ha)	Number of applications	Application Interval	DT ₅₀	LD ₅₀	
	Cereals	1.638	2	14	10.0	>2000.0	
Screening step:							
Acute risk assessment screening step	Indicator species	Shortcut value	Daily Dietary Dose (single)	MAF (90)	Daily Dietary Dose (Multiple)	TER	Please perform first tier risk assessment (see below)
	Small omnivorous bird	158.8	260.11	1.2	312.14	>6.4	
First Tier Risk Assessment:							
	Crop	Generic focal species			Shortcut value	TER	
Calculate TER for each generic focal species	Cereals BBCH 30 -39	Small omnivorous bird “lark” Combination (invertebrates with interception) 25% crop leaves 25% weed seeds 50% ground arthropods			12.0	>84.8	No refinement required
	Cereals BBCH ≥ 40	Small omnivorous bird “lark” Combination (invertebrates with interception) 25% crop leaves 25% weed seeds 50% ground arthropods			7.2	>141.3	

TER values shown in **bold** fall below the relevant trigger

In conclusion, the two risk assessment approaches (combined toxicity of the active substances and formulation toxicity) have resulted in TER_A values that are above the trigger of 10 for acceptability of effects at the screening step and/or tier 1 level. Therefore, the acute dietary risk to birds from BAS 758 00 F is acceptable.

Combined reproductive toxicity

As requested in the summary report of the Steering Committee of the Central Zone Harmonisation workshop in April 2015 and update of October 2016 (Central Zone Harmonisation Workshop, 2016), a long-term combination toxicity tier 1 risk assessment is presented. As proposed there, the calculations follow the concentration addition model. TER_{A combi} values are covered by the virtual compound approach, please see above for details.

The combined TER_{LT} value is calculated according to the following formula:

$$\text{TER}_{\text{LT combi}} = \text{trigger} / ((\text{trigger} / \text{TER}_{\text{LT substance 1}}) + (\text{trigger} / \text{TER}_{\text{LT substance 2}}) + (\text{trigger} / \text{TER}_{\text{LT substance 3}}) +))$$

An acceptable risk is expected when TER_{LT combi} > trigger.

The TER_{LT combi} values are calculated based on the screening step and tier 1 TER values for the active substances. The calculations of the cumulative ecotoxicological effects are summarized in Table 9.2-14.

Table 9.2-14: Combined reproductive toxicity risk assessment for birds due to the use of BAS 758 00 F for the crop group “cereals”

Crop scenario and/or indicator species		TER _{LT} ¹⁾ mefentrifluconazole	TER _{LT} ¹⁾ metrafenone	TER _{LT} ¹⁾ pyraclostrobin	TER _{LT combi}	Trigger
Reproductive (screening step)						
Cereals	Small omnivorous bird	5.3	15.9 17.4	18.2	3.26 3.32	5
Reproductive (tier 1)						
Cereals BBCH 30 -39	Small omnivorous bird “lark”	63.1	190.8 209	218.4	38.96 39.65	5
Cereals BBCH ≥ 40	Small omnivorous bird “lark”	103.3	312.3 330	357.3	63.77 64.47	5

TER values shown in **bold** fall below the relevant trigger

¹⁾ Reproductive TER values are presented in Table 9.2-8, Table 9.2-9 and Table 9.2-10.

The TER_{LT combi} values at tier 1 are all above the trigger value of 5. Thus, it can be concluded that the reproductive risk for birds for the combined exposure to the three active substances in the application of BAS 758 00 F according to good agricultural practice is low and acceptable.

9.2.2.2 Higher-tier risk assessment

Not necessary as acceptable acute and reproductive risks were shown with the screening and/or first-tier risk assessments for all scenarios.

9.2.2.3 Drinking water exposure

Leaf scenario

Since BAS 758 00 F is not intended to be applied on leafy vegetables forming heads or crop plants with comparable water collecting structures at principal growth stage 4 or later, the leaf scenario does not have to be considered.

Puddle scenario

Due to the characteristics of the exposure scenario in connection with the standard assumptions for water uptake by animals, no specific calculations of exposure and TER are necessary when the ratio of effective application rate (in g/ha) to relevant endpoint (in mg/kg bw/d) does not exceed 50 in the case of less sorptive substances ($K_{oc} < 500$ L/kg) or 3000 in the case of more sorptive substances ($K_{oc} \geq 500$ L/kg).

The ratio calculations for effective application rate to relevant endpoint are detailed in Table 9.2-15 (mefentrifluconazole), Table 9.2-16 (metrafenone) and Table 9.2-17 (pyraclostrobin) and are based on worst-case assumptions with regard to the resulting AR_{eff} . The ratios for acute and reproductive endpoints for mefentrifluconazole (0.2 and 7.7, respectively), metrafenone (0.1 and 2.6, respectively) and for pyraclostrobin (0.1 and 1.8, respectively) do not exceed the threshold value of 3000 for the active substances, thus a quantitative drinking water risk assessment for the puddle scenario is not triggered.

Table 9.2-15: Assessment of the risk for birds due to exposure to mefentrifluconazole via contaminated drinking water in puddles

Parameter	Mefentrifluconazole	Reference
K_{oc} (geometric mean, n=8) [L/kg]	3455.6	Chapter 8.9.2 (EFSA Journal 2018; 16(7): 5379)
DT_{50} (soil) (geometric mean, n=6) [days]	200	Chapter 8.9.2 (EFSA Journal 2018; 16(7): 5379)
Number of applications	2	Chapter 9.1
Interval [days]	14	Chapter 9.1
MAF_m ¹⁾	1.95	--
Max use rate [g/ha]	100	Chapter 9.1
AR_{eff} [g/ha] ²⁾	195.0	--
LD_{50} [mg/kg bw]	816	Chapter 9.2.1
Ratio (acute) ³⁾	0.2	--
NO(A)EL [mg/kg bw/d]	25.3	Chapter 9.2.1
Ratio (repro) ³⁾	7.7	--
Trigger	3000	--
Drinking water assessment required [Yes/No]	No	--

¹⁾ $MAF_m = (1 - e^{-nki}) / (1 - e^{-ki})$ with $k = \ln(2)/DT_{50}$ (rate constant), n = number of applications and i = application interval [d]

²⁾ $AR_{eff} = \text{Application rate [g/ha]} \times MAF_m$

³⁾ Ratio of AR_{eff} and relevant toxicity endpoint

Table 9.2-16: Assessment of the risk for birds due to exposure to metrafenone via contaminated drinking water in puddles

Parameter	Metrafenone	Reference
K _{oc} (geometric mean, n=5) [L/kg]	2812	Chapter 8.9.2 (EFSA Scientific Report (2006) 58, 1-72)
DT ₅₀ (soil) (geometric mean, n=5) [days]	250.6	Chapter 8.9.2 (EFSA Scientific Report (2006) 58, 1-72)
Number of applications	2	Chapter 9.1
Interval [days]	14	Chapter 9.1
MAF _m ¹⁾	1.96	--
Max use rate [g/ha]	150	Chapter 9.1
AR _{eff} [g/ha] ²⁾	294.0	--
LD ₅₀ [mg/kg bw]	3807 2025	Chapter 9.2.1
Ratio (acute) ³⁾	0.15	--
NO(A)EL [mg/kg bw/d]	114.7 125.4	Chapter 9.2.1
Ratio (repro) ³⁾	2.6 2.3	--
Trigger	3000	--
Drinking water assessment required [Yes/No]	No	--

¹⁾ $MAF_m = (1 - e^{-nki}) / (1 - e^{-ki})$ with $k = \ln(2)/DT_{50}$ (rate constant), n = number of applications and i = application interval [d]

²⁾ $AR_{eff} = \text{Application rate [g/ha]} \times MAF_m$

³⁾ Ratio of AR_{eff} and relevant toxicity endpoint

Table 9.2-17: Assessment of the risk for birds due to exposure to pyraclostrobin via contaminated drinking water in puddles

Parameter	Pyraclostrobin	Reference
K _{oc} (geometric mean, n=6) [L/kg]	8856	Chapter 8.9.2 (Monograph 12945/ ECCO/BBA/01)
DT ₅₀ (soil) (geometric mean, n=4) [days]	18	Chapter 8.9.2
Number of applications	2	Chapter 9.1
Interval [days]	14	Chapter 9.1
MAF _m ¹⁾	1.58	--
Max use rate [g/ha]	120	Chapter 9.1
AR _{eff} [g/ha] ²⁾	189.6	--
LD ₅₀ [mg/kg b.w.]	1701 2000	Chapter 9.2.1
Ratio (acute) ³⁾	0.1	--
NO(A)EL [mg/kg b.w./d]	105	Chapter 9.2.1
Ratio (repro) ³⁾	1.8	--
Trigger	3000	--
Drinking water assessment required [Yes/No]	No	--

¹⁾ $MAF_m = (1 - e^{-nki}) / (1 - e^{-ki})$ with $k = \ln(2)/DT_{50}$ (rate constant), n = number of applications and i = application interval [d]

²⁾ $AR_{eff} = \text{Application rate [g/ha]} \times MAF_m$

³⁾ Ratio of AR_{eff} and relevant toxicity endpoint

In conclusion, the risk to birds via drinking water from the intended use of BAS 758 00 F according to the proposed use pattern is acceptable.

9.2.2.4 Effects of secondary poisoning

The log P_{ow} of the active substance mefentrifluconazole is 3.4 (EFSA Journal 2018; 16(7): 5379) and thus does exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is required.

The log P_{ow} of the active substance metrafenone is 4.3 (EFSA Scientific Report (2006) 58, 1- 72) and thus does exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is required.

The log P_{ow} of pyraclostrobin is 3.99 (SANCO/1420/2001-final) and thus does exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is required.

Risk assessment for earthworm-eating birds via secondary poisoning

According to EFSA/2009/1438, the risk for vermivorous birds is assessed for a bird of 100 g body weight with a daily food consumption of 104.6 g. Bioaccumulation in earthworms is estimated based on predicted concentrations in soil.

As shown in the following Table 9.2-18 (mefentrifluconazole), Table 9.2-19 (metrafenone) and Table 9.2-20 (pyraclostrobin), the TER_{LT} values for all active substances exceed the trigger of 5 for acceptability of effects, indicating an acceptable risk to earthworm-eating birds via secondary poisoning.

Table 9.2-18: Assessment of the risk for earthworm-eating birds due to exposure to mefentrifluconazole via bioaccumulation in earthworms (secondary poisoning) for the intended uses

Parameter	Mefentrifluconazole	Reference
$PEC_{soil} (accu) [mg/kg \text{ soil}]^{1)}$	0.205	Chapter 8.7
K_{ow}	2350	BASF DocID 2013/1382370
$K_{oc} (geometric \text{ mean}) [L/kg]$	3455.6	Chapter 8.9.2 (EFSA Journal 2018; 16(7): 5379)
$f_{oc} (default)$	0.02	EFSA/2009/1438
$BCF^{2)}$	0.420	--
$PEC_{worm} [mg/kg]^{3)}$	0.086	--
Daily dose $[mg/kg \text{ b.w./d}]^{4)}$	0.090	--
$NO(A)EL [mg/kg \text{ b.w./d}]$	25.3	Chapter 9.2.1
$TER_{LT}^{5)}$	279.7	--

¹⁾ $PEC_{soil} (accu)$ value was calculated for an application scenario of 2 x 100 g a.s./ha with 14-day interval in cereals. For details see chapter 8.7.

²⁾ Bioconcentration factor (BCF) = $(0.84 + 0.012 \times K_{ow}) / (f_{oc} \times K_{oc})$

³⁾ $PEC_{worm} = PEC_{soil} \times BCF$

⁴⁾ Daily dose = $1.05 \times PEC_{worm}$

⁵⁾ $TER_{LT} = NO(A)EL / \text{Daily dose}$.

Table 9.2-19: Assessment of the risk for earthworm-eating birds due to exposure to metrafenone via bioaccumulation in earthworms (secondary poisoning) for the intended use

Parameter	Metrafenone	Reference
PEC _{soil} (accu) [mg/kg soil] ¹⁾	0.158	Chapter 8.7
K _{ow}	19953 ⁶⁾	EFSA Scientific Report (2006) 58, 1-72 (for log P _{ow} = 4.3)
K _{oc} (geometric mean, n=5) [L/kg]	2812	Chapter 8.9.2 (EFSA Scientific Report (2006) 58, 1-72)
f _{oc} (default)	0.02	EFSA/2009/1438
BCF ²⁾	4.272	--
PEC _{worm} [mg/kg] ³⁾	0.675	--
Daily dose [mg/kg b.w./d] ⁴⁾	0.709	--
NO(A)EL [mg/kg b.w./d]	114.7 125.4	Chapter 9.2.1
TER _{LT} ⁵⁾	161.8 176.9	--

¹⁾ PEC_{soil} (accu) value was calculated for an application scenario of 2 x 150 g a.s./ha with 14-day interval in cereals. For details see chapter 8.7.

²⁾ Bioconcentration factor (BCF) = (0.84 + 0.012 x K_{ow}) / (f_{oc} x K_{oc})

³⁾ PEC_{worm} = PEC_{soil} x BCF

⁴⁾ Daily dose = 1.05 x PEC_{worm}

⁵⁾ TER_{LT} = NO(A)EL / Daily dose.

⁶⁾ Calculated based on log P_{ow} of 4.3 (for reference of log P_{ow} = 4.3 see dRAR of Metrafenone Vol. 3, B9, p. 9 and EFSA Scientific report No. 58, 1-72 (2006)).

Table 9.2-20: Assessment of the risk for earthworm-eating birds due to exposure to pyraclostrobin via bioaccumulation in earthworms (secondary poisoning) for the intended use

Parameter	Pyraclostrobin	Reference
PEC _{soil} (twa, 21 days) [mg/kg soil] ¹⁾	0.047	Chapter 8.7
K _{ow}	9772	SANCO/1420/2001-final
K _{oc} (geometric mean, n=6) [L/kg]	8856	Chapter 8.9.2 (Monograph 12945/ ECCO/BBA/01)
f _{oc} (default)	0.02	EFSA/2009/1438
BCF ²⁾	0.667	--
PEC _{worm} [mg/kg] ³⁾	0.031	--
Daily dose [mg/kg b.w./d] ⁴⁾	0.033	--
NO(A)EL [mg/kg b.w./d]	105.0	Chapter 9.2.1
TER _{LT} ⁵⁾	3190.8	--

¹⁾ The PEC_{soil} (twa, 21 days) value was calculated for an application scenario of 2 x 120 g a.s./ha with 14-day interval in cereals. For details see chapter 8.7.

²⁾ Bioconcentration factor (BCF) = (0.84 + 0.012 x K_{ow}) / (f_{oc} x K_{oc})

³⁾ PEC_{worm} = PEC_{soil} x BCF

⁴⁾ Daily dose = 1.05 x PEC_{worm}

⁵⁾ TER_{LT} = NO(A)EL / Daily dose.

Risk assessment for fish-eating birds via secondary poisoning

According to EFSA/2009/1438, the risk for piscivorous birds is assessed for a bird of 1000 g body weight with a daily food consumption of 159 g. Bioaccumulation in fish is estimated based on predicted concentrations in surface water.

As shown in the following Table 9.2-21 (mefentrifluconazole), Table 9.2-22 (metrafenone) and Table 9.2-23 (pyraclostrobin), the TER_{LT} values for all active substances exceed the trigger of 5 for acceptability of effects, indicating an acceptable risk to fish-eating birds via secondary poisoning.

Table 9.2-21: Assessment of the risk for fish-eating birds due to exposure to mefentrifluconazole via bioaccumulation in fish (secondary poisoning) for the intended use

Parameter	Mefentrifluconazole	Reference
PEC _{sw} , (twa, 21 days) [mg/L] ¹⁾	2.020×10^{-3}	Chapter 8.9
BCF fish (max. worst case)	385	EFSA Journal 2018; 16(7): 5379
PEC _{fish} [mg/kg] ²⁾	0.778	--
Daily dose [mg/kg bw/d] ³⁾	0.124	--
NO(A)EL [mg/kg bw/d]	25.3	Chapter 9.2.1
TER _{LT} ⁴⁾	204.6	--

¹⁾ PEC_{sw} (21 d twa) value calculated for a multiple application scenario in spring and winter cereals (2x 100 g/ha with 14-day interval) from FOCUS Step 2 (Northern Europe scenario) as worst-case. For details see chapter 8.9.

²⁾ PEC_{fish} = PEC_{sw}(twa, 21 days) x BCF

³⁾ Daily dose = 0.159 x PEC_{fish}

⁴⁾ TER_{LT} = NO(A)EL / Daily dose.

Table 9.2-22: Assessment of the risk for fish-eating birds due to exposure to metrafenone via bioaccumulation in fish (secondary poisoning) for the intended use

Parameter	Metrafenone	Reference
PEC _{sw} , (twa, 21 days) [mg/L] ¹⁾	2.184×10^{-3}	Chapter 8.9
BCF fish (max. worst case)	530	EFSA Scientific Report (2006) 58, 1- 72
PEC _{fish} [mg/kg] ²⁾	1.158	--
Daily dose [mg/kg bw/d] ³⁾	0.184	--
NO(A)EL [mg/kg bw/d]	114.7 125.4	Chapter 9.2.1
TER _{LT} ⁴⁾	623.2	--

¹⁾ PEC_{sw} (21 d twa) value calculated for a multiple application scenario in spring and winter cereals (2x 150 g/ha with 14-day interval) from FOCUS Step 2 (Northern Europe scenario) as worst-case. For details see chapter 8.9.

²⁾ PEC_{fish} = PEC_{sw}(twa, 21 days) x BCF

³⁾ Daily dose = 0.159 x PEC_{fish}

⁴⁾ TER_{LT} = NO(A)EL / Daily dose.

Table 9.2-23: Assessment of the risk for fish-eating birds due to exposure to pyraclostrobin via bioaccumulation in fish (secondary poisoning) for the intended use

Parameter	Pyraclostrobin	Reference
PEC _{sw} , (twa, 21 days) [mg/L] ¹⁾	0.806×10^{-3}	Chapter 8.9
BCF fish (max. worst case)	736	SANCO/1420/2001-final
PEC _{fish} [mg/kg] ²⁾	0.593	--
Daily dose [mg/kg b.w./d] ³⁾	0.094	--
NO(A)EL [mg/kg b.w./d]	105.0	Chapter 9.2.1
TER _{LT} ⁴⁾	1113.2	--

¹⁾ PEC_{sw} (21 d twa) value calculated for a multiple application scenario in spring and winter cereals (2x 150 g/ha resulting from a risk envelope approach) from FOCUS Step 2 (Northern Europe scenario) as worst-case. For details see chapter 8.9.

²⁾ PEC_{fish} = PEC_{sw}(twa, 21 days) x BCF

³⁾ Daily dose = 0.159 x PEC_{fish}

⁴⁾ TER_{LT} = NO(A)EL / Daily dose.

9.2.2.5 Biomagnification in terrestrial food chains

Low potential for accumulation in animal tissue was concluded in the EU review of mefentrifluconazole (see EFSA Journal 2018;16(7):5379).

Low potential for accumulation in animal tissue was concluded in the EU review of metrafenone (EFSA Scientific Report (2006) 58, 1- 72).

Low potential for accumulation in animal tissue was concluded in the EU review of pyraclostrobin (SANCO/1420/2001-final).

Since the bioaccumulation potential of mefentrifluconazole, metrafenone and pyraclostrobin is low no further assessment on biomagnification is required.

9.2.3 Risk assessment for baits, pellets, granules, prills or treated seed

Not relevant.

9.2.4 Overall conclusions

It can be concluded that the risk to birds from the application of BAS 758 00 F according to good agricultural practice is acceptable.

Review Comments:

The acute and chronic risks of BAS 758 00 F to birds were assessed from toxicity exposure ratios between toxicity endpoints, estimated from study with active ingredients and maximum residues occurring on food items. An acute oral toxicity study with BAS 758 00 F in birds was taken to consideration in the evaluation.

All TER values exceed the relevant triggers in the screening step or Tier 1 risk assessment for mefentrifluconazole, metrafenone, pyraclostrobin and for combined acute (virtual compound and formulation approach) and reproductive risk assessment. Therefore, BAS 758 00 F does not pose an unacceptable risk to birds following applications according to recommended use pattern.

Evaluation of exposing to birds through the drinking water demonstrated the acceptable risk. The potential risk of secondary poisoning is low.

9.3 Effects on terrestrial vertebrates other than birds (KCP 10.1.2)

The risk assessment for mammals is carried out following the latest guidance document by EFSA (EFSA/2009/1438).

9.3.1 Toxicity data

Mammalian toxicity studies have been carried out with mefentrifluconazole, metrafenone and pyraclostrobin. Full details of these studies are provided in the respective EU DARs and related documents.

Active substances

An overview of the EU agreed endpoints is given in Table 9.3-1 for mefentrifluconazole and in Table 9.3-2 for metrafenone. The selection of studies and endpoints for the risk assessment is in line with the results of the EU review process for mefentrifluconazole and the former EU review process for metrafenone.

An overview of the EU agreed endpoints for pyraclostrobin is given in Table 9.3-3. The selection of studies and endpoints for the risk assessment of pyraclostrobin reflects the current status of the ongoing EU renewal process for pyraclostrobin and the latest version (January 2020) of the Renewal Assessment Report (RAR) of pyraclostrobin presented by RMS Germany. In some cases these endpoints deviate from the results of the previous EU review process (SANCO/1420/2001-Final. 2004, 1-24). Justifications are provided below. A full argumentation for the use of the ecologically relevant reproductive endpoint is provided (BASF DocID 2014/1010736). The study is listed in Appendix 1. A short summary is given below.

Table 9.3-1: Mefentrifluconazole (BAS 750 F): Endpoints relevant for the risk assessment for mammals

Species	Substance	Exposure System	Results	Reference [BASF DocID]
Rat	Mefentrifluconazole	Oral, 1 d Acute	LD ₅₀ > 2000 mg a.s./kg bw	EFSA Journal 2018;16(7):5379 [2013/1149656]
Rat	Mefentrifluconazole	Dietary Reproductive toxicity Two-generation study	NOEL _{Reproduction} = 200 mg a.s./kg bw/d NOEL _{Parents} = 25 mg a.s./kg bw/d NOEL _{Offspring} = 75 mg a.s./kg bw/d	EFSA Journal 2018;16(7):5379 [2014/1170754]
Rat	Mefentrifluconazole	Oral Developmental toxicity	NOEL _{Maternal} = 150 mg a.s./kg bw NOEL _{Developmental} = 400 mg a.s./kg bw/d	EFSA Journal 2018;16(7):5379 [2014/1170755]
Rabbit	Mefentrifluconazole	Oral Developmental toxicity	NOEL _{Maternal} = 15 mg a.s./kg bw/d NOEL _{Developmental} = 15 mg a.s./kg bw/d	EFSA Journal 2018;16(7):5379 [2014/1170757]
Endpoint used for acute risk assessment	Mefentrifluconazole	Oral, 1 d Acute	LD ₅₀ > 2000 mg a.s./kg bw	EFSA Journal 2018;16(7):5379 [2013/1149656]
Endpoint used for reproductive risk assessment	Mefentrifluconazole	Dietary Reproductive toxicity – Tier 1	NOEL = 15 mg a.s./kg bw/d	EFSA Journal 2018;16(7):5379 [2014/1170757]

Table 9.3-2: Metrafenone (BAS 560 F): Endpoints relevant for the risk assessment for mammals

Species	Substance	Exposure System	Results	Reference [BASF DocID]
Rat	Metrafenone	Oral, 1 d Acute	LD ₅₀ > 5000 mg a.s./kg bw ¹⁾	EFSA Scientific Report (2006) 58, 1-72; [1999/7000303]
Rat	Metrafenone	Dietary Reproductive toxicity Two-generation study	NOAEL _{Reproduction} = 811 mg a.s./kg bw/d ¹⁾	EFSA Scientific Report (2006) 58, 1-72; [2002/7004752]
Rat	Metrafenone	Oral Prenatal Developmental toxicity	NOAEL _{Maternal} = 1000 mg a.s./kg bw/d NOAEL _{Developmental} = 1000 mg a.s./kg bw/d ²⁾	EFSA Scientific Report (2006) 58, 1-72; [2001/7001372]
Rabbit	Metrafenone	Oral Prenatal Developmental toxicity	NOAEL _{Maternal} = 50 mg a.s./kg bw/d NOAEL _{Developmental} = 50 mg a.s./kg bw/d ²⁾	EFSA Scientific Report (2006) 58, 1-72; [2001/7001288]
Endpoint used for acute assessment	Metrafenone	Oral, 1 d Acute	LD₅₀ > 5000 mg a.s./kg bw	EFSA Scientific Report (2006) 58, 1-72; [1999/7000303]
Endpoint used for reproductive assessment	Metrafenone	Dietary Reproductive toxicity Two-generation study	NOAEL_{Reproduction} = 811 mg a.s./kg bw/d ¹⁾	EFSA Scientific Report (2006) 58, 1-72; [2002/7004752]

¹⁾ Endpoints confirmed in Appendix 1.6, Effects on terrestrial vertebrates of the EFSA Conclusion on the peer review metrafenone. *EFSA Scientific Report* (2006) 58, 1-72.

²⁾ Additional endpoints for mammals from Appendix 1.3, Impact on Human and Animal Health of the EFSA Conclusion on the peer review metrafenone. *EFSA Scientific Report* (2006) 58, 1-72.

Table 9.3-3: Pyraclostrobin (BAS 500 F): Endpoints relevant for the risk assessment for mammals

Species	Substance	Exposure System	Results	Reference [BASF DocID]
Rat	Pyraclostrobin	Oral, 1 d Acute	LD ₅₀ > 5000 mg a.s./kg bw	RAR; Rev.1; 10. Jan. 2020 [1998/10965] SANCO/1420/2001-Final. 2004, 1-24
Mouse	Pyraclostrobin	Oral (single acute) In-vivo micronucleus assay	LD₅₀ = 450 mg a.s./kg bw	RAR; Rev.1; 10. Jan. 2020 [1998/10460, Amendment 2016/1309356]
Rat	Pyraclostrobin	Dietary Reproductive toxicity Two-generation study	NOEL _{Reproduction} = 32.6 mg a.s./kg bw/d NOEL _{Offspring} = 8.2 mg a.s./kg bw/d NOEL _{Parents} = 8.2 mg a.s./kg bw/d	RAR; Rev.1; 10. Jan. 2020 [1999/11869] SANCO/1420/2001-Final. 2004, 1-24
Rat	Pyraclostrobin	Oral Prenatal Developmental toxicity	NOEL _{Maternal} = 10 mg a.s./kg bw/d NOEL _{Developmental} = 50 mg a.s./kg bw/d	[1999/11511] SANCO/1420/2001-Final. 2004, 1-24
Rabbit	Pyraclostrobin	Oral Prenatal Developmental toxicity	NOEL _{Maternal} = 3 mg a.s./kg bw/d NOEL _{Developmental} = 5 mg a.s./kg bw/d ¹⁾ NOEL _{Developmental, ecological relevant} = 10 mg a.s./kg bw/d ²⁾	RAR; Rev.1; 10. Jan. 2020 [2001/1003803 and 1999/11512] SANCO/1420/2001-Final. 2004, 1-24
Endpoint used for acute assessment	Pyraclostrobin	Oral, 1 d Acute	LD ₅₀ > 5000 mg/kg bw	RAR; Rev.1; 10. Jan. 2020 [1998/10965] SANCO/1420/2001-Final. 2004, 1-24
	Pyraclostrobin	Oral (single acute) In-vivo micronucleus assay	LD₅₀ = 450 mg a.s./kg bw	RAR; Rev.1; 10. Jan. 2020 [1998/10460, Amendment 2016/1309356]
Endpoint used for reproductive assessment	Pyraclostrobin	Dietary Reproductive toxicity – Tier 1 / Higher tier	NOEL = 8.2 mg a.s./kg bw/d ¹⁾	RAR; Rev.1; 10. Jan. 2020 [1999/11869] Full argumentation provided in 2014/1010736 SANCO/1420/2001-Final. 2004, 1-24

¹⁾ For details please refer to chapter 9.3.1.1.

²⁾ Ecotoxicologically relevant endpoint for development toxicity (RAR; Rev.1; 10. Jan. 2020, p.53)

Metabolites

Metabolites of mefentrifluconazole, metrafenone and pyraclostrobin

See section 9.2.1 in the bird chapter.

Formulation toxicity

For toxicological classification and labeling purposes, an acute oral toxicity study with the formulation BAS 758 00 F in rats was carried out according to the toxic class method described in OECD 423 (BASF DocID 2020/2101764; see chapter 6.3 and Appendix 2 of chapter 6). No mortality occurred in six animals dosed with 300 mg formulation/kg bw, while four out of six animals dosed with 2000 mg formulation/kg bw died, resulting in $LD_{50} > 300$ and < 2000 mg formulation/kg bw.

9.3.1.1 Justification for new endpoints

Mefentrifluconazole

Acute – Not applicable. Endpoint is EU agreed.

Reproductive – Not applicable. Endpoint is EU agreed.

Metrafenone

Acute – Not applicable. Endpoint is EU agreed.

Reproductive – Not applicable. Endpoint is EU agreed.

Pyraclostrobin

~~Acute – One standard acute oral toxicity test in rats is available with pyraclostrobin (BASF DocID 1998/10965). This study resulted in an LD₅₀ > 5000 mg a.s./kg bw. Additional information on the acute oral toxicity of pyraclostrobin is available from a non-standard study in the mouse: In the in vivo micronucleus assay (BASF DocID 1998/10460 and Amendment 2016/1309356), acute oral toxicity was observed in mice dosed with pyraclostrobin as a solution in olive oil. Even though it was not designed as a study to determine an LD₅₀ in mice, the mortality data in the micronucleus assay were suitable to derive an acute oral toxicity estimate in mice. This estimated endpoint of LD₅₀ ~ 450 mg a.s./kg bw is considered relevant for the ecotoxicological risk assessment for mammals. Both endpoints were evaluated and used in the risk assessment presented by RMS Germany in the latest version (January 2020) of the Renewal Assessment Report (RAR) of pyraclostrobin. For a conservative estimate, the endpoint of LD₅₀ ~ 450 mg a.s./kg bw in mouse is applied in the present risk assessment.~~

Acute – Not applicable. Endpoint is EU agreed.

Reproductive – In the previous EU process (SANCO/1420/2001-Final. 2004, 1-24), no endpoint was agreed on for the wild mammals' risk assessment of pyraclostrobin. However, the reproductive toxicity endpoint for wild mammals was evaluated in the ongoing EU renewal process for pyraclostrobin, in which RMS Germany concluded in the latest version of the Renewal Assessment Report (RAR, January 2020) on the **NOAEL = 8.2 mg/kg bw/d** from the two-generation study in rats as the ecologically relevant reproductive endpoint for the wild mammalian risk assessment of pyraclostrobin. A comprehensive review of the key relevant toxicity studies for the wild mammalian risk assessment, *i.e.*, the two-generation rat study, the rat prenatal development study, and the rabbit prenatal development study is available in a separate document (K-CP 10.1.2/1, BASF DocID 2014/1010736).

Reproductive – Not applicable. Endpoint is EU agreed.

Review Comments:

There is no ecotoxicological EU agreed endpoint as this section of the Review Report has been left blank. In toxicological part of LoEP several endpoints concerning reproductive toxicity of pyraclostrobin to mammals were presented. The endpoint of 75 ppm was the value selected by the RMS - Germany and was considered acceptable to use for the risk assessment by ECCO 126 Peer Review Meeting (7038/ECCO/PSD/02 18 June 2002). Moreover, the NOAEL of 8.2 mg a.s./kg bw/d (75 ppm) was accepted by most of central zone MS for authorization PPP with pyraclostrobin. In zRMS opinion the NOAEL of 8.2 mg a.s./kg bw/d seems to be reasonable to be used in the risk assessment.

According to current state of art, the same toxicity endpoint will be used in all tier risk assessment.

9.3.2 Risk assessment for spray applications

Proposed use pattern for the risk assessments

The proposed use pattern for the use of BAS 758 00 F is summarized in Table 9.2-4. The detailed use pattern table is presented at the beginning of the ecotoxicology chapter (section 9.1).

Table 9.3-4: Proposed use pattern

Crop	Crop group according to EFSA/2009/1438	Application time (BBCH growth stage)	Number of applications	Interval between applications [d]	Application rate per application			
					Mefentrifluconazole [kg/ha]	Metrafenone [kg/ha]	Pyraclostrobin [kg/ha]	BAS 758 00 F [L/ha]
Cereals (winter, spring)	Cereals	30-59	2	14	0.100	0.150	0.120	1.5
		30-59	1	--	0.100	0.150	0.120	1.5
		30-59	2	14	0.067	0.100	0.080	1.0

The application scenario with the highest number of applications and the highest use rate shown in **bold** is used for the risk assessment and covers all possible application scenarios according to the GAP in section 9.1.

9.3.2.1 First-tier assessment (screening/generic focal species)

The dietary TER acute (TER_A) and reproductive (TER_{LT}) values for the screening step and tier 1 risk assessment were calculated with the EFSA calculator tool (version of 9 July 2010, <http://www.efsa.europa.eu/de/efsajournal/pub/1438.htm>) according to EFSA/2009/1438.

Dietary risk assessment for the active substances

Acute risk assessment

The dietary TER acute values for the screening step are presented in Table 9.3-5 (mefentrifluconazole), Table 9.3-6 (metrafenone) and in Table 9.3-7 (pyraclostrobin). All TER_A values for mefentrifluconazole, metrafenone and pyraclostrobin are above the relevant trigger of 10 for acceptability of acute effects at the screening step.

Table 9.3-5: Mefentrifluconazole: Screening step calculations of the acute risk for mammals due to the use of BAS 758 00 F for the crop group “cereals”

Data from Data Entry worksheet	Crop	Application rate (kg a.s./ha)	Number of applications	Application Interval	DT ₅₀	LD ₅₀	
	Cereals	0.1	2	14	10.0	>2000.0	
Screening step:							
Acute risk assessment screening step	Indicator species	Shortcut value	Daily Dietary Dose (single)	MAF (90)	Daily Dietary Dose (Multiple)	TER	No refinement step required
	Small herbivorous mammal	118.4	11.84	1.2	14.2	>140.8	

Table 9.3-6: Metrafenone: Screening step calculations of the acute risk for mammals due to the use of BAS 758 00 F for the crop group “cereals”

Data from Data_Entry worksheet	Crop	Application rate (kg a.s./ha)	Number of applications	Application Interval	DT ₅₀	LD ₅₀	
	Cereals	0.15	2	14	10.0	>5000	
Screening step:							
Acute risk assessment screening step	Indicator species	Shortcut value	Daily Dietary Dose (single)	MAF (90)	Daily Dietary Dose (Multiple)	TER	No refinement step required
	Small herbivorous mammal	118.4	17.76	1.2	21.31	>234.6	

Table 9.3-7: Pyraclostrobin: Screening step calculations of the acute risk for mammals due to the use of BAS 758 00 F for the crop group “cereals”

Data from Data_Entry worksheet	Crop	Application rate (kg a.s./ha)	Number of applications	Application Interval	DT ₅₀	LD ₅₀	
	Cereals	0.12	2	14	10.0	450.0 >5000	
Screening step:							
Acute risk assessment screening step	Indicator species	Shortcut value	Daily Dietary Dose (single)	MAF (90)	Daily Dietary Dose (Multiple)	TER	No refinement step required
	Small herbivorous mammal	118.4	14.21	1.2	17.05	26.4 293.3	

Reproductive risk assessment

The dietary TER reproductive values for the screening step and tier 1 risk assessments are presented in Table 9.3-8 (mefentrifluconazole), Table 9.3-9 (metrafenone) and in Table 9.3-10 (pyraclostrobin).

All TER_{LT} values at the screening step and/or tier 1 risk assessment are above the relevant trigger of 5 for acceptability of reproductive effects, except for pyraclostrobin where the small herbivorous mammal “vole” scenario (BBCH ≥ 40) resulted in a TER_{LT} value below the trigger.

Table 9.3-8: Mefentrifluconazole: Screening step and tier 1 calculations of the long-term/reproductive risk for mammals due to the use of BAS 758 00 F for the crop group “cereals”

Data from Data_Entry worksheet	Crop	Application rate (kg a.s./ha)	Number of applications	Application Interval	DT ₅₀	Reproductive End Point (mg/kg b.w./d)	Time weighted average (TWA)
	Cereals	0.1	2	14	10	15.0	0.53
Screening step:							
Reproductive risk assessment screening step	Indicator species	Shortcut value	Daily Dietary Dose (single)	MAF mean	Daily Dietary Dose (Multiple)	TER	Please perform first tier risk assessment (see below)
	Small herbivorous mammal	48.3	4.83	1.4	3.58	4.2	
First Tier Risk Assessment:							
Calculate TER for each generic focal species	Crop	Generic focal species		Shortcut value	TER	No refinement required	
	Cereals BBCH ≥ 20	Small insectivorous mammal "shrew" ground dwelling invertebrates with interception 100% ground arthropods		1.9	106.4		
	Cereals BBCH 30 - 39	Small omnivorous mammal “mouse” Combination (invertebrates with interception) 25% weeds 50% weed seeds 25% ground arthropods		3.9	51.8		
	Cereals BBCH ≥ 40	Small herbivorous mammal "vole Grass + cereals 100% grass		21.7	9.3		
	Cereals BBCH ≥ 40	Small omnivorous mammal “mouse” Combination (invertebrates with interception) 25% weeds 50% weed seeds 25% ground arthropods		2.3	87.9		

TER values shown in **bold** fall below the relevant trigger

Table 9.3-9: Metrafenone: Screening step and tier 1 calculations of the long-term/reproductive risk for mammals due to the use of BAS 758 00 F for the crop group “cereals”

Data from Data Entry worksheet	Crop	Application rate (kg a.s./ha)	Number of applications	Application Interval	DT ₅₀	Reproductive End Point (mg/kg b.w./d)	Time weighted average (TWA)
	Cereals	0.15	2	14	10	811.0	0.53
Screening step:							
Reproductive risk assessment screening step	Indicator species	Shortcut value	Daily Dietary Dose (single)	MAF mean	Daily Dietary Dose (Multiple)	TER	No refinement step required
	Small herbivorous mammal	48.3	7.25	1.4	5.38	150.9	
First Tier Risk Assessment: ¹⁾							
Calculate TER for each generic focal species selected	Crop	Generic focal species		Shortcut value		TER	No refinement required
	Cereals BBCH ≥ 20	Small insectivorous mammal "shrew" ground dwelling invertebrates with interception 100% ground arthropods		1.9		3835.1	
	Cereals BBCH 30 - 39	Small omnivorous mammal “mouse” Combination (invertebrates with interception) 25% weeds 50% weed seeds 25% ground arthropods		3.9		1868.4	
	Cereals BBCH ≥ 40	Small herbivorous mammal "vole Grass + cereals 100% grass		21.7		335.8	
	Cereals BBCH ≥ 40	Small omnivorous mammal “mouse” Combination (invertebrates with interception) 25% weeds 50% weed seeds 25% ground arthropods		2.3		3168.1	

¹⁾ The screening step resulted in a TER value above the relevant trigger of 5. Hence, a first-tier risk assessment is not necessary. However, first-tier TER calculations are presented as they are required for calculation of combined reproductive toxicity.

Table 9.3-10: Pyraclostrobin: Screening step and tier 1 calculations of the long-term/reproductive risk for mammals due to the use of BAS 758 00 F for the crop group “cereals”

Data from Data_Entry worksheet	Crop	Application rate (kg a.s./ha)	Number of applications	Application Interval	DT ₅₀	Reproductive End Point (mg/kg b.w./d)	Time weighted average (TWA)
	Cereals	0.12	2	14	10	8.2	0.53
Screening step:							
Reproductive risk assessment screening step	Indicator species	Shortcut value	Daily Dietary Dose (single)	MAF mean	Daily Dietary Dose (Multiple)	TER	Please perform first tier risk assessment (see below)
	Small herbivorous mammal	48.3	5.80	1.4	4.30	1.9	
First Tier Risk Assessment:							
Calculate TER for each generic focal species	Crop	Generic focal species		Shortcut value		TER	
	Cereals BBCH ≥ 20	Small insectivorous mammal "shrew" ground dwelling invertebrates with interception 100% ground arthropods		1.9		48.5	No refinement required
	Cereals BBCH 30 - 39	Small omnivorous mammal “mouse” Combination (invertebrates with interception) 25% weeds 50% weed seeds 25% ground arthropods		3.9		23.6	No refinement required
	Cereals BBCH ≥ 40	Small herbivorous mammal "vole Grass + cereals 100% grass		21.7		4.2	Refinement required
	Cereals BBCH ≥ 40	Small omnivorous mammal “mouse” Combination (invertebrates with interception) 25% weeds 50% weed seeds 25% ground arthropods		2.3		40.0	No refinement required

TER values shown in **bold** fall below the relevant trigger

The conclusions for the first-tier dietary risk assessments for each of the active substances are as follows: acceptable acute risks for mammals were shown at the screening step for all three substances. The reproductive risk was acceptable at the screening and/or tier 1 risk assessment for mefentrifluconazole and metrafenone for all scenarios. The reproductive risk for pyraclostrobin was acceptable at the tier 1 risk assessment for all the scenarios, with the exception of the small herbivorous mammal “vole” scenario at BBCH ≥ 40. A higher-tier risk assessment is presented in chapter 9.3.2.2.

Dietary risk assessment for combined effects of simultaneous exposure to several active substances

Combined acute toxicity

According to EFSA/2009/1438 section 2.5 this assessment is relevant for BAS 758 00 F because this formulation contains more than one active substance.

Following Appendix B (step 1) in EFSA/2009/1438 a surrogate LD₅₀ = 1067.2 mg/kg bw is calculated based on the assumption of dose additivity (Table 9.3-11). A combined acute risk assessment is not required if for one active substance the deviation between ‘tox per fraction (a.s.)’ and ‘tox per fraction (mix)’ is ≤ 10% as in that case the risk is covered by the assessment for that active substance. For BAS 758 00 F this does not apply because the deviation for all active substances is more than 10% (Table 9.3-11).

Table 9.3-11: Calculation of surrogate LD₅₀ for the mixture of active substances

Active substance	Concentration a.s. in mixture [g/L]	Fraction a.s. in mixture	LD ₅₀ a.s. [mg/kg bw]	Fraction a.s./ LD ₅₀ a.s.	Surrogate LD ₅₀ [mg/kg bw]	Tox per fraction (a.s.)	Deviation tox per fraction (a.s.) and tox per fraction (mix) [%]
Mefentrifluconazole	66.6	0.27	>2000	0.00014	1067.2 3558.7	7405.4	594 108
Metrafenone	100	0.41	>5000	0.00008		12330.0	1055 246
Pyraclostrobin	80	0.32	450 >5000	0.00072 0.00064		1387.1 15625.0	30 339

A laboratory study on the acute toxicity of formulation BAS 758 00 F to rats (BASF DocID 2020/2101764) has been conducted and resulted in an acute LD₅₀ > 300 mg formulation/kg bw and < 2000 mg formulation/kg bw (see 9.3.1). Since at 2000 mg/kg bw one out of three animals died in the first step and all three animals died in the second step, while no mortality occurred in six animals dosed with 300 mg/kg bw, the LD₅₀ cut-off is 2000 mg/kg bw according to OECD 423, Figure 2d. This LD₅₀ cut-off is used in the risk assessment rather than the lower tested dose of 300 mg/kg bw because no mortality occurred at 300 mg/kg bw, therefore using this dose as an LD₅₀ value would result in an overly conservative risk assessment. Additionally, a dose-response curve was fitted via the probit model according to Finney (1971), resulting in an interpolated LD₅₀ value of 1792 mg formulation/kg bw which is in the same range and thus consistent with the cut-off value of 2000 mg formulation/kg bw.

Appendix B of EFSA/2009/1438 recommends comparing the surrogate LD₅₀ with the experimental LD₅₀ from formulation testing and to run the risk assessment with the lowest of the two values. However, Appendix B does not provide clear recommendations if for the comparison of the two LD₅₀ values and for the calculation of the exposure scenarios only the content of the active substances should be considered as the surrogate LD₅₀ is based on toxicity and concentration of active substances while the experimental LD₅₀ is based on all components of the formulation. Due to this lack of guidance in Appendix B the most comprehensive approach is adopted by the notifier by presenting the two possible risk assessments, one for the virtual compound and another for the formulation.

Exposure and acute risk assessment for combined active substances (virtual compound approach)

The potential exposure to the combined substances follows step 4 of Appendix B of EFSA/2009/1438. The maximum application rate of formulation BAS 758 00 F is 1.5 L product/ha (corresponding to 0.1 kg/ha mefentrifluconazole, 0.15 kg/ha metrafenone and 0.12 kg/ha pyraclostrobin) for the use in cereals; applying the concept for dose additivity to the exposure calculations results in a combined application rate of 0.37 kg virtual compound/ha.

The dietary TER acute value for the screening step presented in Table 9.3-12 is above the trigger of 10. Therefore, the acute risk to mammals from combined effects of the three active substances in BAS 758 00 F is acceptable.

Table 9.3-12: Screening step calculation of the acute risk for mammals due to the use of BAS 758 00 F in “cereals” - exposure to the combined active substances (virtual compound approach)

Data from Data_Entry worksheet	Crop	Application rate (kg virtual compound/ha)	Number of applications	Application Interval	DT ₅₀	LD ₅₀	
	Cereals	0.37	2	14	10.0	1067.2 3558.7	
Screening step:							
Acute risk assessment screening step	Indicator species	Shortcut value	Daily Dietary Dose (single)	MAF (90)	Daily Dietary Dose (Multiple)	TER	No refinement step required
	Small herbivorous mammal	118.4	43.81	1.2	52.57	20.3 67.7	

Note that this virtual compound acute TER was calculated according to the concentration addition approach and thus gives the same value as if calculated using equation $TER_{Acombi} = trigger / ((trigger / TER_{substance\ 1}) + (trigger / TER_{substance\ 2}) + (trigger / TER_{substance\ 3}))$.

Exposure and acute risk assessment for combined active substances (formulation approach)

BAS 758 00 F is intended to be used in the crop groups “cereals” with a maximum single application rate of 1.5 L product/ha. Taking into account the density of the formulation of 1.092 g/cm³, this will result in an application rate of 1.638 kg BAS 758 00 F/ha. The acute dietary risk assessment for mammals is presented in Table 9.3-13.

The dietary TER acute values for the tier 1 risk assessment based on the LD₅₀ cut-off of 2000 mg formulation/kg bw as presented in Table 9.3-13 are above the trigger of 10. Therefore, the acute risk to mammals from effects of BAS 758 00 F is acceptable.

Table 9.3-13: Screening step and tier 1 calculations of the acute risk for mammals due to the use of BAS 758 00 F for the crop group “cereals” – formulation approach

Data from Data_Entry worksheet	Crop	Application rate (kg formulation/ha)	Number of applications	Application Interval	DT ₅₀	LD ₅₀	
	Cereals	1.638	2	14	10.0	2000 1792	
Screening step:							
Acute risk assessment screening step	Indicator species	Shortcut value	Daily Dietary Dose (single)	MAF (90)	Daily Dietary Dose (Multiple)	TER	Please perform first tier risk assessment (see below)
	Small herbivorous mammal	118.4	193.94	1.2	232.73	8.6 7.7	
First Tier Risk Assessment:							
	Crop	Generic focal species		Shortcut value	TER		
Calculate TER for each generic focal species	Cereals BBCH ≥ 20	Small insectivorous mammal "shrew" ground dwelling invertebrates with interception 100% ground arthropods		5.4	188.4 168.9	No refinement required	
	Cereals BBCH 30 - 39	Small omnivorous mammal “mouse” Combination (invertebrates with interception) 25% weeds 50% weed seeds 25% ground arthropods		8.6	118.3 106.0		
	Cereals BBCH ≥ 40	Small herbivorous mammal "vole Grass + cereals 100% grass		40.9	24.9 22.3		
	Cereals BBCH ≥ 40	Small omnivorous mammal “mouse” Combination (invertebrates with interception) 25% weeds 50% weed seeds 25% ground arthropods		5.2	195.7 175.3		

TER values shown in **bold** fall below the relevant trigger

Combined reproductive toxicity

As requested in the summary report of the Steering Committee of the Central Zone Harmonisation workshop in April 2015 and update of October 2016 (Central Zone Harmonisation Workshop, 2016), a long-term combination toxicity tier 1 risk assessment is presented. As proposed there, the calculations follow the concentration addition model. TER_{Acombi} values are covered by the virtual compound approach, please see above for details).

The combined TER_{LT} value is calculated according to the following formula:

$$TER_{LTcombi} = trigger / ((trigger / TER_{substance\ 1}) + (trigger / TER_{substance\ 2}) + (trigger / TER_{substance\ 3}))$$

An acceptable risk is expected when TER_{LTcombi} > trigger.

The TER_{LT combi} values are calculated based on screening step and tier 1 TER values for the active substances. The calculations of the cumulative ecotoxicological effects are summarized in Table 9.3-14.

Table 9.3-14: Combined reproductive toxicity risk assessment for mammals for the crop group “cereals”

Crop scenario and/or indicator species		TER _{LT} ¹⁾ mefentrifluconazole	TER _{LT} ¹⁾ metrafenone	TER _{LT} ¹⁾ pyraclostrobin	TER _{LT} combi	Trigger
Reproductive (screening step)						
Cereals	Small herbivorous mammal	4.2	150.9	1.9	1.3	5
Reproductive (tier 1)						
Cereals BBCH ≥ 20	Small insectivorous mammal "shrew"	106.4	3835.1	48.5	33.0	5
Cereals BBCH 30 - 39	Small omnivorous mammal "mouse"	51.8	1868.4	23.6	16.1	5
Cereals BBCH ≥ 40	Small herbivorous mammal "vole"	9.3	335.8	4.2	2.9	5
Cereals BBCH ≥ 40	Small omnivorous mammal "mouse"	87.9	3168.1	40.0	27.3	5

TER values shown in **bold** fall below the relevant trigger

¹⁾ Reproductive TER values are presented in Table 9.3-8, Table 9.3-9 and Table 9.3-10.

The TER_{LT} combi values are above the relevant trigger of 5 for all scenarios, with the exception of the small herbivorous mammal “vole” scenario at BBCH ≥ 40. A higher-tier risk assessment is presented in chapter 9.3.2.2.

9.3.2.2 Higher-tier risk assessment

Based on the calculations presented in chapter 9.3.2.1 (first-tier risk assessment), higher-tier reproductive risk assessments for dietary exposure are required for the following two scenarios:

(i) Pyraclostrobin

- Reproductive risk assessment for the **small herbivorous mammal “vole” scenario** at BBCH ≥ 40

(ii) Combined reproductive risk assessment

- Reproductive risk assessment for the **small herbivorous mammal “vole” scenario** at BBCH ≥ 40

Relevance of the vole scenario

The common vole (*Microtus arvalis*) is proposed as representative species for the small herbivorous mammal “vole” scenarios by EFSA/2009/1438. However, the relevance of the “vole” scenario is highly discussed by the European Member States. As no harmonization currently exists within Member States on how to deal with the refinement of the risk to voles, the notifier proposes that the decision on and approval implications of any refinement should be conducted at Member State level.

The relevance of the “vole” scenarios for regulatory approvals of PPPs in crops is questionable because of the special biological characteristics of voles, particularly concerning population dynamics and resilience to stressors. Some of those key characteristics are: 1) Arable crops cannot be regarded as primary habitats for common voles; 2) Common vole populations naturally display cyclical changes, and a strong ability to recover from decimation due to their high reproductive potential; 3) Common voles are considered pests in many agricultural areas, since their high biomass consumption can lead to severe crop damage. For more details, see point A 2.1.2.2 in Appendix 2. Due to all uncertainties and discrepancies around the relevance of voles, the risk to small herbivorous mammals should be covered by the assessment of risk to another rodent, i.e., the omnivorous-wood mouse (*Apodemus sylvaticus*).

As some Member States consider the “vole” scenario as relevant, a quantitative higher-tier risk assessment is provided below, although the notifier considers the risk to small herbivorous mammals to be covered by the wood mouse.

The refinement parameters for the quantitative higher-tier reproductive risk assessments (refined TER_{LT} calculations) are:

- Refinement point 1: Foliage residue dissipation (DT₅₀) for pyraclostrobin
- ~~Refinement point 2: Ecological data on PD and FIR/bw for the common vole~~
- Refinement point 3: Relevant deposition factor (DF) for cereals at BBCH ≥ 40
- ~~Refinement point 4: Foliage residue dissipation (DT₅₀) for mefenflupyr (for combined reproductive risk assessment only)~~

(i) Reproductive dietary risk assessment for the small herbivorous mammal “vole” scenario for pyraclostrobin

a) Active ingredient-specific refinement parameter for pyraclostrobin as used in the reproductive higher-tier risk assessments

Refinement point 1: Foliage residue dissipation (DT₅₀) for pyraclostrobin

The geometric mean DT₅₀ = 1.72 days is used for the food item ‘grasses/cereal shoots’ and the geometric mean DT₅₀ = 1.99 days for the food item ‘non-grass herbs’ in the higher-tier TER calculations.

The DT₅₀ values were calculated based on 11 GLP field residue trials with pyraclostrobin in early growth stages of plants in the Northern Europe residue zone. Eight field trials were conducted in the 2012 growing season in pea (BASF DocID 2013/1044539) and wheat (BASF DocID 2013/1045207) at BBCH 12-14, each trial comprising two treated plots, to obtain foliar residue decline data that are fully applicable for Northern Europe. Three additional trials, each comprising one treated plot, were conducted in wheat at BBCH 25-29 in the 2016 growing season (BASF DocID 2017/1029774) and showed a residue decline behavior for pyraclostrobin well in line with that in the studies conducted at earlier BBCH stages of wheat. Based on the field data, DT₅₀ values were calculated and reported for the 11 trials conducted in Northern Europe (BASF DocIDs 2013/1078114 and 2017/1037247). The final reports have not yet been assessed in an EU peer-review evaluation and therefore are presented in Appendix 2 (A.2.1.2.2).

In Table 9.3-15 the DT₅₀ value for each trial and the resulting geometric mean DT₅₀ values for the food items ‘grasses/cereal shoots’ and ‘non-grass herbs’ are presented.

The justification of the use of the geometric mean $DT_{50} = 1.72$ days for the food item ‘grasses/cereal shoots’ and of the geometric mean $DT_{50} = 1.99$ days for the food item ‘non-grass herbs’ as well as the calculation of a “maximum 21-d twa factor” of 0.2293 for the food item ‘grasses/cereal shoots’ and of 0.2614 for the food item ‘non-grass herbs’ using the 21-day moving window approach described in Appendix F of EFSA/2009/1438, are presented in detail under point A 2.1.2.2 of Appendix 2 of this dossier.

Table 9.3-15: Foliar residue decline trials with pyraclostrobin in Northern Europe: DT_{50} in wheat and peas (BASF DocID 2013/1078114 and 2017/1037247)

BASF DocID of field trial data	Crop	BBCH	Location	Trial	Plot	DT ₅₀ [d]	Mean DT ₅₀ [d] of P2 and P3
2013/1045207	Wheat	13/14	DE; Limburgerhof, Rheinland-Pfalz	L120103	P2	2.67	2.02 ¹⁾
	Wheat	13/14	DE; Limburgerhof, Rheinland-Pfalz		P3	1.37	
	Wheat	13/14	DE; Kleve, Nordrhein-Westfalen	L120104	P2	1.40	1.69 ¹⁾
	Wheat	13/14	DE; Kleve, Nordrhein-Westfalen		P3	1.97	
	Wheat	13/14	NL; Gennep, Limburg	L120105	P2	2.18	1.87 ¹⁾
	Wheat	13/14	NL; Gennep, Limburg		P3	1.56	
	Wheat	13/14	UK; Oxfordshire	L120106	P2	1.26	1.26 ¹⁾
	Wheat	13/14	UK; Oxfordshire		P3	1.25	
2017/1029774	Wheat	25-29	DE; Limburgerhof, Rheinland-Pfalz	L160146	P2	2.22	2.22 ²⁾
	Wheat	25-29	DE; Stetten, Baden-Wuerttemberg	L160147	P2	1.17	1.17 ²⁾
	Wheat	25-29	DE; Mauchenheim, Rheinland-Pfalz	L160148	P2	2.11	2.11 ²⁾
Geometric mean (n = 7) DT ₅₀ [d] for monocotyledonous plants (food item grasses/cereal shoots)							1.72
2013/1044539	Peas	12/13	DE; Limburgerhof, Rheinland-Pfalz	L120073	P2	1.28	1.39 ¹⁾
	Peas	12/13	DE; Limburgerhof, Rheinland-Pfalz		P3	1.50	
	Peas	12/13	DE; Kerken, Nordrhein-Westfalen	L120074	P2	1.91	1.73 ¹⁾
	Peas	12/13	DE; Kerken, Nordrhein-Westfalen		P3	1.55	
	Peas	12/13	FR (N); Loir et Cher	L120075	P2	3.76	3.13 ¹⁾
	Peas	12/13	FR (N); Loir et Cher		P3	2.50	
	Peas	12/13	UK; Essex	L120076	P2	2.10	2.08 ¹⁾
	Peas	12/13	UK; Essex		P3	2.06	
Geometric mean (n = 4) DT ₅₀ [d] for dicotyledonous plants (food item non-grass herbs)							1.99

¹⁾ Trial comprises two treated plots

²⁾ Trial comprises one treated plot

Review Comments:

The applicant has provided foliar residue decline data of 16 trials specifically designed for the mammals risk assessment (14 trials conducted in the Central Zone, 2 trials conducted in Northern France). The application rate applied was higher than that for BAS 758 00 F and corresponded to 250 g/ha pyraclostrobin (as opposed to 120 g/ha). The foliar residue decline trials by BASF were specifically targeted to cover the preferred principal growth stages 1 (leaf development) and 2 (side shoots/ tillering). Thus the trials provided by the applicant cover the relevant plant growth stages eaten by wild mammals in accordance with EFSA/2009/1438.

Additionally, the applicant has provided residue decline data at BBCH 25-29.

Due to the high trial number the use of the geomean DT_{50} (for wheat and pea) value in risk assessment seems to be justified as represents a realistic worst-case.

b) Generic refinement parameters as used in the reproductive higher-tier risk assessments

Refinement point 2: Ecological data on PD and FIR/bw for the common vole

~~The 100% monocotyledon diet as assumed by EFSA (2009) is a worst case estimate because it has been shown that voles consume both monocots and dicots.~~

~~Based on data from Rinke (1991) and following CTGB guidance for risk assessment of plant protection products (CTGB, 2020, pp. 26-28, version 2.5, PD for agricultural crops for chronic risk assessments), the following PD values are used for the refined chronic risk assessment for voles in cereals:~~

Grasses	PD = 0.50	
Non-grass herbs	PD = 0.50	PD sum = 1.0

~~These PD values are used for the calculation of the FIR/bw values (see Appendix 2.1.2.2 for details).~~

Review Comments:

For the purposes of this assessment, consideration of refined the PD value parameter is not required. Therefore, new TER calculations without changed of this factor were performed.

Refinement point 3: Relevant deposition factor (DF) for cereals at BBCH ≥ 40

Following EFSA guidance for evaluating laboratory and field dissipation studies to obtain DegT50 values (EFSA Journal 2014; 12(5):3662, p. 28, Table 1.5) and CTGB guidance for risk assessment of plant protection products (CTGB, 2020, pp. 14-17, Table 2, version 2.5, July 2020), a **DF of 0.1** for cereals at BBCH 40-69 will be applied for the small herbivorous mammal scenario at BBCH ≥ 40 in cereals.

Review Comments:

According to “Working document on Risk Assessment of Plant Protection Products in the Central Zone – Ecotoxicology” (May 2021), point 3.2.15, the interception values following EFSA Guidance Document to obtain DegT50 values (EFSA Journal 2014;12(5):3662), can be use in the Tier 2 risk assessment. Therefore, the Applicant proposal was accepted.

Higher-tier TER calculations

The refined TER_{LT} provided in Table 9.3-16 for pyraclostrobin is above the relevant trigger of 5, indicating an acceptable reproductive risk.

Table 9.3-16: Pyraclostrobin: Refined reproductive dietary risk assessment for the “vole” (common vole) scenario in cereals at BBCH \geq 40

Food type	FIR/bw	PD _i , fresh	PT	RUD _{mean} [mg a.s./kg]	Maximum 21-d twa factor	DF	Use rate [kg a.s./ha]	DDD [mg a.s./kg bw/d]
Grasses/ Cereal shoots	1.462	0.50 ¹⁾	1.0	54.2	0.2293 ¹⁾	0.1 ¹⁾	0.120	0.109
Non-grass herbs	1.462	0.50 ¹⁾	1.0	28.7	0.2614 ¹⁾	0.1 ¹⁾	0.120	0.066
Sum DDD [mg a.s./kg bw/d]								0.175
Toxicity endpoint [mg a.s./kg bw/d]								8.2
TER _{LT}								46.9
Food type	FIR/bw	PD _i , fresh	PT	RUD _{mean} [mg a.s./kg]	Maximum 21-d twa factor	DF	Use rate [kg a.s./ha]	DDD [mg a.s./kg bw/d]
Grasses/ Cereal shoots	1.33	1.0	1.0	54.2	0.2293 ¹⁾	0.1 ¹⁾	0.120	0.198
Toxicity endpoint [mg a.s./kg bw/d]								8.2
TER _{LT}								41.4

¹⁾ Refined parameter

Additional qualitative evidence that pyraclostrobin has no adverse effects on free-living wood mouse and common vole populations

Two field effect studies are available to support the low risk from applications of pyraclostrobin to small mammals, as presented in the above quantitative risk assessments for the wood mouse and the common vole.

An initial field effect study on the acute and long term effects of the pyraclostrobin (applied as solo-formulation BAS 500 06 F) on populations of small mammals (BASF DocID 2014/1000041) confirmed the low risk from applications of pyraclostrobin to wood mice. A detailed summary of the study can be found in Appendix 2 of this chapter (A 2.1.2.2). In brief, live trapping (capture-mark-recapture) was carried out from April to October 2013 to compare the abundance and population dynamics of small mammal species in six treated and six untreated winter cereal fields and adjacent off-crop areas in Germany. Eight different parameters (captures/individuals in- and off-crop, trapping efficiency, minimum number alive, population growth rate, percentage of reproductively active individuals, percentage of juveniles, percentage of females, and adult body weight) were monitored for the whole growth period of winter cereals. Though trapping efficiency of common voles was relatively low in this study, no signs of any potential impacts of on voles were observed.

To confirm the initial findings for the common vole, a second field-effect study on the acute and long-term effects of pyraclostrobin (applied as solo-formulation BAS 500 06 F) on populations of common voles (BASF DocID 2015/1126803) was conducted. The study was performed under realistic worst-case field conditions in meadows, which are—in contrast to e.g., arable crop fields—preferred common vole habitats with high abundances of common voles. Meadows were used as a surrogate for other crop types to ensure sufficient numbers of common voles being exposed to the test item pyraclostrobin applied as solo-formulation BAS 500 06 F. A detailed summary of the study can be found in Appendix 2 of this chapter (A 2.1.2.2). In brief, live trapping (capture-mark-recapture) was carried out in ten trapping sessions from May to October 2015. The abundance and population dynamics of common voles was compared in five treated (2 applications of pyraclostrobin applied as BAS 500 06 F following a use pattern of 2 x 250 g pyraclostrobin/ha) and five untreated study fields in Germany. Seven different parameters (trapping success, minimum number alive (MNA), recapture rate, sex ratio, proportion of reproductively active animals, age structure and body weight development) were monitored for the whole study period, which covered the majority of the reproductive season of common voles. Overall, trapping success of common voles was high: 9161 captures of common voles were made, including a total of 2495 individually marked animals. No adverse acute and long-term effects on common vole populations in meadows were detected in any of the parameters investigated.

In conclusion, the studies showed no impacts of the fungicide pyraclostrobin, applied as solo-formulation BAS 500 06 F under field conditions, on the common vole (*Microtus arvalis*) and wood mouse (*Apodemus sylvaticus*).

Review Comments:

For the purposes of this assessment, no further data are required to conclude safe use of BAS 758 00 F.

Based on the higher tier risk assessment, where the deposition factor and foliage residue dissipation (DT₅₀) for pyraclostrobin were modified, all TERs exceed the trigger values set by Commission regulation (EU) 546/2011 for acceptability of effects.

(ii) Reproductive dietary risk assessment for the small herbivorous mammal “vole” scenario for combined toxicity

In the summary report of the Steering Committee of the Central Zone Harmonisation workshop in April 2015 and update of October 2016, the Member States did not provide specific guidance for applicants and risk assessors from the competent regulatory authorities on how to proceed in case the tier-1 calculation

fails the trigger. In the words of the summary report, “*refinement options [for the long-term combined toxicity risk assessment] remain unclear.*” To address the issue in this case, the applicant has decided to also refine the tier 1 risk assessment for mefentrifluconazole and metrafenone and use these refined TER values in a higher tier combined reproductive toxicity risk assessment (Table 9.3-19).

The two generic refinement points as used in the higher tier risk assessment for pyraclostrobin above, i.e., the ecological data on PD and FIR/bw for the common vole (refinement point 2) and the relevant deposition factor (DF) for cereals at BBCH ≥ 40 (refinement point 3) will also be applied in the higher tier reproductive risk assessments for mefentrifluconazole and metrafenone. In addition, foliage residue dissipation data available from studies with mefentrifluconazole will be applied, as summarized below.

Refinement point 4: Foliage residue dissipation (DT₅₀) for mefentrifluconazole

The geometric mean DT₅₀ = 3.72 days is used for the food item ‘grasses/cereal shoots’ and the geometric mean DT₅₀ = 2.67 days for the food item ‘non-grass herbs’ in the higher tier TER calculations.

The DT₅₀ values were calculated based on 15 GLP field residue trials with mefentrifluconazole in early growth stages of plants. Eight field trials in wheat (BASF DocID 2018/1205816) and seven field trials in peas (BASF DocID 2018/1205813) were performed in the 2018 growing season in Southern and Northern Europe residue zones. Based on the field data, DT₅₀ values were calculated and reported for the 15 trials (BASF DocIDs 2019/2034648 and 2019/2034650). The final reports have not yet been assessed in an EU peer review evaluation and therefore are presented in Appendix 2 (A.2.1.2.2).

In Table 9.3-157 the DT₅₀ value for each trial and the resulting geometric mean DT₅₀ values for the food items ‘grasses/cereal shoots’ and ‘non-grass herbs’ are presented. DT₅₀ data are consistent between the Northern and Southern European residue zones for each plant type (DT₅₀ for wheat ‘North’ vs. DT₅₀ for wheat ‘South’: two-sided $p = 0.78$, assuming equal variance; DT₅₀ for pea ‘North’ vs. DT₅₀ for pea ‘South’: two-sided $p = 0.62$, assuming equal variance). For wheat and for peas, residue decline data from the two European residue zones can thus be merged and are considered fully applicable for the Central zone. Details on the calculations of the relevant DT₅₀ and “maximum 21-d two factor” values for mefentrifluconazole are presented under point A 2.1.2.2 of Appendix 2 of this dossier.

Table 9.3-17: Foliar residue decline trials with mefentrifluconazole in Northern and Southern Europe: DT₅₀ in wheat and peas (BASF DocIDs 2019/2034648 and 2019/2034650)

Plant	Trial no.	Country	Zone	Kinetic model	DT ₅₀ [d]
Wheat	L170451	Germany	North	SFO	4.1
	L170452	Germany	North	SFO	6.0
	L170453	Belgium	North	SFO	2.3
	L170454	The Netherlands	North	SFO	2.8
	L170455	Spain	South	SFO	2.4
	L170456	Spain	South	SFO	3.3
	L170457	Italy	South	SFO	5.0
	L170458	Italy	South	SFO	5.8
Geometric mean (n = 8) DT ₅₀ [d] for monocotyledonous plants (food item grasses/cereal shoots)					3.72
Pea	L180497	Germany	North	SFO	3.5
	L170444	Germany	North	SFO	2.5
	L170446	The Netherlands	North	SFO	4.7
	L170447	Spain	South	SFO	2.8
	L170448	Spain	South	SFO	3.5
	L170449	Italy	South	SFO	2.1
	L170450	Italy	South	SFO	3.2
Geometric mean (n = 7) DT ₅₀ [d] for dicotyledonous plants (food item non-grass herbs)					2.67

Higher-tier TER calculations

The refined TER_{LT} value for pyraclostrobin is presented above in Table 9.3-16 and refined TER_{LT} values for mefentrifluconazole and metrafenone are provided in Table 9.3-17 and Table 9.3-18, respectively. Using these refined TER_{LT} values for the active ingredients, the refined $TER_{LT\text{-}combi}$ was calculated and is provided in Table 9.3-19.

Table 9.3-17: Mefentrifluconazole: Refined reproductive dietary risk assessment for the “vole” (common vole) scenario in cereals at BBCH ≥ 40

Food type	FIR/bw	PD_i , fresh	PT	RUD_{mean} [mg a.s./kg]	Maximum 21-d twa factor	DF	Use rate [kg a.s./ha]	DDD [mg a.s./kg bw/d]
Grasses/ Cereal shoots	1.462	0.50 ¹⁾	1.0	54.2	0.4367 ¹⁾²⁾	0.1 ¹⁾	0.1	0.173
Non-grass herbs	1.462	0.50 ¹⁾	1.0	28.7	0.3363 ¹⁾²⁾	0.1 ¹⁾	0.1	0.071
Sum DDD [mg a.s./kg bw/d]								0.244
Toxicity endpoint [mg a.s./kg bw/d]								15
TER_{LT}								61.6

¹⁾— Refined parameter

²⁾— Max. 21-d twa factor based on DT_{50} of 3.72 days from wheat trials used for the food item ‘grasses/cereal shoots’ and DT_{50} of 2.67 days from pea trials used for the food item ‘non-grass herbs’. For study summaries of field trials and DT_{50} calculations for mefentrifluconazole please refer to Appendix A 2.1.2.2.

Table 9.3-18: Metrafenone: Refined reproductive dietary risk assessment for the “vole” (common vole) scenario in cereals at BBCH ≥ 40

Food type	FIR/bw	PD_i , fresh	PT	RUD_{mean} [mg a.s./kg]	Maximum 21-d twa factor	DF	Use rate [kg a.s./ha]	DDD [mg a.s./kg bw/d]
Grasses/ Cereal shoots	1.462	0.50 ¹⁾	1.0	54.2	0.7908	0.1 ¹⁾	0.15	0.470
Non-grass herbs	1.462	0.50 ¹⁾	1.0	28.7	0.7908	0.1 ¹⁾	0.15	0.249
Sum DDD [mg a.s./kg bw/d]								0.719
Toxicity endpoint [mg a.s./kg bw/d]								811
TER_{LT}								1128.3

¹⁾— Refined parameter

Review Comments:

For the purposes of this assessment, no further data are required to conclude safe use of BAS 758 00 F.

Based on the higher tier risk assessment, where the deposition factor and foliage residue dissipation (DT_{50}) for pyraclostrobin were modified, all TERs exceed the trigger values set by Commission regulation (EU) 546/2011 for acceptability of effects.

Table 9.3-19: BAS 758 00 F: Refined combined reproductive toxicity risk assessment for the “vole” (common vole) scenario in cereals at BBCH \geq 40

Crop scenario and/or indicator species		TER _{LT} (higher tier) Mefentrifluconazole	TER _{LT} (higher tier) Metrafenone	TER _{LT} (higher tier) Pyraclostrobin	TER _{LT combi}	Trigger
Reproductive – Higher tier						
Cereals BBCH \geq 40	Small herbivorous mammal "vole Grass + cereals 100% grass	61.6	1128.3	46.9	18.5	5

Crop scenario and/or indicator species		TER _{LT} Mefentrifluconazole	TER _{LT} Metrafenone	TER _{LT} (higher tier) Pyraclostrobin	TER _{LT combi}	Trigger
Reproductive – Higher tier						
Cereals BBCH \geq 40	Small herbivorous mammal "vole Grass + cereals 100% grass	9.3	335.8	41.4	6.3	5

The refined TER_{LT combi} value for the “vole” scenario at BBCH \geq 40 in cereals is above the trigger value of 5. Thus, it can be concluded that the reproductive risk for mammals from the combined exposure to the three active substances in the application of BAS 758 00 F according to good agricultural practice is low and acceptable.

9.3.2.3 Drinking water exposure

Puddle scenario

Due to the characteristics of the exposure scenario in connection with the standard assumptions for water uptake by animals, no specific calculations of exposure and TER are necessary when the ratio of effective application rate (in g/ha) to relevant endpoint (in mg/kg bw/d) does not exceed 50 in the case of less sorptive substances ($K_{oc} < 500$ L/kg) or 3000 in the case of more sorptive substances ($K_{oc} \geq 500$ L/kg).

The ratio calculations for effective application rate to relevant endpoint are detailed in Table 9.3-20 (mefentrifluconazole), Table 9.3-21 (metrafenone) and Table 9.3-22 (pyraclostrobin) and are based on worst-case assumptions with regard to the resulting AR_{eff}. The ratios for acute and reproductive endpoints for mefentrifluconazole (< 0.1 and 13.0 , respectively), metrafenone (< 0.1 and 0.4 , respectively) and for pyraclostrobin (0.4 and 23.1 , respectively) do not exceed the threshold value of 3000 for all active substances, thus no specific calculations of exposure for mammals through drinking water for the puddle scenario are necessary. Therefore, a quantitative drinking water risk assessment for the puddle scenario is not triggered.

Table 9.3-20: Assessment of the risk for mammals due to exposure to mefentrifluconazole via contaminated drinking water in puddles

Parameter	Mefentrifluconazole	Reference
K_{oc} (geometric mean, n=8) [L/kg]	3455.6	Chapter 8.9.2 (EFSA Journal 2018; 16(7): 5379)
DT ₅₀ (soil) [days]	200	Chapter 8.9.2 (EFSA Journal 2018; 16(7): 5379)
Number of applications	2	Chapter 9.1
Interval [days]	14	Chapter 9.1
MAF _m ¹⁾	1.95	--
Max use rate [g/ha]	100	Chapter 9.1
AR _{eff} [g/ha] ²⁾	195.0	--

Parameter	Mefentrifluconazole	Reference
LD ₅₀ [mg/kg bw]	>2000	Chapter 9.3.1
Ratio (acute) ³⁾	<0.1	--
NO(A)EL [mg/kg bw/d]	15	Chapter 9.3.1
Ratio (repro) ³⁾	13.0	--
Trigger	3000	--
Drinking water assessment required [Yes/No]	No	--

¹⁾ $MAF_m = (1 - e^{-nki}) / (1 - e^{-ki})$ with $k = \ln(2)/DT_{50}$ (rate constant), n = number of applications and i = application interval [d]

²⁾ $AR_{eff} = \text{Application rate [g/ha]} \times MAF_m$

³⁾ Ratio of AR_{eff} and relevant toxicity endpoint

Table 9.3-21: Assessment of the risk for mammals due to exposure to metrafenone via contaminated drinking water in puddles

Parameter	Metrafenone	Reference
K _{oc} (geometric mean, n=5) [L/kg]	2812	Chapter 8.9.2 (EFSA Scientific Report (2006) 58, 1-72)
DT ₅₀ (soil) (geometric mean, n=5) [days]	250.6	Chapter 8.9.2 (EFSA Scientific Report (2006) 58, 1-72)
Number of applications	2	Chapter 9.1
Interval [days]	14	Chapter 9.1
MAF _m ¹⁾	1.96	--
Max use rate [g/ha]	150	Chapter 9.1
AR _{eff} [g/ha] ²⁾	294.0	--
LD ₅₀ [mg/kg bw]	>5000	Chapter 9.3.1
Ratio (acute) ³⁾	<0.1	--
NO(A)EL [mg/kg bw/d]	811	Chapter 9.3.1
Ratio (repro) ³⁾	0.4	--
Trigger	3000	--
Drinking water assessment required [Yes/No]	No	--

¹⁾ $MAF_m = (1 - e^{-nki}) / (1 - e^{-ki})$ with $k = \ln(2)/DT_{50}$ (rate constant), n = number of applications and i = application interval [d]

²⁾ AR_{eff} = Application rate [g/ha] x MAF_m

³⁾ Ratio of AR_{eff} and relevant toxicity endpoint

Table 9.3-22: Assessment of the risk for mammals due to exposure to pyraclostrobin via contaminated drinking water in puddles

Parameter	Pyraclostrobin	Reference
K _{oc} (geometric mean, n=6) [L/kg]	8856	Chapter 8.9.2 (Monograph 12945/ ECCO/BBA/01)
DT ₅₀ (soil) [days]	18	Chapter 8.9.2
Number of applications	2	Chapter 9.1
Interval [days]	14	Chapter 9.1
MAF _m ¹⁾	1.58	--
Max use rate [g/ha]	120	Chapter 9.1
AR _{eff} [g/ha] ²⁾	189.6	--
LD ₅₀ [mg/kg bw]	450 >5000	Chapter 9.3.1
Ratio (acute) ³⁾	0.4 0.04	--
NO(A)EL [mg/kg bw/d]	8.2	Chapter 9.3.1
Ratio (repro) ³⁾	23.1	--
Trigger	3000	--
Drinking water assessment required [Yes/No]	No	--

¹⁾ $MAF_m = (1 - e^{-nki}) / (1 - e^{-ki})$ with $k = \ln(2)/DT_{50}$ (rate constant), n = number of applications and i = application interval [d]

²⁾ AR_{eff} = Application rate [g/ha] x MAF_m

³⁾ Ratio of AR_{eff} and relevant toxicity endpoint

In conclusion, the risk to mammals via drinking water from the intended use of BAS 758 00 F according to the proposed use pattern is acceptable.

9.3.2.4 Effects of secondary poisoning

The log P_{ow} of the active substance mefentrifluconazole is 3.4 (EFSA Journal 2018; 16(7): 5379) and thus does exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is required.

The log P_{ow} of the active substance metrafenone is 4.3 (EFSA Scientific Report (2006) 58, 1- 72) and thus does exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is required.

The log P_{ow} of pyraclostrobin is 3.99 (SANCO/1420/2001-final) and thus does exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is required.

Risk assessment for earthworm-eating mammals via secondary poisoning

According to EFSA/2009/1438, the risk for vermivorous mammals is assessed for a small mammal of 10 g body weight with a daily food consumption of 12.8 g. Bioaccumulation in earthworms is estimated based on predicted concentrations in soil is based on experimental data.

As shown in the following Table 9.3-23 (mefentrifluconazole), Table 9.3-24 (metrafenone) and Table 9.3-25 (pyraclostrobin), the TER_{LT} values for all active substances exceed the relevant trigger of 5 for acceptability of effects, indicating an acceptable risk to earthworm-eating mammals via secondary poisoning.

Table 9.3-23: Assessment of the risk for earthworm-eating mammals due to exposure to mefentrifluconazole via bioaccumulation in earthworms (secondary poisoning) for the intended uses

Parameter	Mefentrifluconazole	Reference
PEC_{soil} (accu) [mg/kg soil] ¹⁾	0.205	Chapter 8.7
K_{ow}	2350	BASF DocID 2013/1382370
K_{oc} (geometric mean) [L/kg]	3455.6	Chapter 8.9.2 (EFSA Journal 2018; 16(7): 5379)
f_{oc} (default)	0.02	EFSA/2009/1438
BCF ²⁾	0.420	--
PEC_{worm} [mg/kg] ³⁾	0.086	--
Daily dose [mg/kg bw/d] ⁴⁾	0.110	--
NO(A)EL [mg/kg bw/d]	15	Chapter 9.3.1
TER_{LT} ⁵⁾	136.0	--

¹⁾ PEC_{soil} (accu) value was calculated for an application scenario of 2 x 100 g a.s./ha with 14-day interval in cereals. For details see chapter 8.7.

²⁾ Bioconcentration factor (BCF) = $(0.84 + 0.012 \times K_{ow}) / (f_{oc} \times K_{oc})$

³⁾ $PEC_{worm} = PEC_{soil} \times BCF$

⁴⁾ Daily dose = $1.28 \times PEC_{worm}$

⁵⁾ $TER_{LT} = NO(A)EL / \text{Daily dose}$.

Table 9.3-24: Assessment of the risk for earthworm-eating mammals due to exposure to metrafenone via bioaccumulation in earthworms (secondary poisoning) for the intended use

Parameter	Metrafenone	Reference
PEC _{soil} (accu) [mg/kg soil] ¹⁾	0.158	Chapter 8.7
K _{ow}	19953 ⁶⁾	EFSA Scientific Report (2006) 58, 1-72 (for log P _{ow} =4.3)
K _{oc} (geometric mean, n=5) [L/kg]	2812	Chapter 8.9.2 (EFSA Scientific Report (2006) 58, 1-72)
f _{oc} (default)	0.02	EFSA/2009/1438
BCF ²⁾	4.272	--
PEC _{worm} [mg/kg] ³⁾	0.675	--
Daily dose [mg/kg bw/d]	0.864	--
NO(A)EL [mg/kg bw/d]	811	Chapter 9.3.1
TER _{LT}	938.6	--

¹⁾ PEC_{soil} (accu) value was calculated for an application scenario of 2 x 150 g a.s./ha with 14-day interval in cereals. For details see chapter 8.7.

²⁾ Bioconcentration factor (BCF) = $(0.84 + 0.012 \times K_{ow}) / (f_{oc} \times K_{oc})$

³⁾ PEC_{worm} = PEC_{soil} x BCF

⁴⁾ Daily dose = 1.28 x PEC_{worm}

⁵⁾ TER_{LT} = NO(A)EL / Daily dose.

Table 9.3-25: Assessment of the risk for earthworm-eating mammals due to exposure to pyraclostrobin via bioaccumulation in earthworms (secondary poisoning) for the intended use

Parameter	Pyraclostrobin	Reference
PEC _{soil} (twa, 21 days) [mg/kg soil] ¹⁾	0.047	Chapter 8.7
K _{ow}	9772	SANCO/1420/2001-final
K _{oc} (geometric mean, n=6) [L/kg]	8856	Chapter 8.9.2 (Monograph 12945/ ECCO/BBA/01)
f _{oc} (default)	0.02	EFSA/2009/1438
BCF ²⁾	0.667	--
PEC _{worm} [mg/kg] ³⁾	0.031	--
Daily dose [mg/kg bw/d]	0.040	--
NO(A)EL [mg/kg bw/d]	8.2	Chapter 9.3.1
TER _{LT}	204.4	--

¹⁾ The PEC_{soil} (twa, 21 days) value was calculated for an application scenario of 2 x 120 g a.s./ha with 14-day interval in cereals. For details see chapter 8.7.

²⁾ Bioconcentration factor (BCF) = $(0.84 + 0.012 \times K_{ow}) / (f_{oc} \times K_{oc})$

³⁾ PEC_{worm} = PEC_{soil} x BCF

⁴⁾ Daily dose = 1.28 x PEC_{worm}

⁵⁾ TER_{LT} = NO(A)EL / Daily dose.

Risk assessment for fish-eating mammals via secondary poisoning

According to EFSA/2009/1438, the risk for piscivorous mammals is assessed for a mammal of 3000 g body weight with a daily food consumption of 425 g. Bioaccumulation in fish is estimated based on predicted concentrations in surface water.

As shown in the following Table 9.3-26 (mefentrifluconazole), Table 9.3-27 (metrafenone) and Table 9.3-28 (pyraclostrobin), the TER_{LT} values for all active substances exceed the relevant trigger of 5 for acceptability of effects, indicating an acceptable risk to fish-eating mammals via secondary poisoning.

Table 9.3-26: Assessment of the risk for fish-eating mammals due to exposure to mefentrifluconazole via bioaccumulation in fish (secondary poisoning) for the intended use

Parameter	Mefentrifluconazole	Reference
PEC_{sw} , (tw, 21 days) [mg/L] ¹⁾	2.020×10^{-3}	Chapter 8.9
BCF fish (max. worst case)	385	EFSA Journal 2018; 16(7): 5379
PEC_{fish} [mg/kg] ²⁾	0.778	--
Daily dose [mg/kg b.w./d] ³⁾	0.110	--
NO(A)EL [mg/kg b.w./d]	15	Chapter 9.3.1
TER_{LT} ⁴⁾	135.8	--

¹⁾ PEC_{sw} (21 d tw) value calculated for a multiple application scenario in spring and winter cereals (2x 100 g/ha with 14-day interval) from FOCUS Step 2 (Northern Europe scenario) as worst-case. For details see chapter 8.9.

²⁾ $PEC_{fish} = PEC_{sw}$, (tw, 21 days) x BCF

³⁾ Daily dose = $0.142 \times PEC_{fish}$

⁴⁾ $TER_{LT} = NO(A)EL / \text{Daily dose}$.

Table 9.3-27: Assessment of the risk for fish-eating mammals due to exposure to metrafenone via bioaccumulation in fish (secondary poisoning) for the intended use

Parameter	Metrafenone	Reference
PEC_{sw} , (tw, 21 days) [mg/L] ¹⁾	2.184×10^{-3}	Chapter 8.9
BCF fish (max. worst case)	530	EFSA Scientific Report (2006) 58, 1-72
PEC_{fish} [mg/kg] ²⁾	1.158	
Daily dose [mg/kg b.w./d] ³⁾	0.164	--
NO(A)EL [mg/kg b.w./d]	811	Chapter 9.3.1
TER_{LT} ⁴⁾	4934.1	--

¹⁾ PEC_{sw} (21 d tw) value calculated for a multiple application scenario in spring and winter cereals (2x 150 g/ha with 14-day interval) from FOCUS Step 2 (Northern Europe scenario) as worst-case. For details see chapter 8.9.

²⁾ $PEC_{fish} = PEC_{sw}$, (tw, 21 days) x BCF

³⁾ Daily dose = $0.142 \times PEC_{fish}$

⁴⁾ $TER_{LT} = NO(A)EL / \text{Daily dose}$.

Table 9.3-28: Assessment of the risk for fish-eating mammals due to exposure to pyraclostrobin via bioaccumulation in fish (secondary poisoning) for the intended use

Parameter	Pyraclostrobin	Reference
PEC _{sw} , (twa, 21 days) [mg/L] ¹⁾	0.806×10^{-3}	Chapter 8.9
BCF fish (max. worst case)	736	SANCO/1420/2001-final
PEC _{fish} [mg/kg] ²⁾	0.593	--
Daily dose [mg/kg b.w./d] ³⁾	0.084	--
NO(A)EL [mg/kg b.w./d]	8.2	Chapter 9.3.1
TER _{LT} ⁴⁾	97.3	--

¹⁾ PEC_{sw} (21 d twa) value calculated for a multiple application scenario in spring and winter cereals (2x 150 g/ha resulting from a risk envelope approach) from FOCUS Step 2 (Northern Europe scenario) as worst-case. For details see chapter 8.9.

²⁾ PEC_{fish} = PEC_{sw}, (twa, 21 days) x BCF

³⁾ Daily dose = 0.142 x PEC_{fish}

⁴⁾ TER_{LT} = NO(A)EL / Daily dose.

9.3.2.5 Biomagnification in terrestrial food chains

Low potential for accumulation in animal tissue was concluded in the EU review of mefentrifluconazole (see EFSA Journal 2018;16(7):5379).

Low potential for accumulation in animal tissue was concluded in the EU review of metrafenone (EFSA Scientific Report (2006) 58, 1- 72).

Low potential for accumulation in animal tissue was concluded in the EU review of pyraclostrobin (SANCO/1420/2001-final).

Since the bioaccumulation potential of mefentrifluconazole, metrafenone and pyraclostrobin is low no further assessment on biomagnification is required.

9.3.3 Risk assessment for baits, pellets, granules, prills or treated seed

Not relevant.

9.3.4 Overall conclusions

It can be concluded that the risk to mammals from the application of BAS 758 00 F according to good agricultural practice is acceptable.

Review Comments:

The acute and chronic risks of BAS 758 00 F to mammals were assessed from toxicity exposure ratios between toxicity endpoints, estimated from study with active ingredients and maximum residues occurring on food items. An acute oral toxicity study with BAS 758 00 F in rats was taken to consideration in the evaluation.

All acute TER values exceed the relevant triggers in the screening step or Tier 1 risk assessment for mefentrifluconazole, metrafenone, pyraclostrobin and for combined active substances (virtual compound and formulation approach).

The reproductive risk was acceptable at the screening or Tier 1 risk assessment for mefentrifluconazole and metrafenone for all scenarios. For pyraclostrobin and combined reproductive risk assessment, the TER values, were acceptable at the Tier 1 for all the scenarios, with the exception of the small herbivorous mammal “vole” scenario at BBCH ≥ 40 .

Based on the higher tier risk assessment, where the deposition factor and foliage residue dissipation (DT₅₀) for pyraclostrobin were modified, all TERs exceed the trigger values set by Commission regulation (EU) 546/2011 for acceptability of effects.

Evaluation of exposing to mammals through the drinking water demonstrated the acceptable risk. The potential risk of secondary poisoning is low.

References

- CTGB. 2020. Evaluation Manual for the Authorisation of plant protection products according to Regulation (EC) No 1107/2009. EU part. Plant protection products. Chapter 7 Ecotoxicology: terrestrial; birds and mammals. version 2.5; July 2020. Board for the Authorisation of plant protection products and biocides (ctgb).
- EFSA/2009/1438. Guidance Document on risk assessment for Birds & Mammals. EFSA Journal 2009; 7(12):1438.
- EFSA/2014/3662. EFSA Guidance Document for evaluating laboratory and field dissipation studies to obtain DegT50 values of active substances of plant protection products and transformation products of these active substances in soil. EFSA Journal 2014; 12(5):3662.
- Rinke T. 1991. Percentage of volume versus number of species: Availability and intake of grasses and forbs in *Microtus arvalis*. Folia zoologica 40 (2): 143- 151.

9.4 Effects on other terrestrial vertebrate wildlife (reptiles and amphibians) (KCP 10.1.3)

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 are no guidance or validated regulatory protocols yet available; neither on the type of regulatory testing necessary nor how to conduct a risk assessment for amphibian and reptiles.

In the case of the product BAS 758 00 F and the active substances mefentrifluconazole (BAS 750 F) and metrafenone (BAS 560 F), there are no studies in the open literature on amphibians and reptiles; furthermore, there are no incident reports or other information from the field related to potential negative impacts from the use of this product on amphibians or reptiles. However, there is information in the literature concerning potential toxicity of other pyraclostrobin (BAS 500 F) containing formulations on juvenile terrestrial life stages of amphibians in worst-case overspray laboratory studies.

However, pyraclostrobin-containing products have been used for many years and in many countries worldwide. However, BASF is not aware of findings or (incidence) reports that amphibians or reptiles were affected by pyraclostrobin applications following the label instructions. This lack of effects at field rates was confirmed by semi-field studies in cereals using the solo-EC-formulation BAS 500 06 F. The studies showed no effects on small juvenile common frogs and juvenile common toads at rates of 312.5 and 375 g pyraclostrobin/ha, respectively. The conducted laboratory and semi-field studies are discussed in the renewal process for pyraclostrobin (for more information see the AIR3 dossier).

The use pattern for BAS 758 00 F encompasses a maximum single application rate of 120 g pyraclostrobin/ha. Plant interception will further reduce the amount that can reach the soil and potentially any amphibians occurring there. In the environmental fate section 8, in Table 8.7-1, the interception in cereals is 80%, meaning that 24 g pyraclostrobin/ha could reach the soil surface. Accordingly, the application of BAS 758 00 F at maximum single rates of 120 g pyraclostrobin/ha will be of low risk to amphibians.

In conclusion, there is low risk to terrestrial life stages of amphibians from BAS 758 00 F at a maximum single application rate of 1.5 L product/ha (120 g pyraclostrobin/ha) according to good agricultural practice.

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.

9.5 Effects on aquatic organisms (KCP 10.2)

9.5.1 Toxicity data

Studies on the toxicity to aquatic organisms have been carried out with the formulation BAS 758 00 F, the active substances mefentrifluconazole (BAS 750 F), metrafenone (BAS 560 F), pyraclostrobin (BAS 500 F) and their relevant metabolites. Full details of these studies are provided in the respective EU DAR/RAR and related documents of mefentrifluconazole (DAR, Vol. 3, B.9, January 2018; EFSA Journal 2018;16(7):5379), metrafenone (DAR, Vol. 3, B.9, July 2005; EFSA Scientific report No. 58, 1-72 (2006)) and pyraclostrobin (Monograph, Vol. 3, Annex B.9, August 2001; EU Review Report SANCO/1420/2001-final, September 2004) as well as in Appendix 2 of this document (new studies).

Except for a new acute study on toxicity of mefentrifluconazole to *Pimephales promelas* and a study on toxicity of M750F005 to *Oncorhynchus mykiss*, all studies conducted with the active substance mefentrifluconazole and its metabolites have already been submitted and evaluated during the Annex I inclusion process of mefentrifluconazole.

The selection of studies and endpoints for the risk assessment for metrafenone is generally in line with the results of the EU review process. Justifications for any deviations are provided below.

The selection of studies and endpoints and the risk assessments for pyraclostrobin are in line with the results of the EU review process. In addition to the EU agreed endpoints, new studies and the resulting endpoints are only considered here if essential for the risk assessment or in support of previous evaluations.

Effects on aquatic organisms of product BAS 758 00 F were not evaluated previously as part of the EU assessment of the active substances. New data submitted with this application are listed in Appendix 1 and summarized in Appendix 2.

Appropriate risk assessments for aquatic organisms for the active substances, their major metabolites and the formulated product BAS 758 00 F for the proposed use pattern are provided based on available toxicity data.

Full references to cited literature are given at the end of this document.

Mefentrifluconazole (BAS 750 F) and metabolites

The results from toxicity tests with representative aquatic species conducted with the active substance mefentrifluconazole and its metabolites found in aquatic systems are summarized in Table 9.5.1-1.

Table 9.5.1-1: Endpoints and effect values relevant for the risk assessment for aquatic organisms – mefentrifluconazole and relevant metabolites

Species	Substance	Exposure System	Results	Reference / BASF DocID
<i>Oncorhynchus mykiss</i>	mefentrifluconazole	96 h, f	LC ₅₀ = 0.532 mg a.s./L_{mm}	EFSA Journal 2018;16(7):5379 / 2014/1036951
<i>Cyprinus carpio</i>	mefentrifluconazole	96 h, f	LC ₅₀ = 1.126 mg a.s./L _{mm}	EFSA Journal 2018;16(7):5379 / 2015/1249071
<i>Cyprinodon variegatus</i> ¹⁾	mefentrifluconazole	96 h, ss	LC ₅₀ = 0.761 mg a.s./L _{mm}	EFSA Journal 2018;16(7):5379 / 2014/7002810
<i>Danio rerio</i>	mefentrifluconazole	96 h, s	LC ₅₀ = 0.906 mg a.s./L _{mm}	EFSA Journal 2018;16(7):5379 / 2015/1001581
<i>Pimephales promelas</i>	mefentrifluconazole	96 h, s	LC ₅₀ = 0.65 mg a.s./L _{mm}	New study – not EU evaluated / 2016/1155889
<i>D. rerio</i> (ELS study)	mefentrifluconazole	36 d, f	NOEC = 0.024 mg a.s./L _{nom} * NOEC = 0.027 mg a.s./L _{mm}	EFSA Journal 2018;16(7):5379 / 2014/1262160
<i>C. variegatus</i> ¹⁾ (ELS study)	mefentrifluconazole	35 d, f	NOEC ≥ 0.160 mg a.s./L _{nom} * NOEC ≥ 0.147 mg a.s./L _{mm}	EFSA Journal 2018;16(7):5379 / 2015/7000619
<i>D. rerio</i> (FSDT study)	mefentrifluconazole	69 d, f	NOEC ≥ 0.041 mg a.s./L _{nom} * NOEC ≥ 0.045 mg a.s./L _{mm}	EFSA Journal 2018;16(7):5379 / 2015/1099093
<i>D. rerio</i> (FLC study)	mefentrifluconazole	140 d, f	NOEC = 0.023 mg a.s./L _{nom} * NOEC = 0.022 mg a.s./L_{mm}	EFSA Journal 2018;16(7):5379 / 2016/1042889
<i>O. mykiss</i> (BCF study)	mefentrifluconazole	14 d uptake, 7 d depuration	BCF _{KLg} (whole fish) = 385	EFSA Journal 2018;16(7):5379 / 2015/1122811
<i>Daphnia magna</i>	mefentrifluconazole	48 h, s	EC₅₀ = 0.944 mg a.s./L_{mm}	EFSA Journal 2018;16(7):5379 / 2013/1250866
<i>Americamysis bahia</i> ¹⁾	mefentrifluconazole	48 h, f	LC ₅₀ = 1.53 mg a.s./L _{mm}	EFSA Journal 2018;16(7):5379 / 2014/7002845

Species	Substance	Exposure System	Results	Reference / BASF DocID
<i>Crassostrea virginica</i> ¹⁾	mefentrifluconazole	96 h, f	EC ₅₀ = 0.947 mg a.s./L _{mm}	EFSA Journal 2018;16(7):5379 / 2015/7000021
<i>D. magna</i>	mefentrifluconazole	21 d, ss	NOEC = 0.010 mg a.s./L _{nom} EC ₁₀ = 0.0175 mg a.s./L _{nom} * NOEC = 0.0091 mg a.s./L _{mm} EC₁₀ = 0.0161 mg a.s./L_{mm}	EFSA Journal 2018;16(7):5379 / 2014/1098028
<i>A. bahia</i> ¹⁾	mefentrifluconazole	28 d, f	NOEC ≥ 0.0132 mg a.s./L _{mm}	EFSA Journal 2018;16(7):5379 / 2016/7001293
<i>D. pulex</i>	mefentrifluconazole	21 d, ss	NOEC = 0.0282 mg a.s./L _{nom} EC ₁₀ = 0.0573 mg a.s./L _{nom} * NOEC = 0.0276 mg a.s./L _{mm} EC ₁₀ = 0.0567 mg a.s./L _{mm}	EFSA Journal 2018;16(7):5379 / 2015/1003913
<i>D. longispina</i>	mefentrifluconazole	21 d, ss	NOEC = 0.0338 mg a.s./L _{nom} EC ₁₀ = 0.0558 mg a.s./L _{nom} * NOEC = 0.0342 mg a.s./L _{mm} EC ₁₀ = 0.0564 mg a.s./L _{mm}	EFSA Journal 2018;16(7):5379 / 2015/1003912 + 2015/1251197
Geomean (NOEC/EC ₁₀ -data for 4 crustacean species)	mefentrifluconazole	—	Geomean _{chronic} = 0.0287 mg a.s./L _{mm}	Calculation considering NOEC/EC ₁₀ -data based on mean-measured concentrations
<i>Chironomus dilutus</i> (spiked sediment)	mefentrifluconazole	10 d, ss	NOEC = 7.08 mg a.s./kg dry sediment _{mm} EC ₅₀ > 96 mg a.s./kg dry sediment _{mm}	EFSA Journal 2018;16(7):5379 / 2015/7000621
<i>Hyalella azteca</i> (spiked sediment)	mefentrifluconazole	10 d, ss	NOEC ≥ 100 mg a.s./kg dry sediment _{mm} EC ₅₀ > 100 mg a.s./kg dry sediment _{mm}	EFSA Journal 2018;16(7):5379 / 2015/7000622
<i>Leptocheirus plumulosus</i> ¹⁾ (spiked sediment)	mefentrifluconazole	10 d, s	NOEC ≥ 95 mg a.s./kg dry sediment _{mm} EC ₅₀ > 95 mg a.s./kg dry sediment _{mm}	EFSA Journal 2018;16(7):5379 / 2015/7000623
<i>C. riparius</i> (spiked sediment)	mefentrifluconazole	28 d, s	NOEC ≥ 1.158 mg a.s./kg dry sediment_{im}	EFSA Journal 2018;16(7):5379 / 2014/1243181 + 2017/1044236
<i>C. dilutus</i> (LC study; spiked sediment)	mefentrifluconazole	63 d, ss	NOEC = 5.7 mg a.s./kg dry sediment _{mm} LC ₅₀ > 9.2 mg a.s./kg dry sediment _{mm}	EFSA Journal 2018;16(7):5379 / 2016/7006526
<i>Pseudokirchneriella subcapitata</i> ²⁾	mefentrifluconazole	72 h, s	E _r C ₅₀ = 1.352 mg a.s./L _{mm} E _y C ₅₀ = 0.777 mg a.s./L _{mm}	EFSA Journal 2018;16(7):5379 / 2013/1250865

Species	Substance	Exposure System	Results	Reference / BASF DocID
<i>Skeletonema costatum</i> ^{1), 2)}	mefentrifluconazole	72 h, s	ErC₅₀ = 0.679 mg a.s./L_{mm} EyC ₅₀ = 0.479 mg a.s./L _{mm}	EFSA Journal 2018;16(7):5379 / 2015/7000620 + 2016/1292092 (re-calculation)
<i>Navicula pelliculosa</i> ²⁾	mefentrifluconazole	72 h, s	ErC ₅₀ = 1.347 mg a.s./L _{mm} EyC ₅₀ = 0.671 mg a.s./L _{mm}	EFSA Journal 2018;16(7):5379 / 2015/7000618 + 2016/1292093 (re-calculation)
<i>Anabaena flos-aquae</i> ²⁾	mefentrifluconazole	72 h, s	ErC ₅₀ & EyC ₅₀ > 3.08 mg a.s./L _{mm}	EFSA Journal 2018;16(7):5379 / 2015/7000617
<i>Lemna gibba</i> ²⁾	mefentrifluconazole	7 d, s	ErC₅₀ & EyC₅₀ > 2.017 mg a.s./L_{im}	EFSA Journal 2018;16(7):5379 / 2014/1001322 + 2018/1220943
<i>O. mykiss</i>	1,2,4-triazole (Reg. No. 87084; M750F001)	96 h, s	LC ₅₀ = 498 mg/L _{nom}	EFSA Journal 2018;16(7):5379 / 1983/1000494
<i>O. mykiss</i>	M750F005 (Reg. No. 6003433)	96 h, s	LC ₅₀ > 5 mg/L _{nom}	New study – not EU evaluated / 2019/1022695
<i>O. mykiss</i>	M750F006 (Reg. No. 5863469)	96 h, s	LC ₅₀ = 6.2 mg/L _{mm}	EFSA Journal 2018;16(7):5379 / 2016/1128152
<i>O. mykiss</i>	M750F007 (Reg. No. 6003432)	96 h, s	LC ₅₀ > 7.2 mg/L _{mm}	EFSA Journal 2018;16(7):5379 / 2015/1001489
<i>O. mykiss</i>	1,2,4-triazole	28 d, ss	NOEC = 3.2 mg/L _{nom}	EFSA Journal 2018;16(7):5379 / 2002/1007850
<i>D. magna</i>	1,2,4-triazole	48 h, s	EC ₅₀ > 100 mg/L _{nom}	EFSA Journal 2018;16(7):5379 / 1995/1001851
<i>D. magna</i>	M750F003	48 h, s	EC ₅₀ > 100 mg/L _{nom}	EFSA Journal 2018;16(7):5379 / 2016/1289876
<i>D. magna</i>	M750F005	48 h, s	EC ₅₀ > 8.58 mg/L _{mm}	EFSA Journal 2018;16(7):5379 / 2015/1001490
<i>D. magna</i>	M750F006	48 h, s	EC ₅₀ = 4.42 mg/L _{mm}	EFSA Journal 2018;16(7):5379 / 2015/1001492
<i>D. magna</i>	M750F007	48 h, s	EC ₅₀ > 10 mg/L _{nom} * EC ₅₀ > 9.9 mg/L _{mm}	EFSA Journal 2018;16(7):5379 / 2015/1003915
<i>D. magna</i>	M750F008	48 h, s	EC ₅₀ > 8.07 mg/L _{mm}	EFSA Journal 2018;16(7):5379 / 2015/1001493

Species	Substance	Exposure System	Results	Reference / BASF DocID
<i>C. riparius</i>	M750F003	28 d, s	NOEC \geq 1.944 mg/kg dry sediment _{im}	EFSA Journal 2018;16(7):5379 / 2015/1003916 +2017/1044237
<i>P. subcapitata</i> ²⁾	1,2,4-triazole	72 h, s	E _r C ₅₀ = 22.5 mg/L ⁻³⁾ _{mm}	EFSA Journal 2018;16(7):5379 / 2001/1022266
<i>P. subcapitata</i> ²⁾	M750F003	72 h, s	E _r C ₅₀ > 100 mg/L _{nom}	EFSA Journal 2018;16(7):5379 / 2016/1289875
<i>P. subcapitata</i> ²⁾	M750F005	72 h, s	E _r C ₅₀ > 8.57 mg/L _{mm}	EFSA Journal 2018;16(7):5379 / 2015/1184816
<i>P. subcapitata</i> ²⁾	M750F006	72 h, s	E _r C ₅₀ = 1.42 mg/L _{mm}	EFSA Journal 2018;16(7):5379 / 2015/1184815
<i>P. subcapitata</i> ²⁾	M750F007	72 h, s	E _r C ₅₀ > 10 mg/L _{nom}	EFSA Journal 2018;16(7):5379 / 2015/1003914
<i>P. subcapitata</i> ²⁾	M750F008	72 h, s	E _r C ₅₀ = 4.08 mg/L _{mm}	EFSA Journal 2018;16(7):5379 / 2015/1001491

Abbreviations: s: static; ss: semi-static; f: flow-through; nom: based on nominal concentrations; mm: based on mean measured concentrations; im: based on initial measured concentrations; ELS: early life stage; LC: Life cycle; FLC: full life cycle; FSdT: fish sexual development test; BCF: Bioconcentration factor.

Bold figures: Endpoint used in standard tier 1 risk assessment if more than one endpoint is available for the respective group or organism.

* In addition to the EU agreed endpoints (based on mean measured concentrations), the endpoints based on nominal concentrations are shown here since the measured concentrations were within \pm 20% of nominal throughout the studies. For the risk assessment the mean measured endpoints are used.

¹⁾ Marine species

²⁾ According to the EFSA Aquatic Guidance (EFSA, 2013) as well as according to the PRAPeR meeting (Sept 2015) endpoints based on growth rate are relevant for risk assessment of primary producers.

³⁾ Considering the endpoint for the study on *P. subcapitata* using 1,2,4-triazole, there is a discrepancy in the value reported in the study report (*i.e.* DocID 2001/1022266), between the first EU evaluation (*i.e.* Annex I approval of epoxiconazole (EFSA, 2015), E_rC₅₀ > 31 mg/L) and the endpoint reported in the Annex I approval of mefentrifluconazole (*i.e.* E_rC₅₀ = 22.5 mg/L). For the risk assessment the EU agreed endpoint (E_rC₅₀ > 22.5 mg/L, based on mean measured concentrations) is used.

Metrafenone (BAS 560 F) and metabolites

The results from toxicity tests with representative aquatic species conducted with the active substance metrafenone and its metabolites found in aquatic systems are summarized in Table 9.5.1-2.

Table 9.5.1-2: Endpoints and effect values relevant for the risk assessment for aquatic organisms – metrafenone and relevant metabolites

Species	Substance	Exposure System	Results	Reference / BASF DocID
<i>Oncorhynchus mykiss</i>	metrafenone	96 h, f	LC ₅₀ > 0.82 mg a.s./L _{mm}	EFSA Scientific report No. 58, 1-72 (2006) / 1999/7000289
<i>Cyprinodon variegatus</i> ¹⁾	metrafenone	96 h, f	LC ₅₀ > 0.35 mg a.s./L_{mm} ²⁾	New study – not EU evaluated / 2005/7003439
<i>P. promelas</i>	metrafenone	32 d	NOEC = 0.228 mg a.s./L _{mm}	EFSA Scientific report No. 58, 1-72 (2006) / 2000/7000128
<i>P. promelas</i> (ELS study)	metrafenone	33 d, f	NOEC = 0.204 mg a.s./L_{mm}	New study – not EU evaluated / 2012/1009601
<i>Lepomis macrochirus</i> (BCF study)	metrafenone	28 d uptake, 14 d depuration	BCF _{TRR} (whole fish) = 470 - 530 BCF _{parent} (whole fish) = 140 - 180	EFSA Scientific report No. 58, 1-72 (2006) / 2001/7000274
<i>Daphnia magna</i>	metrafenone	48 h, s	EC ₅₀ > 0.92 mg a.s./L _{mm}	EFSA Scientific report No. 58, 1-72 (2006) / 1999/7000287
<i>Crassostrea virginica</i> ¹⁾	metrafenone	96 h, f	48-h LC₅₀ > 0.33 mg a.s./L_{mm} ^{2) 3)}	New study – not EU evaluated / 2005/7003442
<i>D. magna</i>	metrafenone	21 d, ss	NOEC = 0.225 mg a.s./L _{mm}	EFSA Scientific report No. 58, 1-72 (2006) / 2000/7000130
<i>Americamysis bahia</i> ¹⁾	metrafenone	28 d, f	NOEC = 0.022 mg a.s./L_{mm}	New study – not EU evaluated / 2007/7009454
<i>Chironomus riparius</i>	metrafenone	28 d, s, overlying water	NOEC = 1.0 mg a.s./L_{nom}	EFSA Scientific report No. 58, 1-72 (2006) / 2001/7000462
<i>C. riparius</i>	metrafenone	28 d, s, spiked sediment	NOEC = 296 mg a.s./kg dry sediment_{nom}	New study – not EU evaluated / 2010/1145509
<i>Leptocheirus plumulosus</i> ¹⁾	metrafenone	10 d, s, sediment	EC ₅₀ > 1.7 mg a.s./kg dry sediment _{mm} ⁵⁾	New study – not EU evaluated / 2011/7000373

Species	Substance	Exposure System	Results	Reference / BASF DocID
<i>Selenastrum capricornutum</i> ⁴⁾ (syn. <i>Pseudokirchneriella subcapitata</i>)	metrafenone	72 h, s	$E_rC_{50} > 0.87$ mg a.s./L _{mm} $E_bC_{50} = 0.71$ mg a.s./L _{mm}	EFSA Scientific report No. 58, 1-72 (2006) / 2000/7000122
<i>P. subcapitata</i> ⁴⁾	metrafenone	72 h, s	$E_rC_{50} > 0.339$ mg a.s./L_{mm} $E_bC_{50} > 0.339$ mg a.s./L_{mm}	New study – not EU evaluated / 2011/1254828
<i>Lemna gibba</i> ⁴⁾	metrafenone	7 d, ss	$E_rC_{50} > 0.327$ mg a.s./L_{mm} $E_bC_{50} > 0.327$ mg a.s./L_{mm}	New study – not EU evaluated / 2011/1254832
<i>O. mykiss</i>	CL 375816	96 h, s	$LC_{50} > 99$ mg/L _{mm}	EFSA Scientific report No. 58, 1-72 (2006) / 2002/1004394
<i>O. mykiss</i>	CL 4084564	96 h, s	$LC_{50} = 16.4$ mg/L _{mm}	EFSA Scientific report No. 58, 1-72 (2006) / 2002/1005255
<i>D. magna</i>	CL 375816	48 h, s	$EC_{50} > 100$ mg/L _{nom}	EFSA Scientific report No. 58, 1-72 (2006) / 2002/1004870
<i>D. magna</i>	CL 4084564	48 h, s	$EC_{50} = 46$ mg/L _{mm}	EFSA Scientific report No. 58, 1-72 (2006) / 2002/1004869
<i>P. subcapitata</i> ⁴⁾	CL 375816	72 h, s	$E_rC_{50} > 100$ mg/L _{nom} $E_bC_{50} > 100$ mg/L _{nom}	EFSA Scientific report No. 58, 1-72 (2006) / 2002/1004873
<i>P. subcapitata</i> ⁴⁾	CL 4084564	72 h, s	$E_rC_{50} = 38.7$ mg/L _{mm} $E_bC_{50} = 27.8$ mg/L _{mm}	EFSA Scientific report No. 58, 1-72 (2006) / 2002/1004872

Abbreviations: s: static; ss: semi-static; f: flow-through; nom: based on nominal concentrations; mm: based on mean measured concentrations; im: based on initial measured concentrations; imm: based on initial measured concentrations; ELS: Early life stage; BCF: Bioconcentration factor.

Bold figures: Endpoint used in standard tier 1 risk assessment if more than one endpoint is available for the respective group or organism.

¹⁾ Marine species

²⁾ Based on centrifuged samples.

³⁾ The 96-h EC_{50} based on shell deposition in oyster ($EC_{50} = 0.22$ mg a.s./L) is not considered to be an adequate measure for acute toxicity. Instead, the 48-h LC_{50} value (> 0.33 mg a.s./L) should be considered for the acute RA for *C. virginica*. For detailed justification chapter 9.5.1.1 below.

⁴⁾ According to the EFSA Aquatic Guidance (EFSA, 2013) as well as according to the PRAPeR meeting (Sept 2015) endpoints based on growth rate are relevant for risk assessment of primary producers.

⁵⁾ The 10-day *L. plumulosus* study is an acute study that only assesses mortality (study can be submitted upon request). Since a chronic 28-d study on *C. riparius* is available, the endpoint from this study is the most relevant and used for the risk assessment.

Pyraclostrobin (BAS 500 F) and metabolites

The EU agreed endpoints for the active substance pyraclostrobin (BAS 500 F) and its major metabolites (endpoints from new studies are currently evaluated in the Annex I renewal process of pyraclostrobin) are used for the risk assessment on aquatic organisms.

Besides the standard fish species *O. mykiss*, six further fish species were tested in acute 96 h laboratory studies with pyraclostrobin and an SSD analysis based on the 96 h NOEC and LC₅₀ values was conducted (for details on SSD calculations see "Refined Risk Assessment for pyraclostrobin (BAS 500 F)" which is provided below).

Besides acute and chronic studies with the standard species *D. magna*, a mesocosm study that includes a great number of different and more relevant freshwater invertebrate species is available and used in a higher-tier assessment to address the risk to aquatic invertebrates and the clearly less sensitive primary producers (for details see "Refined Risk Assessment for pyraclostrobin (BAS 500 F)" which is provided below).

In addition to the standard ELS study on *O. mykiss*, used for tier 1 level risk assessment, ELS studies on two other fish species have been performed (i.e. *C. variegatus* and *P. promelas*) and are currently under evaluation in the AIR3 submission process of pyraclostrobin (summaries of these new studies are attached in Appendix 2). The NOECs for the three tested fish species can be used to calculate a geometric mean, which is one of the options for refining the chronic risk to fish. Alternative approaches are also presented (for details see "Refined Risk Assessment for pyraclostrobin (BAS 500 F)" provided below).

Furthermore, spiked sediment *Chironomus* studies were performed with the soil (sediment) metabolites BF 500-3, BF 500-6 and BF 500-7 (BASF DocIDs 2013/1237446, 2014/1001481 and 2014/1001482). These studies were submitted for the Annex I renewal process of pyraclostrobin and are currently in the evaluation phase on EU level. Study summaries are provided in Appendix 2 of this dossier and the results are considered for the risk assessment below.

The results from toxicity tests with representative aquatic species conducted with the active substance pyraclostrobin and its metabolites found in aquatic systems are summarized in Table 9.5.1-3.

Table 9.5.1-3: Endpoints and effect values relevant for the risk assessment for aquatic organisms – pyraclostrobin and relevant metabolites

Species	Substance	Exposure System	Results	Reference / BASF DocID
<i>Oncorhynchus mykiss</i>	pyraclostrobin	96 h, s	LC ₅₀ = 0.00616 mg a.s./L_{mm}	EU Review Report (SANCO/1420/2001-final, September 2004) / 1999/11414
<i>Cyprinus carpio</i>	pyraclostrobin	96 h, s	LC ₅₀ = 0.0177 mg a.s./L _{mm}	Monograph (Vol. 3, Annex B.9, August 2001) / 1998/11580
<i>Danio rerio</i> ³⁾	pyraclostrobin	96 h, s	LC ₅₀ = 0.0619 mg a.s./L _{mm}	Monograph (Vol. 3, Annex B.9, August 2001) / 1999/11834
<i>Lepomis macrochirus</i>	pyraclostrobin	96 h, s	LC ₅₀ = 0.0254 mg a.s./L _{mm}	Monograph (Vol. 3, Annex B.9, August 2001) / 1998/10951

Species	Substance	Exposure System	Results	Reference / BASF DocID
<i>Leuciscus idus melanotus</i> ³⁾	pyraclostrobin	96 h, s	LC ₅₀ = 0.0191 mg a.s./L _{mm}	Monograph (Vol. 3, Annex B.9, August 2001) / 1999/11835
<i>Oryzias latipes</i> ³⁾	pyraclostrobin	96 h, s	LC ₅₀ = 0.0533 mg a.s./L _{mm}	Monograph (Vol. 3, Annex B.9, August 2001) / 1999/11821
<i>Pimephales promelas</i> ³⁾	pyraclostrobin	96 h, s	LC ₅₀ = 0.0161 mg a.s./L _{mm}	Monograph (Vol. 3, Annex B.9, August 2001) / 1999/11833
<i>O. mykiss</i>	pyraclostrobin	28 d, f	NOEC = 0.00464 mg a.s./L _{nom}	EU Review Report (SANCO/1420/2001-final, September 2004) / 1999/11249
<i>O. mykiss</i> (ELS study)	pyraclostrobin	98 d, f	NOEC = 0.00235 mg a.s./L_{mm}	EU Review Report (SANCO/1420/2001-final, September 2004) / 1999/11343
<i>Cyprinodon variegatus</i> ^{1), 2)} (ELS study)	pyraclostrobin	36 d, f	NOEC = 0.0108 mg a.s./L _{mm}	New study—not EU evaluated; requested by US EPA; submitted for evaluation under AIR3 / 2000/5247
<i>P. promelas</i> ¹⁾ (ELS study)	pyraclostrobin	36 d, f	NOEC = 0.00414 mg a.s./L _{mm}	New study—not EU evaluated; requested by US EPA; submitted for evaluation under AIR3 / 2000/5053
<i>O. mykiss</i> (ELS study with multiple exposure)	pyraclostrobin	97 d, f	NOEC = 0.005 mg a.s./L _{nom}	Monograph (Vol. 3, Annex B.9, August 2001) / 1999/11537 + amendment: 2018/1123384
<i>L. macrochirus</i>	pyraclostrobin	BCF (whole fish)	400—535 L/kg (corrected to 5% lipid) 675 (chlorophenyl label) 736 (tolyl label)	Monograph (Vol. 3, Annex B.9, August 2001) / 1999/11348 EU Review Report (SANCO/1420/2001-final, September 2004) / 1999/11348
<i>Daphnia magna</i>	pyraclostrobin	48 h, s	EC₅₀ = 0.0157 mg a.s./L_{nom}	EU Review Report (SANCO/1420/2001-final, September 2004) / 1999/10444 + amendment: 1999/10739

Species	Substance	Exposure System	Results	Reference / BASF DocID
<i>D. magna</i>	pyraclostrobin	21 d, ss	NOEC = 0.0040 mg a.s./L_{nom}	EU Review Report (SANCO/1420/2001-final, September 2004) / 1999/11864
<i>Chironomus riparius</i>	pyraclostrobin	28 d, s, spiked water	NOEC = 0.040 mg a.s./L_{nom} (= 0.12 mg/kg dry sediment #)	EU Review Report (SANCO/1420/2001-final, September 2004) / 2000/1000010 + recalculated endpoint
<i>C. riparius</i> ¹⁾	pyraclostrobin	28 d, spiked sediment	NOEC = 1.37 mg a.s./kg dry sediment	New study – not EU evaluated; submitted for evaluation under AIR3 / 2012/1185699
<i>Pseudokirchneriella subcapitata</i> ⁵⁾	pyraclostrobin	72 h, s	E_rC₅₀ > 0.843 mg a.s./L_{mm} ⁴⁾ E_yC₅₀ = 0.148 mg a.s./L_{mm} ⁴⁾	EU Review Report (SANCO/1420/2001-final, September 2004) / 1999/11020 + supplement: 2009/1037148
<i>Lemna gibba</i> ⁵⁾	pyraclostrobin	14 d	<u>Fond number</u> E_rC₅₀ & E_yC₅₀ > 1.077 mg a.s./L_{mm} <u>Dry weight</u> E_hC₅₀ > 1.077 mg a.s./L_{mm}	New study – not EU evaluated; requested by US-EPA; submitted for evaluation under AIR3 / 2000/5037 +2019/2036269
Outdoor mesocosm (multiple spray application) ³⁾	pyraclostrobin	6 mo, s	NOEC = 0.008 mg a.s./L_{nom}	Monograph (Vol. 3, Annex B.9, August 2001) / 2000/1000011
<i>O. mykiss</i>	BF 500-11	96 h, s	LC₅₀ > 100 mg/L_{mm}	EU Review Report (SANCO/1420/2001-final, September 2004) / 1999/11909
<i>O. mykiss</i>	BF 500-13	96 h, s	LC₅₀ > 50 mg/L_{nom} < 100 mg/L_{nom}	EU Review Report (SANCO/1420/2001-final, September 2004) / 1999/11913
<i>O. mykiss</i>	BF 500-14	96 h, s	LC₅₀ > 39.4 mg/L_{mm} < 82.6 mg/L_{mm}	EU Review Report (SANCO/1420/2001-final, September 2004) / 1999/11837
<i>D. magna</i>	BF 500-11	48 h, s	EC₅₀ > 100 mg/L_{nom}	EU Review Report (SANCO/1420/2001-final, September 2004) / 1999/11917
<i>D. magna</i>	BF 500-13	48 h, s	EC₅₀ > 100 mg/L_{nom}	EU Review Report (SANCO/1420/2001-final, September 2004) / 1999/11921

Species	Substance	Exposure System	Results	Reference / BASF DocID
<i>D. magna</i>	BF 500-14	48 h, s	EC ₅₀ > 60.9 mg/L _{mm}	EU Review Report (SANCO/1420/2001-final, September 2004) / 1999/11910
<i>C. riparius</i> ¹⁾	BF 500-3	28 d, spiked sediment	NOEC ≥ 16.0 mg/kg dry sediment	New study – not EU evaluated; submitted for evaluation under AIR3 / 2013/1237446
<i>C. riparius</i> ⁴⁾	BF 500-6	28 d, spiked sediment	NOEC = 1.2 mg a.s./kg dry sediment	New study – not EU evaluated; submitted for evaluation under AIR3 / 2014/1001481
<i>C. riparius</i> ⁴⁾	BF 500-7	28 d, spiked sediment	NOEC ≥ 123.5 mg/kg dry sediment	New study – not EU evaluated; submitted for evaluation under AIR3 / 2014/1001482
<i>Scenedesmus subspicatus</i> ⁵⁾	BF 500-11	72 h, s	E _r C ₅₀ & E _b C ₅₀ > 100 mg/L _{nom}	EU Review Report (SANCO/1420/2001-final, September 2004) / 1999/11918
<i>S. subspicatus</i> ⁵⁾	BF 500-13	72 h, s	E _r C ₅₀ > 100 mg/L _{nom} E _b C ₅₀ = 66.0 mg/L _{nom}	EU Review Report (SANCO/1420/2001-final, September 2004) / 1999/11922
<i>S. subspicatus</i> ⁵⁾	BF 500-14	72 h, s	E _r C ₅₀ > 100 mg/L _{nom} E _b C ₅₀ = 46.6 mg/L _{nom}	EU Review Report (SANCO/1420/2001-final, September 2004) / 1999/11914

Abbreviations: ELS: early life stage; BCF: bioconcentration factor; s: static; ss: semi-static; f: flow-through; nom: based on nominal concentrations; mm: based on mean measured concentrations; im: based on initial measured concentrations

Note: There are additional new studies with marine species performed for registration in the US. However, a mesocosm study is available, which provides exhaustive information on a large variety of relevant freshwater species.

Bold figures: Endpoint used in standard tier 1 risk assessment if more than one endpoint is available for the respective group or organism.

Based on sediment concentration in spiked water study.

1) Study was not submitted during the Annex I inclusion process of pyraclostrobin, but is currently evaluated in the Annex I renewal process of pyraclostrobin

2) Marine / saltwater species

3) Study was performed with a previous representative solo-formulation BAS 500 00 F; however, results are given in mg a.s./L.

4) In accordance to recent guidelines (EFSA, 2013; OECD, 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 in chapter 8.2 of the MCA dossier part for Annex I renewal.

5) According to the EFSA Aquatic Guidance (EFSA, 2013) as well as according to the PRAPeR meeting (Sept 2015) endpoints based on growth rate are relevant for risk assessment of primary producers.

Formulated product (BAS 758 00 F)

The results from the toxicity tests with representative aquatic species conducted with the formulation BAS 758 00 F found in aquatic systems are summarized in Table 9.5.1-4.

Table 9.5.1-4: Endpoints and effect values relevant for the risk assessment for aquatic organisms – BAS 758 00 F

Species	Substance	Exposure System	Results	Reference / BASF DocID
<i>Oncorhynchus mykiss</i>	BAS 758 00 F	96 h, s	LC ₅₀ = 0.0884 mg/L _{nom}	New study – not EU evaluated 2020/2033900
<i>Daphnia magna</i>	BAS 758 00 F	48 h, s	EC ₅₀ = 0.362 mg/L _{nom}	New study – not EU evaluated 2020/2033902
<i>Pseudokirchneriella subcapitata</i> ¹⁾	BAS 758 00 F	72 h, s	E _r C ₅₀ = 3.82 mg/L _{nom} E _y C ₅₀ = 1.43 mg/L _{nom}	New study – not EU evaluated 2020/2033904

Abbreviations: s: static; nom: based on nominal concentrations

¹⁾ According to the EFSA Aquatic Guidance (EFSA, 2013) as well as according to the PRAPeR meeting (Sept 2015) endpoints based on growth rate are relevant for risk assessment of primary producers.

9.5.1.1 Justification for new endpoints

Mefentrifluconazole and metabolites

In general, for mefentrifluconazole and its metabolites the EU agreed endpoints are used for the risk assessment. A new acute study on *P. promelas* conducted using the active substance is available. Additionally, a new acute study on toxicity of M750F005 (metabolite of mefentrifluconazole) to fish is available. This study was conducted post Annex I inclusion for a different region. The study is provided to support the risk assessment of metabolites.

In line with the EFSA conclusion (EFSA Journal 2018;16(7):5379), the chronic endpoints for fish and invertebrates based on mean measured values are considered for the risk assessment.

Review Comments:

zRMS agrees with Applicant proposal. The acute study on toxicity of M750F00F to fish is essential for the risk assessment.

Metrafenone

Several studies with aquatic organisms exposed to the active substance (metrafenone) were submitted for the first EU review and were considered to be acceptable. However, to ensure that the supporting data base complies with current guidelines, for most aquatic endpoints, either repeat testing of the same species or studies with additional species has produced lower values. Therefore, for the aquatic risk assessment, RAC values have been derived from the lowest endpoints taken from either the original or updated data sets. All studies referred to were submitted to support the renewal of active substance approval for metrafenone.

Furthermore, the 96-h EC₅₀ (based on shell deposition) in oyster is not considered to be an adequate measure for acute toxicity because it is not based on mortality (or immobilization as a surrogate for mortality) but on shell growth. The relevant endpoint of acute studies on aquatic vertebrates and invertebrates (used for acute aquatic RAs) is mortality or immobilization as a surrogate for mortality (*i.e.* in the OECD guidelines acute endpoints do not contain any growth endpoint (e.g. OECD 202 and 203)) and thus using a growth endpoint is not in line with the OECD guidelines. Furthermore, the EU Regulation 283/2013 (European Commission, 2013) defining the data requirements for active substances advises to use a 48-h (not a 96-h) endpoint for acute RA for additional aquatic invertebrate species. In conclusion, the 48-h LC₅₀ value (> 0.33 mg a.s./L) should be considered for the acute RA for *C. virginica*.

Review Comments:

The zRMS is of the opinion that the endpoints given in the current LoEP of metrafenone should be considered for the risk assessment, unless it is necessary to take account of new data to demonstrate the safe application of the measure.

The first EU review of metrafenone was in 2006, resulting in a very limited data set of toxicity to aquatic organisms (tests were performed on four standard species). Therefore, as an exception to the general rule, the Applicant's approach was accepted. New studies with additional species will be considered in the risk assessment (*Cyprinodon variegatus*, *Crassostrea virginica*, *Americamysis bahia*). Additionally, the ELS study with *Pimephales promelas* and *Chironomus riparius* (sediment exposure) will be included for the comprehensive of the evaluation.

Pyraclostrobin and metabolites

All new studies are currently in the evaluation phase of the Annex I renewal process of pyraclostrobin.

In addition to the EU agreed ELS study on *O. mykiss*, ELS studies on two other fish species have been performed (*i.e.* *C. variegatus* (DocID 2000/5247) and *P. promelas* (DocID 2000/5053) as requested by US-EPA. The results for the three tested fish species can be used to calculate a geometric mean of 4.72 µg a.s./L (for details on the Geometric mean calculations see "Refined Risk Assessment for pyraclostrobin (BAS 500 F)", which is provided below).

Also, a *Lemna gibba* study was conducted as part of the submission data package requested by US-EPA (BASF DocID 2000/5037) and resulting data is considered here for the sake of completeness.

Regarding the risk resulting from sediment exposure a new study with *Chironomus riparius* (DocID 2012/1185699) covering exposure via sediment was conducted according to requirements for the active substance. In addition, spiked sediment *Chironomus* studies were performed with the soil (sediment) metabolites BF-500-3, BF-500-6 and BF 500-7 (BASF DocIDs 2013/1237446, 2014/1001481 and 2014/1001482). These studies were submitted for the Annex I renewal process of pyraclostrobin and are currently in the evaluation phase on EU level.

Summaries of these new studies are attached in Appendix 2.

Review Comments:

Regarding the risk resulting from sediment exposure a new studies with *Chironomus* was conducted for the active substance and its metabolites. However, these studies were submitted within AIR 3 (pyraclostrobin) renewal process and are currently in the evaluation phase on EU level. Therefore, only studies essential for the risk assessment were evaluated.

Since pyraclostrobin and BF-500-3 reached > 10 % in sediment, they are considered in the risk assessment by the zRMS.

The new studies with BF-500-6 and BF-500-7 metabolites are available. Regarding the low occurrence and immobility of the metabolites in soil and low water solubility, it can be concluded that BF 500-6 and BF 500-7 will not enter surface water to any relevant amount. Based on this assumption, new studies with BF 500-6 and BF 500-7 are not evaluated by the zRMS in the present dossier.

9.5.2 Risk assessment

The evaluation of the risk for aquatic organisms was performed in accordance with the recommendations of the “Guidance document on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters (EFSA Aquatic GD) in the context of Regulation (EC) No 1107/2009”, as provided by the Commission Services (SANTE-2015-00080, 15 January 2015).

In accordance with the EFSA AGD, risk assessment for algae and higher aquatic plants was performed considering only the more relevant endpoint “growth rate” (E_rC_{50}) where possible.

Furthermore, according to the EFSA Aquatic GD, the risk to aquatic life-stages of amphibians shall be addressed. In general, regarding 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 S. and Thompson H., 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 can be addressed by the currently requested and conducted risk assessment for aquatic organisms. This is also acknowledged in the Aquatic Guidance Document (EFSA, 2013).

Risk assessment for mefentrifluconazole (BAS 750 F)

For mefentrifluconazole EU agreed endpoints are considered for the tier 1 risk assessment. ~~Additionally, the higher tier Geomean RAC of 2.87 µg a.s./L for aquatic crustaceans (resulting from geometric mean calculations based on chronic NOEC/EC₁₀ data for 4 crustacean species) is derived for sake of completeness.~~

Refined risk assessment for mefentrifluconazole (BAS 750 F)

The tier 1 ETR calculations (see below) demonstrate an acceptable risk for all groups of aquatic organisms following all proposed uses of ~~BAS 763 01 F~~ BAS 758 00 F.

~~Furthermore, chronic data on additional crustacean species is available which allow for a refined risk assessment for aquatic crustacean, the most sensitive group of aquatic organisms. Although not used for the risk assessment, the information is provided for completeness.~~

Besides the standard aquatic invertebrate species *D. magna*, three additional crustacean species were tested chronically with mefentrifluconazole (*i.e.* *A. bahia*, *D. pulex* and *D. longispina*). The additional endpoint from the *A. bahia* study is an unbound NOEC (*i.e.* ≥ 0.0132 mg a.s./L).

The presented refinement follows the current recommendations as stated in the Minutes of the Network on Pesticide Steering Consultation for the corrigendum of the Aquatic guidance document (EFSA, 2016) as well as the EFSA Aquatic Guidance document (EFSA, 2013).

The Minutes state: " ...for using the geomean approach, the endpoints should be derived by highly comparable tests (toxicity estimates characterized by a **similar duration** of the tests, a **comparable measurement endpoint**, covering a **similar life stage** of the tested species)."

- The EC₁₀ values from the studies on *D. magna*, *D. pulex* and *D. longispina* are all derived from chronic studies over equal test durations (*i.e.* 21 days) and are based on the same effect parameter, *i.e.* effects on reproduction (most sensitive endpoint: number of offspring/parent). Daphnids were exposed at the same life stage (*i.e.* > 2 and < 24 h at test initiation).
- The exposure duration of the study on *A. bahia* deviates slightly from study durations for *D. magna* (*i.e.* 28 days). However, by including the NOEC derived from the study on *A. bahia* within the geometric mean calculation, the resulting geometric mean value is more conservative than if the endpoint was not included.

The EFSA Aquatic Guidance Document states that the geometric mean approach may be applied for a refined risk assessment, when toxicity data for a limited number of additional test species are available. All preconditions (considering daphnids and *A. bahia*) for using this approach that were set out in the EFSA Guidance (chapter 8.3.2 and 8.3.3) are fulfilled:

- ~~similar endpoints:~~
Endpoints are highly similar as recommendation of the Network on Pesticide Steering Consultation are fulfilled (see above).
- ~~species of the same taxonomic group:~~ aquatic crustaceans (aquatic insects, e.g. *Chironomus* proved to be less sensitive)
- ~~available data exceed the first tier data requirements:~~
additional studies on *D. pulex*, *D. longispina* and *A. bahia*
- ~~most sensitive species should not be more than a factor of 100 below the geometric mean:~~
The study on *A. bahia* provides the lowest endpoint which is a factor of ~2 below the geometric mean of all four chronic species.
- ~~Less than 8 species tested:~~
4 different species
- ~~If the lowest toxicity value is higher than the Geomean-RAC value, it is acceptable to use the Geometric mean approach:~~
The *D. magna* EC₁₀ of 16.1 µg a.s./L is ~5.5 times higher than the RAC_{geomean} of 2.87 µg a.s./L.

Since, requirements of the EFSA AGD and the proposed corrigendum (Network on Pesticide Steering Consultation) are met, the presented geomean approach is considered adequate for refinement of the chronic risk assessment for aquatic crustaceans.

Table 9.5-5: Calculation of the geometric mean based on chronic toxicity data for aquatic invertebrates

Test species	Endpoint [#]	BASF DocID
<i>D. magna</i>	21 d EC ₁₀ = 0.0161 mg a.s./L	2014/1098028
<i>A. bahia</i>	28 d NOEC ≥ 0.0132 mg a.s./L	2016/7001293
<i>D. pulex</i>	21 d EC ₁₀ = 0.0567 mg a.s./L	2015/1003913
<i>D. longispina</i>	21 d EC ₁₀ = 0.0564 mg a.s./L	2015/1003912
Geomean	Geomean_{chronic} = 0.0287 mg a.s./L	-

[#]—The endpoints based on mean measured concentrations are used for the calculation.

The resulting NOEC/EC₁₀ values for the four tested crustacean species can be used to calculate a geometric mean of 0.0287 mg a.s./L. Considering the standard chronic assessment factor of 10 this results in a **Geomean-RAC_{chronic} of 2.87 µg a.s./L.**

Acceptability of risk

The relevant worst-case predicted environmental concentrations in surface water bodies (PEC_{sw} and PEC_{sed}), regulatory acceptable concentrations (RAC) for aquatic organisms and the resulting PEC/RAC ratios (ETR) for the single and twofold application of mefentrifluconazole in 'spring and winter cereals' are given per intended use and each organism group and are presented in Table 9.5-6 - Table 9.5-9. Worst-case PEC values for either 'spring or winter cereals' are considered in a risk envelope approach. For details on the PEC calculations please refer to Part B, Section 8.9.

Table 9.5-6: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for mefentrifluconazole for each organism group based on standard FOCUS Step 1 - 3 calculations for single and twofold application (1x and 2x 67 g a.s./ha) of BAS 758 00 F in 'spring cereals'

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Higher-Plant	Group	Sed. dwell. prolonged
Test species		<i>O. mykiss</i>	<i>D. rerio</i> (FLC study)	<i>D. magna</i>	<i>D. magna</i>	<i>S. costatum</i>	<i>L. gibba</i>	Test species	<i>C. riparius</i>
Endpoint (µg/L)		LC ₅₀ 532	NOEC 22	EC ₅₀ 944	EC ₁₀ 16.1	E _r C ₅₀ 679	E _r C ₅₀ > 2017	Endpoint (µg/kg)	NOEC ≥ 1158
AF		100	10	100	10	10	10	AF	10
RAC (µg/L)		5.32	2.2	9.44	1.61	67.9	> 201.7	RAC (µg/kg)	≥ 115.8
FOCUS Scenario	PEC ^{gl-sw} _{max} (µg/L) ¹⁾	PEC/RAC (= ETR)						PEC ^{gl-sed max} (µg/kg) ¹⁾	PEC/RAC (= ETR)
Step 1									
	13.728	2.6	6.2	1.5	8.5	0.2	< 0.07	420.381	≤ 3.6
Step 2									
N-Europe	2.216	0.4	1.01	0.2	1.4	--	--	72.548	≤ 0.6
S-Europe	4.048	0.8	1.8	0.4	2.5	--	--	135.574	≤ 1.2
Step 3									
D3 ditch	0.424	--	0.2	--	0.3	--	--	0.345	≤ 0.003
D4 pond	0.038	--	0.02	--	0.02	--	--	0.351	≤ 0.003
D4 stream	0.346	--	0.2	--	0.2	--	--	0.126	≤ 0.001
D5 pond	0.021	--	0.01	--	0.01	--	--	0.205	≤ 0.002
D5 stream	0.356	--	0.2	--	0.2	--	--	0.024	≤ 0.0002
R4 stream	0.495	--	0.2	--	0.3	--	--	2.073	≤ 0.02

Abbreviations: FLC: Full life cycle; AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; ETR: exposure-toxicity ratio; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**

¹⁾ Worst-case PECs are derived from either single or twofold application in a risk envelope approach. For details, please refer to Part B, Section 8.9.

For the intended single and twofold application of BAS 758 00 F at 1x and 2x 67 g a.s./ha in 'spring cereals', the calculated PEC/RAC ratios for mefentrifluconazole indicate an acceptable risk for all groups of aquatic organisms based on tier 1 toxicity data and standard FOCUS Step 1-3 $PEC_{sw/sed}$ values. Therefore, no further assessment is necessary.

Table 9.5-7: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for mefentrifluconazole for each organism group based on standard FOCUS Step 1 - 3 calculations for single and twofold application (1x and 2x 67 g a.s./ha) of BAS 758 00 F in ‘winter cereals’

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Higher-Plant	Group	Sed. dwell. prolonged
Test species		<i>O. mykiss</i>	<i>D. rerio</i> (FLC study)	<i>D. magna</i>	<i>D. magna</i>	<i>S. costatum</i>	<i>L. gibba</i>	Test species	<i>C. riparius</i>
Endpoint (µg/L)		LC ₅₀ 532	NOEC 22	EC ₅₀ 944	EC ₁₀ 16.1	E _r C ₅₀ 679	E _r C ₅₀ > 2017	Endpoint (µg/kg)	NOEC ≥ 1158
AF		100	10	100	10	10	10	AF	10
RAC (µg/L)		5.32	2.2	9.44	1.61	67.9	> 201.7	RAC (µg/kg)	≥ 115.8
FOCUS Scenario	PEC ^{gl-sw max} (µg/L) ¹⁾	PEC/RAC (= ETR)						PEC ^{gl-sed max} (µg/kg) ¹⁾	PEC/RAC (= ETR)
Step 1									
	13.728	2.6	6.2	1.5	8.5	0.2	< 0.07	420.381	≤ 3.6
Step 2									
N-Europe	2.216	0.4	1.01	0.2	1.4	--	--	72.548	≤ 0.6
S-Europe	4.048	0.8	1.8	0.4	2.5	--	--	135.574	≤ 1.2
Step 3									
D3 ditch	0.423	--	0.2	--	0.3	--	--	0.327	≤ 0.003
D4 pond	0.039	--	0.02	--	0.02	--	--	0.347	≤ 0.003
D4 stream	0.313	--	0.1	--	0.2	--	--	0.132	≤ 0.001
D5 pond	0.023	--	0.01	--	0.01	--	--	0.211	≤ 0.002
D5 stream	0.338	--	0.2	--	0.2	--	--	0.028	≤ 0.0002
R1 pond	0.079	--	0.04	--	0.05	--	--	1.082	≤ 0.009
R1 stream	0.393	--	0.2	--	0.2	--	--	1.408	≤ 0.01
R3 stream	0.392	--	0.2	--	0.2	--	--	1.292	≤ 0.01
R4 stream	0.551	--	0.3	--	0.3	--	--	1.513	≤ 0.01

Abbreviations: FLC: Full life cycle; AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; ETR: exposure-toxicity ratio; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**

¹⁾ Worst-case PECs are derived from either single or twofold application in a risk envelope approach. For details, please refer to Part B, Section 8.9.

For the intended single and twofold application of BAS 758 00 F at 1x and 2x 67 g a.s./ha in ‘winter cereals’, the calculated PEC/RAC ratios for mefentrifluconazole indicate an acceptable risk for all groups of aquatic organisms based on tier 1 toxicity data and standard FOCUS Step 1-3 PEC_{sw/sed} values. Therefore, no further assessment is necessary.

Table 9.5-8: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for mefentrifluconazole for each organism group based on standard FOCUS Step 1 - 3 calculations for single and twofold application (1x and 2x 100 g a.s./ha) of BAS 758 00 F in ‘spring cereals’

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Higher-Plant	Group	Sed. dwell. prolonged
Test species		<i>O. mykiss</i>	<i>D. rerio</i> (FLC study)	<i>D. magna</i>	<i>D. magna</i>	<i>S. costatum</i>	<i>L. gibba</i>	Test species	<i>C. riparius</i>
Endpoint (µg/L)		LC ₅₀ 532	NOEC 22	EC ₅₀ 944	EC ₁₀ 16.1	E _r C ₅₀ 679	E _r C ₅₀ > 2017	Endpoint (µg/kg)	NOEC ≥ 1158
AF		100	10	100	10	10	10	AF	10
RAC (µg/L)		5.32	2.2	9.44	1.61	67.9	> 201.7	RAC (µg/kg)	≥ 115.8
FOCUS Scenario	PEC ^{gl-sw max} (µg/L) ¹⁾	PEC/RAC (= ETR)						PEC ^{gl-sed max} (µg/kg) ¹⁾	PEC/RAC (= ETR)
Step 1									
	13.728	2.6	6.2	1.5	8.5	0.2	< 0.07	420.381	≤ 3.6
Step 2									
N-Europe	2.216	0.4	1.01	0.2	1.4	--	--	72.548	≤ 0.6
S-Europe	4.048	0.8	1.8	0.4	2.5	--	--	135.574	≤ 1.2
Step 3									
D3 ditch	0.632	--	0.3	--	0.4	--	--	0.514	≤ 0.004
D4 pond	0.057	--	0.03	--	0.04	--	--	0.529	≤ 0.005
D4 stream	0.517	--	0.2	--	0.3	--	--	0.191	≤ 0.002
D5 pond	0.032	--	0.01	--	0.02	--	--	0.309	≤ 0.003
D5 stream	0.531	--	0.2	--	0.3	--	--	0.036	≤ 0.0003
R4 stream	0.742	--	0.3	--	0.5	--	--	3.057	≤ 0.03

Abbreviations: FLC: Full life cycle; AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; ETR: exposure-toxicity ratio; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**

¹⁾ Worst-case PECs are derived from either single or twofold application in a risk envelope approach. For details, please refer to Part B, Section 8.9.

For the intended single and twofold application of BAS 758 00 F at 1x and 2x 100 g a.s./ha in ‘spring cereals’, the calculated PEC/RAC ratios for mefentrifluconazole

indicate an acceptable risk for all groups of aquatic organisms based on tier 1 toxicity data and standard FOCUS Step 1-3 $PEC_{sw/sed}$ values. Therefore, no further assessment is necessary.

Table 9.5-9: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for mefentrifluconazole for each organism group based on standard FOCUS Step 1 - 3 calculations for single and twofold application (1x and 2x 100 g a.s./ha) of BAS 758 00 F in ‘winter cereals’

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Higher-Plant	Group	Sed. dwell. prolonged
Test species		<i>O. mykiss</i>	<i>D. rerio</i> (FLC study)	<i>D. magna</i>	<i>D. magna</i>	<i>S. costatum</i>	<i>L. gibba</i>	Test species	<i>C. riparius</i>
Endpoint (µg/L)		LC ₅₀ 532	NOEC 22	EC ₅₀ 944	EC ₁₀ 16.1	E _r C ₅₀ 679	E _r C ₅₀ > 2017	Endpoint (µg/kg)	NOEC ≥ 1158
AF		100	10	100	10	10	10	AF	10
RAC (µg/L)		5.32	2.2	9.44	1.61	67.9	> 201.7	RAC (µg/kg)	≥ 115.8
FOCUS Scenario	PEC ^{gl-sw max} (µg/L) ¹⁾	PEC/RAC (= ETR)						PEC ^{gl-sed max} (µg/kg) ¹⁾	PEC/RAC (= ETR)
Step 1									
	13.728	2.6	6.2	1.5	8.5	0.2	< 0.07	420.381	≤ 3.6
Step 2									
N-Europe	2.216	0.4	1.01	0.2	1.4	--	--	72.548	≤ 0.6
S-Europe	4.048	0.8	1.8	0.4	2.5	--	--	135.574	≤ 1.2
Step 3									
D3 ditch	0.632	--	0.3	--	0.4	--	--	0.486	≤ 0.004
D4 pond	0.059	--	0.03	--	0.04	--	--	0.522	≤ 0.005
D4 stream	0.467	--	0.2	--	0.3	--	--	0.200	≤ 0.002
D5 pond	0.034	--	0.02	--	0.02	--	--	0.319	≤ 0.003
D5 stream	0.504	--	0.2	--	0.3	--	--	0.041	≤ 0.0004
R1 pond	0.118	--	0.05	--	0.07	--	--	1.612	≤ 0.01
R1 stream	0.589	--	0.3	--	0.4	--	--	2.087	≤ 0.02
R3 stream	0.585	--	0.3	--	0.4	--	--	1.923	≤ 0.02
R4 stream	0.827	--	0.4	--	0.5	--	--	2.240	≤ 0.02

Abbreviations: FLC: Full life cycle; AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; ETR: exposure-toxicity ratio; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**

¹⁾ Worst-case PECs are derived from either single or twofold application in a risk envelope approach. For details, please refer to Part B, Section 8.9.

For the intended single and twofold application of BAS 758 00 F at 1x and 2x 100 g a.s./ha in ‘winter cereals’, the calculated PEC/RAC ratios for mefentrifluconazole indicate an acceptable risk for all groups of aquatic organisms based on tier 1 toxicity data and standard FOCUS Step 1-3 PEC_{sw/sed} values. Therefore, no further assessment is necessary.

Risk assessment for the metabolites of mefentrifluconazole (BAS 750 F)

The acute toxicity to fish of the metabolites M750F003, M750F005, and M750F008 has been estimated using a QSAR (ECOSAR version 1.11) during the Annex I inclusion process to avoid unnecessary vertebrate testing. The QSAR data for fish were assessed as valid in the DAR (please refer to Volume 3 B.9 (AS), Chapter B.9.12.) and using a QSAR model for metabolite risk assessment is in line with the proposed non-testing methods according to the EFSA Aquatic Guidance Document; specifically, to reduce vertebrate toxicity testing (please refer to Chapter 10.1 of the Aquatic GD). Furthermore, there is clear evidence from the available toxicity data for daphnia and algae that the metabolites are less toxic in comparison to the parent. This is further shown by the new available acute toxicity study on *O. mykiss* with M750F005 conducted for a different region post Annex I inclusion. The study shows a ~ 10 times lower toxicity of the metabolite M750F005 (i.e. $LC_{50} > 5$ mg/L) compared to the active substance and therewith confirming the QSAR calculations. Additionally, in some cases in the algae and daphnia studies, metabolites did not show any toxicity up to the solubility limit (in most cases metabolites are 10-times less toxic than the parent). Finally, comparing the available data for daphnia and algae to the QSAR predictions for these groups of organisms, confirms the appropriateness of the approach.

Similarly, for sediment dwellers, there is no indication of increased toxicity from the available data set.

Based on EFSA request during the EU review the aquatic risk assessment for metabolites of mefentrifluconazole was performed assuming a 10-times increased toxicity to fish. Similarly, 10-times increased toxicity to sediment dwellers was assumed. This approach is deemed overly conservative and scientifically not justified as discussed above.

Nevertheless, the risk assessment for metabolites is shown below assuming a 10-times increased toxicity to fish for M750F008 and similar toxicity in comparison to the parent compound for M750F003. For sediment dwelling organisms, similar toxicity in comparison to the parent compound is assumed for M750F001, M750F005, M750F006, M750F007, and M750F008.

Acceptability of risk

In Table 9.5-10, the exposure-toxicity ratios (ETRs) for aquatic organisms are given for the use of BAS 758 00 F in 'spring and winter cereals' and for each organism group for the relevant metabolites of mefentrifluconazole. Worst-case $PEC_{sw/sed}$ values from single and twofold application (1x and 2x 100 g a.s./ha) in 'spring and winter cereals' are used for risk assessment and cover all intended uses.

Table 9.5-10: Aquatic organisms: acceptability of risk ($PEC/RAC < 1$) for metabolites of mefentrifluconazole for each organism group based on worst-case FOCUS Step 1 - 2 calculations following single and twofold application¹⁾ (1x and 2x 100 g a.s./ha) of BAS 758 00 F in 'spring and winter cereals'

Group	Fish acute	Fish prolonged	Inverteb. acute	Algae	Group	Sed. dwell. prolonged
Test species	<i>O. mykiss</i>	<i>O. mykiss</i>	<i>D. magna</i>	<i>P. subcapitata</i>	Test species	<i>C. riparius</i>
AF	100	10	100	10	AF	10
1,2,4-triazole (M750F001)						
Endpoint (µg/L)	LC ₅₀ 498000	NOEC 3200	EC ₅₀ > 100000	E _r C ₅₀ > 22500	Endpoint (µg/kg)	NOEC ≥ 1158 ²⁾
RAC (µg/L)	4980	320	> 1000	> 2250	RAC (µg/kg)	≥ 115.8

Group		Fish acute	Fish prolonged	Inverteb. acute	Algae	Group	Sed. dwell. prolonged
FOCUS Scenario	PEC ^{gl-max, sw} (µg/L)	PEC/RAC ratio (= ETR)				PEC ^{gl-sed max} (µg/kg)	PEC/RAC (= ETR)
Step 1							
	2.154	0.0004	0.007	< 0.002	< 0.001	1.783	≤ 0.02
M750F003							
Endpoint (µg/L)		LC ₅₀ 532 ²⁾	NOEC n.a.	EC ₅₀ > 100000	ErC ₅₀ > 100000	Endpoint (µg/kg)	NOEC ≥ 1944
RAC (µg/L)		5.32	--	> 1000	> 10000	RAC (µg/kg)	≥ 194.4
FOCUS Scenario	PEC ^{gl-max, sw} (µg/L)	PEC/RAC ratio (= ETR)				PEC ^{gl-sed max} (µg/kg)	PEC/RAC (= ETR)
Step 1							
	2.872	0.5	--	< 0.003	< 0.0003	16.854	≤ 0.09
M750F005							
Endpoint (µg/L)		LC ₅₀ > 5000	NOEC n.a.	EC ₅₀ > 8580	ErC ₅₀ > 8570	Endpoint (µg/kg)	NOEC ≥ 1158 ²⁾
RAC (µg/L)		> 50	--	> 85.8	> 857	RAC (µg/kg)	≥ 115.8
FOCUS Scenario	PEC ^{gl-max, sw} (µg/L)	PEC/RAC ratio (= ETR)				PEC ^{gl-sed max} (µg/kg)	PEC/RAC (= ETR)
Step 1							
	2.347	< 0.05	--	< 0.03	< 0.003	143.931	≤ 1.2
Step 2							
N-Europe	0.339	--	--	--	--	24.968	≤ 0.2
S-Europe	0.613	--	--	--	--	46.547	≤ 0.4
M750F006							
Endpoint (µg/L)		LC ₅₀ 6200	NOEC n.a.	EC ₅₀ 4420	ErC ₅₀ 1420	Endpoint (µg/kg)	NOEC ≥ 1158 ²⁾
RAC (µg/L)		62	--	44.2	142	RAC (µg/kg)	≥ 115.8
FOCUS Scenario	PEC ^{gl-max, sw} (µg/L)	PEC/RAC ratio (= ETR)				PEC ^{gl-sed max} (µg/kg)	PEC/RAC (= ETR)
Step 1							
	2.927	0.05	--	0.07	0.02	122.329	≤ 1.06
Step 2							
N-Europe	0.457	--	--	--	--	21.220	≤ 0.2
S-Europe	0.830	--	--	--	--	39.560	≤ 0.3
M750F007							
Endpoint (µg/L)		LC ₅₀ > 7200	NOEC n.a.	EC ₅₀ > 9900	ErC ₅₀ > 10000	Endpoint (µg/kg)	NOEC ≥ 1158 ²⁾
RAC (µg/L)		> 72	--	> 99	> 1000	RAC (µg/kg)	≥ 115.8
FOCUS Scenario	PEC ^{gl-max, sw} (µg/L)	PEC/RAC ratio (= ETR)				PEC ^{gl-sed max} (µg/kg)	PEC/RAC (= ETR)

Group		Fish acute	Fish prolonged	Inverteb. acute	Algae	Group	Sed. dwell. prolonged
Step 1							
	4.655	< 0.06	--	< 0.05	< 0.005	160.566	≤ 1.4
Step 2							
N-Europe	0.747	--	--	--	--	27.853	≤ 0.2
S-Europe	1.358	--	--	--	--	51.926	≤ 0.4
M750F008							
Endpoint (µg/L)		LC ₅₀ 53.2 ³⁾	NOEC n.a.	EC ₅₀ > 8070	E _r C ₅₀ 4080	Endpoint (µg/kg)	NOEC ≥ 1158 ²⁾
RAC (µg/L)		0.532	--	> 80.7	408	RAC (µg/kg)	≥ 115.8
FOCUS Scenario	PEC ^{gl-max, sw} (µg/L)	PEC/RAC ratio (= ETR)				PEC ^{gl-sed max} (µg/kg)	PEC/RAC (= ETR)
Step 1							
	0.302	0.6	--	< 0.004	0.0007	32.129	≤ 0.3

Abbreviations: AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; ETR: Exposure-toxicity ratio; n.a.: no study available; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**

- ¹⁾ Worst-case PECs are derived from either single or twofold application in a risk envelope approach. For details, please refer to Part B, Section 8.9.
- ²⁾ The endpoint for the active substance is used since the toxicity of the metabolite is not expected to be greater than the active substance from supporting data.
- ³⁾ 10-fold higher toxicity compared to the active substance is assumed.

For the intended single and twofold application of BAS 758 00 F in ‘spring and winter cereals’, the calculated PEC/RAC ratios for the mefentrifluconazole metabolites indicate an acceptable risk for all groups of aquatic organisms based on worst-case FOCUS Step 1 - 2 assumptions. Therefore, no further assessment is necessary.

Risk assessment for metrafenone (BAS 560 F)

The selection of studies and endpoints for the risk assessment for metrafenone is generally in line with the results of the EU review process. Justifications for any deviations are provided above.

Acceptability of risk

The relevant worst-case predicted environmental concentrations in surface water bodies (PEC_{sw} and PEC_{sed}), regulatory acceptable concentrations (RAC) for aquatic organisms and the resulting PEC/RAC ratios (ETR) for the single and twofold application of metrafenone in 'spring and winter cereals' are given per intended use and each organism group and are presented in Table 9.5-11 - Table 9.5-14. Worst-case PEC values for either 'spring or winter cereals' are considered in a risk envelope approach. For details on the PEC calculations please refer to Part B, Section 8.9.

Table 9.5-11: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for metrafenone for each organism group based on standard FOCUS Step 1 - 3 calculations for single and twofold application (1x and 2x 100 g a.s./ha) of BAS 758 00 F in 'spring cereals'

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Higher-plant	Sed. dwell. prolonged	Group	Sed. dwell. prolonged
Test species		<i>C. variegatus</i>	<i>P. promelas</i>	<i>C. virginica</i>	<i>A. bahia</i>	<i>P. subcapitata</i>	<i>L. gibba</i>	<i>C. riparius</i>	Test species	<i>C. riparius</i>
Endpoint (µg/L)		LC ₅₀ > 350	NOEC 204	LC ₅₀ > 330	NOEC 22	E _r C ₅₀ > 339 870	E _r C ₅₀ > 327	NOEC 1000	Endpoint (µg/kg)	NOEC 296000
AF		100	10	100	10	10	10	10	AF	10
RAC (µg/L)		> 3.5	20.4	> 3.3	2.20	> 33.9 87	> 32.7	100	RAC (µg/kg)	29600
FOCUS Scenario	PEC ^{gl-sw max} (µg/L) ¹⁾	PEC/RAC (= ETR)							PEC ^{gl-sed max} (µg/kg) ¹⁾	PEC/RAC (= ETR)
Step 1										
	15.876	< 4.5	0.8	< 4.8	7.2	< 0.5 0.2	< 0.5	0.2	394.722	0.01
Step 2										
N-Europe	2.486	< 0.7	--	< 0.8	1.1	--	--	--	66.420	--
S-Europe	4.665	< 1.3	--	< 1.4	2.1	--	--	--	127.694	--
Step 3										
D3 ditch	0.632	< 0.2	--	< 0.2	0.3	--	--	--	0.466	--
D4 pond	0.062	< 0.02	--	< 0.02	0.03	--	--	--	0.351	--
D4 stream	0.516	< 0.1	--	< 0.2	0.2	--	--	--	0.251	--
D5 pond	0.028	< 0.008	--	< 0.008	0.01	--	--	--	0.151	--
D5 stream	0.530	< 0.2	--	< 0.2	0.2	--	--	--	0.033	--
R4 stream	0.663	< 0.2	--	< 0.2	0.3	--	--	--	1.660	--

Abbreviations: AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; ETR: exposure-toxicity ratio; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**

¹⁾ Worst-case PECs are derived from either single or twofold application in a risk envelope approach. For details, please refer to Part B, Section 8.9.

For the intended single and twofold application of BAS 758 00 F at 1x and 2x 100 g a.s./ha in 'spring cereals', the calculated PEC/RAC ratios for metrafenone indicate an acceptable risk for all groups of aquatic organisms based on tier 1 toxicity data and standard FOCUS Step 1-3 PEC_{sw/sed} values. Therefore, no further assessment is necessary.

Table 9.5-12: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for metrafenone for each organism group based on standard FOCUS Step 1 - 3 calculations for single and twofold application (1x and 2x 100 g a.s./ha) of BAS 758 00 F in ‘winter cereals’

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Higher plant	Sed. dwell. prolonged	Group	Sed. dwell. prolonged
Test species		<i>C. variegatus</i>	<i>P. promelas</i>	<i>C. virginica</i>	<i>A. bahia</i>	<i>P. subcapitata</i>	<i>L. gibba</i>	<i>C. riparius</i>	Test species	<i>C. riparius</i>
Endpoint (µg/L)		LC ₅₀ > 350	NOEC 204	LC ₅₀ > 330	NOEC 22	E _r C ₅₀ > 339 870	E _r C ₅₀ > 327	NOEC 1000	Endpoint (µg/kg)	NOEC 296000
AF		100	10	100	10	10	10	10	AF	10
RAC (µg/L)		> 3.5	20.4	> 3.3	2.20	> 33.9 87	> 32.7	100	RAC (µg/kg)	29600
FOCUS Scenario	PEC ^{gl-sw max} (µg/L) ¹⁾	PEC/RAC (= ETR)							PEC ^{gl-sed max} (µg/kg) ¹⁾	PEC/RAC (= ETR)
Step 1										
	15.876	< 4.5	0.8	< 4.8	7.2	< 0.5 0.2	< 0.5	0.2	394.722	0.01
Step 2										
N-Europe	2.486	< 0.7	--	< 0.8	1.1	--	--	--	66.420	--
S-Europe	4.665	< 1.3	--	< 1.4	2.1	--	--	--	127.694	--
Step 3										
D3 ditch	0.631	< 0.2	--	< 0.2	0.3	--	--	--	0.463	--
D4 pond	0.068	< 0.02	--	< 0.02	0.03	--	--	--	0.376	--
D4 stream	0.466	< 0.1	--	< 0.1	0.2	--	--	--	0.274	--
D5 pond	0.031	< 0.009	--	< 0.009	0.01	--	--	--	0.176	--
D5 stream	0.504	< 0.1	--	< 0.2	0.2	--	--	--	0.040	--
R1 pond	0.082	< 0.02	--	< 0.02	0.04	--	--	--	0.448	--
R1 stream	0.515	< 0.1	--	< 0.2	0.2	--	--	--	1.526	--
R3 stream	0.584	< 0.2	--	< 0.2	0.3	--	--	--	1.758	--
R4 stream	0.732	< 0.2	--	< 0.2	0.3	--	--	--	1.421	--

Abbreviations: AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; ETR: exposure-toxicity ratio; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**

¹⁾ Worst-case PECs are derived from either single or twofold application in a risk envelope approach. For details, please refer to Part B, Section 8.9.

For the intended single and twofold application of BAS 758 00 F at 1x and 2x 100 g a.s./ha in ‘winter cereals’, the calculated PEC/RAC ratios for metrafenone indicate an acceptable risk for all groups of aquatic organisms based on tier 1 toxicity data and standard FOCUS Step 1-3 PEC_{sw/sed} values. Therefore, no further assessment is necessary.

Table 9.5-13: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for metrafenone for each organism group based on standard FOCUS Step 1 - 3 calculations for single and twofold application (1x and 2x 150 g a.s./ha) of BAS 758 00 F in ‘spring cereals’

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Higher plant	Sed. dwell. prolonged	Group	Sed. dwell. prolonged
Test species		<i>C. variegatus</i>	<i>P. promelas</i>	<i>C. virginica</i>	<i>A. bahia</i>	<i>P. subcapitata</i>	<i>L. gibba</i>	<i>C. riparius</i>	Test species	<i>C. riparius</i>
Endpoint (µg/L)		LC ₅₀ > 350	NOEC 204	LC ₅₀ > 330	NOEC 22	E _r C ₅₀ > 339 870	E _r C ₅₀ > 327	NOEC 1000	Endpoint (µg/kg)	NOEC 296000
AF		100	10	100	10	10	10	10	AF	10
RAC (µg/L)		> 3.5	20.4	> 3.3	2.20	> 33.9 87	> 32.7	100	RAC (µg/kg)	29600
FOCUS Scenario	PEC ^{gl-sw max} (µg/L) ¹⁾	PEC/RAC (= ETR)							PEC ^{gl-sed max} (µg/kg) ¹⁾	PEC/RAC (= ETR)
Step 1										
	23.815	< 6.8	1.2	< 7.2	11	< 0.5 0.2	< 0.7	0.2	592.083	0.02
Step 2										
N-Europe	3.729	< 1.07	0.2	< 1.1	1.7	--	--	--	99.630	--
S-Europe	6.998	< 2.0	0.3	< 2.1	3.2	--	--	--	191.542	--
Step 3										
D3 ditch	0.948	< 0.3	--	< 0.3	0.4	--	--	--	0.694	--
D4 pond	0.100	< 0.03	--	< 0.03	0.05	--	--	--	0.559	--
D4 stream	0.775	< 0.2	--	< 0.2	0.4	--	--	--	0.400	--
D5 pond	0.043	< 0.01	--	< 0.01	0.02	--	--	--	0.225	--
D5 stream	0.796	< 0.2	--	< 0.2	0.4	--	--	--	0.050	--
R4 stream	1.020	< 0.3	--	< 0.3	0.5	--	--	--	2.457	--

Abbreviations: AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; ETR: exposure-toxicity ratio; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**

¹⁾ Worst-case PECs are derived from either single or twofold application in a risk envelope approach. For details, please refer to Part B, Section 8.9.

For the intended single and twofold application of BAS 758 00 F at 1x and 2x 150 g a.s./ha in 'spring cereals', the calculated PEC/RAC ratios for metrafenone indicate an acceptable risk for all groups of aquatic organisms based on tier 1 toxicity data and standard FOCUS Step 1-3 PEC_{sw/sed} values. Therefore, no further assessment is necessary.

Table 9.5-14: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for metrafenone for each organism group based on standard FOCUS Step 1 - 3 calculations for single and twofold application (1x and 2x 150 g a.s./ha) of BAS 758 00 F in ‘winter cereals’

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Higher plant	Sed. dwell. prolonged	Group	Sed. dwell. prolonged
Test species		<i>C. variegatus</i>	<i>P. promelas</i>	<i>C. virginica</i>	<i>A. bahia</i>	<i>P. subcapitata</i>	<i>L. gibba</i>	<i>C. riparius</i>	Test species	<i>C. riparius</i>
Endpoint (µg/L)		LC ₅₀ > 350	NOEC 204	LC ₅₀ > 330	NOEC 22	E _r C ₅₀ > 339 870	E _r C ₅₀ > 327	NOEC 1000	Endpoint (µg/kg)	NOEC 296000
AF		100	10	100	10	10	10	10	AF	10
RAC (µg/L)		> 3.5	20.4	> 3.3	2.20	> 33.9 87	> 32.7	100	RAC (µg/kg)	29600
FOCUS Scenario	PEC ^{gl-sw max} (µg/L) ¹⁾	PEC/RAC (= ETR)							PEC ^{gl-sed max} (µg/kg) ¹⁾	PEC/RAC (= ETR)
Step 1										
	23.815	< 6.8	1.2	< 7.2	10.8	< 0.5 0.2	< 0.7	0.2	592.083	0.02
Step 2										
N-Europe	3.729	< 1.07	0.2	< 1.1	1.7	--	--	--	99.630	--
S-Europe	6.998	< 2.0	0.3	< 2.1	3.2	--	--	--	191.542	--
Step 3										
D3 ditch	0.947	< 0.3	--	< 0.3	0.4	--	--	--	0.690	--
D4 pond	0.109	< 0.03	--	< 0.03	0.05	--	--	--	0.599	--
D4 stream	0.700	< 0.2	--	< 0.2	0.3	--	--	--	0.438	--
D5 pond	0.047	< 0.01	--	< 0.01	0.02	--	--	--	0.263	--
D5 stream	0.756	< 0.2	--	< 0.2	0.3	--	--	--	0.060	--
R1 pond	0.126	< 0.04	--	< 0.04	0.06	--	--	--	0.674	--
R1 stream	0.792	< 0.2	--	< 0.2	0.4	--	--	--	2.240	--
R3 stream	0.876	< 0.3	--	< 0.3	0.4	--	--	--	2.606	--
R4 stream	1.127	< 0.3	--	< 0.3	0.5	--	--	--	2.072	--

Abbreviations: AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; ETR: exposure-toxicity ratio; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**

¹⁾ Worst-case PECs are derived from either single or twofold application in a risk envelope approach. For details, please refer to Part B, Section 8.9.

For the intended single and twofold application of BAS 758 00 F at 1x and 2x 150 g a.s./ha in ‘winter cereals’, the calculated PEC/RAC ratios for metrafenone indicate an acceptable risk for all groups of aquatic organisms based on tier 1 toxicity data and standard FOCUS Step 1-3 PEC_{sw/sed} values. Therefore, no further assessment is necessary.

Risk assessment for the metabolites of metrafenone (BAS 560 F)

Though the metabolite CL 377160 was observed in soil photolysis studies at concentrations up to 18.9 % AR the first EU evaluation concluded that no metabolites required risk assessment in surface water and sediment.

Acute fish, acute *Daphnia* and algae studies with the potentially relevant metabolites CL 375816 and CL 4084564 were submitted during the initial Annex I review and were considered to be acceptable. The results are presented in Table 9.5.1-2 for completeness. However, no risk assessment is necessary. No additional aquatic studies with metabolites are being submitted.

Risk assessment for pyraclostrobin (BAS 500 F)

Pyraclostrobin shows acute toxicity to fish. Thus, besides the standard fish species *O. mykiss*, six further fish species were tested in acute 96 h laboratory studies with pyraclostrobin and an SSD analysis based on the 96 h NOEC values was conducted (for details on SSD calculations see "Refined Risk Assessment for pyraclostrobin (BAS 500 F)", which is provided below).

~~In addition to the ELS study on *O. mykiss*, used for tier 1 level risk assessment, ELS studies on two other fish species have been performed (i.e. *C. variegatus* and *P. promelas*) for US registration purposes. All studies were performed according to the same test guideline, i.e. OECD TG 210. The obtained NOEC values for the three tested fish species can be used to calculate a geometric mean of 4.72 µg a.s./L (for details on the geomean calculations see "Refined Risk Assessment for pyraclostrobin (BAS 500 F)" provided below).~~

Besides acute and chronic studies with the standard aquatic invertebrate species *D. magna*, a mesocosm study including a large number of different and more relevant freshwater invertebrate species is available and used in a higher-tier assessment to address the risk to aquatic invertebrates (for details see "Refined Risk Assessment for pyraclostrobin (BAS 500 F)" provided below).

Refined risk assessment for pyraclostrobin (BAS 500 F)

Based on standard ETR calculations (see below) a potential risk from the active substance pyraclostrobin cannot be excluded for applications close to surface waters. Therefore, a refined assessment has been performed based on a range of additional and higher-tier studies, which allows reducing potential uncertainties significantly and provides the basis for a sound risk assessment for aquatic organisms with specific consideration of the risk to fish.

First tier risk assessment schemes usually use very cautious, conservative approaches. The lowest endpoint observed in worst-case laboratory tests is compared to an environmental concentration, which itself is based on worst-case assumptions. In Europe, additional uncertainty factors are applied on this ratio between toxicity and exposure (TER). It is 100 for acute and 10 for chronic tests, which is mainly based on the uncertainty that other species/endpoints than those which have been tested in the laboratory studies may be more sensitive to the test item.

Increasing the number of test species can therefore significantly reduce this uncertainty. For acute tests, the TER should be above 100, because in addition to the uncertainty with respect to differential species sensitivity, longer exposure may enhance toxicity and sub-lethal harmful effects are usually observed significantly below the LC₅₀. There are a number of further uncertainties with respect to extrapolating from laboratory results to the field situation; however, due to the conservative nature of such tests, most of these uncertainties are usually more than covered by the worst-case conditions of the laboratory tests.

Pyraclostrobin shows a very low acute-to-chronic ratio (ACR) and a very steep concentration-effect-relationship, which demonstrates that an additional safety factor of three to four is sufficient and safe for extrapolation from acute LC₅₀/EC₅₀ data to chronic NOECs. NOEC values determined in acute studies for sensitive fish species, are almost at the same level as those from long-term chronic studies (rainbow trout: NOEC (96 h) = 4.1 µg a.s./L, NOEC (98 d) = 2.35 µg a.s./L; fathead minnow: NOEC (96 h) = 7 µg a.s./L, NOEC (36 d) = 4.14 µg a.s./L; i.e. the factor between acute and chronic NOEC values is < 2). This is further supported by the higher-tier ELS study with trout simulating 8 contamination events with respective degradation during the exposure peaks. In this case, simulating more realistic worst-case exposure, the NOEC was 5 µg a.s./L, i.e. slightly above the level of the acute 96 h study.

To satisfactorily address remaining uncertainty and allow a more refined risk assessment, a range of additional studies has been performed, such as additional species testing with fish and a complex mesocosm study.

A mesocosm study provides a complex and compelling impression of the potential effects of a test substance under realistic conditions. For regulatory purposes it has been defined as a tool to negate presumptions of unacceptable risks based on comparisons between estimates of Predicted Environmental Concentrations (PEC) and No Observed Effect Concentrations (NOEC) derived from laboratory data and to provide descriptive information on the duration and magnitude of adverse impacts (Touart, 1988). A complex mesocosm study allows a general conclusion on aquatic ecosystems since major direct effects will be quite comparable within different systems (Leeuwangh, 1994).

The results of the mesocosm study showed that pyraclostrobin can have effects on few species at concentrations of 24 µg a.s./L. For all plankton species the effects were found to be reversible; they are thus not considered to pose a significant (ecologically unacceptable) risk to planktonic communities in aquatic ecosystems. However, fish and molluscs may also be affected at this concentration. No effects on any species or endpoint were observed at the second highest test concentration, 8 µg a.s./L, constituting the ecosystem NOEC.

The multitude of endpoints and species and environmental conditions in this mesocosm study show clearly that at this (and lower) concentration no adverse effects on aquatic communities can be expected even after multiple applications. Following the EFSA AGD (2013), an assessment factor of 2 - 3 is applied to the NOEC; employing the AF of 3 results in a **RAC of 2.67 µg a.s./L**, which will be used in the refined risk assessment for invertebrates and primary producers.

Review Comments:

Although, the mesocosms has been provided for the EU evaluation and was adequate to the vineyard application, in zRMS opinion the study should be considered relevant for the present risk assessment. The dose used in the mesocosm experiment clearly cover the present GAP. Application timing is a worst-case in comparison with the present GAP for both number of applications and frequency (8 applications with 14 d intervals and rates increasing from 60 to 160 g a.s./ha vs. 2 applications with 14 days interval at rate of 150 g a.s./ha). Moreover, concentrations corresponding to amounts are multiple greater than the maximum expected PEC values used in the risk assesment.

In original DAR following conclusion was presented: *“The effects of pyraclostrobin (applied as formulated product BAS 500 00 F) on the aquatic environment were investigated in an outdoor mesocosm study under more realistic conditions. Four concentration levels ranging from 0.9 µg a.s./L to 24 µg a.s./L were investigated. Approximately 260 different taxa were determined in the study. For phytoplankton only insignificant transient effects were observed in some of the taxa with recovery taking place until the end of the study. No significant treatment-related long lasting effects were observed on abundance and species diversity of the zooplankton. In case of transient effects, recovery was observed. Whereas for most benthic organisms no clear treatment-related effects were observed, molluscs (Bithynia tentaculata and Valvata sp.) and a mussel species (Dreissena polymorpha) appeared affected at the highest treatment level (24 µg a.s./L). No treatment-related effects appeared with aquatic insects”*

The overall NOEC, accepted on EU level, is 8 µg a.s./L.

Since it is not clear whether the NOEC is from an Effect Class 1 or 2 and the reporting is not clear enough to fully judge the reliability of the study the higher assessment factor of 3 will be used to derive the RAC.

Whereas the aquatic community within the mesocosm study was present with a high diversity of species (in total ca. 260 different taxa), only one species of fish (carp) has been investigated under outdoor conditions in additional ponds in parallel to the mesocosm study. Here the first application with 24 µg a.s./L did not, however the second application (resulting in a measured concentration of 29 µg a.s./L) did cause fish mortality (under concurring adverse conditions, i.e. low oxygen content). Lower concentrations had no effect on fish performance. This is in accordance with the laboratory results for carp, for which the 96-h laboratory LC₅₀ was 17.7 µg a.s./L.

Acute refinement for fish

To address uncertainties regarding differences in species sensitivities of fish, a number of different species has been studied under standard laboratory conditions to allow direct comparison of the species sensitivity distribution (SSD). In addition to three standard fish species (rainbow trout, bluegill sunfish, and carp) tested with the active substance pyraclostrobin, four more fish species were tested in 96 h laboratory tests. The results obtained in these studies are presented in the following table. To ease test item application (due to the low water solubility the addition of solvents is appropriate anyway), the additional tests were conducted using pyraclostrobin in the form of the EC solo formulation BAS 500 00 F (250 g a.s./L).

Table 9.5-15: Sensitivity of different fish species to pyraclostrobin

Species	LC ₅₀ [µg a.s./L]	NOEC/LC ₀ [µg a.s./L]	LC ₅₀ /NOEC	Reference
Rainbow trout <i>Oncorhynchus mykiss</i>	6.2 *	4.5	1.38	1999/11414
Bluegill sunfish <i>Lepomis macrochirus</i>	25.4 * (23.3 - 27.5)	10.9	2.33	1998/10951
Carp <i>Cyprinus carpio</i>	17.7 + >12.1 < 25.8	12.1	1.46	1998/11580
Fathead minnow <i>Pimephales promelas</i>	16.1 * (14.1 - 18.1)	7.0	2.30	1999/11833
Medaka <i>Oryzias latipes</i>	53.3 * (44.4 - 62.2)	16.5	3.23	1999/11821
Zebrafish <i>Danio rerio</i>	61.9 * (55.8 - 68.0)	23.4	2.65	1999/11834
Golden orfe <i>Leuciscus idus melanotus</i>	19.1 + > 13.5 < 27.0	13.5	1.41	1999/11835
mean			2.11	

* Spearman-Kärber estimate of LC₅₀ plus 95% confidence limits.

+ Geometric mean plus corresponding LC₀ (>) and LC₁₀₀ (<) values.

As mentioned above, the concentration-response relationship is very steep. Therefore, in several cases no effect was observed at one concentration whereas 100% mortality was found at the next higher concentration. In the other studies, only one mortality measurement could be made between 0% and 100% mortality. To obtain relevant figures for the LC₅₀ calculations, in the first case the LC₅₀ was determined as geometric mean between the two concentrations (*cf.* OECD TG 203); in the latter case a Spearman-Kärber estimate of the LC₅₀ was made using the original mortality and analytical measurements for the relevant concentrations from the study reports. (Thereby the results may deviate slightly from those given in the reports where no corresponding statistical analysis was performed. The results were corrected for analytically determined concentrations).

These data can be used in a probability distribution; assuming a logistic or normal distribution and a sigmoid curve for the cumulative probability. The following results were obtained using the method of Aldenberg and Slob (1993).

The data were calculated using the RIVM software program "ETX 1.3a". The 95% protection level is called HC₅ (hazardous concentration 5%). The data presented in the following table summarize the 95% protection levels (plus the corresponding 95% confidence limits).

Table 9.5-16: HC₅ calculations for fish

Endpoint	HC ₅
LC ₅₀ [µg a.s./L]	5.9
(95% limit)	(1.49)
NOEC [µg a.s./L]	4.2
(95% limit)	(1.57)

The PPR Panel recommends to use acute NOEC/LC₁₀ values to construct the SSD for fish, since a higher protection level is desired for vertebrates than for invertebrates and plants. Thus, the median HC₅ for fish can be determined to be 4.2 µg a.s./L.

According to the recommendation given in the EFSA Aquatic GD (2013), an assessment factor (AF) of 3 should be applied on the median HC₅ from an SSD constructed with acute NOEC values for fish for derivation of an SSD-RAC (Regulatory Acceptable Concentration), when latency of effects is not to be expected, which is clearly the case for pyraclostrobin (compare chronic risk assessment for fish below). In this case, the use of an AF of 3 is considered warranted also taking into account the various aspects mentioned in the aquatic GD:

- The quality of the acute toxicity data used to construct the SSD is high. All data are based on endpoints from GLP studies meeting all the listed criteria and including analytical support. A wide taxonomical range has been covered with seven species from different fish families (exceeding the minimum requirement of 5 species).
- The SSD-RAC is not higher than the tier 3 RAC derived from the mesocosm study; in fact, it is about six times lower (might indicate the appropriateness of a lower assessment factor).
- Since sufficient information is available for the substance, read-across information for compounds with a similar mode of action is not needed.
- The acute to chronic ratio is significantly smaller than 10 (might indicate the appropriateness of a lower assessment factor).

Accordingly, an assessment factor of three is well justified and still sufficiently conservative.

Following the EFSA AGD (p. 100-101): “Acute LC₁₀ and acute NOEC values may be used to construct the SSD and to calculate the HC₅ and lower limit of the confidence interval of the HC₅ (LLHC₅) for fish (and/or amphibians), since a higher protection level is desired for vertebrates than for invertebrates and plants. Another option is to apply an extra AF to the HC₅ based on acute LC₅₀ or EC₅₀ data.”

The recommended AF on the HC₅ from a **NOEC-based** SSD is 3, leading to an **SSD-RAC_{acute}** of $4.2/3 = 1.4 \mu\text{g a.s./L}$.

The extra AF for an LC₅₀-based SSD is 3 (reflecting the ratio of the LC₅₀ and the acute NOEC (EFSA, 2013, p. 101)) and so an AF of 9 (= 3 x 3) is proposed for use on an HC₅ from an LC₅₀-based SSD. However, the extra AF in the case of pyraclostrobin can be calculated for 7 fish species and is on average 2.11 (see Table 9.5-2). Obviously, the value is lower than 3, due to the steep concentration-response curve. Thus, an AF of 6.33 (= 3 x 2.11) should be applied to the HC₅ from the **LC₅₀-based** SSD, which leads to an **SSD-RAC_{acute}** of $5.9/6.33 = 0.93 \mu\text{g a.s./L}$. As the latter value is lower and thus more conservative than the RAC from the NOEC-based SSD, it will be used in the refined acute risk assessment for fish.

Review Comments:

In line with the current guidance an AF of 9 as stated in the Aquatic Guidance Document was considered for the RAC derivation based on median HC₅ with LC₅₀ values and an AF of 3 was considered for the RAC derivation based on median HC₅ with NOEC values. Taking to consideration, that for most of the acute fish toxicity studies presented in the DAR it was not possible to obtain an accurate LC₅₀ (the results are presented as a range), in zRMS opinion in refined risk assessment HC₅ with NOEC values should be used. Furthermore, considering the very low acute to chronic ratio and fact that the SSD calculation was based on only one cold water species, next to the regular AF presented in the Guidance Document an additional factor of 2 was included.

zRMS agreed to use the SSD-RAC_{acute} of $0.7 \mu\text{g a.s./L}$ in the refined acute risk assessment for fish.

Chronic risk refinement for fish

The long-term risk assessment to fish is based on a number of chronic fish studies. Next to a 28-d juvenile growth test (OECD TG 215) with rainbow trout, a higher-tier ELS study on rainbow trout with a variable exposure pattern covering numerous peaks, there are three standard ELS studies available. In addition to the ELS study on rainbow trout, standard ELS studies on fathead minnow and sheepshead minnow have been performed to satisfy registration requirements in the US (summaries of these new studies are attached

in Appendix 2). Based on this comprehensive data package, we present three possible refinement options here.

All three standard ELS studies were conducted according to the same test guideline (OECD TG 210) and cover the same phase of the life cycle of these three fish species. Also, all three NOECs are based on effects on survival, which is typical for pyraclostrobin due to the very low acute-to-chronic ratio (ACR). Due to the steep concentration-response curves, in which the NOECs had < 10% and the next higher treatments close to 100% mortality, no meaningful EC₁₀ values could be calculated. Since the NOECs have very similar, low effect percentages and the studies employ similar spacing factors of 2 - 3 between the concentrations, the numbers can easily be combined in a geometric mean calculation. Furthermore, since in these studies EC₁₀ values would be higher than NOECs, the NOEC-based geometric mean value (4.72 µg a.s./L) is in fact more conservative than one that would be based on EC₁₀ values. Employing a standard AF of 10, the resulting **geometric mean chronic fish RAC** is **0.472 µg a.s./L**.

The higher-tier ELS study (BASF DocID 1999/11537) was considered to be not valid in the ECCO peer review based on “low survival in the control” (ECCO Peer Review Program, Full Report on Pyraclostrobin, 2002). However, this appears to be based on a misunderstanding. Therefore, a GLP report amendment (BASF DocID 2018/1123384) and associated statement (BASF DocID 2018/1140960) were written to explain the data and the implications on study validity. Based on BASF DocID 2018/1123384, the study is valid for the assessment of chronic toxicity to fish from a realistic worst-case, multiple peak exposure scenario, and covers all relevant chronic endpoints. The higher-tier rainbow trout ELS study can be used to refine the long-term risk assessment for fish. This study employed a slightly more realistic but still worst-case exposure and resulted in a NOEC of 5 µg a.s./L. Applying the standard assessment factor of 10 would derive a **refined exposure chronic fish RAC** of **0.5 µg a.s./L**. Regardless of the study validity, the information can always be used in a weight-of-evidence approach (see below).

As a third possibility for refinement, a weight-of-evidence (WoE) approach can be used to derive a chronic RAC for fish. This approach combines all available data on fish: the 3 standard ELS studies, the juvenile growth test, the higher-tier ELS, the carp observations in the mesocosm study and the acute data on seven different fish species from different families. Such an approach does justice to the wealth of information and recognizes the added value of these vertebrate studies in the risk assessment.

Since the acute NOECs were within a factor of 2 from the chronic NOECs, they provide valuable information on comparative acute and chronic fish sensitivity and confirm the low acute-to-chronic ratio (ACR). Furthermore, both the acute and chronic data confirm that rainbow trout is the most sensitive species. Considering the extensive information regarding the fish species sensitivity distribution and the variety of chronic studies, it seems justified to apply a 2-fold reduced assessment factor on the lowest standard chronic tier 1 endpoint, *i.e.* applying a factor of 5 on the rainbow trout ELS NOEC of 2.35 µg a.s./L, which generates a **WoE chronic fish RAC** of **0.47 µg a.s./L**. It should also be noted that with 98 days, the trout ELS is in fact the longest study in the fish data package and that such long exposure durations are not expected for pyraclostrobin, due to adsorption and degradation of this lipophilic, non-persistent substance.

That all three chronic risk refinement options lead to a very similar RAC, lends further credibility to the derived values and confirms their robustness. The lowest chronic RAC proposal of 0.47 µg a.s./L will be used in the refined risk assessment.

In conclusion, peak concentrations of up to 8 µg pyraclostrobin/L will not have a negative impact on aquatic invertebrates and aquatic plants. A significant number of additional and higher-tier studies with fish show, that no acute or chronic risk to fish is to be expected at concentrations below 0.93 µg a.s./L or 0.47 µg a.s./L, respectively.

Review Comments:

According the recommendation of “Working document on Risk Assessment of Plant Protection Products in Central Zone Ecotoxicology” (May 2021), point 3.3.12, is no agreement for using a geometric mean for chronic data. Therefore, the risk assessment will be based on lowest available endpoint of 2.35 µg/L.

Acceptability of risk

The relevant worst-case predicted environmental concentrations in surface water bodies (PEC_{sw} and PEC_{sed}), regulatory acceptable concentrations (RAC) for aquatic organisms and the resulting PEC/RAC ratios (ETR) for the single and twofold application of pyraclostrobin in 'spring and winter cereals' are given per intended use and each organism group and are presented in Table 9.5-17 - Table 9.5-24. Worst-case PEC values for either 'spring or winter cereals' are considered in a risk envelope approach. For details on the PEC calculations please refer to Part B, Section 8.9.

The chronic risk to aquatic insects resulting from water exposure can be addressed with a *C. riparius* study (BASF DocID 2000/1000010) that obtained a NOEC of 40 µg a.s./L. To assess the risk from sediment exposure, the concentration of pyraclostrobin in sediment at the NOEC treatment in the spiked water study was estimated considering the partitioning of the active substance between sediment and the overlying water as observed in the water-sediment study with ^{14}C -labelled pyraclostrobin. After 7 days of exposure to 80 µg a.s./L and 320 µg a.s./L (initial water concentrations), the parent substance was found at 68.3% and 63.1% of the total applied radioactivity in the sediment, respectively. Considering that on average about 65.7% of the applied parent substance was found in the sediment, the concentration of pyraclostrobin at the NOEC concentration in the *Chironomus* spiked water study is about 120 µg a.s./kg dry sediment. Instead of using the extrapolated sediment value for *C. riparius*, a more reliable NOEC can be obtained from a spiked sediment study, which is now available. This study provides a NOEC of 1.37 mg a.s./kg dry sediment, which is used in the risk assessment.

Table 9.5-17: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for pyraclostrobin for each organism group based on standard FOCUS Step 1 - 3 calculations for single and twofold application (1x and 2x 80 g a.s./ha) of BAS 758 00 F in 'spring cereals'

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Higher plant	Sed. dwell. prolonged	Higher-tier information			Group	Sed. dwell. prolonged	
Test species		<i>O. mykiss</i>	<i>O. mykiss</i> (ELS study)	<i>D. magna</i>	<i>D. magna</i>	<i>P. subcapitata</i>	<i>L. gibba</i>	<i>C. riparius</i>	Fish acute: SSD based on 96 h LC ₅₀ NOECs for 7 fish species	Fish chronic: WoE-approach (modified ELS and geomean yield-very similar RACs)	Inverteb.: Outdoor mesocosm (multiple spray appl.)	Test species	<i>C. riparius</i>	
Endpoint (µg/L)		LC ₅₀ 6.16	NOEC 2.35	EC ₅₀ 15.7	NOEC 4.0	E _r C ₅₀ > 843	E _r C ₅₀ > 1077	NOEC 40	HC ₅ 5.9 4.2	NOEC 2.35	NOEC 8.0	Endpoint (µg/kg)	NOEC 1370	
AF		100	10	100	10	10	10	10	6.33 6	5	3	AF	10	
RAC (µg/L)		0.0616	0.235	0.157	0.4	> 84.3	> 107.7	4.0	0.93 0.7	0.47-1	2.67	RAC (µg/kg)	137	
FOCUS Scenario	PEC ^{gl-sw} max (µg/L) ²⁾	PEC/RAC (= ETR)									PEC ^{gl-sed} max (µg/kg) ²⁾	PEC/RAC (= ETR)		
Step 1 ³⁾														
	10.567	172	45	67	26	< 0.1	< 0.1	2.6	11	15.1	22	4.0	693.739	5.1
Step 2 ³⁾														
N-Europe	1.380	22	5.9	8.8	3.5	--	--	0.3	1.5	2.0	2.9	0.5	88.061	0.6
S-Europe	1.934	31	8.2	12	4.8	--	--	0.5	2.1	2.8	4.1	0.7	163.138	1.2
Step 3														
D3 ditch	0.502	8.1	2.1	3.2	1.3	--	--	--	0.5	0.7	1.07	--	0.507	0.004
D4 pond	0.023	0.4	0.1	0.1	0.06	--	--	--	0.02	0.03	0.05	--	0.208	0.002
D4 stream	0.434	7.0	1.8	2.8	1.09	--	--	--	0.5	0.06	0.9	--	0.094	0.0007

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Higher plant	Sed. dwell. prolonged	Higher-tier information				Group	Sed. dwell. prolonged
D5 pond	0.021	0.3	0.09	0.1	0.05	--	--	--	0.02	0.03	0.04	--	0.203	0.001
D5 stream	0.464	7.5	2.0	3.0	1.2	--	--	--	0.5	0.7	0.99	--	0.100	0.0007
R4 stream	0.332	5.4	1.4	2.1	0.8	--	--	--	0.4	0.5	0.7	--	3.346	0.02

Abbreviations: ELS: Early life stage; AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; ETR: exposure-toxicity ratio; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**

- 1) More details on the derivation of the refined chronic fish RAC can be found under “Refined risk assessment for pyraclostrobin (BAS 500 F)” above.
- 2) Worst-case PECs are derived from either single or twofold application in a risk envelope approach. For details, please refer to Part B, Section 8.9.
- 3) Worst-case Step 1 - 2 PECs are derived from either single or twofold application of 120 g a.s./ha in ‘spring or winter cereals’ in a risk envelope approach. For details, please refer to Part B, Section 8.9.

For the intended single and twofold application of BAS 758 00 F at 1x and 2x 80 g a.s./ha in ‘spring cereals’, the calculated PEC/RAC ratios for pyraclostrobin did not indicate an acceptable acute and chronic risk for fish and invertebrates based on tier 1 toxicity data and standard FOCUS Step 1-3 PEC_{sw/seed} values. Therefore, further PEC/RAC ratios were calculated considering the available higher-tier information. Based on these approaches, acceptable acute risk for fish (based on SSD) and aquatic invertebrates (based on mesocosm data) can be demonstrated considering FOCUS Step 3 PEC_{sw/seed} values. The calculated PEC/RAC ratios for chronic risk for fish indicate the need for some mitigation measures ~~for the D3 ditch scenario~~. Respective FOCUS Step 4 calculations for pyraclostrobin were carried out considering ~~5 m non-sprayed buffer zones (drift buffer) and drift-reducing nozzles as~~ mitigation measures, considering the overall aquatic RAC of ~~0.47~~ **0.235** µg a.s./L based on chronic fish ~~(WoE approach)~~.

Table 9.5-18: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for pyraclostrobin based on FOCUS Step 4 calculations and the overall aquatic RAC of ~~0.47~~ 0.235 µg a.s./L with mitigation of spray drift for single and twofold application (1x and 2x 80 g a.s./ha) of BAS 758 00 F in 'spring cereals'

Intended use		‘spring cereals’	
Active substance		pyraclostrobin	
Application rate (g/ha)		1x and 2x 80 g a.s./ha ¹⁾	
		PEC _{sw} (µg/L)	
Nozzle reduction	No-spray buffer (m)	Edge of field	5
None	D3 ditch	0.502	0.162 0.136
50 %		0.222 0.251	0.081
75%		0.126	--
RAC (µg/L)		PEC/RAC ratio	
0.47 0.235			
None	D3 ditch	1.07 2.14	0.3 0.58
50 75%		0.5 0.54	--
		PEC _{sw} (µg/L)	
Nozzle reduction	No-spray buffer (m)	Edge of field	5
None	D4 stream	--	0.159
50 %		0.217	--
RAC (µg/L)		PEC/RAC ratio	
0.235			
None	D4 stream	--	0.68
50 %		0.92	--
		PEC _{sw} (µg/L)	
Nozzle reduction	No-spray buffer (m)	Edge of field	5
None	D5 stream	--	0.169
50 %		0.232	--
RAC (µg/L)		PEC/RAC ratio	
0.235			
None	D5 stream	--	0.72
50 %		0.99	--
		PEC _{sw} (µg/L)	
Nozzle reduction	No-spray buffer and vegetative filter strip (m)	Edge of field	10 + 10
None	R4 stream	--	0.132
RAC (µg/L)		PEC/RAC ratio	
0.235			

None	R4 stream	--	0.56
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4Abbreviations: PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**

¹⁾ Worst-case PECs are derived from either single or twofold application in a risk envelope approach. For details, please refer to Part B, Section 8.9.

Based on the overall higher-tier RAC of ~~0.47~~ **0.235** µg a.s./L, the calculated higher-tier PEC/RAC ratios indicate an acceptable risk for all groups of aquatic organisms in the critical FOCUS Step 4 scenario if a non-sprayed buffer zone of 5 m or 50% drift reducing nozzles are considered for the intended use of BAS 758 00 F in 'spring cereals' for scenarios D4 and D5. A non-sprayed buffer zone of 5 m or 75% drift reducing nozzles are considered for the intended use of BAS 758 00 F in 'spring cereals' for scenarios D3 For scenario R4 non-sprayed, vegetated buffer zone of 10 m is required. For details on the chronic risk for fish, see "Refined risk assessment for pyraclostrobin (BAS 500 F)", which is provided above.

Table 9.5-19: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for pyraclostrobin for each organism group based on standard FOCUS Step 1 - 3 calculations for single and twofold application (1x and 2x 80 g a.s./ha) of BAS 758 00 F in 'winter cereals'

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Higher plant	Sed. dwell. prolonged	Higher-tier information			Group	Sed. dwell. prolonged	
Test species		<i>O. mykiss</i>	<i>O. mykiss</i> (ELS study)	<i>D. magna</i>	<i>D. magna</i>	<i>P. subcapitata</i>	<i>L. gibba</i>	<i>C. riparius</i>	Fish acute: SSD based on 96 h LC ₅₀ NOECs for 7 fish species	Fish chronic: WoE approach (modified ELS and geometric mean yield very similar RACs)	Inverteb.: Outdoor mesocosm (multiple spray appl.)	Test species	<i>C. riparius</i>	
Endpoint (µg/L)		LC ₅₀ 6.16	NOEC 2.35	EC ₅₀ 15.7	NOEC 4.0	E _r C ₅₀ > 843	E _r C ₅₀ > 1077	NOEC 40	HC ₅ 5.9 4.2	NOEC 2.35	NOEC 8.0	Endpoint (µg/kg)	NOEC 1370	
AF		100	10	100	10	10	10	10	6.33 6	5	3	AF	10	
RAC (µg/L)		0.0616	0.235	0.157	0.4	> 84.3	> 107.7	4.0	0.93 0.7	0.47 0.4	2.67	RAC (µg/kg)	137	
FOCUS Scenario	PEC ^{gl-sw max} (µg/L) ²⁾	PEC/RAC (= ETR)										PEC ^{gl-sed max} (µg/kg) ²⁾	PEC/RA C (= ETR)	
Step 1 ³⁾														
	10.567	172	45	67	26	< 0.1	< 0.1	2.6	11	15.1	22	4.0	693.739	5.1
Step 2 ³⁾														
N-Europe	1.380	22	5.9	8.8	3.5	--	--	0.3	1.5	2.0	2.9	0.5	88.061	0.6
S-Europe	1.934	31	8.2	12	4.8	--	--	0.5	2.1	2.8	4.1	0.7	163.138	1.2
Step 3														
D3 ditch	0.502	8.1	2.1	3.2	1.3	--	--	--	0.5	0.72	1.07	--	0.487	0.004
D4 pond	0.021	0.3	0.09	0.1	0.05	--	--	--	0.02	0.03	0.04	--	0.228	0.002
D4 stream	0.371	6.0	1.6	2.4	0.9	--	--	--	0.4	0.53	0.8	--	0.016	0.0001

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Higher plant	Sed. dwell. prolonged	Higher-tier information				Group	Sed. dwell. prolonged
D5 pond	0.024	0.4	0.1	0.2	0.06	--	--	--	0.03	0.03	0.05	--	0.228	0.002
D5 stream	0.401	6.5	1.7	2.6	1.003	--	--	--	0.4	0.57	0.9	--	0.034	0.0002
R1 pond	0.032	0.5	0.1	0.2	0.08	--	--	--	0.03	0.05	0.07	--	0.472	0.003
R1 stream	0.331	5.4	1.4	2.1	0.8	--	--	--	0.4	0.47	0.7	--	3.010	0.02
R3 stream	0.464	7.5	2.0	3.0	1.2	--	--	--	0.5	0.66	0.99	--	2.525	0.02
R4 stream	0.332	5.4	1.4	2.1	0.8	--	--	--	0.4	0.47	0.7	--	4.006	0.03

Abbreviations: ELS: Early life stage; AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; ETR: exposure-toxicity ratio; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**

- 1) More details on the derivation of the refined chronic fish RAC can be found under “Refined risk assessment for pyraclostrobin (BAS 500 F)” above.
- 2) Worst-case PECs are derived from either single or twofold application in a risk envelope approach. For details, please refer to Part B, Section 8.9.
- 3) Worst-case Step 1 - 2 PECs are derived from either single or twofold application of 120 g a.s./ha in ‘spring or winter cereals’ in a risk envelope approach. For details, please refer to Part B, Section 8.9.

For the intended single and twofold application of BAS 758 00 F at 1x and 2x 80 g a.s./ha in ‘winter cereals’, the calculated PEC/RAC ratios for pyraclostrobin did not indicate an acceptable acute and chronic risk for fish and invertebrates based on tier 1 toxicity data and standard FOCUS Step 1-3 PEC_{sw/sed} values. Therefore, further PEC/RAC ratios were calculated considering the available higher-tier information. Based on these approaches, acceptable acute risk for fish (based on SSD) and aquatic invertebrates (based on mesocosm data) can be demonstrated considering FOCUS Step 3 PEC_{sw/sed} values. The calculated PEC/RAC ratios for chronic risk for fish indicate the need for some mitigation measures ~~for the D3 ditch scenario~~. Respective FOCUS Step 4 calculations for pyraclostrobin were carried out considering ~~5 m non-sprayed buffer zones (drift buffer) and drift-reducing nozzles as~~ mitigation measures, considering the overall aquatic RAC of ~~0.47~~ **0.235** µg a.s./L based on chronic fish ~~(WoE approach)~~.

Table 9.5-20: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for pyraclostrobin based on FOCUS Step 4 calculations and the overall aquatic RAC of ~~0.47~~ 0.235 µg a.s./L with mitigation of spray drift for single and twofold application (1x and 2x 80 g a.s./ha) of BAS 758 00 F in 'winter cereals'

Intended use		‘winter cereals’	
Active substance		pyraclostrobin	
Application rate (g/ha)		1x and 2x 80 g a.s./ha ¹⁾	
		PEC _{sw} (µg/L)	
Nozzle reduction	No-spray buffer (m)	Edge of field	5
None	D3 ditch	0.502	0.136
50 75%		0.252 0.126	0.068
RAC (µg/L)		PEC/RAC ratio	
0.47 0.235			
None	D3 ditch	1.07	0.3 0.69
50 75%		0.54	--
		PEC _{sw} (µg/L)	
Nozzle reduction	No-spray buffer (m)	Edge of field	5
None	D4 stream	--	0.135
50 %		0.185	--
RAC (µg/L)		PEC/RAC ratio	
0.235			
None	D4 stream	--	0.57
50 %		0.79	--
		PEC _{sw} (µg/L)	
Nozzle reduction	No-spray buffer (m)	Edge of field	5
None	D5 stream	--	0.146
50 %		0.200	--
RAC (µg/L)		PEC/RAC ratio	
0.235			
None	D5 stream	--	0.62
50 %		0.85	--
		PEC _{sw} (µg/L)	
Nozzle reduction	No-spray buffer (m)	Edge of field	5
None	R1 stream	--	0.171
50%		0.171	
RAC (µg/L)		PEC/RAC ratio	
0.235			

None	R1 stream	--	0.73
50%		0.73	--
		PEC _{sw} (µg/L)	
Nozzle reduction	No-spray buffer (m)	Edge of field	5
None	R3 stream	--	0.187
50%		0.232	
RAC (µg/L)		PEC/RAC ratio	
0.235			
None	R3 stream	--	0.80
50%		0.99	
		PEC _{sw} (µg/L)	
Nozzle reduction	No-spray buffer and vegetative filter strip (m)	Edge of field	10 + 10
None	R4 stream	--	0.136
RAC (µg/L)		PEC/RAC ratio	
0.235			
None	R4 stream	--	0.58

Abbreviations: PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**

- ¹⁾ Worst-case PECs are derived from either single or twofold application in a risk envelope approach. For details, please refer to Part B, Section 8.9.

Based on the overall higher-tier RAC of ~~0.47~~ **0.235** µg a.s./L, the calculated higher-tier PEC/RAC ratios indicate an acceptable risk for all groups of aquatic organisms in the critical FOCUS Step 4 scenario if a non-sprayed buffer zone of 5 m or 50% drift reducing nozzles are considered for the intended use of BAS 758 00 F in 'winter cereals' for scenarios D4, D5, R1 and R3. A non-sprayed buffer zone of 5 m or 75% drift reducing nozzles are considered for the intended use of BAS 758 00 F in 'winter cereals' for scenarios D3. For scenario R4 non-sprayed, vegetated buffer zone of 10 m is required. For details on the chronic risk for fish, see "Refined risk assessment for pyraclostrobin (BAS 500 F)", which is provided above.

Table 9.5-21: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for pyraclostrobin for each organism group based on standard FOCUS Step 1 - 3 calculations for single and twofold application (1x and 2x 120 g a.s./ha) of BAS 758 00 F in 'spring cereals'

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Higher plant	Sed. dwell. prolonged	Higher-tier information			Group	Sed. dwell. prolonged	
Test species		<i>O. mykiss</i>	<i>O. mykiss</i> (ELS study)	<i>D. magna</i>	<i>D. magna</i>	<i>P. subcapitata</i>	<i>L. gibba</i>	<i>C. riparius</i>	Fish acute: SSD based on 96 h LC ₅₀ NOECs for 7 fish species	Fish chronic: WoE-approach (modified ELS and geometric mean yield very similar RACs)	Inverteb.: Outdoor mesocosm (multiple spray appl.)	Test species	<i>C. riparius</i>	
Endpoint (µg/L)		LC ₅₀ 6.16	NOEC 2.35	EC ₅₀ 15.7	NOEC 4.0	E _r C ₅₀ > 843	E _r C ₅₀ > 1077	NOEC 40	HC ₅ 5.9 4.2	NOEC 2.35	NOEC 8.0	Endpoint (µg/kg)	NOEC 1370	
AF		100	10	100	10	10	10	10	6.33 6	5	3	AF	10	
RAC (µg/L)		0.0616	0.235	0.157	0.4	> 84.3	> 107.7	4.0	0.93 0.7	0.47-1.1	2.67	RAC (µg/kg)	137	
FOCUS Scenario	PEC ^{gl-sw max} (µg/L) ²⁾	PEC/RAC (= ETR)										PEC ^{gl-sed max} (µg/kg) ²⁾	PEC/RAC (= ETR)	
Step 1 ³⁾														
	10.567	172	45	67	26	< 0.1	< 0.1	2.6	11	15.1	22	4.0	693.739	5.1
Step 2 ³⁾														
N-Europe	1.380	22	5.9	8.8	3.5	--	--	0.3	1.5	2.0	2.9	0.5	88.061	0.6
S-Europe	1.934	31	8.2	12	4.8	--	--	0.5	2.1	2.8	4.1	0.7	163.138	1.2
Step 3														
D3 ditch	0.754	12	3.2	4.8	1.9	--	--	--	0.8	1.01	1.6	--	0.758	0.006
D4 pond	0.035	0.6	0.1	0.2	0.09	--	--	--	0.04	0.05	0.07	--	0.311	0.002
D4 stream	0.616	10	2.6	3.9	1.5	--	--	--	0.7	0.88	1.3	--	0.081	0.0006

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Higher plant	Sed. dwell. prolonged	Higher-tier information				Group	Sed. dwell. prolonged
D5 pond	0.032	0.5	0.1	0.2	0.08	--	--	--	0.03	0.05	0.07	--	0.293	0.002
D5 stream	0.633	10	2.7	4.0	1.6	--	--	--	0.7	0.90	1.3	--	0.173	0.001
R4 stream	0.498	8.1	2.1	3.2	1.2	--	--	--	0.5	0.71	1.06	--	4.928	0.04

Abbreviations: ELS: Early life stage; AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; ETR: exposure-toxicity ratio; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**

- 1) More details on the derivation of the refined chronic fish RAC can be found under “Refined risk assessment for pyraclostrobin (BAS 500 F)” above.
- 2) Worst-case PECs are derived from either single or twofold application in a risk envelope approach. For details, please refer to Part B, Section 8.9.
- 3) Worst-case Step 1 - 2 PECs are derived from either single or twofold application of 120 g a.s./ha in ‘spring or winter cereals’ in a risk envelope approach. For details, please refer to Part B, Section 8.9.

For the intended single and twofold application of BAS 758 00 F at 1x and 2x 120 g a.s./ha in ‘spring cereals’, the calculated PEC/RAC ratios for pyraclostrobin did not indicate an acceptable acute and chronic risk for fish and invertebrates based on tier 1 toxicity data and standard FOCUS Step 1-3 PEC_{sw/sed} values. Therefore, further PEC/RAC ratios were calculated considering the available higher-tier information. Based on these approaches, acceptable acute risk for fish (based on SSD) and aquatic invertebrates (based on mesocosm data) can be demonstrated considering FOCUS Step 3 PEC_{sw/sed} values. The calculated PEC/RAC ratios for chronic risk for fish indicate the need for some mitigation measures for D3 ditch and D4, D5 and R4 stream scenarios. Respective FOCUS Step 4 calculations for pyraclostrobin were carried out considering ~~5-m non-sprayed buffer zones (drift buffer) and drift-reducing nozzles as~~ mitigation measures, considering the overall aquatic RAC of ~~0.47~~ **0.235** µg a.s./L based on chronic fish (WoE approach).

Table 9.5-22: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for pyraclostrobin based on FOCUS Step 4 calculations and the overall aquatic RAC of ~~0.47~~ **0.235 µg a.s./L with mitigation of spray drift for single and twofold application (1x and 2x 120 g a.s./ha) of BAS 758 00 F in ‘spring cereals’**

Intended use		‘spring cereals’			
Active substance		pyraclostrobin			
Application rate (g/ha)		1x and 2x 120 g a.s./ha ¹⁾			
		PEC _{sw} (µg/L)			
Nozzle reduction	No-spray buffer (m)	Edge of field		5	
None	D3 ditch	0.754		0.204	
50 %		0.377		0.102	
75%		0.188		--	
None	D4 stream	0.616		0.225	
50 %		0.308		0.112	
75%		0.154		--	
None	D5 stream	0.633		0.231	
50 %		0.316		0.116	
75%		0.158		--	
None	R4 stream	0.498		0.450	
50 %		0.450		0.450	
Nozzle reduction	No-spray buffer and vegetative filter strip (m)	Edge of field		10 + 10	
None	R4 stream	--		0.114	
RAC (µg/L)		PEC/RAC ratio			
0.47 0.235					
None	D3 ditch	1.6	--	0.4	0.87
50 75 %		0.8	0.80	—	--
None	D4 stream	1.3	--	0.5	0.96
50 75 %		0.7	0.66	—	--
None	D5 stream	1.3	--	0.5	0.98
50 75 %		0.7		—	--
Nozzle reduction	No-spray buffer and vegetative filter strip (m)	Edge of field		10 + 10	
None	R4 stream	1.06	--	0.96	0.49
50 %		0.96		—	

Abbreviations: PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**

¹⁾ Worst-case PECs are derived from either single or twofold application in a risk envelope approach. For details, please refer to Part B, Section 8.9.

Based on the overall higher-tier RAC of ~~0.47~~ 0.235 µg a.s./L, the calculated higher-tier PEC/RAC ratios indicate an acceptable risk for all groups of aquatic organisms in the critical FOCUS Step 4 scenario if a non-sprayed buffer zone of 5 m or 75 50% drift reducing nozzles are considered for the intended use of BAS 758 00 F in 'spring cereals' for scenarios D3, D4 and D5. For scenario R4 non-sprayed, vegetated buffer zone of 10 m is required.. For details on the chronic risk for fish, see "Refined risk assessment for pyraclostrobin (BAS 500 F)", which is provided above.

Table 9.5-23: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for pyraclostrobin for each organism group based on standard FOCUS Step 1 - 3 calculations for single and twofold application (1x and 2x 120 g a.s./ha) of BAS 758 00 F in 'winter cereals'

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Higher plant	Sed. dwell. prolonged	Higher-tier information			Group	Sed. dwell. prolonged	
Test species		<i>O. mykiss</i>	<i>O. mykiss</i> (ELS study)	<i>D. magna</i>	<i>D. magna</i>	<i>P. subcapitata</i>	<i>L. gibba</i>	<i>C. riparius</i>	Fish acute: SSD based on 96 h LC ₅₀ NOECs for 7 fish species	<i>Fish chronic: WoE-approach (modified ELS and geometric mean yield very similar RACs)</i>	Inverteb.: Outdoor mesocosm (multiple spray appl.)	Test species	<i>C. riparius</i>	
Endpoint (µg/L)		LC ₅₀ 6.16	NOEC 2.35	EC ₅₀ 15.7	NOEC 4.0	E _r C ₅₀ > 843	E _r C ₅₀ > 1077	NOEC 40	HC ₅ 5.9 4.2	NOEC 2.35	NOEC 8.0	Endpoint (µg/kg)	NOEC 1370	
AF		100	10	100	10	10	10	10	6.33 6	5	3	AF	10	
RAC (µg/L)		0.0616	0.235	0.157	0.4	> 84.3	> 107.7	4.0	0.93 0.7	0.47-1	2.67	RAC (µg/kg)	137	
FOCUS Scenario	PEC ^{gl-sw max} (µg/L) ²⁾	PEC/RAC (= ETR)										PEC ^{gl-sed max} (µg/kg) ²⁾	PEC/RA C (= ETR)	
Step 1 ³⁾														
	10.567	172	45	67	26	< 0.1	< 0.1	2.6	11	15.1	22	4.0	693.739	5.1
Step 2 ³⁾														
N-Europe	1.380	22	5.9	8.8	3.5	--	--	0.3	1.5	2.0	2.9	0.5	88.061	0.6
S-Europe	1.934	31	8.2	12	4.8	--	--	0.5	2.1	2.8	4.1	0.7	163.138	1.2
Step 3														
D3 ditch	0.753	12	3.2	4.8	1.9	--	--	--	0.8	1.08	1.6	--	0.728	0.005
D4 pond	0.031	0.5	0.1	0.2	0.08	--	--	--	0.03	0.04	0.07	--	0.340	0.002
D4 stream	0.556	9.0	2.4	3.5	1.4	--	--	--	0.6	0.79	1.2	--	0.023	0.0002

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Higher plant	Sed. dwell. prolonged	Higher-tier information				Group	Sed. dwell. prolonged
D5 pond	0.036	0.6	0.2	0.2	0.09	--	--	--	0.04	0.05	0.08	--	0.340	0.002
D5 stream	0.601	9.8	2.6	3.8	1.5	--	--	--	0.6	0.86	1.3	--	0.051	0.0004
R1 pond	0.048	0.8	0.2	0.3	0.1	--	--	--	0.05	0.07	0.1	--	0.710	0.005
R1 stream	0.496	8.1	2.1	3.2	1.2	--	--	--	0.5	0.71	1.06	--	4.473	0.03
R3 stream	0.697	11	3.0	4.4	1.7	--	--	--	0.7	0.10	1.5	--	3.777	0.03
R4 stream	0.498	8.1	2.1	3.2	1.2	--	--	--	0.5	0.71	1.06	--	5.934	0.04

Abbreviations: ELS: Early life stage; AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; ETR: exposure-toxicity ratio; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**

- 1) More details on the derivation of the refined chronic fish RAC can be found under “Refined risk assessment for pyraclostrobin (BAS 500 F)” above.
- 2) Worst-case PECs are derived from either single or twofold application in a risk envelope approach. For details, please refer to Part B, Section 8.9.
- 3) Worst-case Step 1 - 2 PECs are derived from either single or twofold application of 120 g a.s./ha in ‘spring or winter cereals’ in a risk envelope approach. For details, please refer to Part B, Section 8.9.

For the intended single and twofold application of BAS 758 00 F at 1x and 2x 120 g a.s./ha in ‘winter cereals’, the calculated PEC/RAC ratios for pyraclostrobin did not indicate an acceptable acute and chronic risk for fish and invertebrates based on tier 1 toxicity data and standard FOCUS Step 1-3 PEC_{sw/sed} values. Therefore, further PEC/RAC ratios were calculated considering the available higher-tier information. Based on these approaches, acceptable acute risk for fish (based on SSD) and aquatic invertebrates (based on mesocosm data) can be demonstrated considering FOCUS Step 3 PEC_{sw/sed} values. The calculated PEC/RAC ratios for chronic risk for fish indicate the need for some mitigation measures for the D3 ditch and D4, D5, R1, R3 and R4 stream scenarios. Respective FOCUS Step 4 calculations for pyraclostrobin were carried out considering ~~5 m non-sprayed buffer zones (drift buffer) and drift reducing nozzles as~~ mitigation measures, considering the overall aquatic RAC of ~~0.47~~ **0.235** µg a.s./L based on chronic fish ~~(WoE approach)~~.

Table 9.5-24: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for pyraclostrobin based on FOCUS Step 4 calculations and the overall aquatic RAC of ~~0.47~~ 0.235 µg a.s./L with mitigation of spray drift for single and twofold application (1x and 2x 120 g a.s./ha) of BAS 758 00 F in ‘winter cereals’

Intended use		‘winter cereals’			
Active substance		pyraclostrobin			
Application rate (g/ha)		1x and 2x 120 g a.s./ha ¹⁾			
		PEC _{sw} (µg/L)			
Nozzle reduction	No-spray buffer (m)	Edge of field		5	
None	D3 ditch	0.753		0.204	
50 %		0.376		0.102	
75%		0.188		--	
None	D4 stream	0.556		0.203	
50 %		0.278		0.102	
75%		0.139		--	
None	D5 stream	0.601		0.219	
50 %		0.301		0.110	
75%		0.150		--	
None	R1 stream	0.496		0.261	
50 %		0.261		0.261	
None	R3 stream	0.697		0.287	
50 %		0.287		0.287	
None	R4 stream	0.498		0.458	
50 %		0.458		0.458	
Nozzle reduction	No-spray buffer and vegetative filter strip (m)	Edge of field		10 + 10	
None	R1 stream	--		0.119	
None	R2 stream	--		0.135	
None	R4 stream	--		0.208	
RAC (µg/L)		PEC/RAC ratio			
0.47 0.235					
None	D3 ditch	1.6	--	0.4	0.87
50 75 %		0.8	0.80	--	
None	D4 stream	1.2	--	0.4	0.86
50 75 %		0.6	0.59	--	
None	D5 stream	1.3	--	0.5	0.93
50 75 %		0.6	0.64	--	
None	R1 stream	1.06		0.6	

50 %		0.6	—
None	R3 stream	1.5	0.6
50 %		0.6	—
None	R4 stream	1.06	0.97
50 %		0.97	—
Nozzle reduction	No-spray buffer and vegetative filter strip (m)	Edge of field	10 + 10
None	R1 stream	--	0.51
None	R2 stream	--	0.57
None	R4 stream	--	0.89

Abbreviations: PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**

- ¹⁾ Worst-case PECs are derived from either single or twofold application in a risk envelope approach. For details, please refer to Part B, Section 8.9.

Based on the overall higher-tier RAC of ~~0.47~~ 0.235 µg a.s./L, the calculated higher-tier PEC/RAC ratios indicate an acceptable risk for all groups of aquatic organisms in the critical FOCUS Step 4 scenario if a non-sprayed buffer zone of 5 m or ~~50~~ 75 % drift reducing nozzles are considered for the intended use of BAS 758 00 F in 'winter cereals' for scenarios D3, D4 and D5. For scenarios R1, R3 and R4 non-sprayed, vegetated buffer zone of 10 m is required. For details on the chronic risk for fish, see "Refined risk assessment for pyraclostrobin (BAS 500 F)", which is provided above.

Risk assessment for the metabolites of pyraclostrobin (BAS 500 F)

Next to the parent, the metabolites BF 500-3, BF 500-6 and BF 500-7 occurred as major metabolites (see section B08) in the dark water/sediment study. Additionally, BF 500-3, BF 500-11, BF 500-13 and BF 500-14 occurred in relevant amounts in the aquatic photolysis, respectively the irradiated water/sediment study.

The aerobic soil metabolites BF 500-6 and BF 500-7 show very high sorption indicating that they are non-mobile in soil (BF 500-6: $K_{oc} > 3300$ mL/g; BF 500-7: $K_{oc} > 4000$ mL/g); (see section B08). Considering the high sorption, the low mobility in soil and the very low water solubility, it can be concluded that BF 500-6 and BF 500-7 will not enter surface waters in any ecotoxicologically relevant amount. Furthermore, studies with these metabolites and soil organisms indicate very low toxicity and overall low ecotoxicological potential. ~~To assess the risk to sediment dwelling organisms, 28-d spiked sediment studies on *C. riparius* have been conducted with both metabolites. These results are considered for the risk assessment below.~~

The pyraclostrobin metabolite BF 500-3 was not observed in standard aerobic soil metabolism studies, but only in soil under anaerobic conditions (see section B08). However, aerobic conditions are predominant in soil. The sorption behavior of BF 500-3 was investigated in the context of the EU review of pyraclostrobin and K_{oc} -values ranged from 4240 to 12000 mL/g (see section B08) with an arithmetic mean K_{oc} of 9315 mL/g. Due to its non-occurrence under more relevant aerobic conditions and its high K_{oc} , this metabolite will not be relevant for drainage or runoff entry into surface waters. BF 500-3 was observed in sediment with a maximum occurrence of 16.9% TAR in an irradiated water/sediment study (please refer to section B08). The highest amount of BF 500-3 in the water phase never exceeds 5.0% TAR and thus, the metabolite is only relevant in the sediment phase. As a major sediment metabolite, it has been tested in a 28-d spiked sediment study with *Chironomus riparius* and the results are used in the respective ETR calculations (see below).

Acceptability of risk

In Table 9.5-25, the exposure-toxicity ratios (ETRs) for aquatic organisms are given for the use of BAS 758 00 F in 'spring and winter cereals' and for each organism group for the relevant metabolites of pyraclostrobin. Worst-case $PEC_{sw/sed}$ values from single and twofold application (1x and 2x 150 g a.s./ha) in 'winter cereals' are used for risk assessment and cover all intended uses.

Table 9.5-25: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for metabolites of pyraclostrobin for each organism group based on worst-case FOCUS Step 1 - 2 calculations following single and twofold application ¹⁾ (1x and 2x 150 g a.s./ha) of BAS 758 00 F in 'winter cereals' ²⁾, covering all intended uses

Group		Fish acute	Inverteb. acute	Algae	Group	Sed. dwell. prolonged
Test species		<i>O. mykiss</i>	<i>D. magna</i>	<i>S. subspicatus</i>	Test species	<i>C. riparius</i>
AF		100	100	10	AF	10
BF 500-3						
Endpoint (µg/L)		LC ₅₀ n.a.	EC ₅₀ n.a.	E _r C ₅₀ n.a.	Endpoint (µg/kg)	NOEC ≥ 16000
RAC (µg/L)		--	--	--	RAC (µg/kg)	≥ 1600
FOCUS Scenario	PEC _{gl-max, sw} (µg/L)	PEC/RAC ratio (= ETR)			PEC _{gl-sed max} (µg/kg)	PEC/RAC (= ETR)
Step 1						
	n.c.	--	--	--	440.173 443.116	0.3
BF 500-6						
Endpoint (µg/L)		LC ₅₀ n.a.	EC ₅₀ n.a.	E _r C ₅₀ n.a.	Endpoint (µg/kg)	NOEC 1200
RAC (µg/L)		--	--	--	RAC (µg/kg)	120
FOCUS Scenario	PEC _{gl-max, sw} (µg/L)	PEC/RAC ratio (= ETR)			PEC _{gl-sed max} (µg/kg)	PEC/RAC (= ETR)
Step 1						
	n.c.	--	--	--	216.075	1.8
Step 2						
N-Europe	n.c.	--	--	--	33.180	0.3
S-Europe	n.c.	--	--	--	65.472	0.5
BF 500-7						
Endpoint (µg/L)		LC ₅₀ n.a.	EC ₅₀ n.a.	E _r C ₅₀ n.a.	Endpoint (µg/kg)	NOEC ≥ 123500
RAC (µg/L)		--	--	--	RAC (µg/kg)	≥ 12350
FOCUS Scenario	PEC _{gl-max, sw} (µg/L)	PEC/RAC ratio (= ETR)			PEC _{gl-sed max} (µg/kg)	PEC/RAC (= ETR)
Step 1						
	n.c.	--	--	--	106.830	0.009
BF 500-11						
Endpoint (µg/L)		LC ₅₀ > 100000	EC ₅₀ > 100000	E _r C ₅₀ > 100000	Endpoint (µg/kg)	NOEC n.a.
RAC (µg/L)		> 1000	> 1000	> 10000	RAC (µg/kg)	--
FOCUS Scenario	PEC _{gl-max, sw} (µg/L)	PEC/RAC ratio (= ETR)			PEC _{gl-sed max} (µg/kg)	PEC/RAC (= ETR)
Step 1						
	8.824	< 0.009	< 0.009	< 0.0009	n.c.	--

Group		Fish acute	Inverteb. acute	Algae	Group	Sed. dwell. prolonged
	8.818	< 0.008	< 0.008	< 0.0008		
BF 500-13						
Endpoint (µg/L)		LC ₅₀ > 50000 < 100000	EC ₅₀ > 100000	E _r C ₅₀ > 100000	Endpoint (µg/kg)	NOEC n.a.
RAC (µg/L)		> 500 < 1000	> 1000	> 10000	RAC (µg/kg)	--
FOCUS Scenario	PEC _{gl-max, sw} (µg/L)	PEC/RAC ratio (= ETR)			PEC _{gl-sed max} (µg/kg)	PEC/RAC (= ETR)
Step 1						
	11.668 11.664	< 0.02 > 0.01	< 0.01	< 0.001	n.c.	--
BF 500-14						
Endpoint (µg/L)		LC ₅₀ > 39400 < 82600	EC ₅₀ > 60900	E _r C ₅₀ > 100000	Endpoint (µg/kg)	NOEC n.a.
RAC (µg/L)		> 394 < 826	> 609	> 10000	RAC (µg/kg)	--
FOCUS Scenario	PEC _{gl-max, sw} (µg/L)	PEC/RAC ratio (= ETR)			PEC _{gl-sed max} (µg/kg)	PEC/RAC (= ETR)
Step 1						
	12.444 12.434	< 0.03 > 0.02	< 0.02	< 0.001	n.c.	--

Abbreviations: AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; ETR: Exposure-toxicity ratio; n.a.: no study available; n.c.: not calculated; PEC/RAC ratios above the relevant trigger of 1 are shown in **bold**

- 1) Worst-case PECs are derived from either single or twofold application in a risk envelope approach. For details, please refer to Part B, Section 8.9.
- 2) At Steps 1 and 2 only the crop 'winter cereals' was considered, representing the worst-case in the context of a risk envelope approach. For details, please refer to Part B, Section 8.9.

For the intended single and twofold application of BAS 758 00 F in 'winter cereals' (covering all intended uses), the calculated PEC/RAC ratios for the pyraclostrobin metabolites indicate an acceptable risk for all groups of aquatic organisms based on worst-case FOCUS Step 1 - 2 assumptions. Therefore, no further assessment is necessary.

Risk assessment for the formulated product (BAS 758 00 F)

Studies with the formulated product (BAS 758 00 F) were conducted with fish, *Daphnia* and algae. A fish study was conducted, because fish are the most sensitive group of aquatic organisms based on the available acute toxicity data for the active ingredients mefentrifluconazole and pyraclostrobin. Furthermore, in case of another EC formulation containing mefentrifluconazole (BAS 750 01 F), increased toxicity of the formulation to fish in comparison to the toxicity data available for the active substance was observed. As the other groups of aquatic organisms (*i.e.* invertebrates and algae) were not clearly more sensitive than fish based on the active substance data and to exclude potential increase in toxicity due to co-formulants, an acute toxicity study with fish and the formulated product BAS 758 00 F is deemed justified.

In the following a mixture toxicity RA for the formulated product is conducted in accordance with the EFSA AGD (2013). Measured and calculated mixture toxicity should be compared to determine synergistic, additive or antagonistic effects of the formulation. In the following the concentration addition (CA) model is used. To determine the respective formulation effect, EFSA proposes to calculate the model deviation ratio (MDR), which divides the calculated mixture toxicity ($LC_{50 \text{ mix-CA}}$; $EC_{50 \text{ mix-CA}}$) by the measured mixture toxicity ($LC_{50 \text{ PPP}}$; $EC_{50 \text{ PPP}}$). If the MDR is between 0.2 and 5 the observed and calculated mixture toxicities are considered in agreement. Respective MDR calculations for BAS 758 00 F are presented in Table 9.5.2-26.

Table 9.5.2-26: Comparison of the measured toxicity of the formulated product BAS 758 00 F and the calculated mixture toxicity of the active substances mefentrifluconazole, metrafenone and pyraclostrobin

Test species	Test system	Endpoint	Measured toxicity of the a.s. ($EC_{x \text{ a.s.}}$) [$\mu\text{g a.s./L}$]		Measured toxicity of BAS 758 00 F ($EC_{x \text{ PPP}}$) [$\mu\text{g product/L}$]	Calculated mixture toxicity ($EC_{x \text{ mix-CA}}$) [$\mu\text{g mixture/L}$] ¹⁾	MDR ($EC_{x \text{ mix-CA}}$ / $EC_{x \text{ PPP}}$)
<i>O. mykiss</i>	acute	LC_{50} (96 h)	mefentrifluconazole	532	88.4 (20.0 $\mu\text{g a.s./L}$)	82.5 (22.2 $\mu\text{g a.s./L}$)	0.9
			metrafenone	> 820 ²⁾			
			pyraclostrobin	6.16			
<i>D. magna</i>	acute	EC_{50} (48 h)	mefentrifluconazole	944	362 (81.8 $\mu\text{g a.s./L}$)	207 (46.8 $\mu\text{g a.s./L}$)	0.6
			metrafenone	> 920 ³⁾			
			pyraclostrobin	15.7			
<i>P. subcapitata</i>	--	E_rC_{50} (72 h)	mefentrifluconazole	1352 ⁴⁾	3820 (863 $\mu\text{g a.s./L}$)	2486 (562 $\mu\text{g a.s./L}$)	0.7
			metrafenone	> 339			
			pyraclostrobin	> 843			

Abbreviations: PPP: Plant Protection Product; CA: concentration addition; MDR: model deviation ratio

- ¹⁾ The theoretical mixture toxicity of the formulation was re-calculated assuming concentration addition based on the measured toxicity data of the active substances, their nominal contents within the formulation (*i.e.* 66.7 g mefentrifluconazole/L, 100 g metrafenone/L and 80 g pyraclostrobin/L) and the product density of 1.092 g/cm³.
- ²⁾ For metrafenone, the slightly higher fish endpoint of the study with *O. mykiss* is considered, since the formulation study and the studies with mefentrifluconazole and pyraclostrobin (providing the lowest endpoint for fish) were conducted with this fish species as well.
- ³⁾ For metrafenone, the slightly higher invertebrate endpoint of the study with *D. magna* is considered, since the formulation study and the studies with mefentrifluconazole and pyraclostrobin (providing the lowest endpoint for invertebrates) were conducted with this fish species as well.
- ⁴⁾ For mefentrifluconazole, the slightly higher algae endpoint of the study with the green algae *P. subcapitata* is considered, since the formulation studies and the study with pyraclostrobin and metrafenone (providing the lowest endpoint for algae) were conducted with this green alga species as well.

The calculated MDR values are between 0.6 and 0.9 for all organisms, indicating that the mixture does not show synergistic or antagonistic toxicity compared to the active substances but instead follows the expected toxicity for all groups of aquatic organisms (*i.e.* the CA model provides a reliable estimate of the toxicity of the given mixture). Furthermore, based on the calculations it can be concluded that chronic studies on fish and invertebrates using the formulations are not required, since the product is not by a factor ≥ 10 acutely more toxic than the active substances. As the formulation will break down rapidly once in the environment, no chronic exposure to the formulated product is expected. Therefore, the chronic risk to fish and invertebrates is sufficiently addressed by the risk assessment for the active substances and no further chronic risk assessment is necessary.

With regard to the mixture risk assessment, the EFSA Aquatic GD further states that if the toxicity of the mixture is largely explained by the toxicity of a single active substance and the CA model provides a reliable estimate of the toxicity of the given mixture, a sufficient protection level might be achieved by simply basing the risk assessment on the toxicity data for that “single driver”. Whether one a.s. is driving the toxicity of the given mixture can be verified by the “Toxic Unit (TU)” approach. The EFSA Aquatic GD states that if more than 90% of the sum of toxic units calculated for the formulation comes from a single a.s., the risk assessment is sufficiently addressed by the risk assessment for the active substances. TU calculations for BAS 758 00 F are presented in Table 9.5.2-27.

Table 9.5.2-27: Toxic Unit calculations for BAS 758 00 F based on the content of the active substances mefentrifluconazole, metrafenone and pyraclostrobin in the formulated product and the toxicity of the active substances

Group	Test substance	Test system	Nominal content of a.s. in BAS 758 00 F [g/L]	Measured toxicity of the a.s. (LC _{50a.s.} / EC _{xa.s.}) [μ g a.s./L]	Toxic Unit (TU)	Toxic Unit [%]
Fish, acute	mefentrifluconazole	96-h LC ₅₀ <i>O. mykiss</i>	66.7	532	125376	1.0
	metrafenone	96-h LC ₅₀ <i>O. mykiss</i>	100	> 820	121951	0.9
	pyraclostrobin	96-h LC ₅₀ <i>O. mykiss</i>	80	6.16	12987013	98.1
SUM TU					13234340	
Invertebrate, acute	mefentrifluconazole	48-h EC ₅₀ <i>D. magna</i>	66.7	944	70657	1.3
	metrafenone	48-h EC ₅₀ <i>D. magna</i>	100	> 920	108696	2.1
	pyraclostrobin	48-h EC ₅₀ <i>D. magna</i>	80	15.7	5095541	96.6
SUM TU					5274894	
Algae	mefentrifluconazole	72-h E _r C ₅₀ <i>P. subcapitata</i>	66.7	1352	49334	11.2
	metrafenone	72-h E _r C ₅₀ <i>P. subcapitata</i>	100	> 339	294985	67.2
	pyraclostrobin	72-h E _r C ₅₀ <i>P. subcapitata</i>	80	> 843	94899	21.6
SUM TU					439219	

Group	Test substance	Test system	Nominal content of a.s. in BAS 758 00 F [g/L]	Measured toxicity of the a.s. (LC _{50a.s.} / EC _{xa.s.}) [µg a.s./L]	Toxic Unit (TU)	Toxic Unit [%]
Aquatic plants	mefentrifluconazole	7-d ErC ₅₀ <i>L. gibba</i>	66.7	> 2017	33069	8.0
	metrafenone	7-d ErC ₅₀ <i>L. gibba</i>	100	> 327	305810	74.0
	pyraclostrobin	14-d ErC ₅₀ <i>L. gibba</i>	80	> 1077	74280	18.0
SUM TU					413160	

Bold values: Toxic units > 90% for a single a.s.

TU calculations for algae and aquatic plants indicate that none of the active substances solely accounts for the toxicity of the formulated product BAS 758 00 F. However, for the risk to fish and invertebrates, pyraclostrobin is driving the toxicity (*i.e.* ≥ 96.6%) of the formulated product. Therefore, in line with the EFSA Aquatic GD (2013), the risk assessment for the risk to fish and invertebrates is sufficiently addressed by the risk assessment for the active substance that drives the toxicity (*i.e.* pyraclostrobin).

For algae and aquatic plants, a tier 1 risk assessment considering PEC_{mix} values and measured endpoints from formulation studies (recalculated to content of a.s.) and calculated mixture toxicity following concentration addition (ErC_{50 mix-CA}) is provided in Table 9.5-28 and Table 9.5-29.

Table 9.5-28: Aquatic organisms: acceptability of risk (PEC_{mix}/RAC_{PPP / mix-CA} < 1) for the formulation BAS 758 00 F for algae and aquatic plants based on worst-case PEC_{mix} values following single and twofold application (1x and 2x 1.0 L product/ha) of BAS 758 00 F in ‘spring and winter cereals’

Group		Algae	Aquatic plants
Test species		<i>P. subcapitata</i>	<i>I. gibba</i>
Endpoint (µg sum a.s./L)		ErC ₅₀ PPP 863	ErC ₅₀ mix-CA 597 ¹⁾
AF		10	10
RAC (µg sum a.s./L)		86.3	59.7
FOCUS Scenario	PEC _{mix-max} (µg/L) ²⁾	PEC/RAC (= ETR)	
Step 1			
	40.171	0.5	0.7

Abbreviations: AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; ETR: Exposure-toxicity ratio; PEC/RAC ratios (= ETR) above the relevant trigger of 1 are shown in **bold**

¹⁾ The theoretical mixture toxicity of the formulation was re-calculated assuming concentration addition based on the measured toxicity data of the active substances, their nominal contents within the formulation (*i.e.* 66.7 g mefentrifluconazole/L, 100 g metrafenone/L and 80 g pyraclostrobin/L) and the product density of 1.092 g/cm³.

²⁾ Worst-case PECs are derived from either single or twofold application in ‘spring and winter cereals’ in a risk envelope approach. For details, please refer to Part B, Section 8.9.

For the intended single and twofold application of BAS 758 00 F at 1x and 2x 1.0 L product/ha in ‘spring and winter cereals’ the calculated $PEC_{mix}/RAC_{PPP/mix-CA}$ ratios indicate an acceptable risk for all algae and aquatic plants based on tier 1 toxicity data and Step 1 $PEC_{sw\ mix}$ values. Therefore, no further assessment is necessary.

Table 9.5-29: Aquatic organisms: acceptability of risk ($PEC_{mix}/RAC_{PPP/mix-CA} < 1$) for the formulation BAS 758 00 F for algae and aquatic plants based on worst-case PEC_{mix} values following single and twofold application (1x and 2x 1.5 L product/ha) of BAS 758 00 F in ‘spring and winter cereals’

Group		Algae	Aquatic plants
Test species		<i>P. subcapitata</i>	<i>I. gibba</i>
Endpoint (µg sum a.s./L)		ErC50 PPP 863	ErC50 mix-CA 597 ¹⁾
AF		10	10
RAC (µg sum a.s./L)		86.3	59.7
FOCUS Scenario	PEC _{mix-max} (µg/L) ²⁾	PEC/RAC (= ETR)	
Step 1			
	48.110	0.6	0.8

Abbreviations: AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; ETR: Exposure-toxicity ratio; PEC/RAC ratios (= ETR) above the relevant trigger of 1 are shown in **bold**

- ¹⁾ The theoretical mixture toxicity of the formulation was re-calculated assuming concentration addition based on the measured toxicity data of the active substances, their nominal contents within the formulation (*i.e.* 66.7 g mefentrifluconazole/L, 100 g metrafenone/L and 80 g pyraclostrobin/L) and the product density of 1.092 g/cm³.
- ²⁾ Worst-case PECs are derived from either single or twofold application in ‘spring and winter cereals’ in a risk envelope approach. For details, please refer to Part B, Section 8.9.

For the intended single and twofold application of BAS 758 00 F at 1x and 2x 1.5 L product/ha in ‘spring and winter cereals’ the calculated $PEC_{mix}/RAC_{PPP/mix-CA}$ ratios indicate an acceptable risk for all algae and aquatic plants based on tier 1 toxicity data and Step 1 $PEC_{sw\ mix}$ values. Therefore, no further assessment is necessary.

Residue data in fish

Mefentrifluconazole

The log P_{ow} of the active substance mefentrifluconazole was determined to be 3.34. In the BCF study (BASF DocID 2015/1122811) the steady state after exposure of *O. mykiss* to mefentrifluconazole at a nominal exposure level of 0.01 mg/L, was reached after 2.6 days. After exposure termination, radioactivity levels in fish tissues decreased rapidly with a half-life of *ca.* 0.59 days. After 7 days in clean water the whole-body residues in fish had declined to 3% of the mean steady state concentration (CF_{ss}). The BCF_{KLg} (lipid content and growth corrected) was determined to be 385.

Despite the relatively high lipophilicity of mefentrifluconazole, it is concluded that there is no risk of bioaccumulation due to the low accumulation and rapid excretion of the active substance from fish. Thus, residues of mefentrifluconazole in fish are of no concern and no accumulation in the food chain is to be expected.

Metrafenone

The log P_{ow} of the active substance metrafenone was determined to be 4.3 (EFSA Scientific report No. 58, 1-72 (2006)). Hence, a bioconcentration study in fish has been performed (BASF DocID 2001/7000274). The BCF for the total [^{14}C] metrafenone-derived radioactivity and the parent metrafenone in whole fish were in the range of 470 and 530 and 140 to 180, respectively. The time to reach 95% clearance (CT_{95}) in the total [^{14}C] metrafenone-derived radioactivity and the parent metrafenone in whole fish was 1.8 to 2.3 days and 2.3 to 2.7 days, respectively. There was no bioconcentration of the metrafenone metabolites and degradation products, which indicated an intensive metabolic clearance of metrafenone. The potential for accumulation in fish is low, because of the rapid excretion of the parent compound and its metabolites.

Pyraclostrobin

The log P_{ow} of the active substance pyraclostrobin was determined to be 3.99 (EU Review Report, SANCO/1420/2001-final, September 2004). Hence, a bioconcentration study in fish has been performed (BASF DocID 1999/11348). An apparent steady state was reached after 2 - 4 days of exposure. The bioconcentration factors for parent compound in whole fish were 379 and 507 L/kg (two labels). The half-life for elimination was 0.9 days. The time for elimination of 90% of the activity varied between 2.8 and 3.0 days. The nature of radioactivity in fish tissues after 28 days of exposure consisted of the parent substance (39 - 74%) and 4 metabolites (2 - 9%). Due to the limited bioaccumulation and the rapid metabolization and excretion of the active substance (and its metabolites), there is no risk of bioaccumulation. In addition, pyraclostrobin dissipates rapidly in water preventing continuous exposure.

Thus, residues of the active substances of BAS 758 00 F in fish are not of concern and no accumulation in the food chain is to be expected.

9.5.3 Overall conclusions

The standard and refined risk assessment for the fungicidal product BAS 758 00 F, the active substances mefentrifluconazole, metrafenone and pyraclostrobin as well as their major metabolites demonstrates that the application of BAS 758 00 F in 'spring and winter cereals' according to good agricultural practice is of low risk to aquatic ecosystems if a ~~non-sprayed buffer zone of 5 m or 50% drift-reducing nozzles~~ risk mitigation measures are employed.

Review Comments:

The relevant predicted environmental concentrations in water (PEC_{sw}) for risk assessments covering the proposed use pattern are taken from Part B Section 8 (Environmental Fate). The initial risk assessment was based on the worst case PEC_{sw} values and the results of laboratory toxicity testing.

For active substances and relevant metabolites PEC_{sw} calculations were performed with FOCUS STEPS 1-2 (active substances and metabolites) and FOCUS STEP 3 (all active substance) and STEP 4 (pyraclostrobin).

For pyraclostrobin the mesocosms study was taken to consideration in the refined risk assessment.

For all active substances and their metabolites for the intended single and twofold application of BAS 758 00 F in cereals, the calculated PEC/RAC ratios indicate an acceptable risk for all groups of aquatic organisms without any mitigation measures.

Based on the mixture toxicity assessment, it can be concluded that the mitigation measures based on the risk assessment of pyraclostrobin will be sufficient to protect aquatic organisms.

Single and twofold application (1x and 2 x 1 L/ha) of BAS 758 00 F

A non-sprayed buffer zone of 5 m or 50% drift reducing nozzles are considered for the intended use of BAS 758 00 F in 'spring cereals' for scenarios D4 and D5. A non-sprayed buffer zone of 5 m or 75% drift reducing nozzles are considered for the intended use of BAS 758 00 F in 'spring cereals' for scenarios D3. For scenario R4 non-sprayed, vegetated buffer zone of 10 m is required.

Single and twofold application (1x and 2 x 1.5 L/ha) of BAS 758 00 F

A non-sprayed buffer zone of 5 m or 75% drift reducing nozzles are considered for the intended use of BAS 758 00 F in 'spring cereals' for scenarios D3, D4 and D5. For scenario R4 non-sprayed, vegetated buffer zone of 10 m is required.

Single and twofold application (1x and 2 x 1 L/ha) of BAS 758 00 F

A non-sprayed buffer zone of 5 m or 50% drift reducing nozzles are considered for the intended use of BAS 758 00 F in 'winter cereals' for scenarios D4, D5, R1 and R3. A non-sprayed buffer zone of 5 m or 75% drift reducing nozzles are considered for the intended use of BAS 758 00 F in 'winter cereals' for scenarios D3. For scenario R4 non-sprayed, vegetated buffer zone of 10 m is required.

Single and twofold application (1x and 2 x 1.5 L/ha) of BAS 758 00 F

A non-sprayed buffer zone of 5 m or 75 % drift reducing nozzles are considered for the intended use of BAS 758 00 F in 'winter cereals' for scenarios D3, D4 and D5. For scenarios R1, R3 and R4 non-sprayed, vegetated buffer zone of 10 m is required.

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9.5.4 Effects on bees (KCP 10.3.1)

9.5.5 Toxicity data

Acute contact and oral toxicity studies on honey bees have been carried out with BAS 758 00 F, the active substances mefentrifluconazole (BAS 750 F), metrafenone (BAS 560 F) and pyraclostrobin (BAS 500 F) and relevant metabolites.

~~Furthermore, a chronic oral toxicity study on honey bees, a single exposure as well as two repeated exposure toxicity studies on honey bee larvae and an acute oral and a contact toxicity study on bumble bees have been carried out with the active substance mefentrifluconazole. Chronic toxicity studies with honey bees and a bee larvae study are submitted for metrafenone (tested as BAS 560 00 F) that have not been evaluated previously.~~

~~For pyraclostrobin, a new dossier for EU re-registration within the AIR 3 process has been recently submitted and the new data therein are also used in the present risk assessment. In addition to the already EU evaluated acute studies on honey bees, new acute contact and acute oral toxicity studies on honey bees and bumble bees have been carried out with pyraclostrobin. Moreover, new data from a chronic toxicity study on adult honey bees and a repeated exposure toxicity study on honey bee larvae are available.~~

Additionally, chronic toxicity studies on adult honey bees and toxicity studies on honey bee larvae have been carried out with BAS 758 00 F.

All studies are listed in Table 9.5-30, Table 9.5-31, Table 9.5-32 and Table 9.5-33. Full details of already EU evaluated studies can be found in the respective EU documents of the active substances. New data submitted with this application are listed in Appendix 1 and summarized in Appendix 2.

Table 9.5-30: Endpoints and effect values for mefentrifluconazole relevant for the risk assessment for bees

Species	Substance	Exposure System	Results	Reference
<i>Apis mellifera</i> (adults)	mefentrifluconazole	acute oral	LD ₅₀ (48 h) > 100 µg a.s./bee	EFSA Journal 2018;16(7):5379 2015/1128674
<i>Apis mellifera</i> (adults)	mefentrifluconazole	acute contact	LD ₅₀ (48 h) > 100 µg a.s./bee	EFSA Journal 2018;16(7):5379 2015/1128674
<i>Apis mellifera</i> (adults)	mefentrifluconazole	chronic oral	LDD ₅₀ (10 d) > 110.5 µg a.s./bee/day NOEDD (10 d) ≥ 110.5 µg a.s./bee/day	EFSA Journal 2018;16(7):5379 2013/1235086
<i>Apis mellifera</i> (larvae)	mefentrifluconazole	single exposure	NOED (8 d) = 29.7 µg a.s./larva LD ₅₀ (8 d) = 43.9 µg a.s./larva	EFSA Journal 2018;16(7):5379 2013/1235087
<i>Apis mellifera</i> (larvae)	mefentrifluconazole	repeated exposure	NOED (21 d) ≥ 50.1 µg a.s./larva ED ₅₀ (21 d) > 50.1 µg a.s./larva	Draft Assessment Report (DAR) of mefentrifluconazole (Apr. 2017), Vol. 3, B.9 2014/1327676 #
<i>Apis mellifera</i> (larvae)	mefentrifluconazole	repeated exposure	NOED (22 d) = 25 µg a.s./larva ED ₅₀ (22 d) > 50 µg a.s./larva NOEC (22 d) = 162.4 µg a.s./kg food EC ₅₀ (22 d) > 324.8 mg a.s./kg food	EFSA Journal 2018;16(7):5379 2017/1045562 Draft Assessment Report (DAR) of mefentrifluconazole (January 2018), Vol. 3CA, B.9
<i>Bombus terrestris</i> (adults)	mefentrifluconazole	acute oral	LD ₅₀ (96 h) > 195.4 µg a.s./bumble bee	EFSA Journal 2018;16(7):5379 2014/1275250
<i>Bombus terrestris</i> (adults)	mefentrifluconazole	acute contact	LD ₅₀ (96 h) > 200.0 µg a.s./bumble bee	EFSA Journal 2018;16(7):5379 2014/1275250

According to the Draft Assessment Report (DAR) of mefentrifluconazole (April 2017), Vol. 3, B.9, the study is not reliable

Table 9.5-31: Endpoints and effect values for metrafenone relevant for the risk assessment for bees

Species	Substance	Exposure System	Results	Reference
<i>Apis mellifera</i> (adults)	metrafenone	acute oral	LD ₅₀ > 114 µg a.s./bee	EFSA Scientific report No. 58, 1-72 (2006)
<i>Apis mellifera</i> (adults)	metrafenone	acute contact	LD ₅₀ > 100 µg a.s./bee	EFSA Scientific report No. 58, 1-72 (2006)
<i>Apis mellifera</i> (adults)	metrafenone (tested as BAS 560 02 F)	chronic oral	LDD ₅₀ (10 d) > 291 µg a.s./bee/day NOEDD (10 d) ≥ 291 µg a.s./bee/day	not EU evaluated 2014/1093920
<i>Apis mellifera</i> (larvae)	metrafenone (tested as BAS 560 02 F)	repeated exposure ¹⁾	LD ₅₀ (8 d) = 115.61 µg a.s./larva NOED (8 d) = 49.98 µg a.s./larvae	not EU evaluated 2014/1093921

¹⁾ ~~Study comprised repeated exposure of honey bee larvae over a period of 4 days (i.e. crafting day 3 to 6). However, the study duration was 8 days only (i.e. no assessment of adult emergence was conducted).~~

Table 9.5-32: Endpoints and effect values for pyraclostrobin relevant for the risk assessment for bees

Species	Substance	Exposure System	Results	Reference
<i>Apis mellifera</i> (adults)	pyraclostrobin	acute oral	LD ₅₀ (48 h) > 73.1 µg/bee	EC Review report, SANCO/1420/2001- 2004 1999/11457
<i>Apis mellifera</i> (adults)	pyraclostrobin	acute contact	LD ₅₀ (48 h) > 100.0 µg/bee	EC Review report, SANCO/1420/2001- 2004 1999/11457
<i>Apis mellifera</i> (adults)	pyraclostrobin	acute-oral	LD ₅₀ (48 h) > 110.0 µg/bee	not EU-evaluated 2013/1003210
<i>Apis mellifera</i> (adults)	pyraclostrobin	acute-contact	LD ₅₀ (48 h) > 100.0 µg/bee	not EU-evaluated 2013/1003210
<i>Apis mellifera</i> (adults)	pyraclostrobin	chronic-oral	LDD ₅₀ (10 d) = 3.4 µg/bee/day NOEDD (10 d) = 1.0 µg/bee/day	not EU-evaluated 2017/1142796 amendment 2019/1024628
<i>Apis mellifera</i> (larvae)	pyraclostrobin	repeated exposure	ED ₅₀ (22 d) = 20.1 µg/larva NOED (22 d) = 12.8 µg/larva	not EU-evaluated 2017/1142794 amendment 2017/1142798
<i>Bombus terrestris</i> (adults)	pyraclostrobin	acute-oral	LD ₅₀ (96 h) > 97.0 µg/bee	not EU-evaluated 2016/1000530
<i>Bombus terrestris</i> (adults)	pyraclostrobin	acute-contact	LD ₅₀ (96 h) > 100.0 µg/bee	not EU-evaluated 2016/1000530

Table 9.5-33: Endpoints and effect values of BAS 758 00 F relevant for the risk assessment for bees

Species	Product	Exposure System	Results	Reference
<i>Apis mellifera</i> (adults)	BAS 758 00 F	acute oral	LD ₅₀ (48 h) = 249 µg/bee (corresponding to 56.2 µg total a.s./bee)	not EU evaluated 2020/2037657
<i>Apis mellifera</i> (adults)	BAS 758 00 F	acute contact	LD ₅₀ (48 h) = 700 µg/bee (corresponding to 158.1 µg total a.s./bee)	not EU evaluated 2020/2037657
<i>Apis mellifera</i> (adults)	BAS 758 00 F	chronic oral	LDD ₅₀ (10 d) = 21.4 µg/bee/day (corresponding to 4.84 µg total a.s./bee/day) NOEDD (10 d) = 12.7 µg/bee/day (corresponding to 2.86 µg total a.s./bee/day)	not EU evaluated 2021/2008152
<i>Apis mellifera</i> (larvae)	BAS 758 00 F	repeated exposure	ED ₅₀ (22 d) > 97.7 µg/larva (corresponding to 22.1 µg total a.s./larva) NOED (22 d) ≥ 97.7 µg/larva (corresponding to 22.1 µg total a.s./larva)	not EU evaluated 2021/2008153
Higher-tier studies (tunnel test, field studies)				
<i>Apis mellifera</i> (colonies)	BAS 758 00 F	semi-field	Semi-field tunnel test (<i>Phacelia tanacetifolia</i>): no unacceptable lethal or sublethal effects on honey bee colonies exposed to 407 g total a.s./ha	not EU evaluated 2021/2047630

9.5.5.1 Justification for new endpoints

Effects of the formulation BAS 758 00 F on honey bees were not evaluated as part of the EU assessment of the active substances mefentrifluconazole, metrafenone or pyraclostrobin. Hence, all relevant data and assessments considering this formulation are provided here and are considered adequate.

~~A honey bee larvae and a chronic oral honey bee study, both with metrafenone, have been included to address the new data requirements according to Commission Regulation (EU) 1107/2009. New acute oral and contact honey bee studies, conducted with the active substance pyraclostrobin, are used in the risk assessment in place of the EU agreed endpoints. In the original study submitted in the Annex I inclusion, the effects of the reference item were slightly out of range. Therefore, the study was repeated. In addition, a chronic study on adult honey bees, a study with repeated exposure to honey bee larvae as well as acute toxicity studies to bumble bees are submitted.~~

All chronic studies on bees which were previously not evaluated on EU level, were checked for their potential to calculate L/EC_{10/20} values in accordance with Commission Regulations (EU) 283/2013 and 284/2013, respectively. If a calculation was possible, the L/EC_{10/20} are provided in the corresponding study summary in Appendix 2. However, since these values are not relevant for the risk assessment, they are not listed in chapter.

9.5.6 Risk assessment

The evaluation of the risk for bees was performed in accordance with the recommendations of the “Guidance Document on Terrestrial Ecotoxicology”, as provided by the Commission Services (SANCO/10329/2002 rev.2 (final), October 17, 2002) and the EPPO 2010 risk assessment scheme (OEPP/EPPO, 2010: *Environmental risk assessment scheme for plant protection products, Chapter 10: Honey bees, PP 3/10 (3), Bulletin OEPP/EPPO Bulletin 40, 323–331*). The EFSA bee guidance document (EFSA Journal 2013; 11(7):3295) was not used as it has not been adopted by the Standing Committee on Plants, Animals, Food and Feed at the time of application.

The application of BAS 758 00 F is envisioned in cereals. The following risk assessment is based on the worst-case maximum single application rate of 1.5 L BAS 758 00 F/ha (equivalent to 0.100 kg mefentrifluconazole /ha, 0.150 kg metrafenone/ha and 0.120 kg pyraclostrobin/ha; see Section 9 Chapter 9.1 for details).

9.5.6.1 Hazard quotients for bees

The risk to honey bees from the use of mefentrifluconazole, metrafenone, pyraclostrobin and BAS 758 00 F was assessed using the maximum single application rate and the LD₅₀ values to calculate hazard quotients (HQ) for oral exposure (Q_{HO}) and contact exposure (Q_{HC}) (OEPP/EPPO, 2010: Chapter 10: Honey bees, PP 3/10 (3)) as follows.

$$\text{Hazard Quotient (HQ)} = \frac{\text{Maximum application rate [g/ha]}}{\text{Acute LD}_{50} [\mu\text{g/bee}]}$$

A hazard quotient of less than 50 indicates a low risk to honey bees colonies in the field (see Table 9.5-34 to Table 9.5-37).

Table 9.5-34: First-tier assessment of the risk for bees due to the use of mefentrifluconazole as contained in BAS 758 00 F according to the proposed use pattern

Intended use	cereals		
Active substance	mefentrifluconazole		
Application rate (g a.s./ha)	2 x 100		
Test design	LD ₅₀ (lab.) (μg a.s./bee)	Single application rate (g a.s./ha)	Q _{HO} , Q _{HC} criterion: Q _H ≤ 50
Oral toxicity	> 100	100	< 1.0
Contact toxicity	> 100		< 1.0

Q_{HO}, Q_{HC}: Hazard quotients for oral and contact exposure.

Table 9.5-35: First-tier assessment of the risk for bees due to the use of metrafenone as contained in BAS 758 00 F according to the proposed use pattern

Intended use	cereals		
Product	metrafenone		
Application rate (g a.s./ha)	2 x 150		
Test design	LD₅₀ (lab.) (µg/bee)	Single application rate (g/ha)	Q_{HO}, Q_{HC} criterion: Q_H ≤ 50
Oral toxicity	> 114	150	< 1.3
Contact toxicity	> 100		< 1.5

Table 9.5-36: First-tier assessment of the risk for bees due to the use of pyraclostrobin as contained in BAS 758 00 F according to the proposed use pattern

Intended use	cereals		
Active substance	pyraclostrobin		
Application rate (g a.s./ha)	1 x 120		
Test design	LD₅₀ (lab.) (µg a.s./bee)	Single application rate (g/ha)	Q_{HO}, Q_{HC} criterion: Q_H ≤ 50
Oral toxicity	> 110.0 73.1	120	< 1.1 1.6
Contact toxicity	> 100		< 1.2

Table 9.5-37: First-tier assessment of the risk for bees due to the use of BAS 758 00 F according to the proposed use pattern

Intended use	cereals		
Product	BAS 758 00 F		
Application rate (L/ha)	2 x 1.5		
Test design	LD₅₀ (lab.) (µg/bee)	Single application rate (g/ha)	Q_{HO}, Q_{HC} criterion: Q_H ≤ 50
Oral toxicity	249	1638 ¹⁾	6.6
Contact toxicity	700		2.3

Q_{HO}, Q_{HC}: Hazard quotients for oral and contact exposure.

¹⁾ Taking into account a single application of 1.5 L product/ha and the density of BAS 758 00 F of 1.092 g/cm³.

Under Regulation (EC) No 1107/2009, no adopted risk assessment scheme currently exists for chronic honey bee or honey bee larvae studies. Nevertheless, additional studies were carried out with mefentrifluconazole (BAS 750 F), ~~metrafenone (BAS 560 F) and pyraclostrobin (BAS 500 F)~~ as well as BAS 758 00 F. For mefentrifluconazole, chronic toxicity study on honey bees resulted in a NOEDD $\geq 110.5 \mu\text{g a.s./bee/day}$. The NOED derived from the repeated exposure study on honey bee larvae was $25 \mu\text{g a.s./larva}$. ~~For metrafenone, chronic toxicity study on honey bees resulted in a NOEDD $\geq 291 \mu\text{g a.s./bee/day}$. The NOED derived from the repeated exposure study on honey bee larvae was $49.98 \mu\text{g a.s./larva}$. For pyraclostrobin, the chronic toxicity study on honey bees resulted in a NOEDD of $1.0 \mu\text{g a.s./bee/day}$. The NOED derived from the repeated exposure study on honey bee larvae was $12.8 \mu\text{g a.s./larva}$.~~ For BAS 758 00 F, the chronic toxicity study on honey bees resulted in a NOEDD of $2.86 \mu\text{g total a.s./bee/day}$. The NOED derived from the repeated exposure study on honey bee larvae was $\geq 22.1 \mu\text{g total a.s./larva}$. In the absence of clear guidance (noted and agreed by member states) a preliminary risk assessment according to the current legal requirements (SANCO/10329/2002 and EPPO 2010) has been conducted and is presented below.

Repeated exposure of adult honey bees and immature life stages within the hive is realistic for active substances but not for the formulated product (formulants have different physical and chemical properties with different dissipation/degradation). Conclusively, chronic exposure of adult bees for 10 days and repeated exposure of honey bee larvae to the original product at a constant dose is unlikely. Hence, focus in the risk assessment provided below should be on the active substance. The data obtained for BAS 758 00 F is considered less relevant for the risk assessment but is presented as a worst-case scenario. Please note that the intended uses of BAS 758 00 F are in cereals which are a non-bee attractive crops (USDA (2017), *Attractiveness of Agricultural Crops to Pollinating Bees for the Collection of Nectar and/or Pollen*). Therefore, the following assessment is considered an unrealistic worst case.

For the **chronic risk assessment for adult honey bees and honey bee larvae**, the revised EPPO scheme (2010) suggests calculating the ratio between the NOEL (oral) and the exposure. This approach has been originally proposed for seed treatments, but can be directly applied to foliar applications as well. For adult bees, the exposure is assessed through the amount of residues that may be ingested by a bee in one day. The ratio between the NOEL (= NOED in $\mu\text{g a.s./bee/day}$) and the exposure (also in $\mu\text{g a.s./bee/day}$) is then calculated as follows:

$$TER_{\text{chronic,adult}} = \frac{NOED_{\text{oral}} [\mu\text{g a.s./bee/day}]}{\text{Amount of residues ingested by a bee in one day} [\mu\text{g a.s./bee/day}]}$$

For the risk assessment the exposure of larvae is estimated as the amount of residues that may be ingested by the larvae during their complete larval stage (feeding period of five days) as a worst case assumption. For larvae, the ratio between the NOEL (in $\mu\text{g a.s./larva}$) and the exposure (residues ingested over the five-day feeding period in $\mu\text{g a.s./larva}$) is calculated by the following equation:

$$TER_{\text{chronic,larvae}} = \frac{NOEL_{\text{oral}} [\mu\text{g a.s./larva}]}{\text{Amount of residues ingested by a larva} [\mu\text{g a.s./larva}]}$$

Following EPPO (2010) the expected worst-case residue consumption of larvae and adult bees was calculated. For pyraclostrobin, RUD residue values reported in the recently published external EFSA supporting publication on residues in bee relevant matrices (EFSA 2017) have been used to estimate the exposure. For mefentrifluconazole and metrafenone, no specific RUD values are reported. Therefore, overall RUD residue values for spray applications have been used for exposure estimation as reported in EFSA (2017). In order to be protective, we suggest using the 3rd Quantile data which are well above the more realistic median values. Expected residues in nectar and pollen are calculated using the maximum single application rate of BAS 758 00 F (100 g mefentrifluconazole/ha, 150 g metrafenone/ha and 120 g pyraclostrobin/ha; see Table 9.5-38).

Table 9.5-38: Residue values of the active substances in pollen and nectar

	3rd quartile RUD	Expected residues based on proposed GAP
Pollen		
mefentrifluconazole (Application rate 100 g a.s./ha)	63.70 mg a.s./kg ²⁾	6.37 mg a.s./kg
metrafenone (Application rate 150 g a.s./ha)	63.70 mg a.s./kg ²⁾	9.56 mg a.s./kg
pyraclostrobin (Application rate 120 g a.s./ha)	60.88 mg a.s./kg ¹⁾	7.31 mg a.s./kg
BAS 758 00 F (Application rate 100 g mefentrifluconazole/ha, 150 g metrafenone/ha and 120 g pyraclostrobin/ha)	63.70 mg a.s./kg for mefentrifluconazole and metrafenone ²⁾ 60.88 mg a.s./kg for pyraclostrobin ¹⁾	23.23 mg total a.s./kg
Nectar		
mefentrifluconazole (Application rate 100 g a.s./ha)	3.99 mg a.s./kg ²⁾	0.40 mg a.s./kg
metrafenone (Application rate 150 g a.s./ha)	3.99 mg a.s./kg ²⁾	0.6 mg a.s./kg
pyraclostrobin (Application rate 120 g a.s./ha)	2.16 mg a.s./kg ¹⁾	0.26 mg a.s./kg
BAS 758 00 F (Application rate 100 g mefentrifluconazole/ha, 150 g metrafenone/ha and 120 g pyraclostrobin/ha)	3.99 mg a.s./kg for mefentrifluconazole and metrafenone ²⁾ 2.16 mg a.s./kg for pyraclostrobin ¹⁾	1.26 mg total a.s./kg

¹⁾ Specific RUD values for pyraclostrobin from EFSA supporting publication on residues in bee relevant matrices (EFSA 2017).

²⁾ Overall RUD values from EFSA supporting publication on residues in bee relevant matrices (EFSA 2017).

To calculate the expected consumption of the relevant matrixes EPPO 2010 refers to a review by Rortais *et al.* (2005). For adult honey bees, only nectar consumption is relevant as adult bees do not consume pollen. In Rortais *et al.* (2005) the maximum amount of sugar an adult bee consumes per day is given as 128 mg/bee/day. Based on nectar sugar concentration of 30% this corresponds to a total consumption of approximately 426.7 mg/bee/day, which can be considered an unrealistic worst-case scenario. In the absence of clear guidance, the nectar sugar concentration was taken from Rortais *et al.* (2005), which cite a range of sugar concentrations in nectars between 5-80% specifically mentioning 40% as representative in bee attractive crops. This range suggests that 30% sugar concentration can be considered conservative for crop plants, which is well supported by the literature (Pamminger *et al.* 2019). For honey bee larvae Rortais *et al.* (2005) gives a maximum of 59.4 mg sugar/5days, which corresponds to a nectar consumption of 198 mg/5days based on 30% sugar concentration in nectar. In addition to their nectar requirements honey bee larvae consume up to 2 mg pollen/5days (Babendreier *et al.* 2004). It is to be noted that the pollen consumption values mentioned in Rortais *et al.* (2005) based on a citation of Babendreier *et al.* (2004) are not the values which are mentioned in the original publication Babendreier *et al.* (2004).

To calculate the residue intake of mefentrifluconazole (BAS 750 F), metrafenone (BAS 560 F), pyraclostrobin (BAS 500 F) and BAS 758 00 F by adult honey bees and honey bee larvae, the consumed amounts of pollen and nectar are multiplied with relevant measured residue in nectar and pollen after application of BAS 758 00 F (see Table 9.5-39 to Table 9.5-42). The calculated chronic TER values are given in Table 9.5-43 to Table 9.5-46. These TERs are compared to the trigger of 1 as proposed in the revised EPPO scheme (2010). **Given the protective worst-case assumptions underlying this risk assessment (detailed above), as well as the fact that all calculated TERs far exceed the suggested trigger by at least a factor of 5, it can be concluded that the risk for chronic adult and developmental exposure to honey bees can be considered acceptable.**

Table 9.5-39: Total residue intake for adult honey bees and larvae following exposure to BAS 750 F according to the proposed uses

Honey bee stage	Adult	Larva (over 5 days)
Residue in pollen	6.37 mg a.s./kg (= 0.00637 µg a.s./mg)	6.37 mg a.s./kg (= 0.00637 µg a.s./mg)
Pollen consumption	0	2.0 mg/larva
Residue intake through pollen	0 µg a.s./bee/day	0.013 µg a.s./larva
Residue in nectar	0.40 mg a.s./kg (= 0.00040 µg a.s./mg)	0.40 mg a.s./kg (= 0.00040 µg a.s./mg)
Nectar consumption	426.7 mg/bee/day	198.0 mg/larva
Residue intake through nectar	0.17 µg a.s./bee/day	0.079 µg a.s./larva
Total residue intake	0.17 µg a.s./bee/day	0.092 µg a.s./larva

Table 9.5-40: Total residue intake for adult honey bees and larvae following exposure to BAS 560 F according to the proposed uses

Honey bee stage	Adult	Larva (over 5 days)
Residue in pollen	9.56 mg a.s./kg (= 0.00956 µg a.s./mg)	9.56 mg a.s./kg (= 0.00956 µg a.s./mg)
Pollen consumption	0	2.0 mg/larva
Residue intake through pollen	0 µg a.s./bee/day	0.019 µg a.s./larva
Residue in nectar	0.60 mg a.s./kg (= 0.00060 µg a.s./mg)	0.60 mg a.s./kg (= 0.00060 µg a.s./mg)
Nectar consumption	426.7 mg/bee/day	198.0 mg/larva
Residue intake through nectar	0.26 µg a.s./bee/day	0.13 µg a.s./larva
Total residue intake	0.26 µg a.s./bee/day	0.14 µg a.s./larva

Table 9.5-41: Total residue intake for adult honey bees and larvae following exposure to BAS 500 F according to the proposed uses

Honey bee stage	Adult	Larva (over 5 days)
Residue in pollen	7.31 mg a.s./kg (= 0.00731 µg a.s./mg)	7.31 mg a.s./kg (= 0.00731 µg a.s./mg)
Pollen consumption	0	2.0 mg/larva
Residue intake through pollen	0 µg a.s./bee/day	0.015 µg a.s./larva
Residue in nectar	0.26 mg a.s./kg (= 0.00026 µg a.s./mg)	0.26 mg a.s./kg (= 0.00026 µg a.s./mg)
Nectar consumption	426.7 mg/bee/day	198.0 mg/larva
Residue intake through nectar	0.11 µg a.s./bee/day	0.051 µg a.s./larva
Total residue intake	0.11 µg a.s./bee/day	0.066 µg a.s./larva

Table 9.5-42: Total residue intake for adult honey bees and larvae following exposure to BAS 758 00 F according to the proposed uses

Honey bee stage	Adult	Larva (over 5 days)
Residue in pollen	23.23 mg total a.s./kg (= 0.02323 µg total a.s./mg)	23.23 mg total a.s./kg (= 0.02323 µg total a.s./mg)
Pollen consumption	0	2.0 mg/larva
Residue intake through pollen	0 µg total a.s./bee/day	0.046 µg a.s./larva
Residue in nectar	1.26 mg total a.s./kg (= 0.00126 µg total a.s./mg)	1.26 mg total a.s./kg (= 0.00126 µg total a.s./mg)
Nectar consumption	426.7 mg/bee/day	198.0 mg/larva
Residue intake through nectar	0.54 µg a.s./bee/day	0.25 µg a.s./larva
Total residue intake	0.54 µg a.s./bee/day	0.30 µg a.s./larva

Table 9.5-43: Chronic risk to adult bees and larvae following the use of BAS 750 F in cereals using TER approach

Honey bee stage	Exposure route	NOED	Worst case residue intake	TER _{ch}	Trigger value
Adult	Oral	≥ 110.5 µg a.s./bee/day	0.17 µg a.s./bee/day	≥ 650	1
Larvae	Oral	25 µg a.s./larva	0.092 µg a.s./larva	272	1

~~Table 9.5-44: Chronic risk to adult bees and larvae following the use of BAS 560 F in cereals using TER approach~~

Honey-bee stage	Exposure route	NOED	Worst-case residue intake	TER _{ch}	Trigger value
Adult	Oral	≥ 291 µg a.s./bee/day	0.26 µg a.s./bee/day	≥ 1119	1
Larvae	Oral	49.98 µg a.s./larva	0.14 µg a.s./larva	357	1

~~Table 9.5-45: Chronic risk to adult bees and larvae following the use of BAS 500 F in cereals using TER approach~~

Honey-bee stage	Exposure route	NOED		Worst-case residue intake	TER _{ch}	Trigger value
Adult	Oral	1.0 µg a.s./bee/day		0.11 µg a.s./bee/day	9	1
Larvae	Oral	12.8 µg a.s./larva		0.066 µg a.s./larva	194	1

Table 9.5-46: Chronic risk to adult bees and larvae following the use of BAS 758 00 F in cereals using TER approach

Honey bee stage	Exposure route	NOED	Worst case residue intake	TER _{ch}	Trigger value
Adult	Oral	2.86 µg total a.s./bee/day	0.54 µg a.s./bee/day	5	1
Larvae	Oral	≥ 22.1 µg total a.s./larva	0.30 µg a.s./larva	≥ 74	1

The underlying assumptions of the revised EPPO (2010) risk assessment for chronic adult bees and honey bee larvae largely comply with the proposals presented in the EFSA bee guidance document:

- in both approaches the chronic adult and larvae endpoints are set into relation to exposure which is based on pollen and nectar consumption.
- in both approaches the assumed amount of pollen and nectar consumption and the relevant time-frame is identical as it is based on the same literature references.
- the RUD values used from the EFSA supporting publication 2017 are based on a review and quality evaluation of available residue studies. The request of EFSA for the supporting publication was the limited availability of residue data at the time of the finalization of the EFSA bee guidance document. The EFSA supporting publication 2017 reflects therefore the current knowledge status.
- the possibility to refine exposure by using a time-weighted-average factor is a common refinement option for risk assessment of non-target organisms which is also mentioned in the EFSA bee guidance document.

However, in some respects this proposal deviates from the EFSA bee guidance document. Main differences lie in the endpoints and triggers used. In the EFSA bee guidance document it is proposed to use the LDD₅₀ endpoint for chronic adult and the NOED for honey bee larvae. At the same time the proposed chronic adult trigger in the EFSA bee guidance document for the ETR (exposure toxicity ratio) based on LDD₅₀ is 0.03, corresponding to a TER trigger (toxicity exposure ratio) of 33.3. The ETR trigger for the larvae risk assessment in the EFSA bee guidance document is 0.2 (corresponding to a TER trigger of 5). In EPPO 2010 the proposed TER trigger is 1 based on NOED endpoint for the chronic adult and the larvae risk assessment.

9.5.6.2 Higher-tier risk assessment for bees (tunnel test, field studies)

Under Regulation (EC) No 1107/2009, no risk assessment scheme exists currently for chronic honey bee or honey bee larvae studies. Based on the preliminary risk assessment according to SANCO/10329/2002 and EPPO 2010 that BASF provided, all TER values exceed the trigger of 1.

Nevertheless, in addition to the laboratory studies, a higher-tier semi-field tunnel test with BAS 758 00 F (BASF DocID 2021/2047630) has been performed according to OECD guidance document No. 75 (2007), EPPO Standard PP 1/170(3) (2010), OCSPP 850.3040: Field Testing for Pollinators (2012), recommendations of the AG Bienenschutz (2011), ICPPR (2014), recommendations of the EFSA Guidance Document on the risk assessment of plant protection products on bees (2013) and Pistorius et al. (2012), SANTE/2020/12830 rev. 1 (14/02/21), OECD ENV/JM/MONO(2007)17-Guidance Document on Pesticide Residue Analytical Methods. The study was carried out to gain additional information about the potential toxicity of BAS 758 00 F covering effects on honey bee larvae and bee brood development under more realistic conditions. BAS 758 00 F was applied at a rate of 1.65 L/ha (407 g total a.s./ha) during active foraging of the honey bees onto flowering *Phacelia tanacetifolia* enclosed within a tunnel. A study summary is presented in Appendix 2.

The application of BAS 758 00 F caused no unacceptable effects on honey bee mortality, foraging conditions, behaviour, colony development, colony strength and bee brood development when applied at a rate of 1.65 L/ha (equivalent to 407 g total a.s./ha) under semi-field conditions (tunnel) to *P. tanacetifolia* during active foraging conditions.

The results of this higher tier study confirms the results of the laboratory studies and demonstrates no unacceptable risk from BAS 758 00 F to honey bees at all life stages. Therefore, the proposed use of BAS 758 00 F, according to good agricultural practice, presents low risk to honey bees at all life stages and will not adversely affect honey bee colonies.

9.5.7 Effects on bumble bees

For bumble bees no specific data requirement exists under regulation (EC) No 1107/2009. Nevertheless, to support the application an acute oral and contact study was conducted with the active substance mefentrifluconazole and pyraclostrobin. The oral and contact LD₅₀ were determined to be > 195.4 µg a.s./bumble bee and > 200 µg a.s./bumble bee, respectively for mefentrifluconazole. Whereas the oral and contact LD₅₀ were determined to be > 97.0 µg a.s./bumble bee and > 100.0 µg a.s./bumble bee, respectively for pyraclostrobin. For metrafenone no acute laboratory data are available but the evaluated honey bee data indicate low acute toxicity. Considering this information and the low acute risk indicated for honeybees by a high margin of safety in the risk assessment for all three active substance (see above), it can be assumed that BAS 758 00 F poses no unacceptable risk to bumble bees at the proposed use rate.

9.5.8 Effects on solitary bees

No reliable and validated testing methods for solitary bees are currently available and no specific data requirement exists under regulation (EC) No 1107/2009. The EFSA bee guidance document (EFSA Journal 2013; 11(7):3295) has not been adopted at the time of application. Therefore, no studies with solitary bees have been performed.

9.5.9 Overall conclusions

The hazard quotients for BAS 758 00 F and the active substances mefentrifluconazole, metrafenone and pyraclostrobin for acute oral and acute contact exposure of honey bees are considerably below the Commission Regulation (EU) 546/2011 trigger value of 50. Based on the available information it can be concluded that no unacceptable risk to honey bees is expected from applications of BAS 758 00 F according to the proposed uses. This is confirmed by a risk assessment following EPPO (2010) for chronic exposure to adult honey bees and repeated exposure to honey bee larvae.

Review Comments:

The evaluation of the acute risk for bees was performed in accordance with the recommendations of the “Guidance Document on Terrestrial Ecotoxicology”, as provided by the Commission Services (SANCO/10329/2002 rev.2 (final), October 17, 2002). The submitted risk assessment, based on laboratory studies, has been accepted. It can therefore be concluded that there will be negligible acute risk associated with the exposure of *Apis mellifera* to BAS 758 00 F.

The data requirements in accordance with Commission Regulation (EU) No 284/2013 for the chronic toxicity to adult honeybees and honeybee larvae are fulfilled.

There is not harmonized approach for the chronic risk assessment for bees, therefore, Concerned Member States must decide on the acceptability of EPPO 2010 approach at national level.

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9.6 Effects on arthropods other than bees (KCP 10.3.2)

9.6.1 Toxicity data

The toxicity of BAS 758 00 F to non-target arthropods has been investigated by carrying out Tier I tests on *Typhlodromus pyri* and *Aphidius rhopalosiphi* and Tier II tests on *A. rhopalosiphi* and *Chrysoperla carnea* as well as an aged residue study on *C. carnea*. All studies are listed in Table 9.6-1. New data submitted with this application are listed in Appendix 1 and summarized in Appendix 2.

Table 9.6-1: Endpoints and effect values for BAS 758 00 F relevant for the risk assessment for non-target arthropods

Species	Product	Exposure System	Results	Reference
<i>Typhlodromus pyri</i> (protonymphs)	BAS 758 00 F	laboratory test glass plates 2D exposure	LR ₅₀ = 1.748 L/ha Corrected mortality: ¹⁾ -1.0% at 0.28125 L/ha 1.0% at 0.5625 L/ha 20.4% at 1.125 L/ha 72.4% at 2.25 L/ha 93.9% at 4.5 L/ha	not EU evaluated 2020/2037663
<i>Aphidius rhopalosiphi</i> (adults)	BAS 758 00 F	laboratory test glass plates 2D exposure	LR ₅₀ = 0.61621 L/ha Corrected mortality: 0% at 0.28125 L/ha 36.8% at 0.5625 L/ha 100% at 1.125 L/ha 100% at 2.25 L/ha 100% at 4.5 L/ha	not EU evaluated 2020/2037662
<i>Aphidius rhopalosiphi</i> (adults)	BAS 758 00 F	extended laboratory test barley plants, 3D exposure	LR ₅₀ > 3.0 L/ha ER ₅₀ > 3.0 L/ha Corrected mortality: ¹⁾ 0% at 0.5 L/ha -3.6% at 0.75 L/ha -3.6% at 1.0 L/ha 0% at 1.5 L/ha 3.6% at 2.0 L/ha 0% at 3.0 L/ha Effects on reproduction: ¹⁾ -3.3% at 0.5 L/ha 5.6% at 0.75 L/ha 3.7% at 1.0 L/ha -4.2% at 1.5 L/ha 6.1% at 2.0 L/ha 11.7% at 3.0 L/ha	not EU evaluated 2021/2015154

Species	Product	Exposure System	Results	Reference
<i>Chrysoperla carnea</i> (larvae)	BAS 758 00 F	extended laboratory test bean leaves, 2D exposure	<p>LR₅₀ = 1.37 L/ha ER₅₀ > 1.5 L/ha</p> <p>Corrected mortality: 12.8% at 0.5 L/ha 25.5% at 0.75 L/ha 40.4% at 1.0 L/ha 55.3% at 1.5 L/ha 74.5% at 2.0 L/ha 70.2% at 3.0 L/ha</p> <p>Hatching rate: 74.7% in the control 74.6% at 0.5 L/ha 75.0% at 0.75 L/ha 73.0% at 1.0 L/ha 72.7% at 1.5 L/ha</p>	not EU evaluated 2021/2015155
<i>Chrysoperla carnea</i> (larvae)	BAS 758 00 F	aged residue test bean plants, 3D exposure	<p><u>0 DAT:</u> LR₅₀ > 3.0 L/ha ER₅₀ > 3.0 L/ha</p> <p>Corrected mortality: 20.9% at 1.5 L/ha 32.6% at 2.0 L/ha 39.5% at 3.0 L/ha</p> <p>Hatching rate: 74.4% in the control 74.3% at 1.5 L/ha 73.7% at 2.0 L/ha 72.3% at 3.0 L/ha</p> <p><u>7 DAT:</u> LR₅₀ > 3.0 L/ha ER₅₀ > 3.0 L/ha</p> <p>Corrected mortality: 2.2% at 2.0 L/ha 4.4% at 3.0 L/ha</p> <p>Hatching rate: 73.8% in the control 74.1% at 2.0 L/ha 74.0% at 3.0 L/ha</p>	not EU evaluated 2021/2027050

¹⁾ Positive values indicate a decrease in survival or reproduction; negative values indicate an increase in survival or reproduction, compared to the control.

9.6.1.1 Justification for new endpoints

Effects of BAS 758 00 F on non-target arthropods other than bees were not evaluated as part of the EU assessment of the active substances mefentrifluconazole, pyraclostrobin and metrafenone. Hence, all relevant data and assessments considering this formulation are provided here and are considered adequate.

9.6.2 Risk assessment

The testing and risk assessment strategy used here follow the approach recommended in the ESCORT 2 guidance document, ESCORT 3, and the EC Guidance Document on Terrestrial Ecotoxicology (SANCO/10329, 17 October 2002).

9.6.2.1 Risk assessment for in-field exposure

The application of BAS 758 00 F is envisioned in cereals. The following risk assessment is based on the worst-case field application rate of 2×1.5 L/ha (see Section 9 Chapter 9.1 for details).

The in-field exposure (Predicted Environmental Rate, PER) is calculated according to the ESCORT 2 Guidance Document using the following equation:

$$PER_{\text{in-field}} = \text{Application rate [L/ha]} * \text{MAF}$$

Default foliar and soil MAF values following multiple applications are given in the ESCORT 2 Guidance Document and are the following for BAS 758 00 F and its application scheme.

MAF (leaf substrate) = 1.7

MAF (soil) = 1.9

As a pre-emergence or early post-emergence application is not intended for the use of BAS 758 00 F (see Section 9 Chapter 9.1 for details), the MAF (soil) will not be considered in the following risk assessment. Thus, the $PER_{\text{in-field}}$ is 2.55 L/ha.

The potential risk for non-target arthropods exposed in-field to BAS 758 00 F was assessed by calculating the hazard quotient ($HQ = \text{exposure/toxicity}$, see Table 9.6-2) for tier I standard laboratory studies according to the formula:

$$HQ_{\text{in-field}} = \frac{PER_{\text{in-field}} [\text{L/ha}]}{LR_{50} [\text{L/ha}]}$$

For higher tier laboratory studies risk is acceptable if the $PER_{\text{in-field}}$ is below the relevant endpoint (see Table 9.6-2).

Table 9.6-2: First- and higher-tier assessment of the in-field risk for non-target arthropods due to the use of BAS 758 00 F according to the proposed use pattern

Intended use	cereals		
Product	BAS 758 00 F		
Application rate (L/ha)	2 x 1.5		
MAF	1.7 (vegetation)		
Test species	Tier I		
	LR ₅₀ (lab.) [L/ha]	PER _{in-field} [L/ha]	HQ _{in-field} criterion: HQ ≤ 2
<i>Typhlodromus pyri</i>	1.748	2.55	1.46
<i>Aphidius rhopalosiphi</i>	0.61621		4.14
Test species	Higher-tier		
	Endpoint [L/ha]	PER _{in-field} [L/ha]	PER _{in-field} below rate with ≤ 50% effect?
<i>Aphidius rhopalosiphi</i>	LR ₅₀ > 3.0 ER ₅₀ > 3.0	2.55	yes yes
<i>Chrysoperla carnea</i>	LR ₅₀ = 1.37 ER ₅₀ > 1.5		no no
<i>Chrysoperla carnea</i>	no unacceptable effects on survival and reproduction on DAT 0 at 3.0 L/ha and DAT 7 at 3.0 L/ha		yes acceptable risk at DAT 0

Criteria values shown in **bold** reach the relevant trigger.

For *C. carnea*, in-field risk cannot be excluded based on the results of the extended laboratory study. However, an aged residue study is available that resulted in no unacceptable effects on survival and reproduction at a rate higher than the PER_{in-field} after exposure to freshly dried residues (*i.e.* DAT0). Therefore, no unacceptable risk in in-field habitats is to be expected if BAS 758 00 F is applied according to the proposed uses.

9.6.2.2 Risk assessment for off-field exposure

Exposure of non-target arthropods living in off-field areas to BAS 758 00 F will mainly be due to spray drift from field applications. Off-field areas are assumed to be densely vegetated and thus spray drift is unlikely to reach bare ground. Therefore, evaluation of exposure via soil residues in off-field areas was not considered. Off-field foliar PER values were calculated from in-field foliar PER values in conjunction with drift values listed in Appendix IV of the ESCORT 2 guidance document:

$$\text{PER}_{\text{off-field}} = \frac{\text{maximum PER}_{\text{in-field}} * (\% \text{ drift}/100)}{\text{vegetation distribution factor}}$$

A vegetation distribution or dilution factor is included in the equation when calculating PER values from toxicity endpoints derived from two-dimensional studies (Table 9.6-3). A dilution factor of 10 is recommended by ESCORT 2.

For 2 applications of BAS 758 00 F in cereals, the drift value at 1 m distance is 2.38% of the application rate (82nd percentile drift). The drift factor (% drift/100) is therefore 2.38/100 = 0.0238.

Table 9.6-3: PER_{off-field} values following application of BAS 758 00 F

Study type [Exposure scenario]	Maximum PER _{in-field} [L/ha]	Drift factor [% drift/100]	Vegetation distribution factor	PER _{off-field} [L/ha]
2D	2.55	0.0238	10/ 5	0.00607 /0.01214
3D			--	0.0607

To assess the potential risk of BAS 758 00 F to off-field non-target arthropods (see Table 9.6-4), the PER_{off-field} (Table 9.6-3) is compared to the toxicity endpoints of tier I standard laboratory studies according to the following equation:

$$HQ_{\text{off-field}} = \frac{\text{PER}_{\text{off-field}} [\text{L/ha}]}{\text{LR}_{50} [\text{L/ha}]} * \text{correction factor}$$

For higher tier laboratory studies risk is acceptable if the PER_{off-field} is below the relevant endpoint.

ESCORT 2 recommends a correction factor of 10 for Tier I and 5 for higher Tier data in the off-field risk assessment to account for extrapolation from testing just few representative species to the species diversity expected in off-field areas.

Table 9.6-4: First- and higher-tier assessment of the off-field risk for non-target arthropods due to the use of BAS 758 00 F according to the proposed use pattern

Intended use	cereals				
Product	BAS 758 00 F				
Application rate (L/ha)	2 x 1.5				
MAF	1.7 (vegetation)				
vdf	10 or 5 (2D exposure) / - (3D exposure)				
Test species	Tier I				
	LR ₅₀ (lab.) [L/ha]	Drift rate (%)	PER _{off-field} [L/ha]	CF	HQ _{off-field} criterion: HQ ≤ 2
<i>Typhlodromus pyri</i>	1.748	2.38	0.00607	10	0.03
<i>Aphidius rhopalosiphi</i>	0.61621				0.1
Test species	Higher-tier				
	Endpoint [L/ha]	Drift rate (%)	PER _{off-field} [L/ha]	CF	corr. PER _{off-field} below rate with ≤ 50 % effect?
<i>Aphidius rhopalosiphi</i>	LR ₅₀ > 3.0 ER ₅₀ > 3.0	2.38	0.0607	5	yes yes
<i>Chrysoperla carnea</i>	LR ₅₀ = 1.37 ER ₅₀ > 1.5		0.00607/0.01214		yes yes
<i>Chrysoperla carnea</i>	no unacceptable effects on survival and repro on DAT 7 at 3.0 L/ha (aged residue)		0.0607		yes

MAF: Multiple application factor; vdf: Vegetation distribution factor; (corr.) PER: (corrected) Predicted environmental rate; CF: Correction factor; HQ: Hazard quotient.

9.6.2.3 Additional higher-tier risk assessment

Not relevant.

9.6.2.4 Risk mitigation measures

No risk mitigation needed.

9.6.3 Overall conclusions

Based on the results of the conducted first and higher tier risk assessments it can be concluded that low risk for non-target arthropods is expected from the use of BAS 758 00 F according to the proposed use pattern. No unacceptable effects on non-target arthropods are expected in in-field and off-field habitats.

Review Comments:

Based on the results of the conducted risk assessment it can be concluded that low risk for non-target arthropods is expected from the use of BAS 758 00 F according to the proposed use pattern. No unacceptable effects on non-target arthropods are expected in in-field and off-field habitats.

9.7 Effects on non-target soil meso- and macrofauna (KCP 10.4)

9.7.1 Toxicity data

Studies on the toxicity to earthworms and other non-target soil organisms (meso- and macrofauna) have been carried out with mefentrifluconazole, metrafenone, pyraclostrobin and relevant metabolites. Full details of these studies are provided in the respective EU documents. Additionally, new toxicity studies on earthworms and other non-target meso- and macrofauna have been conducted with metrafenone, pyraclostrobin and their relevant metabolites as well as BAS 758 00 F. All studies are listed in Table 9.7-1, Table 9.7-2, Table 9.7-3 and Table 9.7-4.

New data submitted with this application are listed in Appendix 1 and summarized in Appendix 2.

Table 9.7-1: Endpoints and effect values of mefentrifluconazole and metabolites relevant for the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna)

Species	Substance/metabolite	Exposure System	Results	Reference
Acute #				
<i>Eisenia fetida</i>	mefentrifluconazole	Mixed into substrate 14 d 10% peat content	LC ₅₀ > 1000 mg/kg dry soil LC _{50, CORR} = 500 mg/kg dry soil *	EFSA Journal 2018;16(7):5379 2015/1003342
Chronic				
<i>Eisenia fetida</i>	mefentrifluconazole	Mixed into substrate 56 d 10% peat content	NOEC = 8.0 mg/kg dry soil EC ₁₀ = 5.3 mg/kg dry soil NOEC _{CORR} = 4.0 mg/kg dry soil * EC_{10, CORR} = 2.65 mg/kg dry soil *	EFSA Journal 2018;16(7):5379 2013/1235075
<i>Eisenia fetida</i>	Metabolite, Reg. No. 87 084 1,2,4-triazole	Mixed into substrate 56 d 10% peat content	NOEC ≥ 1.0 mg/kg dry soil	EFSA Journal 2018;16(7):5379 2004/1041154
<i>Folsomia candida</i>	mefentrifluconazole	Mixed into substrate 28 d 5% peat content	NOEC ≥ 400 mg/kg dry soil NOEC _{CORR} ≥ 200 mg/kg dry soil *	EFSA Journal 2018;16(7):5379 2013/1235081
<i>Folsomia candida</i>	Metabolite, Reg. No. 87 084 1,2,4-triazole	Mixed into substrate 28 d 10% peat content	NOEC = 1.8 mg/kg dry soil	EFSA Journal 2018;16(7):5379 2002/1007851
<i>Hypoaspis aculeifer</i>	mefentrifluconazole	Mixed into substrate 14 d 5% peat content	NOEC ≥ 1000 mg/kg dry soil NOEC _{CORR} ≥ 500 mg/kg dry soil *	EFSA Journal 2018;16(7):5379 2013/1235082
<i>Hypoaspis aculeifer</i>	Metabolite, Reg. No. 87 084 1,2,4-triazole	Mixed into substrate 14 d 5% peat content	NOEC = 171 mg/kg dry soil	EFSA Journal 2018;16(7):5379 2014/1326895

Values shown in **bold** are relevant for the conclusion of the risk assessment.

Acute studies listed for reference only but not used in the risk assessment according to Commission Regulation (EU) 283/2013.

* Corrected value derived by dividing the endpoint by a factor of 2 due to a log Pow >2.

Table 9.7-2: Endpoints and effect values of metrafenone and metabolites relevant for the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna)

Species	Substance/metabolite	Exposure System	Results	Reference
Acute #				
<i>Eisenia fetida</i>	metrafenone	Mixed into substrate 14 d 10% peat content	LC _{50, CORR} > 500 mg/kg dry soil*	EFSA Scientific report No. 58, 1-72 (2006)
<i>Eisenia fetida</i>	metrafenone (tested as BAS 560 00 F) ¹⁾	Mixed into substrate 14 d acute 10% peat content	LC _{50, CORR} > 150 mg a.s./kg dry soil*	EFSA Scientific report No. 58, 1-72 (2006)
<i>Eisenia fetida</i>	Metabolite, CL 377160	Mixed into substrate 14 d 10% peat content	LC _{50, CORR} > 500 mg/kg dry soil*	EFSA Scientific report No. 58, 1-72 (2006)
Chronic				
<i>Eisenia fetida</i>	metrafenone (tested as BAS 560 02 F) ¹⁾	Mixed into substrate 56 d 5% peat content	NOEC ≥ 240 mg/kg dry soil (equivalent to 101.61 mg a.s./kg dry soil) ²⁾ NOEC _{CORR} ≥ 50.8 mg a.s./kg dry soil ²⁾	not EU evaluated 2011/1000384
<i>Eisenia fetida</i>	Metabolite, CL 377160	Mixed into substrate 56 d 5% peat content	NOEC ≥ 1000 mg/kg dry soil NOEC _{CORR} ≥ 500 mg/kg dry soil ²⁾	not EU evaluated 2014/1093923
<i>Eisenia andrei</i>	Metabolite, CL 3000402 ³⁾	Mixed into substrate 56 d 5% peat content	NOEC = 27.78 mg/kg dry soil NOEC _{CORR} = 13.89 mg/kg dry soil ²⁾	not EU evaluated 2015/1041971
<i>Folsomia candida</i>	metrafenone (tested as BAS 560 02 F) ¹⁾	Mixed into substrate 28 d 5% peat content	NOEC ≥ 1000 mg/kg dry soil (equivalent to 422.65 mg a.s./kg dry soil) ²⁾ NOEC _{CORR} ≥ 211.3 mg a.s./kg dry soil ²⁾	not EU evaluated 2013/1003203
<i>Eisenia fetida</i>	metrafenone (tested as BAS 560 00 F)	--	NOEC _{CORR} = 3.0 mg/kg dry soil*	EFSA Scientific report No. 58, 1-72 (2006)
<i>Eisenia fetida</i>	metrafenone (tested as BAS 560 02 F) ¹⁾	--	NOEC _{CORR} = 10.44 mg/kg dry soil*	EFSA Scientific report No. 58, 1-72 (2006)

Values shown in **bold** are relevant for the conclusion of the risk assessment.

Acute studies listed for reference only but not used in the risk assessment according to Commission Regulation (EU) 283/2013.

* Corrected value derived by dividing the endpoint by a factor of 2 due to a log Pow >2.

¹⁾ BAS 560 02 F is the representative solo-formulation in the ongoing active substance renewal process of metrafenone containing 500 g metrafenone/L (nominal).

²⁾ In accordance with the ongoing AIR process, endpoint based on the content of the active substance (nominal) and taking into account a density of the test batch.

³⁾ Metabolite not considered relevant according to the evaluation in section 8 (E-fate). For details please refer to chapter 9.1.3.

Table 9.7-3: Endpoints and effect values of pyraclostrobin and metabolites relevant for the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna)

Species	Substance/metabolite	Exposure System	Results	Reference
Acute #				
<i>Eisenia fetida</i>	pyraclostrobin	Mixed into substrate 14 d, acute 10% peat content	LC ₅₀ = 567 mg/kg dry soil LC _{50, CORR} = 283 mg/kg dry soil *	EC Review report, SANCO/1420/2001-2004 Monograph (2001) 1,1-651 1999/10708
<i>Eisenia fetida</i>	Metabolite, Reg. No. 364 380 500M01 (BF 500-6)	Mixed into substrate 14 d, acute 10% peat content	LC ₅₀ > 1000 mg/kg dry soil LC _{50, CORR} > 500 mg/kg dry soil *	EC Review report, SANCO/1420/2001-2004 Monograph (2001) 1,1-651 1999/11308
<i>Eisenia fetida</i>	Metabolite, Reg. No. 369 315 500M02 (BF 500-7)	Mixed into substrate 14 d, acute 10 % peat content	LC ₅₀ > 1000 mg/kg dry soil LC _{50, CORR} > 500 mg/kg dry soil *	EC Review report, SANCO/1420/2001-2004 Monograph (2001) 1,1-651 1999/11309
Chronic				
<i>Eisenia fetida</i>	pyraclostrobin	Mixed into substrate 56 d, chronic 10% peat content	NOEC = 23.1 mg/kg dry soil NOEC _{CORR} = 11.6 mg/kg dry soil * EC ₁₀ = 22.2 mg/kg dry soil EC_{10, CORR} = 11.1 mg/kg dry soil *	not EU evaluated 2014/1000461
<i>Eisenia fetida</i>	Metabolite, Reg. No. 364 380 500M01 (BF 500-6)	Mixed into substrate 56 d, chronic 10% peat content	NOEC ≥ 320 mg/kg dry soil NOEC _{CORR} ≥ 160 mg/kg dry soil ^a	not EU evaluated 2013/1003174
<i>Eisenia fetida</i>	Metabolite, Reg. No. 369 315 500M02 (BF 500-7)	Mixed into substrate 56 d, chronic 10% peat content	NOEC ≥ 320 mg/kg dry soil NOEC _{CORR} ≥ 160 mg/kg dry soil ^a	not EU evaluated 2013/1224029
<i>Folsomia candida</i>	Metabolite, Reg. No. 364 380 500M01 (BF 500-6)	Mixed into substrate 28 d, chronic 5% peat content	NOEC ≥ 1000 mg/kg dry soil NOEC _{CORR} ≥ 500 mg/kg dry soil ^a EC ₁₀ = n.d. ¹⁾	not EU evaluated 2013/1068054 statistical recalculation 2019/2034464
<i>Folsomia candida</i>	Metabolite, Reg. No. 369 315 500M02 (BF 500-7)	Mixed into substrate 28 d, chronic 5% peat content	NOEC ≥ 800 mg/kg dry soil NOEC _{CORR} ≥ 400 mg/kg dry soil ^a EC ₁₀ = n.d. ¹⁾	not EU evaluated 2013/1224030 statistical recalculation 2019/2034467

Values shown in **bold** are relevant for the conclusion of the risk assessment.

Acute studies listed for reference only but not used in the risk assessment according to Commission Regulation (EU) 283/2013.

* Corrected value derived by dividing the endpoint by a factor of 2 due to a log Pow >2.

¹⁾ A statistical re-evaluation was conducted confirming that the calculation of an L/EC₁₀ is not feasible.

Table 9.7-4: Endpoints and effect values of BAS 758 00 F relevant for the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna)

Species	Product	Exposure System	Results	Reference
Chronic				
<i>Eisenia andrei</i>	BAS 758 00 F	Mixed into substrate, 56 d 10% peat content	NOEC = 84.4 mg/kg dry soil (equivalent to 5.1 mg mefentrifluconazole, 7.7 mg metrafenone and 6.2 mg pyraclostrobin/kg dry soil) EC ₁₀ = 86.6 mg/kg dry soil (equivalent to 5.3 mg mefentrifluconazole, 7.9 mg metrafenone and 6.3 mg pyraclostrobin/kg dry soil) NOEC _{CORR} = 9.5 mg total a.s./kg dry soil ^{1) *} EC₁₀ CORR = 9.8 mg total a.s./kg dry soil^{1) *}	not EU evaluated 2020/2037658
<i>Folsomia candida</i>	BAS 758 00 F	Mixed into substrate, 28 d 5% peat content	NOEC = 151.9 mg/kg dry soil (equivalent to 9.3 mg mefentrifluconazole, 13.9 mg metrafenone and 11.1 mg pyraclostrobin/kg dry soil) EC₁₀ = 191.7 mg/kg dry soil (equivalent to 11.7 mg mefentrifluconazole, 17.6 mg metrafenone and 14.0 mg pyraclostrobin/kg dry soil) NOEC _{CORR} = 17.2 mg total a.s./kg dry soil ^{1) *} EC ₁₀ CORR = 21.6 mg total a.s./kg dry soil ^{1) *}	not EU evaluated 2020/2037659
<i>Hypoaspis aculeifer</i>	BAS 758 00 F	Mixed into substrate, 14 d 5% peat content	NOEC = 262.1 mg/kg dry soil (equivalent to 16.0 mg mefentrifluconazole, 24.0 mg metrafenone and 19.2 mg pyraclostrobin/kg dry soil) EC₁₀ = 397.5 mg/kg dry soil (equivalent to 24.2 mg mefentrifluconazole, 36.4 mg metrafenone and 29.1 mg pyraclostrobin/kg dry soil) NOEC _{CORR} = 29.6 mg total a.s./kg dry soil ^{1) *} EC ₁₀ CORR = 44.9 mg total a.s./kg dry soil ^{1) *}	not EU evaluated 2020/2037660

Values shown in **bold** are relevant for the conclusion of the risk assessment.

* Corrected value derived by dividing the endpoint by a factor of 2 due to a log Pow >2.

¹⁾ Endpoint based on sum of active substances (nominal) and taking into account a density of BAS 758 00 F of 1.092 g/cm³.

9.7.1.1 Justification for new endpoints

Effects of the formulation BAS 758 00 F on earthworms and other non-target soil organisms (meso- and macrofauna) were not evaluated as part of the EU assessment of the active substances mefentrifluconazole, metrafenone or pyraclostrobin. Hence, all relevant data and assessments considering this formulation are provided here and are considered adequate.

The endpoints for *Folsomia* and *Hypoaspis* were corrected in the EFSA conclusion for mefentrifluconazole. This is not in accordance with the current guidance (EPPO scheme 2002) because the tests were conducted with a substrate carbon content of 5%. EFSA proposed the correction in its technical report on the outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology (EFSA supporting publication 2015: EN 924. 62 pp.). However, this correction is not justified by specific data and is not adopted by all member states. Therefore, both values are given in the following risk assessment and the conclusion are based on the non-corrected values.

~~For metrafenone (tested as BAS 560 02 F) and its relevant metabolites, new chronic studies on earthworms which were not evaluated as part of the EU assessment of the active substances metrafenone on earthworms have been carried out. In addition, a new chronic *Folsomia candida* study on metrafenone (tested as BAS 560 02 F) is available. All studies are currently evaluated in the ongoing active substance renewal process of the active substance. The data are provided here and considered adequate.~~

~~For the active substance pyraclostrobin and the metabolites BF 500-6 and BF 500-7, new chronic studies which were not evaluated as part of the EU assessment of the active substances pyraclostrobin on earthworms have been carried out. The studies are currently evaluated in the ongoing active substance renewal process of the active substance. The data are provided here and considered adequate.~~

All chronic studies on earthworms and collembolans after guidelines OECD 222 and OECD 232 respectively, were checked for their potential to calculate EC_{10/20} values. If a calculation was possible, the EC_{10/20} are provided in the corresponding study summary in Appendix 2 and the EC₁₀ is listed in Chapter 9.7.1.

For the risk assessment, both values (if available) are used. The conclusion, however, will be based on the EC₁₀ if reliable. If the EC₁₀ is not reliable or could not be calculated the NOEC is considered the relevant endpoint for the risk assessment.

9.7.2 Risk assessment

The evaluation of the risk for earthworms and other non-target soil organisms (meso- and macrofauna) was performed in accordance with the recommendations of the “Guidance Document on Terrestrial Ecotoxicology”, as provided by the Commission Services (SANCO/10329/2002 rev 2 (final), October 17, 2002).

For substances with a $\log P_{ow} > 2$ an endpoint correction by a soil factor of 2 (f_{oc}) must be considered. The EPPO earthworm scheme 2002 recommends an endpoint correction for earthworm studies with 10% peat content only. According to EPPO there is no need to correct endpoints derived from studies conducted with 5% peat. It should be noted that EFSA proposed an endpoint correction for 5% peat studies in its technical report on the outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology (EFSA supporting publication 2015: EN 924. 62 pp.). However, this correction is not justified by specific data and is not adopted by all member states. Therefore, corrected and uncorrected values are given in the following risk assessment and the conclusion is based on endpoints which are in line with the EPPO earthworm scheme 2002.

The $\log P_{ow}$ values of the mefentrifluconazole metabolite 1,2,4-triazole is < 2 . Therefore, the endpoint was not corrected. The active substances mefentrifluconazole, metrafenone, pyraclostrobin and the relevant metabolites of the latter two active substances have a $\log P_{ow} > 2$.

9.7.2.1 First-tier risk assessment

The relevant predicted environmental concentrations in soil (PEC_{soil}) for risk assessments covering the proposed use pattern are taken from Part B Section 8 (Environmental Fate), Chapter 8.7.2. According to the assessment of environmental-fate data, multi-annual accumulation in soil does not need to be considered for pyraclostrobin and the metrafenone metabolite CL 377160. In contrast, multi-annual accumulation needs to be considered for the metrafenone, mefentrifluconazole and its metabolite 1,2,4-triazole and the metabolites of pyraclostrobin, BF 500-6 and BF 500-7.

The potential risk of BAS 758 00 F, mefentrifluconazole, metrafenone, pyraclostrobin and relevant metabolites to earthworms and other non-target soil macro-organisms was assessed by comparing the maximum PEC_{soil} values with NOEC or EC_{10} values, to generate long-term TER values (TER_{lt} , Table 9.7-5 to Table 9.7-8).

The TER was calculated as follows:

$$TER = \frac{\text{Endpoint [mg/kg dry soil]}}{PEC_{soil} \text{ [mg/kg dry soil]}}$$

Table 9.7-5: First-tier assessment of the chronic risk for earthworms and other non-target soil organisms (meso- and macrofauna) due to the use of mefentrifluconazole as contained in BAS 758 00 F according to the proposed use pattern

Intended use	2 x 100 g mefentrifluconazole/ha in cereals		
Chronic effects on earthworms			
Active substance/metabolite	Endpoint (mg/kg dry soil)	PEC _{soil} (mg/kg dry soil)	TER _{lt} (criterion TER ≥ 5)
mefentrifluconazole	NOEC = 8.0 EC ₁₀ = 5.3 NOEC _{CORR} = 4.0 <u>EC_{10,CORR} = 2.65</u>	0.205 *	39 26 20 <u>13</u>
Metabolite, Reg. No. 87 084 1,2,4-triazole	<u>NOEC ≥ 1.0</u>	0.001 *	<u>≥ 1000</u>
Chronic effects on other soil meso- and macrofauna			
Collembola (<i>Folsomia candida</i>)			
Active substance/metabolite	Endpoint (mg/kg dry soil)	PEC _{soil} (mg/kg dry soil)	TER _{lt} (criterion TER ≥ 5)
mefentrifluconazole	<u>NOEC ≥ 400</u> NOEC _{CORR} ≥ 200	0.205 *	<u>≥ 1951</u> ≥ 976
Metabolite, Reg. No. 87 084 1,2,4-triazole	<u>NOEC = 1.8</u>	0.001 *	<u>1800</u>
Springtail (<i>Hypoaspis aculeifer</i>)			
mefentrifluconazole	<u>NOEC ≥ 1000</u> NOEC _{CORR} ≥ 500	0.205 *	<u>≥ 4878</u> ≥ 2439
Metabolite, Reg. No. 87 084 1,2,4-triazole	<u>NOEC = 171</u>	0.001 *	<u>171000</u>

Underlined values are relevant for the conclusion of the risk assessment.

* PEC_{soil, accu}. For details please refer to section 8, chapter 8.7.

Table 9.7-6: First-tier assessment of the chronic risk for earthworms and other non-target soil organisms (meso- and macrofauna) due to the use of metrafenone as contained in BAS 758 00 F according to the proposed use pattern

Intended use	2 x 150 g metrafenone/ha in cereals		
Chronic effects on earthworms			
Product/active substance	Endpoint (mg/kg dry soil)	PEC _{soil} (mg/kg dry soil)	TER _{It} (criterion TER ≥ 5)
metrafenone (based on BAS 560 02 F)	NOEC ≥ 101.61¹⁾ NOEC_{CORR} ≥ 50.8¹⁾	0.158 [*]	≥ 643 ≥ 322
metrafenone (based on BAS 560 00 F)	NOEC _{CORR} ≥ 3.0	0.158 *	≥ 19
Metabolite, CL 377160	NOEC ≥ 1000 NOEC_{CORR} ≥ 500	<0.001 [#]	≥ 1000000 ≥ 500000
Chronic effects on other soil macro- and mesofauna			
Product/active substance	Endpoint (mg/kg dry soil)	PEC _{soil} (mg/kg dry soil)	TER _{It} (criterion TER ≥ 5)
Collembola (<i>Folsomia candida</i>)			
metrafenone (based on BAS 560 02 F)	NOEC ≥ 422.65¹⁾ NOEC_{CORR} ≥ 211.3¹⁾	0.158 [*]	≥ 2675 ≥ 1337

Underlined values are relevant for the conclusion of the risk assessment.

* PEC_{soil, accu}. For details please refer to section 8, chapter 8.7.

PEC_{soil, ini}. For details please refer to section 8, chapter 8.7.

1) In accordance with the ongoing AIR process, endpoint based on the content of the active substance (nominal) and taking into account a density of the test batch

Table 9.7-7: First-tier assessment of the chronic risk for earthworms and other non-target soil organisms (meso- and macrofauna) due to the use of pyraclostrobin as contained in BAS 758 00 F according to the proposed use pattern

Intended use	2 × 120 g pyraclostrobin/ha in cereals		
Chronic effects on earthworms			
Active substance/metabolite	Endpoint (mg/kg dry soil)	PEC _{soil} (mg/kg dry soil)	TER _{It} (criterion TER ≥ 5)
pyraclostrobin	NOEC = 23.1 NOEC _{CORR} = 11.6 EC ₁₀ = 22.2 <u>EC₁₀ CORR = 11.1</u>	0.057 [*] 0.059	405 392 204 197 389 376 <u>195 188</u>
Metabolite, Reg.No 364 380 500M01 (BF 500-6)	NOEC ≥ 320 <u>NOEC_{CORR} ≥ 160</u>	0.065 [#]	≥ 4923 <u>≥ 2462</u>
Metabolite, Reg. No. 369 315 500M02 (BF 500-7)	NOEC ≥ 320 <u>NOEC_{CORR} ≥ 160</u>	0.026 [#]	≥ 12308 <u>≥ 6154</u>
Chronic effects on other soil meso- and macrofauna			
Active substance/metabolite	Endpoint (mg/kg dry soil)	PEC _{soil} (mg/kg dry soil)	TER _{It} (criterion TER ≥ 5)
Collembola (<i>Folsomia candida</i>)			
Metabolite, Reg.No 364 380 500M01 (BF 500-6)	<u>NOEC ≥ 1000</u> NOEC _{CORR} ≥ 500	0.065 [#]	<u>≥ 15385</u> ≥ 7692
Metabolite, Reg. No. 369 315 500M02 (BF 500-7)	<u>NOEC ≥ 800</u> NOEC _{CORR} ≥ 400	0.026 [#]	<u>≥ 30769</u> ≥ 15385

Underlined values are relevant for the conclusion of the risk assessment.

* PEC_{soil, ini}. For details see chapter 8.7.

PEC_{soil accu}. For details see chapter 8.7.

Table 9.7-8: First-tier assessment of the chronic risk for earthworms and other non-target soil organisms (meso- and macrofauna) due to the use of BAS 758 00 F according to the proposed use pattern

Intended use	2 x 1.5 kg BAS 758 00 F/ha in cereals		
Chronic effects on earthworms			
Product	Endpoint (mg a.s./kg dry soil) ¹⁾	PEC _{soil} (mg a.s./kg dry soil)	TER _{lt} (criterion TER ≥ 5)
(total) a.s. in BAS 758 00 F	NOEC = 19.1 EC ₁₀ = 19.6 NOEC _{CORR} = 9.6 <u>EC₁₀ CORR = 9.8</u>	0.420 ²⁾ 0.422 ²⁾	45 47-46 23 <u>23</u>
Chronic effects on other soil meso- and macrofauna			
Product	Endpoint (mg a.s./kg dry soil) ¹⁾	PEC _{soil} (mg a.s./kg dry soil)	TER _{lt} (criterion TER ≥ 5)
Collembola (<i>Folsomia candida</i>)			
(total) a.s. in BAS 758 00 F	NOEC = 34.3 <u>EC₁₀ = 43.3</u> NOEC _{CORR} = 17.2 EC ₁₀ CORR = 21.6	0.420 ²⁾	82 <u>103</u> 41 51
Soil mites (<i>Hypoaspis aculeifer</i>)			
(total) a.s. in BAS 758 00 F	NOEC = 59.2 <u>EC₁₀ = 89.8</u> NOEC _{CORR} = 29.6 EC ₁₀ CORR = 44.9	0.420 ²⁾	141 <u>214</u> 70 107

Underlined values are relevant for the conclusion of the risk assessment.

¹⁾ Endpoint based on the content of the active substances (nominal) and taking into account a density of BAS 758 00 F of 1.092 g/cm³.

²⁾ Based on the sum of the worst-case active substance PEC_{soil} values. PEC_{soil, accu} for mefentrifluconazole and metrafenone, PEC_{soil, ini} for pyraclostrobin.

9.7.2.2 Higher-tier risk assessment

Not relevant.

9.7.3 Overall conclusions

All TER values for BAS 758 00 F, the active substances mefentrifluconazole, metrafenone, pyraclostrobin and relevant metabolites for chronic exposure of earthworms and other non-target soil organisms (meso- and macrofauna) are considerably higher than the Commission Regulation (EU) 546/2011 trigger value of 5. This indicates that BAS 758 00 F poses no unacceptable risk to earthworms and other non-target soil organisms (meso- and macrofauna) when applied according to the proposed use pattern.

Review Comments:

The long-term risks of BAS 758 00 F to soil meso- and macro-organisms were assessed from toxicity exposure ratios between toxicity endpoints and maximum PEC_{soil} . The relevant predicted environmental concentration in soil (PEC_{soil}) for risk assessment covering the proposed use pattern was taken from Part B Section 8 (Environmental Fate).

Safe use of BAS 758 00 F was confirmed based on TER_{LT} calculations for formulation (endpoints based on the nominal content of the active substances) and active substances.

9.8 Effects on soil microbial activity (KCP 10.5)

9.8.1 Toxicity data

Studies on the effects on soil microorganisms have been carried out with the active substances mefentrifluconazole, metrafenone and pyraclostrobin and their relevant metabolites. Full details of these studies are provided in the respective EU documents.

New data submitted with this application are listed in Appendix 1 and summarized in Appendix 2.

All studies are listed in Table 9.8-1, Table 9.8-3, Table 9.8-2 and Table 9.8-4.

Table 9.8-1: Endpoints and effect values of mefentrifluconazole and relevant metabolites relevant for the risk assessment for soil microorganisms

Endpoint	Substance/metabolite	Exposure System	Results	Reference
N-mineralization	mefentrifluconazole	28 d, aerobic loamy sand	Nitrate formation rate at 2.53 mg/kg dry soil +2.1%	EFSA Journal 2018;16(7):5379 2015/1108623
	Metabolite, Reg. No. 87 084 1,2,4-triazole	28 d, aerobic sandy loam	Nitrate formation rate at 0.333 mg/kg dry soil +8.3%	EFSA Journal 2018;16(7):5379 2000/1021861
C-mineralization ¹⁾	mefentrifluconazole	28 d, aerobic loamy sand	CO ₂ formation rate or O ₂ consumption at 2.53 mg/kg dry soil -1.1%	EFSA Journal 2018;16(7):5379 2015/1108621

+ = stimulation, - = inhibition

¹⁾ Carbon transformation studies are listed for reference only but are not used in the risk assessment according to Commission Regulation (EU) No 283/2013.

Table 9.8-2: Endpoints and effect values of metrafenone and relevant metabolite relevant for the risk assessment for soil microorganisms

Endpoint	Substance	Exposure System	Results	Reference
N-mineralisation	metrafenone (tested as BAS 560 00 F) ¹⁾	28 d, aerobic sandy loam	Nitrate formation rate at 2.00 mg a.s./kg dry soil (equivalent to 8.19 mg product/ha) < 25%	EFSA Scientific report No. 58, 1-72 (2006)
	Metabolite, CL 377160	28 d, aerobic loamy sand	Nitrate formation rate at 0.31 mg/kg dry soil < 25%	EFSA Scientific report No. 58, 1-72 (2006)
	Metabolite, CL 3000402 ²⁾	28 d, aerobic loamy sand	Nitrate formation rate at 1.00 mg/kg dry soil -17.2%	not EU evaluated 2015/1132053

¹⁾ Study was conducted with the metrafenone solo-formulation BAS 560 00 F (300.0 g metrafenone/L (nominal), please refer to Part C).

²⁾ Metabolite not considered relevant according to the evaluation in section 8 (E-fate). For details, please refer to chapter 9.1.3. However, the results are shown as additional information.

Table 9.8-3: Endpoints and effect values of pyraclostrobin and relevant metabolites relevant for the risk assessment for soil microorganisms

Endpoint	Substance/metabolite	Exposure System	Results	Reference
N-mineralization	pyraclostrobin (tested as BAS 500 00 F) ¹⁾	49 d, aerobic loamy sand	Nitrate formation rate at 3.33 mg/kg dry soil +5.0%	EC Review report, SANCO/1420/2001-2004 1998/11260
	<i>tested in combination:</i> Metabolite, Reg. No. 364 380 500M01 (BF 500-6) Metabolite, Reg. No. 369 315 500M02 (BF 500-7)	28 d, aerobic loamy sand	Nitrogen turnover 1.0 mg/kg dry soil (BF500-6) 0.5 mg/kg dry soil (BF500-7) -9.8%	EC Review report, SANCO/1420/2001-2004 1999/11311
C-mineralization ²⁾	<i>tested in combination:</i> Metabolite, Reg. No. 364 380 500M01 (BF 500-6) Metabolite, Reg. No. 369 315 500M02 (BF 500-7)	28 d, aerobic loamy sand	CO ₂ formation 1.0 mg/kg dry soil (BF500-6) 0.5 mg/kg dry soil (BF500-7) 0%	EC Review report, SANCO/1420/2001-2004 1999/11120

+ = stimulation, - = inhibition

¹⁾ Study was conducted with the pyraclostrobin solo-formulation BAS 500 00 F (250.0 g pyraclostrobin/L (nominal), please refer to Part C).

²⁾ Carbon transformation studies are listed for reference only but are not used in the risk assessment according to Commission Regulation (EU) No 283/2013.

Table 9.8-4: Endpoints and effect values of BAS 758 00 F relevant for the risk assessment for soil microorganisms

Endpoint	Product	Exposure System	Results	Reference
N-mineralization	BAS 758 00 F	28 d, aerobic loamy sand	Nitrate formation rate 40 mg/kg dry soil (equivalent to 9.03 mg total a.s./kg dry soil) ¹⁾ +4.4%	not EU evaluated 2020/2037661

+ = stimulation, - = inhibition

¹⁾ Calculated, based on the nominal content of the a.s. and considering a density of BAS 758 00 F of 1.092 g/cm³.

9.8.1.1 Justification for new endpoints

Effects on soil microbial activity of BAS 758 00 F were not evaluated as part of the EU review of mefentrifluconazole, metrafenone or pyraclostrobin. Therefore, all relevant data and assessments are provided here and are considered adequate.

9.8.2 Risk assessment

The evaluation of the risk for soil microorganisms was performed in accordance with the recommendations of the “Guidance Document on Terrestrial Ecotoxicology”, as provided by the Commission Services (SANCO/10329/2002 rev 2 (final), October 17, 2002).

The relevant predicted environmental concentrations in soil (PEC_{soil}) for risk assessments covering the proposed use pattern are taken from Section 8 (Environmental Fate), Chapter 8.7.2, and were already used in the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna) (see Section 9 Chapter 9.8).

The potential risk of BAS 758 00 F, mefentrifluconazole, metrafenone, pyraclostrobin and relevant metabolites to soil micro-organisms was assessed by comparing the maximum PEC_{soil} values with the maximum concentration with effects ≤ 25 % (see Table 9.8-5, Table 9.8-7, Table 9.8-6 and Table 9.8-8).

Table 9.8-5: Assessment of the risk for effects on soil micro-organisms due to the use of mefentrifluconazole as contained in BAS 758 00 F according to the proposed use pattern

Intended use	cereals		
Active substance	mefentrifluconazole		
Application rate (g a.s./ha)	2 x 100		
N-mineralization			
Active substance/metabolite	Max. conc. with effects ≤ 25 % (mg/kg dry soil)	PEC _{soil} (mg/kg dry soil)	Risk acceptable?
mefentrifluconazole	2.53 (at 28 d)	0.205 *	yes
Metabolite, Reg. No. 87 084 1,2,4-triazole	0.333 (at 28 d)	0.001 *	yes

* PEC_{soil, accu}

Table 9.8-6: Assessment of the risk for effects on soil micro-organisms due to the use of metrafenone as contained in BAS 758 00 F according to the proposed use pattern

Intended use	cereals		
Active substance	metrafenone		
Application rate (g a.s./ha)	2 x 150		
N-mineralization			
Active substance/metabolite	Max. conc. with effects ≤ 25 % (mg/kg dry soil)	PEC _{soil} (mg/kg dry soil)	Risk acceptable?
metrafenone (tested as BAS 560 00 F)	2.00	0.158 *	yes
Metabolite, CL 377160	0.31	< 0.001 #	yes

* PEC_{soil, accu}

PEC_{soil, ini}

Table 9.8-7: Assessment of the risk for effects on soil micro-organisms due to the use of pyraclostrobin as contained in BAS 758 00 F according to the proposed use pattern

Intended use	cereals		
Active substance	pyraclostrobin		
Application rate (g a.s./ha)	2 x 120		
N-mineralization			
Active substance/metabolite	Max. conc. with effects ≤ 25 % (mg/kg dry soil)	PEC _{soil} (mg/kg dry soil)	Risk acceptable?
pyraclostrobin	3.33 (at 49 d)	0.057 * 0.059 #	yes
Metabolite, Reg. No. 364 380 500M01 (BF 500-6)	1.0 (at 28 d)	0.065 *	yes
Metabolite, Reg. No. 369 315 500M02 (BF 500-7)	0.5 (at 28 d)	0.026 *	yes

PEC_{soil, ini}

* PEC_{soil, accu}

Table 9.8-8: Assessment of the risk for effects on soil micro-organisms due to the use of BAS 758 00 F according to the proposed use pattern

BAS 758 00 F according to the proposed use pattern			
Intended use	cereals		
Product	BAS 758 00 F		
Application rate (L/ha)	2 x 1.5		
N-mineralization			
Product	Max. conc. with effects ≤ 25 % (mg/kg dry soil)	PEC _{soil} (mg/kg dry soil)	Risk acceptable?
BAS 758 00 F	9.03 (at 28 d) ¹⁾	0.420 ²⁾ 0.422 ²⁾	yes

¹⁾ Calculated, based on the nominal content of the a.s. and considering a density of BAS 758 00 F of 1.092 g/cm³.

²⁾ Based on the sum of the worst-case active substance PEC_{soil} values. PEC_{soil, accu} for mefentrifluconazole and metrafenone, PEC_{soil, ini} for pyraclostrobin.

9.8.3 Overall conclusions

For the formulation BAS 758 00 F, the active substances mefentrifluconazole, metrafenone and pyraclostrobin as well as for the relevant metabolites, the maximum concentration with effects < 25% (SANCO/10329/2002 trigger) are all above the maximum PEC_{soil} values. Therefore, it is concluded that the use of BAS 758 00 F will not pose an unacceptable risk to non-target soil micro-organisms, if applied according to good agricultural practice.

Review Comments:

BAS 758 00 F had no significant effect on soil micro-organisms at 9.03 mg sum a.s./kg dry soil. This is approximately 21 times higher than the maximum PEC_{soil} of 0.422 mg sum a.s./kg dry soil following the worst-case application to cereals. Moreover, the acceptable risk was confirmed for mefentrifluconazole, metrafenone, pyraclostrobin and their relevant metabolites. This supports the conclusion that under field conditions, use of BAS 751 00 F at the proposed rates poses no unacceptable risk to non-target soil micro-organisms.

9.9 Effects on non-target terrestrial plants (KCP 10.6)

9.9.1 Toxicity data

Vegetative vigour and seedling emergence studies have been conducted with BAS 758 00 F. New data submitted with this application are listed in Appendix 1 and summarized in Appendix 2.

An overview of the endpoints and effects values of the formulation BAS 758 00 F is provided in Table 9.9-1 below.

Table 9.9-1: Endpoints and effect values of BAS 758 00 F relevant for the risk assessment for non-target terrestrial plants

Species	Product	Exposure System	Results	Reference
<i>Daucus carota</i> _d (carrot) <i>Lactuca sativa</i> _d (lettuce) <i>Brassica napus</i> _d (oilseed rape) <i>Brassica oleracea</i> _d (cabbage) <i>Glycine max</i> _d (soybean) <i>Solanum lycopersicum</i> _d (tomato) <i>Allium cepa</i> _m (onion) <i>Lolium multiflorum</i> _m (ryegrass) <i>Triticum aestivum</i> _m (wheat) <i>Zea mays</i> _m (corn)	BAS 758 00 F	21 d Seedling emergence	ER ₅₀ emergence > 1.5 L/ha ER ₅₀ plant weight > 1.5 L/ha ER ₅₀ plant height > 1.5 L/ha	not EU evaluated 2020/2037664
<i>Daucus carota</i> _d (carrot) <i>Lactuca sativa</i> _d (lettuce) <i>Brassica napus</i> _d (oilseed rape) <i>Brassica oleracea</i> _d (cabbage) <i>Glycine max</i> _d (soybean) <i>Solanum lycopersicum</i> _d (tomato) <i>Allium cepa</i> _m (onion) <i>Lolium multiflorum</i> _m (ryegrass) <i>Triticum aestivum</i> _m (wheat) <i>Zea mays</i> _m (corn)	BAS 758 00 F	21 d Vegetative vigour	ER ₅₀ plant weight > 1.5 L/ha ER ₅₀ plant height > 1.5 L/ha	not EU evaluated 2020/2037665

m: monocotyledonous; d: dicotyledonous

9.9.1.1 Justification for new endpoints

Effects on non-target plants of BAS 758 00 F were not evaluated as part of the initial Annex I inclusion or the Annex I renewal process of mefentrifluconazole, pyraclostrobin or metrafenone. Hence, all relevant data and assessments considering this formulation are provided here and are considered adequate.

9.9.2 Risk assessment

9.9.2.1 Tier-1 risk assessment (based screening data)

The risk assessment is based on the “Guidance Document on Terrestrial Ecotoxicology”, (SANCO/10329/2002 rev.2 final, 2002). It is restricted to off-field areas, as non-target plants are non-crop plants located outside the treated area.

The application of BAS 758 00 F is envisioned in cereals. The following risk assessment is based on the worst-case single application rate of 1.5 L BAS 758 00 F/ha (see Section 9 Chapter 9.1 for details).

The amount of spray drift reaching off-crop habitats is calculated using the 90th percentile estimates in Appendix IV of ESCORT 2. The predicted rate reaching the off-crop environment (PER_{off-field}) is calculated as:

$$\text{PER}_{\text{off-field}} = \text{maximum single application rate (L/ha)} * (\% \text{ drift}/100)$$

For a single application to field crops (i.e. cereals), 2.77% of the application rate was assumed to reach areas at 1 m from the edge of the field (worst-case scenario). The highest single application rate of BAS 758 00 F is 1.5 L product/ha. The maximum off-field predicted environmental rate (PER_{off-field}) is thus calculated to be 0.0416 L product/ha.

The potential risk of BAS 758 00 F to non-target plants was assessed by comparing the calculated PER value to the ER₅₀ values in order to generate the toxicity exposure ratio (TER) as follows.

$$\text{TER} = \frac{\text{Endpoint [L/ha]}}{\text{PER}_{\text{off-field [L/ha]}}}$$

The results of the risk assessment are presented in Table 9.9-2.

Table 9.9-2: Assessment of the risk for non-target plants due to the use of BAS 758 00 F according to the proposed use pattern

Intended use		cereals		
Product		BAS 758 00 F		
Application rate (L/ha)		2 x 1.5		
MAF		n/a		
Test species	ER₅₀ (L/ha) ¹⁾	Drift rate (%)	PER_{off-field} (L/ha)	TER criterion: TER ≥ 5
<i>Daucus carota</i> _d (carrot) <i>Lactuca sativa</i> _d (lettuce) <i>Brassica napus</i> _d (oilseed rape) <i>Brassica oleracea</i> _d (cabbage) <i>Glycine max</i> _d (soybean) <i>Solanum lycopersicum</i> _d (tomato) <i>Allium cepa</i> _m (onion) <i>Lolium multiflorum</i> _m (ryegrass) <i>Triticum aestivum</i> _m (wheat) <i>Zea mays</i> _m (corn)	> 1.5	2.77	0.0416	> 36

MAF: Multiple application factor; PER: Predicted environmental rate; TER: toxicity to exposure ratio.

¹⁾ Worst case endpoint derived from vegetative vigour and seedling emergence.

9.9.2.2 Tier-2 risk assessment (based on dose-response data)

Not relevant.

9.9.2.3 Higher-tier risk assessment

Not relevant.

9.9.2.4 Risk mitigation measures

No risk mitigation needed.

9.9.3 Overall conclusions

Based on the risk assessment it can be concluded that BAS 758 00 F poses no unacceptable risk to non-target plants, if applied according to the recommended use pattern. Particular precautions to reduce the environmental concentrations resulting from BAS 758 00 F applications are not required for the protection of terrestrial non-target plants.

Review Comments:

The risk assessment is based on the “Guidance Document on Terrestrial Ecotoxicology”, (SANCO/10329/2002 rev.2 final, 2002).

Based on the risk assessment it can be concluded that the proposed use of BAS 758 00 F poses no unacceptable risk to non-target plants, if applied according to the recommended use pattern. Particular precautions to reduce the environmental concentrations resulting from BAS 758 00 F applications are not required for the protection of terrestrial non-target plants.

9.10 Effects on other terrestrial organisms (flora and fauna) (KCP 10.7)

Not relevant.

9.11 Monitoring data (KCP 10.8)

According to the knowledge of the applicant, there are currently no monitoring studies available which assess ecotoxicological effects on BAS 758 00 F or one of the active substances.

9.12 Classification and Labelling

According to (EC) No 1272/2008 (CLP) plant protection products must be classified for their environmental hazard (acute and chronic). Classification is based on acute and chronic product data if adequate data is available. When sufficient product data is not available, the summation method is carried out instead.

For the product BAS 758 00 F acute data (LC₅₀/EC₅₀) are available for all trophic levels. Regarding chronic toxicity, adequate data are available for algae only, thus chronic classification will be based on the summation method using data on the active substance. In Annex IV of Commission Regulation (EC) 1272/2008 (CLP), mefentrifluconazole is listed with chronic hazard category 1 (H410), and M = 1. Pyraclostrobin is also listed in Annex IV with the chronic category 1 (H410) and M = 100. Metrafenone is currently not listed in Annex IV, thus chronic classification is based on the lowest endpoint for the active substance. The relevant data for classification purposes has been shown in Table 9.12-1.

Table 9.12-1: Ecotoxicology/Environment data relevant for classification of BAS 758 00 F

Substance tested	Study Type (duration)	Findings	Triggered classification and labelling	Reference
Acute (short-term) aquatic hazard				
BAS 758 00 F	<i>Oncorhynchus mykiss</i> (96 h)	LC ₅₀ = 0.0884 mg/L	Aquatic acute hazard cat. 1 (H400)	BASF DocID 2020/2033900
BAS 758 00 F	<i>Daphnia magna</i> (48 h)	EC ₅₀ = 0.362 mg/L	No aquatic acute hazard cat.	BASF DocID 2020/2033902
BAS 758 00 F	<i>Pseudokirchneriella subcapitata</i> (72 h)	ErC ₅₀ = 3.82 mg/L	No aquatic acute hazard cat.	BASF DocID 2020/2033904
		NOEC = 0.16 mg/L	Aquatic chronic hazard cat. 2 (H411)	
Chronic (long-term) aquatic hazard				
Mefentrifluconazole (BAS 750 F) ¹⁾	--	--	Aquatic chronic hazard cat. 1 (H410); M = 1	Legal classification according to Annex IV of (EC) 1272/2008
	Biodegradation	not readily biodegradable	--	BASF DocID 2014/1239574
Pyraclostrobin (BAS 500 F) ²⁾	--	--	Aquatic chronic hazard cat. 1 (H410); M = 100	Legal classification according to Annex IV of (EC) 1272/2008
	Biodegradation	not readily biodegradable	--	BASF DocID 1999/10655
Metrafenone (BAS 560 F) ³⁾	<i>Americamysis bahia</i> (28 d)	NOEC = 0.022 mg/L	Aquatic chronic hazard cat. 1 (H410); M = 1	BASF DocID 2007/7009454
	Biodegradation	not readily biodegradable	--	EFSA Scientific Report (2006) 58, 1- 72

¹⁾ Nominal contents within the formulated product: **66.6 g/L (6.1% w/w)**.

²⁾ Nominal contents within the formulated product: **80.0 g/L (7.33% w/w)**.

³⁾ Nominal contents within the formulated product: **100.0 g/L (9.16% w/w)**.

Based on the lowest acute aquatic toxicity endpoint obtained with BAS 758 00 F aquatic acute hazard category 1 (H400) is given according to (EC) No 1272/2008 (CLP).

Regarding chronic classification, mefentrifluconazole (a.s. content of 6.1% w/w within the product) classified as chronic hazard cat. 1 (M=1), pyraclostrobin (a.s. content of 7.33% w/w within the product) classified as chronic hazard cat. 1 (M=100) and metrafenone (a.s. content of 9.16% w/w within the product) classified as chronic hazard cat. 1 (M=1), are considered for the summation method in the 1st equation according to CLP ($M \times \text{hazard cat. 1}$), yielding a value which is above the trigger of 25%. Hence, BAS 758 00 F is classified as aquatic chronic hazard category 1 (H410). Chronic classification of BAS 758 00 F using the summation method is summarized in Table 9.12-2.

Table 9.12-2: Chronic classification of BAS 758 00 F using the summation method according to (EC) No 1272/2008

Chronic classification of BAS 758 00 F						
Formulation component				Result (% Content x M-Factor)		
Name	Chronic Category	M-Factor	Content in BAS CODE [%]			
BAS 750 F	1	1	6.1	6.1		
BAS 500 F	1	100	7.33	733		
BAS 560 F	1	1	9.16	9.16		
1 st equation	SUM (<i>M x Chronic 1</i>)			748.26	≥ 25 %	BAS 758 00 F: Aquatic Chronic Hazard Category 1

Conclusion

Based on the data obtained with the product and the lowest chronic aquatic toxicity endpoints of the classified compounds within the formulated product the following classification and labelling is proposed for BAS 758 00 F: **aquatic acute hazard category 1 (H400); aquatic chronic hazard category 1 (H410)** according to GHS following Regulation (EC) No 1272/2008.

Appendix 1 Lists of data considered in support of the evaluation

Tables considered not relevant can be deleted as appropriate.

MS to blacken authors of vertebrate studies in the version made available to third parties/public.

List of data submitted by the applicant and relied on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 40.1.1.1/1	XXXXXXXXXX	2014	BAS 500 F (Pyraclostrobin)—Acute toxicity in the canary (Serinus canaria) after single oral administration (LD50) 2013/1400375 XXXXXXXXXXXXXXXXXXXXX. yes Unpublished	Yes	BASF
KCP 40.1.1.1/2	XXXXXXXXXX	2011	BAS 560 F (Metrafenone)—Acute toxicity in the zebra finch (Taeniopygia guttata) after single oral administration (LD50) 2011/1263863 XXXXXXXXXXXXXXXXXXXXX yes Unpublished	Yes	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.1.1.1/3	XXXXXXXXXX	2021	BAS 758 00 F: An Acute Oral Toxicity Study with the Northern Bobwhite Using a Sequential Testing Procedure 2021/2037989 XXXXXXXXXXXXXXXXXXXXX yes Unpublished	Yes	BASF
KCP 10.1.1.2/4	XXXXXXXXXX	2006	BAS 560 F—1 generation reproduction study on the mallard duck (Anas platyrhynchos) by administration in the diet 2006/1018046 XXXXXXXXXXXXXXXXXXXXX yes Unpublished	Yes	BASF
KCP 10.1.2/1	Anonymous	2014	Pyraclostrobin - Ecologically relevant reproductive toxicity endpoint for the wild mammal risk assessment 2014/1010736 BASF SE, Limburgerhof, Germany Fed.Rep. no Unpublished	No	BASF
KCP 10.1.2.2/1	Moreno, S.	2013	Study on the residue behaviour of Pyraclostrobin (BAS 500 F) on wheat (young plants) after treatment with BAS 500 06 F under field conditions in North and South Europe, season 2012 2013/1045207 Agricultura y Ensayo S.L., Alcala de Guadaira, Spain yes Unpublished	No	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.1.2.2/2	Martin, T.	2013	Study on the residue behavior of Pyraclostrobin (BAS 500 F) on pea (young plants) after the application of BAS 500 06 F under field conditions in France (North), Germany, United Kingdom, Italy and Spain, 2012 2013/1044539 Agrologia SL, Utrera, Spain yes Unpublished	No	BASF
KCP 10.1.2.2/3	Erzgraeber, B.	2013	Dissipation of BAS 500 F - Pyraclostrobin on young plants (wheat and peas) - Trials conducted in the Northern Zone of Europe - Calculation of DT50 / DT90 dissipation times 2013/1078114 BASF SE, Limburgerhof, Germany Fed.Rep. no Unpublished	No	BASF
KCP 10.1.2.2/4	Kramm, R.	2017	Study on the residue behaviour of BAS 500 F (Pyraclostrobin) in young wheat plants after a foliar application with BAS 500 06 F at growth stage 25/29 under field conditions in Northern Europe, in spring 2016 2017/1029774 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 10.1.2.2/5	Schroeder, T.	2017	Dissipation of BAS 500 F - Pyraclostrobin on young wheat plants from field trials conducted in Northern zone of Europe - Calculation of DT50 dissipation times 2017/1037247 BASF SE, Limburgerhof, Germany Fed.Rep. no Unpublished	No	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.1.2.2/6	Galvez, O., Moreno, S.	2019	Study on the residue behaviour of Mefentrifluconazole (BAS 750 F) on wheat (young plants) after treatment with BAS 750 05 F under field conditions in North and South Europe, season 2018 2018/1205816 Agricultura y Ensayo S.L., Alcala de Guadaira, Spain yes Unpublished	No	BASF
KCP 10.1.2.2/7	Moreno, S.	2019	Study on the residue behaviour of Mefentrifluconazole (BAS 750 F) on pea (young plants) after treatment with BAS 750 05 F under field conditions in North and South Europe, season 2018 2018/1205813 Agricultura y Ensayo S.L., Alcala de Guadaira, Spain yes Unpublished	No	BASF
KCP 10.1.2.2/8	Szegedi, K.	2019	Calculation of DT50 dissipation times for BAS 750 F—Mefentrifluconazole on wheat plants under field conditions in North and South Europe 2019/2034648 BASF SE, Limburgerhof, Germany Fed.Rep. no Unpublished	No	BASF
KCP 10.1.2.2/9	Szegedi, K.	2019	Calculation of DT50 dissipation times for BAS 750 F—Mefentrifluconazole on pea plants under field conditions in North and South Europe 2019/2034650 BASF SE, Limburgerhof, Germany Fed.Rep. no Unpublished	No	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.1.2.2/10	XXXXXXXXXX	2014	Field study on the acute and long term effects of a Pyraclostrobin formulation (BAS 500 06 F) applied as foliar spray in spring to cereals on populations of small mammals (wood mice and common voles) in Central Europe (Germany) 2014/1000041 XXXXXXXXXXXXXXXXXXXXXXXXXXXX yes Unpublished	No	BASF
KCP 10.1.2.2/11	XXXXXXXXXX	2016	Field study on the acute and long term effects of a Pyraclostrobin formulation (BAS 500 06 F) applied as foliar spray on meadows to populations of common voles in Central Europe (Germany) 2015/1126803 XXXXXXXXXXXXXXXXXXXXXXXXXXXX yes Unpublished	No	BASF
KCP 10.2/4	Janson, G.	2015	Report Amendment No.1—Chronic toxicity of BAS 750 F (Reg.No. 5834378) to Daphnia longispina in a 21-day semi-static test 2015/1251197 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.2/2	Backfisch, K., Kusebauch, B.	2017	Report Amendment 1: Chronic toxicity of Reg.No. 5834378 to the non-biting midge Chironomus riparius – A spiked sediment study 2017/1044236 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 10.2/3	Backfisch, K., Kusebauch, B.	2017	Amendment No. 1: Chronic toxicity of Reg.No. 5924326 (M750F003; metabolite of BAS 750 F) to the non-biting midge Chironomus riparius - A spiked sediment study 2017/1044237 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 10.2/4	Rzodeczko, H.	2018	Amendment No. 1: BAS 750 F (Reg.No. 5834378) – Lemna gibba CPCC 310, Growth inhibition test 2018/1220943 Institute of Industrial Organic Chemistry, Pszczyna, Poland yes Unpublished	No	BASF
KCP 10.2.1/1	XXXXXXXXXX	2016	BAS 750 F – Acute toxicity study in the fathead minnow (Pimephales promelas) 2016/1155889 XXXXXXXXXXXXXXXXXXXXXXXXXXXX yes Unpublished	Yes	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.2.1/2	XXXXXXXXXX	2019	Reg.No. 6003433 (metabolite of BAS 750 F) - Acute toxicity study in the rainbow trout (<i>Oncorhynchus mykiss</i>) 2019/1022695 XXXXXXXXXXXXXXXXXXXXXXXXXXXX yes Unpublished	Yes	BASF
KCP 10.2.1/3	XXXXXXXXXX	2005	BAS 560 F: A 96-hour flow-through acute toxicity test with the sheepshead minnow (<i>Cyprinodon variegatus</i>) 2005/7003439 XXXXXXXXXXXXXXXXXXXXXXXXXXXX yes Unpublished	Yes	BASF
KCP 10.2.1/4	XXXXXXXXXX	2021	BAS 758 00 F – Acute Toxicity to Rainbow trout (<i>Oncorhynchus mykiss</i>) in a static 96-Hour Test 2020/2033900 XXXXXXXXXXXXXXXXXXXXXXXXXXXX yes Unpublished	No Yes	BASF
KCP 10.2.1/5	Palmer, S.	2005	BAS 560 F: A 96-hour shell deposition test with the eastern oyster (<i>Crassostrea virginica</i>) 2005/7003442 Wildlife International Ltd., Easton MD, United States of America yes Unpublished	No	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.2.1/6	Eckenstein, H.	2021	BAS 758 00 F - Effect on Daphnia magna in a static 48-Hour Immobilization Test 2020/2033902 IES - Innovative Environmental Services Ltd., Witterswil, Switzerland yes Unpublished	No	BASF
KCP 10.2.1/7	Hoffmann, F.	2012	Effect of BAS 560 F (Metrafenone, Reg.No. 4037710) on the growth of the green alga Pseudokirchneriella subcapitata 2011/1254828 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 10.2.1/8	Eckenstein, H.	2021	BAS 758 00 F - Effect on Pseudokirchneriella subcapitata in a 72 Hour Algal Growth Inhibition Test 2020/2033904 IES - Innovative Environmental Services Ltd., Witterswil, Switzerland yes Unpublished	No	BASF
KCP 10.2.1/9	Boeri, R., Ward, T., Magazu, J.	2000	Growth and reproduction toxicity test with BAS 500 F and the duckweed, Lemna gibba G3 2000/5037 T.R. Wilbury Laboratories Inc., Marblehead MA, United States of America yes Unpublished	No	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.2.1/10	Anonymous	2019	Addendum to study BASF DocID: 2000/5037 – Growth and Reproduction Toxicity with BAS 500 F and the Duckweed, Lemna gibba G3 2019/2036269 BASF SE, Limburgerhof, Germany Fed.Rep. no Unpublished	No	BASF
KCP 10.2.1/11	Hoffmann, F.	2012	Effect of BAS 560 F (Metrafenone, Reg.No. 4037710) on the growth of Lemna gibba 2011/1254832 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 10.2.2/1	XXXXXXXXXX	1999	BAS 500 F – Early life stage toxicity test on the rainbow trout (Oncorhynchus mykiss WALBAUM 1792) in a flow through system with variable concentrations 1999/11537 XXXXXXXXXXXXXXXXXXXXXXXXXXXX yes Unpublished	Yes	BASF
KCP 10.2.2/2	XXXXXXXXXX	2018	Amendment 1: BAS 500 F – Early life stage toxicity test on the rainbow trout (Oncorhynchus mykiss WALBAUM 1792) in a flow through system with variable concentrations 2018/1123384 XXXXXXXXXXXXXXXXXXXXXXXXXXXX yes Unpublished	Yes	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.2.2/3	XXXXXXXXXX	2001	Early life stage toxicity of BAS 500 F to the sheepshead minnow, <i>Cyprinodon variegatus</i> 2000/5247 XXXXXXXXXXXXXXXXXXXXXXXXXXXX yes Unpublished	Yes	BASF
KCP 10.2.2/4	XXXXXXXXXX	2000	Early life stage toxicity of BAS 500 F to the fathead minnow, <i>Pimephales promelas</i> 2000/5053 XXXXXXXXXXXXXXXXXXXXXXXXXXXX yes Unpublished	Yes	BASF
KCP 10.2.2/5	XXXXXXXXXX	2012	BAS 560 F (Metrafenone) — Early life stage toxicity test on the fathead minnow (<i>Pimephales promelas</i>) in a flow through system 2012/1009601 XXXXXXXXXXXXXXXXXXXXXXXXXXXX yes Unpublished	Yes	BASF
KCP 10.2.2/6	Obermann, M.	2012	Concentration control analysis of BAS 560 F (Metrafenone) in mixing water, GV/T project no. 50F0437/01E002 2012/1016030 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.2.2/7	Cafarella, M.	2007	BAS 560 F - Life-cycle toxicity test with mysids (Americamysis bahia) 2007/7009454 Springborn Smithers Laboratories, Wareham MA, United States of America yes Unpublished	No	BASF
KCP 10.2.2/8	Kuhl, R.	2013	Effects of BAS 500 F (Pyraclostrobin) on the development of sediment dwelling larvae of Chironomus riparius in a sediment-water system - Exposed via spiked sediment 2012/1185699 Institut fuer Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 10.2.2/9	Kuhl, R.	2013	Effects of Reg.No. 340266 (metabolite of BAS 500 F (Pyraclostrobin), synonymous: 500M07, BF 500-3) on the development of sediment dwelling larvae of Chironomus riparius in a sediment-water system - exposed via spiked sediment 2013/1237446 Institut fuer Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 10.2.2/10	Backfisch, K.	2014	Chronic toxicity of Reg. No. 364380 (BF 500-6; metabolite of Pyraclostrobin) to the non-biting midge Chironomus riparius - A spiked sediment study 2014/1001481 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.2.2/11	Backfisch, K.	2014	Chronic toxicity of Reg.No. 369315 (BF 500 7; Metabolite of Pyraclostrobin) to the non-biting midge Chironomus riparius – A spiked sediment study 2014/1001482 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 10.2.2/12	Backfisch, K., Weltje, L.	2011	Chronic toxicity of Reg.No. 4037710 (BAS 560 F; Metrafenone) to the non-biting midge Chironomus riparius - A spiked sediment study 2010/1145509 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 10.3.1.1.1/1	Sekine, T.	2013	Effects of BAS 500 F (Acute Contact and Oral) on Honey Bees (Apis mellifera L.) in the Laboratory 2013/1003210 IBACON GmbH yes Unpublished	No	
KCP 10.3.1.1.1/2	Amsel, K.	2016	Acute toxicity of BAS 500 F to the bumblebee Bombus terrestris L. under laboratory conditions 2016/1000530 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished	No	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.3.1.1.1/3	Franke, M.	2020	Acute toxicity of BAS 758 00 F to the honeybee <i>Apis mellifera</i> L. under laboratory conditions 2020/2037657 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 10.3.1.1.2/4	Sekine, T.	2013	Effects of BAS 500 F (Acute Contact and Oral) on Honey Bees (<i>Apis mellifera</i> L.) in the Laboratory 2013/1003210 IBACON GmbH yes Unpublished	No	
KCP 10.3.1.1.2/2	Amsel, K.	2016	Acute toxicity of BAS 500 F to the bumblebee <i>Bombus terrestris</i> L. under laboratory conditions 2016/1000530 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 10.3.1.1.2/3	Franke, M.	2020	Acute toxicity of BAS 758 00 F to the honeybee <i>Apis mellifera</i> L. under laboratory conditions 2020/2037657 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished	No	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.3.1.2/1	Altenburg, M., Obermann, M.	2017	Honey bee (Apis mellifera), chronic oral toxicity test with BAS 500 F (Pyraclostrobin) under laboratory conditions 2017/1142796 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 10.3.1.2/2	Altenburg, M., Obermann, M.	2019	Amendment 1: Honey bee (Apis mellifera), chronic oral toxicity test with BAS 500 F (Pyraclostrobin) under laboratory conditions 2019/1024628 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 10.3.1.2/3	Verge, E.	2014	BAS 560 02 F and BAS 560 AA F blank formulation—Assessment of effects on the adult honey bee, Apis mellifera L., in 10 days chronic feeding test under laboratory conditions 2014/1093920 Eurofins Agrosience Services EcoChem GmbH, Niefern-Oeschelbronn, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 10.3.1.2/4	Dressler, K.	2021	Chronic toxicity of BAS 758 00 F to the honey bee Apis mellifera L. under laboratory conditions 2021/2008152 BioChem agrar GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished	No	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.3.1.3/1	Royer, S.	2017	Repeated exposure of BAS 500 F (Pyraclostrobin) to honey bee (Apis mellifera) larvae under laboratory conditions (in vitro) 2017/1142794 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 10.3.1.3/2	Royer, S.	2017	Amendment No. 1: Repeated exposure of BAS 500 F (Pyraclostrobin) to honey bee (Apis mellifera) larvae under laboratory conditions (in vitro) 2017/1142798 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 10.3.1.3/3	Eckert, J.	2015	BAS 560 02 F and BAS 560 AA F blank formulation—Honey bee (Apis mellifera L.) larval toxicity test (repeated feeding exposure) 2014/1093921 Eurofins Agrosience Services EcoChem GmbH, Niefern-Oeschelbronn, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 10.3.1.3/4	Schmidt, K.	2022	Repeated exposure of honey bee (Apis mellifera L.) larvae - to BAS 758 00 F under laboratory conditions 2021/2008153 BioChem agrar GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished	No	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.3.1.5/1	Schnurr, A.	2022	Effects of BAS 758 00 F on the honeybee <i>Apis mellifera</i> L. under semi-field conditions (tunnel test) with additional assessments on colony and brood development 2021/2047630 BioChem agrar GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 10.3.2.1/1	Roehlig, U.	2020	Effects of BAS 758 00 F on the predatory mite <i>Typhlodromus pyri</i> Scheuten in a laboratory test 2020/2037663 BioChem agrar GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 10.3.2.1/2	Roehlig, U.	2020	Effects of BAS 758 00 F on the parasitic wasp <i>Aphidius rhopalosiphi</i> (DESTEFANI-PEREZ) in a laboratory test 2020/2037662 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Machern OT Gerichshain, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 10.3.2.2/1	Roehlig, U.	2021	Effects of BAS 758 00 F on the parasitic wasp <i>Aphidius rhopalosiphi</i> (DESTEFANI-PEREZ) in an extended laboratory test 2021/2015154 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished	No	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.3.2.2/2	Roehlig, U.	2021	Effects of BAS 758 00 F on the green lacewing Chrysoperla carnea STEPH. in an extended laboratory test 2021/2015155 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 10.3.2.2/3	Roehlig, U.	2021	Effects of BAS 758 00 F on the green lacewing Chrysoperla carnea STEPH. In an extended laboratory test (under semi-field conditions aged residues on potted bean plants) 2021/2027050 BioChem agrar GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 10.4.1.1/1	Friedrich, S.	2014	Sublethal toxicity of BAS 500 F (Pyraclostrobin) to the earthworm Eisenia fetida in artificial soil 2014/1000461 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 10.4.1.1/2	Ganssmann, M.	2013	Effects of Reg.No. 364380 (metabolite of BAS 500 F, Pyraclostrobin) on reproduction and growth of earthworms Eisenia fetida in artificial soil 2013/1003174 Institut fuer Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany Fed.Rep. yes Unpublished	No	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.4.1.1/3	Ganssmann, M.	2013	Effects of Reg.No. 369315 (metabolite of BAS 500 F, Pyraclostrobin) on reproduction and growth of earthworms Eisenia fetida in artificial soil with 10% peat 2013/1224029 Institut fuer Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 10.4.1.1/4	Witte, B.	2011	Effects of BAS 560 02 F on reproduction and growth of earthworms Eisenia fetida in artificial soil with 5% peat 2011/1000384 Institut fuer Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 10.4.1.1/5	McCormac, A.	2014	CL377160—Determination of chronic toxicity to the earthworm Eisenia andrei in an artificial soil substrate 2014/1093923 Mambo Tox Ltd., Southampton SO16 7NP, United Kingdom yes Unpublished	No	BASF
KCP 10.4.1.1/6	McCormac, A.	2015	CL 3000402—Determination of chronic toxicity to the earthworm Eisenia andrei in an artificial soil substrate 2015/1041971 Mambo Tox Ltd., Southampton SO16 7NP, United Kingdom yes Unpublished	No	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.4.1.1/7	Friedrich, S.	2021	Effects of BAS 758 00 F on the reproduction of the earthworm Eisenia andrei in artificial soil 2020/2037658 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 10.4.2.1/4	Ganssmann, M.	2013	Effects of Reg.No. 364380 (metabolite of BAS 500 F, Pyraclostrobin) on reproduction of the collembola Folsomia candida in artificial soil with 5% peat 2013/1068054 Institut fuer Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 10.4.2.1/2	Braaker, S.	2019	Addendum to study BASF DocID: 2013/1068054— Effects of Reg. No. 364380 (Metabolite of BAS 500 F, Pyraclostrobin) on Reproduction of the Collembola Folsomia candida in Artificial Soil with 5% Peat 2019/2034464 BASF SE, Limburgerhof, Germany Fed.Rep. no Unpublished	No	BASF
KCP 10.4.2.1/3	Ganssmann, M.	2013	Effects of Reg.No. 369315 (metabolite of BAS 500 F, Pyraclostrobin) on reproduction of the collembola Folsomia candida in artificial soil with 5% peat 2013/1224030 Institut fuer Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany Fed.Rep. yes Unpublished	No	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.4.2.1/4	Braaker, S.	2019	Addendum to study BASF DocID: 2013/1224030— Effects of Reg. No 369315 (Metabolite of BAS 500 F, pyraclostrobin) on Reproduction of the Collembola Folsomia candida in Artificial Soil with 5% Peat 2019/2034467 BASF SE, Limburgerhof, Germany Fed.Rep. no Unpublished	No	BASF
KCP 10.4.2.1/5	Ganssmann, M.	2013	Effects of BAS 560 02 F on reproduction of the Collembola Folsomia candida in artificial soil with 5% peat 2013/1003203 Institut fuer Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 10.4.2.1/6	Friedrich, S.	2020	Effects of BAS 758 00 F on the reproduction of the collembolan Folsomia candida 2020/2037659 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 10.4.2.1/7	Schulz, L.	2020	Effects of BAS 758 00 F on the reproduction of the predatory mite Hypoaspis aculeifer 2020/2037660 BioChem agrar GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished	No	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.5/1	Schoebinger, U.	2015	CL3000402: Effects on the activity of the soil microflora under laboratory conditions (nitrogen transformation) 2015/1132053 Eurofins Agrosience Services EcoChem GmbH, Niefern-Oeschelbronn, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 10.5/2	Schulz, L.	2020	Effects of BAS 758 00 F on the activity of soil microflora (Nitrogen Transformation test) 2020/2037661 BioChem agrar GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 10.6.2/1	Teresiak-Baumgart, P.	2020	Effects of BAS 758 00 F on vegetative vigour of ten species of terrestrial plants under greenhouse conditions 2020/2037665 Agro-Check Dr. Teresiak & Erdmann GbR, Lentzke, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 10.6.2/2	Teresiak-Baumgart, P.	2020	Effect of BAS 758 00 F on seedling emergence and seedling growth of ten species of terrestrial plants under greenhouse conditions 2020/2037664 Agro-Check Dr. Teresiak & Erdmann GbR, Lentzke, Germany Fed.Rep. yes Unpublished	No	BASF

List of data submitted or referred to by the applicant and relied on, but already evaluated at EU peer review

Please refer to Part A

The following tables are to be completed by MS

List of data submitted by the applicant and not relied on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.1.1.1/1	XXXXXXXXXX	2014	BAS 500 F (Pyraclostrobin) - Acute toxicity in the canary (Serinus canaria) after single oral administration (LD50) 2013/1400375 XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX yes Unpublished	Yes	BASF
KCP 10.1.1.1/2	XXXXXXXXXX	2011	BAS 560 F (Metrafenone) - Acute toxicity in the zebra finch (Taeniopygia guttata) after single oral administration (LD50) 2011/1263863 XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX yes Unpublished	Yes	BASF
KCP 10.1.1.2/1	XXXXXXXXXX	2006	BAS 560 F - 1-generation reproduction study on the mallard duck (Anas platyrhynchos) by administration in the diet 2006/1018046 XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX yes Unpublished	Yes	BASF
KCP 10.1.2.2/6	Galvez, O., Moreno, S.	2019	Study on the residue behaviour of Mefentrifluconazole (BAS 750 F) on wheat (young plants) after treatment with BAS 750 05 F under field conditions in North and South Europe, season 2018 2018/1205816	No	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			Agricultura y Ensayo S.L., Alcala de Guadaira, Spain yes Unpublished		
KCP 10.1.2.2/7	Moreno, S.	2019	Study on the residue behaviour of Mefentrifluconazole (BAS 750 F) on pea (young plants) after treatment with BAS 750 05 F under field conditions in North and South Europe, season 2018 2018/1205813 Agricultura y Ensayo S.L., Alcala de Guadaira, Spain yes Unpublished	No	BASF
KCP 10.1.2.2/8	Szegedi, K.	2019	Calculation of DT50 dissipation times for BAS 750 F - Mefentrifluconazole on wheat plants under field conditions in North and South Europe 2019/2034648 BASF SE, Limburgerhof, Germany Fed.Rep. no Unpublished	No	BASF
KCP 10.1.2.2/9	Szegedi, K.	2019	Calculation of DT50 dissipation times for BAS 750 F - Mefentrifluconazole on pea plants under field conditions in North and South Europe 2019/2034650 BASF SE, Limburgerhof, Germany Fed.Rep. no Unpublished	No	BASF
KCP 10.1.2.2/10	XXXXXXXXXX	2014	Field study on the acute and long-term effects of a Pyraclostrobin formulation (BAS 500 06 F) applied as foliar spray in spring to cereals on populations of small mammals (wood mice and common voles) in Central Europe (Germany)	Yes	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			2014/1000041 XXXXXXXXXXXXXXXXXXXXXXXXXXXX yes Unpublished		
KCP 10.1.2.2/11	XXXXXXXXXX	2016	Field study on the acute and long-term effects of a Pyraclostrobin formulation (BAS 500 06 F) applied as foliar spray on meadows to populations of common voles in Central Europe (Germany) 2015/1126803 XXXXXXXXXXXXXXXXXXXXXXXXXXXX yes Unpublished	Yes	BASF
KCP 10.2/1	Janson, G.	2015	Report Amendment No.1 - Chronic toxicity of BAS 750 F (Reg.No. 5834378) to Daphnia longispina in a 21 day semi-static test 2015/1251197 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 10.2/2	Backfisch, K., Kusebauch, B.	2017	Report Amendment 1: Chronic toxicity of Reg.No. 5834378 to the non-biting midge Chironomus riparius - A spiked sediment study 2017/1044236 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 10.2/4	Rzodeczko, H.	2018	Amendment No. 1: BAS 750 F (Reg.No. 5834378) - Lemna gibba CPCC 310, Growth inhibition test	No	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			2018/1220943 Institute of Industrial Organic Chemistry, Pszczyna, Poland yes Unpublished		
KCP 10.2.1/1	XXXXXXXXXX	2016	BAS 750 F - Acute toxicity study in the fathead minnow (Pimephales promelas) 2016/1155889 XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX yes Unpublished	Yes	BASF
KCP 10.2.1/7	Hoffmann, F.	2012	Effect of BAS 560 F (Metrafenone, Reg.No. 4037710) on the growth of the green alga Pseudokirchneriella subcapitata 2011/1254828 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 10.2.1/9	Boeri, R., Ward, T., Magazu, J.	2000	Growth and reproduction toxicity test with BAS 500 F and the duckweed, Lemna gibba G3 2000/5037 T.R. Wilbury Laboratories Inc., Marblehead MA, United States of America yes Unpublished	No	BASF
KCP 10.2.1/10	Anonymous	2019	Addendum to study BASF DocID: 2000/5037 - Growth and Reproduction Toxicity with BAS 500 F and the Duckweed, Lemna gibba G3	No	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			2019/2036269 BASF SE, Limburgerhof, Germany Fed.Rep. no Unpublished		
KCP 10.2.1/11	Hoffmann, F.	2012	Effect of BAS 560 F (Metrafenone, Reg.No. 4037710) on the growth of Lemna gibba 2011/1254832 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 10.2.2/1	XXXXXXXXXX	1999	BAS 500 F - Early life-stage toxicity test on the rainbow trout (Oncorhynchus mykiss WALBAUM 1792) in a flow through system with variable concentrations 1999/11537 XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX yes Unpublished	Yes	BASF
KCP 10.2.2/2	XXXXXXXXXX	2018	Amendment 1: BAS 500 F - Early life-stage toxicity test on the rainbow trout (Oncorhynchus mykiss WALBAUM 1792) in a flow through system with variable concentrations 2018/1123384 XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX yes Unpublished	Yes	BASF
KCP 10.2.2/3	XXXXXXXXXX	2001	Early life stage toxicity of BAS 500 F to the sheepshead minnow, Cyprinodon variegatus	Yes	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			2000/5247 XXXXXXXXXXXXXXXXXXXXXXXXXXXX yes Unpublished		
KCP 10.2.2/4	XXXXXXXXXX	2000	Early life stage toxicity of BAS 500 F to the fathead minnow, Pimephales promelas 2000/5053 XXXXXXXXXXXXXXXXXXXXXXXXXXXX yes Unpublished	Yes	BASF
KCP 10.2.2/5	XXXXXXXXXX	2012	BAS 560 F (Metrafenone) - Early life-stage toxicity test on the fathead minnow (Pimephales promelas) in a flow through system 2012/1009601 XXXXXXXXXXXXXXXXXXXXXXXXXXXX yes Unpublished	Yes	BASF
KCP 10.2.2/6	Obermann, M.	2012	Concentration control analysis of BAS 560 F (Metrafenone) in mixing-water, GV/T project-no. 50F0437/01E002 2012/1016030 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 10.2.2/8	Kuhl, R.	2013	Effects of BAS 500 F (Pyraclostrobin) on the development of sediment dwelling larvae of Chironomus riparius in a sediment-water system - Exposed via spiked sediment	No	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			2012/1185699 Institut fuer Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany Fed.Rep. yes Unpublished		
KCP 10.2.2/10	Backfisch, K.	2014	Chronic toxicity of Reg. No. 364380 (BF 500-6; metabolite of Pyraclostrobin) to the non-biting midge Chironomus riparius - A spiked sediment study 2014/1001481 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 10.2.2/11	Backfisch, K.	2014	Chronic toxicity of Reg.No. 369315 (BF 500-7; Metabolite of Pyraclostrobin) to the non-biting midge Chironomus riparius - A spiked sediment study 2014/1001482 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 10.3.1.1.1/1	Sekine, T.	2013	Effects of BAS 500 F (Acute Contact and Oral) on Honey Bees (Apis mellifera L.) in the Laboratory 2013/1003210 IBACON GmbH yes Unpublished	No	
KCP 10.3.1.1.1/2	Amsel, K.	2016	Acute toxicity of BAS 500 F to the bumblebee Bombus terrestris L. under laboratory conditions	No	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			2016/1000530 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished		
KCP 10.3.1.1.2/1	Sekine, T.	2013	Effects of BAS 500 F (Acute Contact and Oral) on Honey Bees (Apis mellifera L.) in the Laboratory 2013/1003210 IBACON GmbH yes Unpublished	No	
KCP 10.3.1.1.2/2	Amsel, K.	2016	Acute toxicity of BAS 500 F to the bumblebee Bombus terrestris L. under laboratory conditions 2016/1000530 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 10.3.1.2/1	Altenburg, M., Obermann, M.	2017	Honey bee (Apis mellifera), chronic oral toxicity test with BAS 500 F (Pyraclostrobin) under laboratory conditions 2017/1142796 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 10.3.1.2/2	Altenburg, M., Obermann, M.	2019	Amendment 1: Honey bee (Apis mellifera), chronic oral toxicity test with BAS 500 F (Pyraclostrobin) under laboratory conditions 2019/1024628	No	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished		
KCP 10.3.1.2/3	Verge, E.	2014	BAS 560 02 F and BAS 560 AA F blank formulation - Assessment of effects on the adult honey bee, Apis mellifera L., in 10 days chronic feeding test under laboratory conditions 2014/1093920 Eurofins Agrosience Services EcoChem GmbH, Niefern-Oeschelbronn, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 10.3.1.3/1	Royer, S.	2017	Repeated exposure of BAS 500 F (Pyraclostrobin) to honey bee (Apis mellifera) larvae under laboratory conditions (in vitro) 2017/1142794 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 10.3.1.3/2	Royer, S.	2017	Amendment No. 1: Repeated exposure of BAS 500 F (Pyraclostrobin) to honey bee (Apis mellifera) larvae under laboratory conditions (in vitro) 2017/1142798 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 10.3.1.3/3	Eckert, J.	2015	BAS 560 02 F and BAS 560 AA F blank formulation - Honey bee (Apis mellifera L.) larval toxicity test (repeated feeding exposure)	No	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			2014/1093921 Eurofins Agroscience Services EcoChem GmbH, Niefern-Oeschelbronn, Germany Fed.Rep. yes Unpublished		
KCP 10.4.1.1/2	Ganssmann, M.	2013	Effects of Reg.No. 364380 (metabolite of BAS 500 F, Pyraclostrobin) on reproduction and growth of earthworms Eisenia fetida in artificial soil 2013/1003174 Institut fuer Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 10.4.1.1/3	Ganssmann, M.	2013	Effects of Reg.No. 369315 (metabolite of BAS 500 F, Pyraclostrobin) on reproduction and growth of earthworms Eisenia fetida in artificial soil with 10% peat 2013/1224029 Institut fuer Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 10.4.1.1/4	Witte, B.	2011	Effects of BAS 560 02 F on reproduction and growth of earthworms Eisenia fetida in artificial soil with 5% peat 2011/1000384 Institut fuer Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 10.4.1.1/5	McCormac, A.	2014	CL377160 - Determination of chronic toxicity to the earthworm Eisenia andrei in an artificial soil substrate	No	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			2014/1093923 Mambo-Tox Ltd., Southampton SO16 7NP, United Kingdom yes Unpublished		
KCP 10.4.1.1/6	McCormac, A.	2015	CL 3000402 - Determination of chronic toxicity to the earthworm <i>Eisenia andrei</i> in an artificial soil substrate 2015/1041971 Mambo-Tox Ltd., Southampton SO16 7NP, United Kingdom yes Unpublished	No	BASF
KCP 10.4.2.1/1	Ganssmann, M.	2013	Effects of Reg.No. 364380 (metabolite of BAS 500 F, Pyraclostrobin) on reproduction of the collembola <i>Folsomia candida</i> in artificial soil with 5% peat 2013/1068054 Institut fuer Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 10.4.2.1/2	Braaker, S.	2019	Addendum to study BASF DocID: 2013/1068054 - Effects of Reg. No. 364380 (Metabolite of BAS 500 F, Pyraclostrobin) on Reproduction of the <i>Collembola Folsomia candida</i> in Artificial Soil with 5% Peat 2019/2034464 BASF SE, Limburgerhof, Germany Fed.Rep. no Unpublished	No	BASF
KCP 10.4.1.1/2	Ganssmann, M.	2013	Effects of Reg.No. 364380 (metabolite of BAS 500 F, Pyraclostrobin) on reproduction and growth of earthworms <i>Eisenia fetida</i> in artificial soil	No	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			2013/1003174 Institut fuer Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany Fed.Rep. yes Unpublished		
KCP 10.4.1.1/3	Ganssmann, M.	2013	Effects of Reg.No. 369315 (metabolite of BAS 500 F, Pyraclostrobin) on reproduction and growth of earthworms Eisenia fetida in artificial soil with 10% peat 2013/1224029 Institut fuer Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 10.4.1.1/4	Witte, B.	2011	Effects of BAS 560 02 F on reproduction and growth of earthworms Eisenia fetida in artificial soil with 5% peat 2011/1000384 Institut fuer Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 10.4.1.1/5	McCormac, A.	2014	CL377160 - Determination of chronic toxicity to the earthworm Eisenia andrei in an artificial soil substrate 2014/1093923 Mambo-Tox Ltd., Southampton SO16 7NP, United Kingdom yes Unpublished	No	BASF
KCP 10.4.1.1/6	McCormac, A.	2015	CL 3000402 - Determination of chronic toxicity to the earthworm Eisenia andrei in an artificial soil substrate 2015/1041971	No	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			Mambo-Tox Ltd., Southampton SO16 7NP, United Kingdom yes Unpublished		
KCP 10.4.2.1/4	Braaker, S.	2019	Addendum to study BASF DocID: 2013/1224030 - Effects of Reg. No 369315 (Metabolite of BAS 500 F, pyraclostrobin) on Reproduction of the Collembola Folsomia candida in Artificial Soil with 5% Peat 2019/2034467 BASF SE, Limburgerhof, Germany Fed.Rep. no Unpublished	No	BASF
KCP 10.4.2.1/5	Ganssmann, M.	2013	Effects of BAS 560 02 F on reproduction of the Collembola Folsomia candida in artificial soil with 5% peat 2013/1003203 Institut fuer Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 10.5/1	Schoebinger, U.	2015	CL3000402: Effects on the activity of the soil microflora under laboratory conditions (nitrogen transformation) 2015/1132053 Eurofins Agrosience Services EcoChem GmbH, Niefern-Oeschelbronn, Germany Fed.Rep. yes Unpublished	No	BASF

List of data relied on not submitted by the applicant but necessary for evaluation

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP XX	Author	YYYY	Title Company Report N Source GLP/non GLP/GEP/non GEP Published/Unpublished	Y/N	Owner

Appendix 2 Detailed evaluation of the new studies

A 2.1 KCP 10.1 Effects on birds and other terrestrial vertebrates

A 2.1.1 KCP 10.1.1 Effects on birds

A 2.1.1.1 KCP 10.1.1.1 Acute oral toxicity

A 2.1.1.1.1 Study 1

The study is currently under evaluation in the course of the EU renewal process of pyraclostrobin (RAR of pyraclostrobin Rev. 1 – 10 January 2020 Vol. 3 – B9).

Comments of zRMS:	Study not evaluated.
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Reference: CP 10.1.1.1/1

Report: BAS 500 F (Pyraclostrobin) - Acute toxicity in the canary (*Serinus canaria*) after single oral administration (LD₅₀),
XXXXXXXXXX
Report No 15W0494/96W003
BASF DocID 2013/1400375
Authority registration No

Guideline(s): EPA OCSPP 850.2100, EPA 850.2000, EPA 712-C-025

Deviations: No

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

Acceptability: Yes

Duplication (if vertebrate study): No

Executive Summary

An avian acute oral toxicity test with the active substance pyraclostrobin (BAS 500 F) was conducted. The objective of the study was to evaluate the acute toxicity of the substance in canaries (*Serinus canaria*) and to determine the oral LD₅₀ in the canary after a single oral administration of the test substance and a 14-day post observation period.

The test substance was administered via a single oral dose of 0 (control), 210, 340, 551, 893 and 1446 mg a.s./kg body weight to groups of 28 weeks old canaries. Ten birds (5 males and 5 females) were used in each test substance group. The doses were administered undiluted in two gelatin capsules directly into the crop. Birds that have been fasted for about 3 to 5 hours were administered the test substance. Birds of all groups received food and water *ad libitum* throughout the test. All groups were observed for mortality, signs of clinical toxicity, impact on food consumption and body weight for 14 consecutive days post dosing. Gross-pathological examinations were conducted on all birds sacrificed at the termination of the test.

No regurgitation was observed during the first hour after dosing in any of the dose groups, thus all birds received the full dose. No mortality occurred throughout the duration of the study in the control and in the 210, 893 and 1446 mg a.s./kg b.w. dose groups. There was one male mortality in each of the dose groups of 340 and 551 mg a.s./kg b.w, which was considered not to be treatment related as there was no mortality in the higher dose groups. No substance-related impairment of food uptake in comparison to the control was observed in any of the dose groups. No statistically significant substance-related reduction of the body weights in the male and female birds was observed in any of the dose groups compared to the control. The body weight development of the females was not statistically different from the control in any of the dose groups. In males the body weight development on some occasions was higher than in the control group, however, without any dose-response pattern and it was not considered to be treatment-related. All birds were examined macroscopically (post-mortem) after study termination. No abnormalities caused by the test substance were observed in birds that died or in sacrificed birds.

In an acute oral toxicity test with the canary (*Serinus canaria*), the LD₅₀ of pyraclostrobin was >1446 mg a.s./kg b.w. The NOEL was ≥ 1446 mg a.s./kg b.w.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 500 F (Reg. No. 304428), batch no. COD-001236, purity: 99.02% (tolerance $\pm 1.0\%$).

B. STUDY DESIGN

Test species: Canary (*Serinus canaria*), visually indistinguishable from wild birds; adults, age: approx. 28 weeks old (before beginning of first egg-laying period); supplier: Zoowelt, Bechtheim, Germany.

Test design: Birds were administered single doses of 210, 340, 551, 893 and 1446 mg a.s./kg body weight of the test substance pyraclostrobin (BAS 500 F) undiluted in two gelatin capsules directly into the crop; 5 males and 5 females per dose group were used. The birds were observed for regurgitation for 1 - 3 hours after dosing. An observation period of 14 days followed, during which mortalities and signs of toxicity were recorded, four times on day of dosing and daily thereafter; assessment of body weight was carried out on the day before dosing and on days 7 and 14 after dosing; mean food consumption (g/bird/day) was calculated from the daily food consumption per cage separately for male and female birds after dosing. Gross-pathological post-mortem examinations of all birds at study termination on day 14 after dosing.

Endpoints: Mortality, clinical signs, feed consumption, body weight (b.w.), and gross-pathological examinations were conducted on all birds sacrificed at the termination of the test. Calculation of LD₅₀ and NOEL.

Test concentrations: 0 (Control), 210, 340, 551, 893, and 1446 mg a.s./kg body weight (nominal).

Test conditions: Birds fasted for about 3 h to 5 h before administration of the test substance; temperature: 19.9°C \pm 0.4°C; relative humidity: 51.3% \pm 8.5%; photoperiod: 8 hours light : 16 hours dark, light intensity: 20 - 38 lux.

Analytics: No analytical determinations of the test substance in the carrier were necessary since the test substance was applied without carrier.

Statistics: No statistical calculation of the LD₅₀ was performed since no substance-related mortality was observed in the tested doses. Dunnett's test was used for body weight data and Fisher's exact test for pair-wise comparison of mortality data.

C. DESCRIPTION OF THE ANALYTICAL PROCEDURES

Not applicable, no analysis of the test-substance preparations was carried out.

II. RESULTS AND DISCUSSION

Biological results:

No mortality occurred throughout the duration of the study in the control and in the 210, 893 and 1446 mg a.s./kg b.w. dose groups. There was one male mortality in each of the dose groups of 340 and 551 mg a.s./kg b.w, which was considered not to be treatment related as there was no mortality in the higher dose groups. No regurgitation was observed during the first hour after dosing in any of the dose groups, thus all birds received the full dose. No substance-related impairment of food uptake in comparison to the control was observed in any of the dose groups. No statistically significant substance-related reduction of the body weights in the male and female birds was observed on day 7 and at day 14 (sacrifice) in any of the dose groups compared to the control. The body weight development of the females was not statistically different from the control in any of the dose groups. In males the body weight development on some occasions was higher than in the control group, however, without any dose-response pattern and it was not considered to be treatment-related. All birds were examined macroscopically (post-mortem) after study termination. No abnormalities caused by the test substance were observed in birds that died or sacrificed birds. The relevant data and endpoints are summarized in the table below.

Table A 1: Acute toxicity of pyraclostrobin (BAS 500 F) to the canary (*Serinus canaria*)

	Dose rate [mg a.s./kg b.w.]					
	0 (control)	210	340	551	893	1446
Number of birds per dose group	10	10	10	10	10	10
Number of dead birds	0	0	1 ¹⁾	1 ¹⁾	0	0
Dead birds percentage [%]	0	0	10	10	0	0
Endpoints [mg a.s./kg b.w.]						
Highest dose causing no substance-related mortality	1446					
LD ₅₀ (14 d)	>1446					
NOEL	≥ 1446					

b.w. = body weight

¹⁾ One male bird at 340 mg/kg bw died five days after dosing and one male bird at 551 mg/kg bw died one day after dosing. According to the test guideline, 10% mortality is acceptable for the control group. Because the mortality in both these groups did not exceed 10% and in the higher dose groups (893 and 1446 mg/kg body weight) no mortality was observed, it was concluded that the mortality can be clearly not attributed to the test substance.

Validity criteria:

Criteria	Trigger value	Study value and page no.	Criteria met? Y/N	Guideline
Birds assignment to treatment and control pens	Random	Random p. 19	Y	OCSPP 850.2100
Control birds died or became moribund	≤ 10%	0% p. 27	Y	OCSPP 850.2100
Number of birds used for each dose level of the test substance and control	≥ 10	10 p. 22	Y	OCSPP 850.2100
Test substance method of administration	Orally, by capsule or gavage	Capsule p. 23	Y	OCSPP 850.2100
In a definitive test only, number of treatments tested	≥ 5 + control	5 dose levels + control, p. 22	Y	OCSPP 850.2100
Non-incident control mortality ¹⁾	0%	0% p. 27	Y	OECD 223
Incidental control mortality ¹⁾	≤ 10%	0% p. 27	Y	OECD 223

¹⁾ Incidental = self-inflicted, e.g., abrasions or broken legs. Non-incident mortality is things like disease or mishandling of animals that indicate poor health of the test population or poor study conduct.

In Commission Regulation (EU) No. 283/2013, acute bird studies are required to be conducted using either US EPA OCSPP 850.2100 (2012) or OECD 223 (2010). This study was conducted under OCSPP 850.2100. All validity criteria for OCSPP 850.2100 and OECD 223 (included for completeness) are fulfilled.

III. CONCLUSION

In an acute oral toxicity test with the canary (*Serinus canaria*), the LD₅₀ of pyraclostrobin was >1446 mg a.s./kg b.w. The NOEL was ≥ 1446 mg a.s./kg b.w.

A 2.1.1.1.2 Study 2

The study is currently under evaluation in the course of the EU renewal process of metrafenone (RAR of metrafenone – October 2018 Vol. 3 – B9).

Comments of zRMS:	Study not evaluated.
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Reference:	CP 10.1.1.1/2
Report:	BAS 560 F (Metrafenone) - Acute toxicity in the zebra finch (<i>Taeniopygia guttata</i>) after single oral administration (LD ₅₀), XXXXXXXXXX Report No EU-376971,EU-15W0437/01W001 BASF DocID 2011/1263863 Authority registration No
Guideline(s):	EPA 71-1, EPA 850.2100
Deviations:	No
GLP:	Yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany),
Acceptability:	Yes
Duplication (if vertebrate study):	No

Executive Summary

A laboratory study was conducted to evaluate the acute toxicity of BAS 560 F (metrafenone technical) administered to the zebra finch (*Taeniopygia guttata*) as a single oral dose. Ten zebra finch, five males and five females, were assigned to each of the treatment groups (500, 1000 or 2000 mg a.s./kg bw) and the control group. Birds were observed for mortality, signs of clinical toxicity, impact on food consumption and body weight for 14 consecutive days after exposure.

Analytical verification of the test substance concentration in the diet demonstrated that measured concentrations were within a range of 92.3% to 97.5% of the nominal concentrations during the test. The biological results are therefore based on the nominal concentrations.

No substance-related mortality, impairment of feed uptake or reduction in body weights occurred throughout the duration of the study in all treatment groups. Clinical signs were only observed in the highest test group, where five birds per sex were observed to be tumbling during the first hour of observation only. All birds were free of substance-related findings during the post-mortem examinations. Although one bird died, in the treatment group exposed to 1000 mg a.s./kg, this was not considered substance related. Hence, the highest dose causing no substance related mortality was 2000 mg a.s./kg bw for males and females.

In an acute oral toxicity test with the zebra finch (*Taeniopygia guttata*), the LD₅₀ of metrafenone was found to be > 2000 mg a.s./kg bw. The highest dose without mortality was determined to be 2000 mg a.s./kg bw. A no-observed-effect level (NOEL) based on signs of toxicity (tumbling) was established to be 1000 mg a.s./kg bw.

I. MATERIALS AND METHODS

A. MATERIALS

- 1. Test material:** BAS 560 F
Batch number: AC12053-29
Purity: 94.2% metrafenone
Description: Solid, beige
- 2. Test concentrations:** 0 (control), 500, 1000 or 2000 mg a.s./kg body weight (bw)
- 3. Vehicle:** 0.5% aqueous carboxy methyl cellulose
- 4. Test organism**
Species: Zebra finch (*Taeniopygia guttata*)
Age: Approximately two months old, before their first breeding season
Source: Kölle-Zoo, Ludwigshafen, Germany
Acclimation period: Approximately six weeks
Diet: Commercial diet for exotic birds in grain form, supplied by 'Versale-Laganv', Deinze, Belgium. The birds received the food *ad libitum*, except for a fasting period of 19 to 21 hours prior to dosing
Housing: Cages composed of stainless steel with stainless steel floors and covered with card board, measuring 1.67 m deep x 0.76 m wide x 0.76 m high (total area: 1.3 m²)

B. STUDY DESIGN

1. Environmental conditions:

Temperature: 19.8-26.1°C

Relative humidity: 42-99%

Photoperiod: 8h light : 16h darkness, with light intensity between 52 and 138 lux

2. Animal assignment and treatment:

Fifteen days prior to dosing, birds were weighed individually and then randomly allocated to the test groups. Per test group, ten birds were included; five males and five females. At test initiation, birds were administered the test substance by gavage into the crop. The birds were observed for at least one hour after dosing for regurgitation and then the next fourteen days. During these fourteen days, the birds were offered feed *ad libitum*.

3. Dose preparation:

For each test item group, 50 g of a preparation of the test substance in carboxy methyl cellulose (CMC) in drinking water was prepared separately. The test substance was suspended in 0.5% CMC with an ultra turrax stirrer. The birds were administered single doses of 500, 1000 or 2000 mg a.s./kg bw. The control group received CMC dispersion only.

4. Measurements/observations:

During the observation period of fourteen days, assessment of mortality and signs of clinical toxicity was carried out four times on the day of dosing and daily thereafter. Assessment of body weight was carried out on the day of dosing and on day seven and fourteen. Mean food consumption was calculated from the weekly food consumption, separately for male and female birds. Gross-pathological post-mortem examinations of all birds were performed at study termination on day fourteen after dosing.

For analysis of test item concentration, a sample was obtained from each test item concentration and the control dispersions. The analysis was performed using HPLC.

5. Statistics:

No statistical calculation of the LD₅₀ was performed, since no substance related mortality (above 50%) was observed. Food consumption was not examined statistically, since the food consumption was evaluated only per cage and not per bird. Body weight data were statistically analyzed using the Dunnett's test, with the program ToxData of the PDS Pathology Data Systems Ltd.

II. RESULTS AND DISCUSSION

MORTALITIES AND BEHAVIOURAL OBSERVATIONS

No substance related mortality occurred throughout the duration of the study in the control and in all test substance groups. The single mortality (female bird on Day 13) in the test group exposed to 1000 mg a.s./kg b.w. was considered not substance related. The highest concentration causing no substance related mortality was 2000 mg a.s./kg bw for males and females. No substance related clinical signs were observed in the control and in all test substance concentration groups, except in the highest test group where five birds per sex were observed to be tumbling during the first hour of observation. These symptoms were no longer noted after the 2-hour post dosing observation period.

FOOD CONSUMPTION AND BODY WEIGHT

There was no substance-related impairment of feed uptake in comparison to the control observed in any of the dose groups. There was no statistically significant substance-related reduction of the body weights in any of the dose groups at day 7 and day 14 (sacrifice), and the body weight development was not impaired in comparison to the control group. The results are presented in the table below.

Table A 2: Mean food consumption per day and body weight change of zebra finch (*Taeniopygia guttata*) exposed to BAS 560 F

Nominal concentration (mg a.s./kg bw)	Mean food consumption (g/bird/day)		Overall mean body weight gain (g) compared between day 0 and day 14	
	Males	Females	Males	Females
Negative control	3.8	3.9	1.0	1.1
500	4.1	3.8	1.5	0.8
1000	3.9	3.6	1.5	0.2
2000	3.6	4.0	1.0	1.1

POST-MORTEM EXAMINATIONS

One surviving female of the control group had a high-grade enlarged liver. All other surviving birds were free of findings. The bird that died on day 13 (dose group 1000 mg/kg bw) had a grey white encrustation on the liver, which was not considered to be treatment-related.

ANALYSIS

The results of the analytical verification of the test substance concentration in the diet were within a range of 92.3% to 97.5% of the nominal concentrations during the test. The biological results are therefore based on the nominal concentrations. The concentrations of the test substance BAS 560 F in stock solutions observed in the analysis are shown in the table below.

Table A 3: Measured concentrations of BAS 560 F in stock solutions of test samples

Nominal concentration (g a.s./50 g)	Nominal concentration (g test substance/50 g)*	Measured concentration (mg test substance/L)	Measured as % of nominal
2.500	2.650	2.445	92.3
5.000	5.300	4.999	94.3
10.000	10.600	10.331	97.5

* The concentration of the test substance BAS 560 F, containing 94.2% of the active substance metrafenone

DEFICIENCIES

None.

III. CONCLUSION

The LD₅₀ for the zebra finch (*Taeniopygia guttata*) exposed to BAS 560 F was found to be > 2000 mg a.s./kg body weight. The no-observed-effect level (NOEL) was 1000 mg a.s./kg body weight, based on signs of toxicity (tumbling).

A 2.1.1.1.3 Study 3

Comments of zRMS:	The study was conducted according to OECD 223 and the principles of GLP. No deviations to the guideline were noted. The study is considered to be reliable and suitable for the risk assessment.
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Reference: CP 10.1.1.1/3

Report: BAS 758 00 F: An Acute Oral Toxicity Study with the Northern Bobwhite Using a Sequential Testing Procedure,
XXXXXXXXXX
Report No 147B-391, 876355, S21-05617
BASF DocID 2021/2037989
Authority registration No

Guideline(s): OECD 223

Deviations: No. All validity criteria are fulfilled. Study is valid for use in risk assessment.

GLP: Yes
(certified by United States Environmental Protection Agency)

Acceptability: Yes

Duplication (if vertebrate study): No

Executive Summary

An acute oral avian toxicity test with the formulation BAS 758 00 F containing the active substances mefentrifluconazole, metrafenone and pyraclostrobin was conducted to evaluate the acute toxicity of BAS 758 00 F administered orally to the northern bobwhite (*Colinus virginianus*).

The test substance was administered via a single oral dose of 0 (control) and 2000 mg/kg bw to 47-week-old bobwhite quails. Five birds (mixed sex) were used in each test group. After fasting for about 17 hours, the test substance was administered once by gavage into the crop of each bird. The animals of the control group received reverse osmosis deionized water only. Each bird was carefully observed five times on Day 0 after dosing and two times during the remaining period for regurgitation (Day 0), mortality, general condition, overt signs of toxicity, and abnormal behavior. The birds were offered food and water *ad libitum*. The test was terminated after 14 days. Mortality, general condition, overt signs of toxicity, and abnormal behavior were recorded once daily during the observation period. Gross-pathological examinations were conducted on all birds.

No regurgitation was observed among the birds in the control or treatment group. When compared to the control group, there was an apparent treatment-related loss of mean body weight in the 2000 mg/kg dosage level from Day 0 to Day 3. From Day 3 to Day 7 and Day 7 to Day 14 the treatment group had greater gains in mean body weight than the control group but it was not large enough to fully compensate for the previous loss. Overall, from Day 0 to Day 14, the 2000 mg/kg treatment group had a loss of mean body weight when compared to the control group. When compared to the control group, there was a reduction in feed consumption for each of the first three days for the 2000 mg/kg dosage level.

For all feeding intervals from Day 3 to Day 14 the feed consumption for the 2000 mg/kg dosage group was comparable to the control group.

A gross necropsy was performed on all birds from the control group and all birds from the 2000 mg/kg treatment level at test termination. There were no remarkable findings for any of the birds in the control or treatment group.

No mortality was observed during the study. The LD₅₀ was empirically estimated to be > 2000 mg/kg bw, the highest concentration tested.

In an acute toxicity test with the bobwhite quail (*Colinus virginianus*), the 14-day LD₅₀ of BAS 758 00 F was > 2000 mg BAS 758 00 F/kg bw.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 758 00 F; batch No. FD-200124-0004; content of a.s.: metrafenone (BAS 760 F, Reg. No. 4037710): 94.9 g/L (nominal: 100.0 g/L), mefentrifluconazole (BAS 750 F, Reg. No. 5834378): 66.7 g/L (nominal: 66.6 g/L), pyraclostrobin (BAS 500 F, Reg. No. 304428): 81.0 g/L (nominal: 80.0 g/L); density: 1.092 g/cm³.

B. STUDY DESIGN

Test species: Northern Bobwhite (*Colinus virginianus*), young adults before their first breeding season, visually indistinguishable from wild birds; all birds were from the same hatch, pen-reared; age: 47 weeks old at dosing; source: Trace Pheasantry, Douglassville, PA 19518, United States.

Test design: Five birds of mixed sex (2 males and 3 females) were randomly assigned each to the treatment group and the control group. Prior to the administration, the birds were fasted for about 17 hours. Thereafter birds were administered a single dose of the test item BAS 758 00 F once by gavage into the crop. Controls received reverse osmosis deionized water only. Each animal was carefully observed post-dosing for regurgitation, mortality, general condition, over signs of toxicity and abnormal behavior, then at five time points on Day 0. An observation period of 14 days followed, with observations conducted twice daily. The birds were offered food and water *ad libitum*. Body weights were measured individually on the day of dosing (Day 0) and on Days 3, 7 and 14 post-dosing. Mean food consumption (g/animal/day) was then determined from Days 3 to 7 and Days 7 to 14. All animals were sacrificed at the termination of the observation period. The post-mortem examination included evaluation of general physical condition, and an examination of the thoracic and abdominal cavities, including cardiovascular and respiratory systems, liver, spleen, gastrointestinal tract, and urogenital system.

Endpoints: Mortality, clinical signs, feed consumption, body weight (bw), and gross-pathological examinations were conducted. Determination of LD₅₀ was conducted.

Test concentrations: 0 (control) and 2000 mg BAS 758 00 F/kg bw

Test conditions:	Birds fasted for about 17 hours before administration of the test substance. Mean temperature: 22.4°C (21.7 - 23.0°C); mean relative humidity: 73% (61 - 82%). Photoperiod: 8 hours light, 16 hours dark, lighting provided by fluorescent bulbs; light intensity: approx. 357 lux.
Analytics:	The analysis of the test item preparations has not been performed.
Statistics:	Descriptive statistics; no statistical calculation of the LD ₅₀ was performed since no bird died.

C. DESCRIPTION OF THE ANALYTICAL PROCEDURES

Not applicable, no analysis of the test-substance preparations was carried out.

II. RESULTS AND DISCUSSION

Biological results:

No mortality occurred during the study in the control or treatment group. No regurgitation was observed among the birds in the control or treatment group.

When compared to the control group, there was an apparent treatment-related loss of mean body weight in the 2000 mg/kg dosage level from Day 0 to Day 3. From Day 3 to Day 7 and Day 7 to Day 14 the treatment group had greater gains in mean body weight than the control group but it was not large enough to fully compensate for the previous loss. Overall, from Day 0 to Day 14, the 2000 mg/kg treatment group had a loss of mean body weight when compared to the control group.

When compared to the control group, there was a reduction in feed consumption for each of the first three days for the 2000 mg/kg dosage level. For all feeding intervals from Day 3 to Day 14 the feed consumption for the 2000 mg/kg dosage group was comparable to the control group.

A gross necropsy was performed on all birds from the control group and all birds from the 2000 mg/kg treatment level at test termination. There were no remarkable findings for any of the birds in the control or treatment group.

Table A 4: Acute toxicity of BAS 758 00 F to the bobwhite quail (*Colinus virginianus*)

Mortality	Dose [mg BAS 758 00 F/kg bw]
LD ₅₀ (14 d)	> 2000

Abbreviations: bw = body weight

Validity criteria:

Criteria	Trigger value	Study value and page no.	Criteria met? Y/N	Guideline
Non-incidental control mortality ¹⁾	0%	0% p. 11-12	Y	OECD 223
Incidental control mortality ¹⁾	≤ 10%	0% p. 11-12	Y	OECD 223

¹⁾ Incidental = self-inflicted, e.g., abrasions or broken legs. Non-incidental mortality is things like disease or mishandling of animals that indicate poor health of the test population or poor study conduct.

In Commission Regulation (EU) No. 283/2013, acute bird studies are required to be conducted using either US EPA OCSPP 850.2100 (2012) or OECD 223 (2016). This study was conducted under OECD 223 (2016) and met all validity criteria for OECD 223. Therefore, this study should be considered fully valid and reliable for use in the risk assessment.

III. CONCLUSION

In an acute toxicity test with the bobwhite quail, the 14-day LD₅₀ of BAS 758 00 F was > 2000 mg BAS 758 00 F/kg bw.

A 2.1.1.2 KCP 10.1.1.2 Higher tier data on birds

The study is currently under evaluation in the course of the EU renewal process of metrafenone (RAR of metrafenone – October 2018 Vol. 3 – B9).

Comments of zRMS:	Study not evaluated.
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Reference:	CP 10.1.1.2/1
Report:	BAS 560 F - 1-generation reproduction study on the mallard duck (<i>Anas platyrhynchos</i>) by administration in the diet, XXXXXXXXXX Report No EU-72W0437/015097 BASF DocID 2006/1018046 Authority registration No
Guideline(s):	EPA 540/9-82-024, EPA 540/9-86-139, EPA 71-4, EPA 850.2300, OECD 206
Deviations:	No
GLP:	Yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)
Acceptability:	Yes
Duplication (if vertebrate study):	No

Executive Summary

The reproductive toxicity of BAS 560 F to mallard duck (*Anas platyrhynchos*) was determined in a one-generation reproduction study, for 22 weeks. Groups of sixteen male/female pairs were exposed to dietary concentrations of 450, 900 and 1350 mg a.s./kg (corresponding to 50.9, 114.7, 167.7 mg a.s./kg bw), in parallel with a control. During the study, the effects of adult exposure to the test item were evaluated for adult health, body weight and feed consumption. In addition, the number of eggs laid, eggshell thickness and egg fertility, embryo viability, hatch rates, offspring survival and offspring weight were assessed.

The measured concentration of test substance in the diet ranged from 92.7% to 98.9% of the nominal concentration. Hence, the biological results were based on the nominal concentrations.

No substance-related effects in the parent generation on mortality, birds' health, food consumption and body weight could be detected in any treatment group. Avoidance of feed was not observed. In the offspring, no biologically relevant substance-related effects were observed in the 450 and 900 mg a.s./kg diet groups. In the highest test group, effects noted were a reduction in the number of eggs laid per female per week and the survival during hatching, and an increase of the number of chicks found dead in shell.

The no-observed-effect concentration (NOEC) during this study was 900 mg/kg feed (114.7 mg a.s./kg bw/d) and the lowest-observable-effect concentration (LOEC) was 1350 mg/kg diet (167.7 mg a.s./kg bw/d), based on reproductive performance.

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test material:** BAS 560 F
CAS number/ Batch number: 220899-03-6/ COD-000610
Purity: 99.4% metrafenone
Description: Solid, beige
2. **Test concentrations:** 0 (control), 450, 900 and 1350 mg a.s./kg diet (corresponding to 0, 50.9, 114.7 and 167.7 mg a.s./kg bw)
3. **Vehicle:** None, as the test item was immediately mixed through the diet
4. **Test organism**
Species: Mallard duck (*Anas platyrhynchos*)
Age: Approximately five months old and approaching their first breeding season
Source: Geflügelhof Knerr, Rieschweiler-Mühlback, Germany
Acclimation period: Two weeks
Diet: Experimental diet, feed for ducks in meal form, supplied by Provimi kliba SA, Basel, Switzerland. Additionally, adult birds were offered mussel shell grit *ad libitum*. Municipal drinking water was offered *ad libitum* and during the exposure period additionally with a water bath.
Housing: Adults: Cages composed of galvanized or stainless steel wire, with stainless steel wire floors, measuring 1.3 m deep x 0.65 m width x 1.3 m high (total area: 0.85 m²). Hatchlings: Pens made of galvanized or stainless steel wire with stainless steel wire mesh.

B. STUDY DESIGN

1. Environmental conditions:

Temperature:	Adults: 16.3-22.7°C; Eggs: $37.8 \pm 0.1^\circ\text{C}$; Hatchlings: 37.7-38.1°C
Relative humidity:	Adults: 28-93%; Eggs: 60-70%; Hatchlings: 80-90%
Photoperiod:	Adults: 7h light : 17h darkness (week one to seven); 14h light : 10h darkness (week eight and nine); 17h light : 7h darkness (week ten to twenty-two). Hatchlings: 17h light : 7h darkness

2. Animal assignment and treatment:

Sixteen replicates per concentration group, with one male and one female per replicate, were exposed during a pre-egg period of ten weeks and a subsequent egg-laying period of twelve weeks. During these twenty-two weeks, the birds were offered feed with test substance *ad libitum*. The eggs were incubated using a commercial incubator with automatic egg rotation. After hatching, the chicks were moved to pens and observed for fourteen days. These chicks received the same feed as the adult birds (without test item) in pelleted form, *ad libitum*.

3. Dose preparation:

The concentrations used in the test were 450, 900 and 1350 mg a.s./kg diet, which were mixed separately with diet. The test substance was weighed and then mixed with the diet using a laboratory mixer, for ten minutes. The diet/test substance mixtures were prepared once a week. The control group received feed without test substance.

4. Measurements/observations:

For the adult birds, observations were made daily for mortality and clinical observations (general condition, signs of toxic effects and abnormal behavior). Food consumption was determined weekly, along with routine observations concerning palatability. In addition, body weights were recorded at test initiation, at the end of weeks two, four, six and eight of the pre-egg production period, and at test termination. All birds which died during the test or were sacrificed at the end of the test were necropsied and subjected to gross-pathological assessment.

All eggs produced were collected daily during the 12-week egg-laying period starting at the beginning of week 11 and ending at the end of week 22. These eggs were also weighed per cage and examined visually for their quality. One egg per pair was collected in the weeks 1, 3, 5, 7, 9 and 11 of the egg-laying period and was examined for egg shell thickness. All eggs (not cracked, broken, abnormal or taken for egg shell measurements) were placed in an incubator at weekly intervals and were candled on days 14 and 21 of the incubation period for evaluation of infertilities, as well as early and late embryonic deaths.

For the chicks, the number hatched and any abnormalities were recorded. These chicks were reared until fourteen days old, and mortality and symptoms (toxic signs) were checked daily during this period. In addition, their body weights were recorded when the chicks were removed from the hatcher and fourteen days after hatching.

To verify if the samples were homogeneously mixed, samples were collected at the beginning of the exposure period from the upper, middle and lower layer of the highest and lowest test item concentration diets. Otherwise, samples (from freshly made diets) were obtained from each test item concentration and the control diets, at three different time points (1: test initiation (start of substance feeding), 2: start of egg-laying period (ten weeks after test initiation) and 3: eighteen weeks after test initiation), for analysis of test item concentration. The analysis was performed using HPLC with variable wavelength detection set at 205 nm.

5. Statistics:

For body weight and food consumption of the adult birds, as well as for egg weight, egg shell thickness and chicks' body weight, a comparison between each test item concentration group and the control group was performed using the Dunnett's test. For count data (i.e. number of eggs and hatched chicks) and proportions (number of fertile eggs of eggs initially set) the Wilcoxon test was used. The SAS-System was used for the statistical analyses. For the analyses of body weight and food consumption of the adult birds, the DATATOX F1-System was used.

II. RESULTS AND DISCUSSION

A. MORTALITIES AND BEHAVIOURAL OBSERVATIONS

Only one bird died during the pre-egg-laying period; a female exposed to 900 mg a.s./kg diet. This was not considered to be related to the test substance exposure. No abnormalities in appearance and behavior were observed in any of the groups during the study.

B. FOOD CONSUMPTION AND BODY WEIGHT

The food consumption was in the normal range during both the pre-egg and the egg-laying period. The uptake was only statistically significantly different (increased) from the control group for adult birds exposed to 900 mg a.s./kg diet during weeks 4 and 7. No rejection of food was observed. Body weight did not differ significantly between male and female birds exposed to BAS 560 F and the birds in the control group. The results are presented in the table below.

Table A 5: Mean food consumption and body weight change of mallard duck (*Anas platyrhynchos*) exposed to BAS 560 F for 22 days

Nominal concentration (mg a.s./kg diet)	Mean feed consumption (g/bird/day)	Overall mean body weight change (%) compared between day 0 and day 22	
		Males	Females
Negative control	139.0	-5.66	+1.85
450	133.6	-3.91	+4.56
900	150.9	-4.26	+2.63
1350	146.0	-1.50	-0.90

C. REPRODUCTIVE RESULTS

The groups exposed to 450 and 900 mg a.s./kg diet did not show statistically significant differences to the control group for any of the reproduction parameters. For the group exposed to 1350 mg a.s./kg diet, statistically significant differences to the control group were observed for number of eggs laid/female/week, survival during hatch and number of chicks 'dead-in-shell'. The results are presented in the following tables:

Table A 6: Reproductive performance of mallard duck (*Anas platyrhynchos*) exposed to BAS 560 F

Reproductive parameter	Experimental group (mg a.s./kg diet)			
	Control	450	900	1350
No. of eggs laid/group	870	819	811	640
No. of cracked and broken eggs/group	14	17	2	5
Mean egg weight (g)	56.5	55.4	57.5	58.2
Mean egg shell thickness (mm)	0.39	0.39	0.39	0.40
No. of eggs incubated/group	785	739	742	583
No. of fertile eggs/group	577	568	538	524
No. of infertile eggs/group	208	171	204	59
No. of early embryonic mortalities/group	26	11	35	19
No. of viable 14-day old embryos/group	551	557	504	505
No. of late embryonic mortalities/group	4	5	14	8
No. of viable 21-day old embryos/group	547	552	490	497
No. of total embryonic mortalities/group	30	16	48	27
No. of 'dead-in-shell'/group	109	120	90	158
No. of chicks hatched/group	438	432	400	339
No of 14-day surviving chicks/group	438	422	396	336
No. of eggs laid/female/week	4.5	4.3	4.2	3.3*
No of chicks hatched/female/week	2.3	2.3	2.1	1.8
No of 14-day surviving chicks/female/week	2.3	2.2	2.1	1.8
Mean body weight of chicks at hatching (g)	33.8	32.8	34.5	33.3
Mean body weight of chicks 14 days after hatching (g)	280.9	283.5	286.8	290.2

* Statistically significantly different to the control group (p<0.05)

Table A 7: Reproductive performance of mallard duck (*Anas platyrhynchos*) exposed to BAS 560 F expressed as percentages

Reproductive parameter	Experimental group (mg a.s./kg diet)			
	Control	450	900	1350
% fertile eggs of incubated eggs	72.4	82.0	69.2	89.7
% viable 14-day old embryos of eggs incubated	68.9	80.2	65.1	85.5
% viable eggs at day 21 of eggs incubated at day 14	99.4	99.2	96.8	98.0
% hatched chicks of eggs incubated at day 21	83.1	70.5	79.0	60.4*
% 14-day survivors of chicks hatched	99.4	97.5	99.2	99.2
% cracked and broken eggs of eggs laid	1.7	1.8	0.2	1.0
% early embryonic mortalities of fertile eggs	6.7	2.2	6.4	4.3
% late embryonic mortalities of fertile eggs	0.5	0.7	2.5	1.9
% 'dead-in-shell' of fertile eggs	15.7	28.6	16.7	36.8*
Hatchability (% chicks hatched of total eggs incubated)	55.5	56.3	51.9	52.6
Hatchability (% chicks hatched of fertile eggs)	77.0	68.5	74.4	57.0*

* Statistically significantly different to the control group (p<0.05)

D. ANALYSIS

Analysis of the samples collected from the upper, middle and lower layer of the diets showed measured concentrations of 445.2, 434.6 and 448.4 mg test substance/kg for the lowest test substance concentration and 1307.5, 1349.2 and 1302.7 mg/kg for the highest test substance concentration for the respective layers. These values demonstrate a homogeneous distribution of the test substance in the diet.

The measured concentration of test substance in the diet (with samples taken at three time points) ranged from 92.7% to 98.9% of the nominal concentrations. The biological results are therefore based on the nominal concentrations. The concentrations of the test substance BAS 560 F observed in the analysis are shown in the table below.

Table A 8: Measured concentrations of BAS 560 F in test samples

Nominal concentration (mg a.s./kg)	Nominal concentration* (mg test substance/kg)	Time point**	Measured concentration (mg test substance/L)	Measured as % of nominal test substance concentration	Mean measured as % of nominal test substance concentration
0 (negative control)	0 (negative control)	1 2 3	< LOQ	-	-
450	453	1 2 3	442.7 448 433	97.7 98.9 95.6	97.4
900	905	1 2 3	850.8 839 869	94.0 92.7 98.6	94.2
1350	1358	1 2 3	1319.8 1312 1339	97.2 96.6 98.6	97.5

* The concentration of the test substance BAS 560 F, containing 99.4% of the active substance metrafenone

** Samples were taken from freshly made diets at three different time points (1: test initiation (start of substance feeding), 2: start of egg-laying period (ten weeks after test initiation) and 3: eighteen weeks after test initiation)

LOQ = Limit of quantification (1.86 mg/L, corresponding to 18.6 mg/kg feed)

E. DEFICIENCIES

None.

III. CONCLUSION

In the study with the mallard duck (2006/1018046) 3 concentrations were tested besides control: 450 ppm, 900 ppm and 1350 ppm. The LOEC is at the highest concentration tested. As only 3 concentrations are available (and several of these concentrations show no effect) an EC_{10/20} calculation is not appropriate.

The no-observed-effect concentration (NOEC) for the mallard duck (*Anas platyrhynchos*) exposed to BAS 560 F was 900 mg a.s./kg diet (114.7 mg a.s./kg bw/d) and the lowest-observable-effect concentration (LOEC) was 1350 mg a.s./kg diet (167.7 mg a.s./kg bw/d), based on reproductive performance.

A 2.2 KCP 10.1.2 Effects on terrestrial vertebrates other than birds

A 2.2.1 KCP 10.1.2.1 Acute oral toxicity to mammals

No studies conducted.

A 2.2.1.1 KCP 10.1.2.2 Higher tier data on mammals

A 2.2.1.1.1 Study 1

The study is currently under evaluation in the course of the EU renewal process of pyraclostrobin (RAR of pyraclostrobin Rev. 1 – 10 January 2020 Vol. 3 – B9).

Comments of zRMS:	Study was evaluated and accepted for the regulatory use in RR for BAS 751 00 F.
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Reference:	CP 10.1.2.2/1
Report	Study on the residue behaviour of Pyraclostrobin (BAS 500 F) on wheat (young plants) after treatment with BAS 500 06 F under field conditions in North and South Europe, season 2012, Moreno S., 2013 Report No: 421980 BASF DocID 2013/1045207 Authority registration No
Guideline(s):	EEC 87/18 (No. L 15/29) 1986, International guidelines for distribution and pesticides application AEPLA FAO 1985, EC 1107/2009 of the European Parliament and of the Council of 21 Oct 2009, EEC 79/117, EEC 91/414, EEC 7029/VI/95 rev. 5 Appendix B, EEC 7525/VI/95 rev. 9 (March 2011)
Deviations:	No
GLP:	Yes (certified by ENAC, Entidad Nacional de Acreditación, Madrid, Spain)
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive Summary

The objective of the study is to determine the magnitude of residues of pyraclostrobin (BAS 500 F) and the analyte 500M07 after one application of BAS 500 06 F carried out on wheat. The selected application rates, frequency and spray interval cover the Good Agricultural Practice (critical GAP).

I. MATERIAL AND METHODS

A. MATERIALS

Test item and application

The trial consisted of a control plot (untreated) and two treated plots (plot 2 and plot 3) without replication. No product containing the test item was used on the test plots during the season 2012.

The test item BAS 500 06 F (EC) was foliar applied on plot 2 at a nominal rate of 250 g pyraclostrobin/ha in a nominal spray volume of 200 L/ha at three leaves unfolded growth stage (BBCH 13) according to Good Laboratory Practice. The test item BAS 500 06 F (EC) was also foliar applied on plot 3 at a nominal rate of 100 g pyraclostrobin/ha in a nominal spray volume of 200 L/ha at three leaves unfolded growth stage (BBCH 13) according to Good Laboratory Practice.

B. STUDY DESIGN

Study site

During the 2012 growing season a total of nine trials were conducted in representative wheat growing areas in Germany, The Netherlands, The United Kingdom, Spain and Italy.

Sampling information

For this study specimens were collected as wheat whole plants without roots 1 hour after application as well as 1, 2, 3, 4, 5, 7, 10, 12 and 14 days thereafter. All specimens were sampled from the untreated, and from both treated plots. Untreated specimens were obtained prior to treated specimens at each sampling occasion. All specimens were transferred to freezer storage on the day of sampling and were then stored frozen ($\leq -18^{\circ}\text{C}$).

Residue analysis

All specimens were analyzed for pyraclostrobin (BAS 500 F) and its metabolite 500M07 (BF 500-3) using BASF method no. 535/1 (L0076/01). The method has a limit of quantitation of 0.01 mg/kg for both analyses.

C. Description of the analytical procedures

For analysis of plant materials, BASF method no. 535/1 (L0076/01) was used, which determines the analyte by means of HPLC-MS/MS. Validation of the analytical method was performed on plant matrices in a separate study (BASF DocID 2006/1039427). Pyraclostrobin and its metabolite 500M07 (BF 500-3) are extracted with a mixture of methanol, water and hydrochloric acid. An aliquot of the extract is centrifuged and partitioned at alkaline conditions against cyclohexane. The method has a limit of quantitation (LOQ) of 0.01 mg/kg. The maximum storage interval from harvest until analysis was 229 days. It was investigated earlier (BASF DocID 2001/5000232) that residues were stable during the period of frozen storage prior to analysis.

Procedural recoveries (overall mean) were 90.6% for pyraclostrobin and 89.5% for the metabolite 500M07 (BF 500-3) in wheat plants using fortification levels between 0.01–100 mg/kg. The results are summarized in the following table.

Table A 9: Summary of wheat plant matrix recoveries for pyraclostrobin and its metabolite 500M07 (BF 500-3)

Test System	Matrix	Fortification Level [mg/kg]	Mean [%]	SD [±]	RSD [%]	n
Wheat	Whole plant no roots	0.01, 0.10, 1.0, 10 and 100	BAS 500 F			
			90.6	7.0	7.7	29
			500M07 (BF 500-3)			
			89.5	7.4	8.2	29

II. RESULTS AND DISCUSSION

Table A 10: Summary of Residues of pyraclostrobin (BAS 500 F) and 500M07 in wheat

Plot 2							
Sampling No.	Portion analyzed	DALA ¹⁾	Growth stage	n	BAS 500 F [mg/kg]	500M07 (BF 500-3) ²⁾ [mg/kg]	Sum ³⁾ [mg/kg]
1	Wheat, Whole plant no roots	1 ⁴⁾	13-14	9	13 - 35	0.036 - 1.5	14 - 36
2		1	13-14	9	2.4 - 26	0.41 - 3.0	2.8 - 27
3		2	13-14	9	4.2 - 25	1.0 - 2.9	5.2 - 28
4		3	13-21	9	2.5 - 18	0.83 - 3.2	3.3 - 22
5		4	13-21	9	1.7 - 22	0.64 - 4.6	2.3 - 26
6		5	13-21	9	1.4 - 19	0.59 - 5.4	2.0 - 24
7		7	13-23	9	0.38 - 12	0.17 - 4.3	0.55 - 17
8		10	13-30	9	0.13 - 10	0.040 - 4.2	0.17 - 14
9		12	13-31	9	0.043 - 5.0	0.021 - 2.7	0.067 - 7.6
10		14	13-31	9	0.028 - 5.5	0.012 - 2.5	0.040 - 8.0
Plot 3							
Sampling No.	Portion analyzed	DALA ¹⁾	Growth stage	n	BAS 500 F [mg/kg]	500M07 (BF 500-3) ²⁾ [mg/kg]	Sum ³⁾ [mg/kg]
1	Wheat, Whole plant no roots	1 ⁴⁾	13-14	9	4.6 - 11	0.021 - 0.33	4.9 - 12
2		1	13-14	9	3.0 - 10	0.21 - 0.82	3.4 - 11
3		2	13-14	9	2.0 - 9.6	0.41 - 1.3	2.6 - 11
4		3	13-21	9	1.5 - 7.0	0.35 - 1.7	2.0 - 8.7
5		4	13-21	9	0.95 - 8.3	0.28 - 2.0	1.3 - 10
6		5	13-21	9	0.45 - 6.5	0.19 - 2.0	0.64 - 8.5
7		7	13-23	9	0.23 - 4.5	0.091 - 1.7	0.32 - 6.2
8		10	13-30	9	0.079 - 3.3	0.028 - 1.5	0.11 - 4.8
9		12	13-31	9	0.028 - 1.9	< 0.01 - 1.1	0.038 - 3.0
10		14	13-31	9	< 0.01 - 1.8	< 0.01 - 1.0	< 0.02 - 2.8

1 days after last application

2 conversion factor for calculation of 500M07 (BF 500-3) to parent BAS 500 F is 1.084

3 for residues < 0.010 mg/kg, value was set to 0.010 mg/kg for calculation of sum

4 HALA: hours after last application

III. CONCLUSION

In whole plant samples collected from plot 2 directly after the application (BBCH 13-14) residues of pyraclostrobin (BAS 500 F) ranged between 13 and 35 mg/kg. At the last sampling at 14 DALA (BBCH 13-31) residues decreased to a range of 0.028 – 5.5 mg/kg.

In whole plant samples collected from plot 3 directly after the application (BBCH 13-14) residues of pyraclostrobin ranged between 4.6 and 11 mg/kg. At the last sampling at 14 DALA (BBCH 13-31) residues decreased to a range of < 0.01 – 1.8 mg/kg. No residues of pyraclostrobin ≥ 0.01 mg/kg were present in control specimens.

A 2.2.1.1.2 Study 2

The study is currently under evaluation in the course of the EU renewal process of pyraclostrobin (RAR of pyraclostrobin Rev. 1 – 10 January 2020 Vol. 3 – B9).

Comments of zRMS:	Study was evaluated and accepted for the regulatory use in RR for BAS 751 00 F.
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Reference:	CP 10.1.2.2/2
Report	Study on the residue behavior of Pyraclostrobin (BAS 500 F) on pea (young plants) after the application of BAS 500 06 F under field conditions in France (North), Germany, United Kingdom, Italy and Spain, 2012, Martin T., 2013 Report No: 421989 BASF DocID 2013/1044539 Authority registration No
Guideline(s):	EC 1107/2009 of the European Parliament and of the Council of 21 Oct 2009, EEC 79/117, EEC 91/414, EEC 7029/VI/95 rev. 5, EEC 7525/VI/95 rev. 7
Deviations:	No
GLP:	Yes (certified by ENAC, Entidad Nacional de Acreditación, Madrid Spain)
Acceptability:	Yes
Duplication (if vertebrate study)	--

Executive Summary

The objective of the study was to determine the residue level of pyraclostrobin (BAS 500 F) in or on young pea plants after one application of BAS 500 06 F.

I. MATERIAL AND METHODS

A. MATERIALS

Test item and application

Each trial consisted of a control plot (untreated) and two treated plots. All applications were made as foliar sprays, using commercial ground equipment or equipment which simulated commercial applications. No product containing the test item was used on the test plots during the year 2012. BAS 500 06 F was the test item used in this study. The actual application rates were within 10% of the nominal values.

The selected application rates, frequency and spray interval cover the Good Agricultural Practice (critical GAP), which will be defined by the label directions.

BAS 500 06 F (200 g/L of pyraclostrobin, EC) was foliar applied once on plot 2 at a rate equivalent to 250 g/ha of pyraclostrobin (1.25 L of formulated product /ha) and once on plot 3 at a rate equivalent to 100 g/ha of pyraclostrobin (0.5 L of formulated product /ha). The spray volume used was 200 L/ha and the application timing was at BBCH 12 - 13.

B. STUDY DESIGN

Study site

During the 2012 growing season, eight trials in peas were conducted in different representative growing areas in France (North), Germany, The United Kingdom, Italy and Spain.

Sampling information

Specimens of whole plant without roots were collected on plot 1 at the day of the application and 5 and 14 days thereafter. Specimens of whole plant without roots were collected on plot 2 and plot 3, 1 hour after the application and 1, 2, 3, 4, 5, 7, 10, 12 and 14 days thereafter.

Control (untreated) specimens were taken at every time point, and were collected prior to collection of the treated specimens to avoid contamination. Generally the specimens were frozen within 6 hours of being taken, and remained frozen at or below -18°C, including during transportation, until analysis. The maximum storage interval from harvest until analysis was 188 days. Data indicate that residues were stable during the period of frozen storage prior to analysis.

Residue analysis

Specimens were analysed for pyraclostrobin (BAS 500 F) and its metabolite 500M07 (BF 500-3) using BASF method No. 535/1 (L0076/01). The method has a limit of quantitation of 0.01 mg/kg for both analytes.

C. Description of the analytical procedures

For analysis of plant materials, BASF method no. 535/1 (L0076/01) was used, which determines the analyte by means of HPLC-MS/MS. Validation of the analytical method was performed on plant matrices in a separate study (BASF DocID 2006/1039427). Pyraclostrobin and its metabolite 500M07 (BF 500-3) are extracted with a mixture of methanol, water and hydrochloric acid. An aliquot of the extract is centrifuged and partitioned at alkaline conditions against cyclohexane. The method has a limit of quantitation (LOQ) of 0.01 mg/kg. The maximum storage interval from harvest until analysis was 188 days. It was investigated earlier (BASF DocID 2001/5000232) that residues were stable during the period of frozen storage prior to analysis.

Procedural recoveries (overall mean) were 95.5% for pyraclostrobin and 94.6% for the metabolite 500M07 (BF 500-3) in young pea plants using fortification levels between 0.01–100 mg/kg. The results are summarized in the following table.

Table A 11: Summary of pea plant matrix recoveries for pyraclostrobin and its metabolite 500M07 (BF 500-3)

Test System	Matrix	Fortification Level [mg/kg]	Mean [%]	SD [±]	RSD [%]	n
Pea	Whole plant no roots	0.01, 0.10, 1.0, 10 and 100	BAS 500 F			
			95.5	5.8	6.1	29
			500M07 (BF 500-3)			
			94.6	11	12	29

II. RESULTS AND DISCUSSION

Table A 12: Summary of Residues of pyraclostrobin (BAS 500 F) and 500M07 in peas

Plot 2							
Samp. No.	Portion analysed	DALA ¹⁾	Growth stage	n	Residues expressed as parent equivalents		
					BAS 500 F [mg/kg]	500M07 (BF 500-3) ²⁾ [mg/kg]	Sum ³⁾ [mg/kg]
1	Pea, whole plant no roots	1 ⁴⁾	12-13	8	11 - 23	0.026 - 0.41	11 - 23
2		1	12-13	8	9.4 - 19	0.53 - 2.5	10 - 22
3		2	12-14	8	2.9 - 11	0.64 - 3.6	3.5 - 15
4		3	12-14	8	3.2 - 8.7	1.2 - 3.7	4.8 - 12
5		4	13-15	8	2.7 - 5.9	1.1 - 2.8	4.1 - 8.0
6		5	13-16	8	2.1 - 5.1	0.83 - 3.1	3.6 - 8.1
7		7	13-17	8	1.4 - 3.6	0.68 - 2.6	2.7 - 6.3
8		10	13-19	8	0.16 - 2.2	0.13 - 1.4	0.29 - 3.6
9		12	14-21	8	0.38 - 2.0	0.23 - 1.4	0.71 - 3.5
10		14	14-23	8	0.14 - 1.4	0.062 - 0.89	0.20 - 2.1
Plot 3							
Samp. No.	Portion analysed	DALA ¹⁾	Growth stage	n	Residues expressed as parent equivalents		
					BAS 500 F [mg/kg]	500M07 (BF 500-3) ²⁾ [mg/kg]	Sum ³⁾ [mg/kg]
1	Pea, whole plant no roots	1 ⁴⁾	12-13	8	3.9 - 6.2	0 017 - 0.11	4.0 - 6.3
2		1	12-13	8	1.4 - 5.3	0.12 - 0.78	1.5 5.6
3		2	12-14	8	1.6 - 8.0	0.40 - 1.9	2.1 - 9.9
4		3	12-14	8	1.0 - 2.4	0.46 - 1.1	1.5 - 3.5
5		4	13-15	8	0.95 - 1.5	0.43 - 0.73	1.5 - 2.0
6		5	13-16	8	0.60 - 1.6	0.41 - 0.94	1.1 - 2.4
7		7	13-17	8	0.34 - 0.82	0.25 - 0.62	0.61 - 1.4
8		10	13-19	8	0.24 - 0.69	0.13 - 0.58	0.37 - 1.2
9		12	13-21	8	0.10 - 0.43	0.040 - 0.37	0.14 - 0.80
10		14	14-23	8	0.043 - 0.29	0.023 - 0.27	0.066 - 0.53

¹⁾ days after last application

²⁾ conversion factor for calculation of 500M07 (BF 500-3) to parent BAS 500 F is 1.084

³⁾ for residues < 0.010 mg/kg, value was set to 0.010 mg/kg for calculation of sum

⁴⁾ HALA: hours after last application

III. CONCLUSION

In whole plant samples collected from plot 2 directly after the application (BBCH 12-13) residues of pyraclostrobin (BAS 500 F) ranged between 11 - 23 mg/kg. Residues has been decreasing over the whole trial period and at last sampling at 14 DALA (BBCH 14-23) residues ranged between 0.14 - 1.4 mg/kg.

In whole plant samples collected from plot 3 directly after the application (BBCH 12-13) residues of pyraclostrobin ranged between 3.9 - 6.2 mg/kg. Residues has been decreasing over the whole trial period and at last sampling at 14 DALA (BBCH 14-23) residues ranged between 0.043 - 0.29 mg/kg.

No residues of pyraclostrobin ≥ 0.01 mg/kg were present in control specimens.

A 2.2.1.1.3 Study 3

The study is currently under evaluation in the course of the EU renewal process of pyraclostrobin (RAR of pyraclostrobin Rev. 1 – 10 January 2020 Vol. 3 – B9).

Comments of zRMS:	The report was evaluated and accepted for the regulatory use in RR for BAS 751 00 F.
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Reference:	CP 10.1.2.2/3
Report	Dissipation of BAS 500 F - Pyraclostrobin on young plants (wheat and peas) - Trials conducted in the Northern Zone of Europe - Calculation of DT50 / DT90 dissipation times, Erzgraeber B., 2013 Report No not given BASF DocID 2013/1078114 Authority registration No
Guideline(s):	No Justification: No guidelines available
Deviations:	No
GLP:	No Justification: for modelling calculations of DT ₅₀ dissipation times GLP is not required
Acceptability:	Yes
Duplication (if vertebrate study)	--

Executive Summary

This modelling report provides kinetic analysis and estimation of dissipation times (DT₅₀, DT₉₀ values) for pyraclostrobin on young plants.

The decline of pyraclostrobin residues on young plants was well described by this first order kinetics. The DT₅₀ values of pyraclostrobin in young plants derived for the individual trials and plots ranged between 1.25 and 3.75days.

I. MATERIAL AND METHODS

Calculation of DT₅₀ and DT₉₀ values

The concentration time curves were described by a single first order (SFO) kinetic model, and this was fitted against the results of the individual trials using non-linear parameter estimation methods.

The goodness-of-fit of the kinetic models was assessed by visual inspection and statistical measures. For visual inspection, the recommended graphical representations of observed and modeled decline curves versus time and the residuals versus time are presented. As goodness-of-fit measures, the χ^2 minimum error level is provided.

II. RESULTS AND DISCUSSION

Table A 13: DT₅₀ values of pyraclostrobin in young wheat plants and peas

Plant	Trial	Plot	DT ₅₀ [d]	Kinetic model	χ^2 error	r^2
wheat	L120103	P2 ¹⁾	2.67	SFO	10.3	0.97787
wheat	L120103	P3 ²⁾	1.37	SFO	19.9	0.95564
wheat	L120104	P2	1.40	SFO	11.2	0.98795
wheat	L120104	P3	1.97	SFO	12.5	0.97825
wheat	L120105	P2	2.18	SFO	9.3	0.98535
wheat	L120105	P3	1.56	SFO	10.2	0.98748
wheat	L120106	P2	1.26	SFO	7.0	0.99658
wheat	L120106	P3	1.25	SFO	7.9	0.99416
peas	L120073	P2	1.28	SFO	7.0	0.99504
peas	L120073	P3	1.50	SFO	9.9	0.98735
peas	L120074	P2	1.91	SFO	8.4	0.98946
peas	L120074	P3	1.55	SFO	8.3	0.99175
peas	L120075	P2	3.76	SFO	5.1	0.99011
peas	L120075	P3	2.50	SFO	12.6	0.96317
peas	L120076	P2	2.10	SFO	12.7	0.97268
peas	L120076	P3	2.06	SFO	16.0	0.95846

¹⁾ and ²⁾ In 2012, BASF conducted 8 GLP field residue trials, with two individual plots P2 and P3 of different application rates per trial, in the Northern Zone of Europe to obtain residue decline data in wheat and peas at early growth stages of the crops. Wheat and peas were treated with either nominally 1.25 L (P2) or 0.5 L (P3) product BAS 500 06 F/ha. BAS 500 06 F contains 200 g pyraclostrobin/L, thus 250 g a.s./ha (P2) or 100 g a.s./ha were applied at BBCH 13 or 13/14 onto wheat and at BBCH 12-13 or 13 onto peas.

III. CONCLUSION

The decline of pyraclostrobin residues on young plants was well described by this first order kinetics. The DT₅₀ values of pyraclostrobin in young plants derived for the individual trials and plots ranged between 1.25 and 3.75 days.

A 2.2.1.1.4 Study 4

The study is currently under evaluation in the course of the EU renewal process of pyraclostrobin.

Comments of zRMS:	Three trials on young wheat plants under field conditions were conducted in Germany. Each field trial consisted two plots. The application rate was 0.25 kg a.s./ha. The samplings were carried out at 0, then 1, 2, 3, 4, 5, 7, 10, 12 and 14 days after the application. The sampling schedule gave 9 data points for each trial, which is sufficient to perform the reliable kinetic analysis. The study is considered to be reliable and suitable for the risk assessment.
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Reference:	CP 10.1.2.2/4
Report	Study on the residue behaviour of BAS 500 F (Pyraclostrobin) in young wheat plants after a foliar application with BAS 500 06 F at growth stage 25/29 under field conditions in Northern Europe, in spring 2016, Kramm R., 2017 report No EU-809785 BASF DocID 2017/1029774 Authority registration No
Guideline(s):	EEC 7029/VI/95 rev. 5 Appendix B, EC 1107/2009 of the European Parliament and of the Council of 21 Oct 2009, EEC 7525/VI/95 rev. 9 (March 2011), OECD 509 Crop Field Trial (2009), SANCO/3029/99 rev. 4 (11 July 2000), OECD-ENV/JM/MONO/(2007)17
Deviations:	No
GLP:	Yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)
Acceptability:	Yes
Duplication (if vertebrate study)	--

Executive Summary

The objective of the study was to determine the magnitude of residues of pyraclostrobin (BAS 500 F) after one application of BAS 500 06 F carried out on wheat at the growth stage “end of tillering” (BBCH 25-29). The selected application rates, frequency and spray interval cover the Good Agricultural Practice (critical GAP).

In whole plant samples collected from plot 2 directly after the application at the growth stage “end of tillering” (BBCH 25-29) residues of pyraclostrobin (BAS 500 F) ranged between 18 and 25 mg/kg. At the last sampling at 14 DALA (BBCH 31-32) residues decreased to a range of 0.35 - 0.87 mg/kg.

I. MATERIAL AND METHODS

A. MATERIAL

Test item and application

The trial consisted of a control plot (untreated) and one treated plot (plot 2) without replication. No product containing the test item was used on the test plots during season 2015 and 2016.

The test item BAS 500 06 F (EC) was foliar applied on plot 2 at a nominal rate of 250 g pyraclostrobin/ha in a nominal spray volume of 200 L/ha at growth stage “end of tillering” (BBCH 25-29) according to Good Laboratory Practice.

B. STUDY DESIGN

Study site

During the 2016 growing season a total of three trials were conducted in representative wheat growing areas in Germany.

Table 12: Trial Site Description

Trial No.	Crop	Variety*	Region°	Country / Region	Name of Owner / Address of Field Test Site
L160146	Wheat	Kadrilj	Europe North	Germany / Palatinate	BASF SE Versuchsgarten 5 67117 Limburgerhof
L160147		Chamsin		Germany / Kraichgau	Jürgen Wintterle Field No. 2261 74193 Stetten
L160148		Chamsin		Germany / Rheinhessen	Mathias Lehn Field No. 3006 67294 Mauchenheim

*Non-GLP data

° Climatic zone according to European Community Guideline SANCO 7525/VI/95 - rev.10.1, December 2015

Table 18: Average Air Temperature and Precipitation (Non GLP data)

Trial No.	Weather Station	Distance from Test Location [km]	Recorded Date	Average Air Temperature [°C]	Precipitation (monthly Σ) [mm]
L160146	Agrarmeteorologie Rheinland-Pfalz; Station Schifferstadt	~1.0	01.-31.05.2016	15.1	97.3
L160147	Agrarmeteorologie Baden-Württemberg; Station Stetten	~1.5	01.-31.05.2016	13.8	84.2
L160148	Agrarmeteorologie Rheinland-Pfalz; Station Kettenheim	~5.0	06.-20.05.2016	15.3*	12.1*

*Based on 06.-20.05.2016 only

Table 19: Precipitation (Data from pluviometer set at trial site – Non GLP)

Trial No.	Rainfall from application until last sampling									
L160146	DALA*	1	2	3	4	5	7	10	12	14
	l/m ²	0	0	0	0	0	5.0	11.8	0	0
L160147	DALA	1	2	3	4	5	7	10	12	14
	l/m ²	0	0	0	0	3.5	0.5	10.0	0	2.5
L160148	DALA	1	2	3	4	5	7	10	12	14
	l/m ²	0	0	0	0	4.0	5.0	1.0	0	2.0

*DALA= Days After Last Treatment -> calculated values

Sampling information

For this study specimens were collected as wheat whole plants without roots 1 hour after application as well as 1, 2, 3, 4, 5, 7, 10, 12 and 14 days thereafter. All specimens were sampled from the untreated and from the treated plots. Untreated specimens were obtained prior to treated specimens at each sampling occasion. All specimens were transferred to freezer storage on the day of sampling and were then stored frozen ($\leq -18^{\circ}\text{C}$).

Residue analysis

All specimens were analyzed for pyraclostrobin (BAS 500 F) using BASF method no. 535/1 (L0076/01). The method has a limit of quantitation of 0.01 mg/kg for both analyses.

C. Description of the analytical procedures

For analysis of plant materials, BASF method no. 535/1 (L0076/01) was used, which determines the analyte by means of HPLC-MS/MS. Validation of the analytical method was performed on plant matrices in a separate study (BASF DocID 2006/1039427). Pyraclostrobin was extracted with a mixture of methanol, water and hydrochloric acid. An aliquot of the extract is centrifuged and partitioned at alkaline conditions against cyclohexane. The method has a limit of quantitation (LOQ) of 0.01 mg/kg. The maximum storage interval from harvest until analysis was 103 days. It was investigated earlier (BASF DocID 2001/5000232) that residues were stable during the period of frozen storage prior to analysis.

Procedural recoveries (overall mean) were 106% for pyraclostrobin in wheat plants using fortification levels between 0.01–50 mg/kg. The results are summarized in the following table.

Table A 14: Summary of wheat plant matrix recoveries for pyraclostrobin

Test System	Matrix	Fortification Level [mg/kg]	Mean [%]	SD [±]	RSD [%]	n
			BAS 500 F			
Wheat	Whole plant no roots	0.01, 0.10 and 50	106	3.9	3.7	8

II. RESULTS AND DISCUSSION

Table A 7: Summary of residues of pyraclostrobin (BAS 500 F) in wheat

Sampling No.	Portion analyzed	DALA ¹⁾	Growth stage	n	BAS 500 F [mg/kg]
1	Wheat, (whole plant no roots)	1 ²⁾	25 - 29	3	18 - 25
2		1	25 - 29	3	11 - 13
3		2	27 - 30	3	7.1 - 10
4		3	29 - 30	3	6.3 - 7.8
5		4	30 - 31	3	2.3 - 4.9
6		5	30 - 31	3	1.2 - 3.6
7		7	30 - 31	3	1.9 - 2.0
8		10	31	3	0.8 - 1.2
9		12	31 - 32	3	0.39 - 1.1
10		14	31 - 32	3	0.1 – 0.46 (0.35 - 0.87) *

¹⁾ days after last application

²⁾ HALA: hours after last application

* The range of residue values, i.e. 0.35 – 0.87 mg /kg for sampling date at 14 DALA given in the table on page 6 of the study report is incorrect. Correct values, i.e. 0.1 – 0.46, are presented in the table on page 17 of the study report.

III. CONCLUSION

In whole plant samples collected from plot 2 directly after the application at the growth stage “end of tillering” (BBCH 25-29) residues of pyraclostrobin (BAS 500 F) ranged between 18 and 25 mg/kg. At the last sampling at 14 DALA (BBCH 31-32) residues decreased to a range of 0.35 - 0.87 mg/kg. No residues of pyraclostrobin ≥ 0.01 mg/kg were present in control specimens.

A 2.2.1.1.5 Study 5

Comments of zRMS:	<p>The calculation is considered valid and acceptable for regulatory use.</p> <p>FOCUS (2006, 2014) degradation kinetics guidance was applied to calculate DT₅₀ endpoints for pyraclostrobin modelling from residues measured in three plant residue trials in Europe (L160146, L160147, L160148). The data were described reasonably well by SFO kinetics and acceptable endpoints were derived for all studies.</p> <p>The error value for all trial is below 15%. Therefore, the results were considered to be reliable and suitable for the risk assessment.</p> <p>The calculation is considered valid and acceptable for regulatory use.</p>
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Reference:	CP 10.1.2.2/5
Report	<p>Dissipation of BAS 500 F - Pyraclostrobin on young wheat plants from field trials conducted in Northern zone of Europe - Calculation of DT₅₀ dissipation times,</p> <p>Schroeder T., 2017</p> <p>report No EU-CALC-2133</p> <p>BASF DocID 2017/1037247</p> <p>Authority registration No</p>
Guideline(s):	FOCUS degradation kinetics (2006 and 2014)
Deviations:	No
GLP:	<p>No</p> <p>Justification: for modelling calculations of DT₅₀ dissipation times GLP is not required</p>
Acceptability:	Yes
Duplication (if vertebrate study)	--

Executive Summary

The residue decline of BAS 500 F - pyraclostrobin in young wheat plants was investigated in a range of field trials at different sites in Northern Europe during the 2016 growing season (Kramm, 2017, BASF DocID 2017/1029774). This modeling report provides kinetic analysis and estimation of the dissipation times (DT₅₀ values) for pyraclostrobin for each field data set. The DT₅₀ values ranged between 1.17 – 2.22 d.

I. MATERIAL AND METHODS

Kinetic modeling strategy

Kinetic evaluation was performed in order to derive dissipation parameters for the residues of pyraclostrobin in wheat plants. Since no specific recommendation is available how to carry out the kinetic evaluation for plant residue decline studies, guidance of the FOCUS workgroup on degradation kinetics was used in order to derive degradation parameters for modeling purposes. Recommended estimation methods were applied in order to derive SFO (single first order) dissipation parameters for modeling purposes. The appropriate kinetic model was identified based on visual and statistical assessment.

According to FOCUS, a SFO kinetic model is considered appropriate, if the fit is visually acceptable and passes the χ^2 -test at an error level of 15% or less. Furthermore, the error term required to pass the χ^2 -test may be larger if there is a large scatter in the data like for field studies. In this case, a decision should be based on visual assessment. If the overall pattern of decline in pesticide concentrations is represented adequately by the model and the distribution of the residuals is random, the half-life from the SFO model may be used for modeling.

The goodness-of-fit of the kinetic models was assessed by visual inspection and statistical measures, as recommended by the FOCUS guidance (FOCUS, 2006) on degradation kinetics. For visual inspection, the recommended graphical representations of observed and modeled decline curves versus time and the residuals versus time are presented. As goodness-of-fit measures, the χ^2 minimum error level is provided.

II. RESULTS

The presented DT₅₀ values derived under consideration of guidance from the FOCUS kinetics working group are suitable endpoints for modeling purposes. The degradation rate constants of the different fits were estimated significantly different from zero as indicated by low P values. The visual assessment of the different fits showed that the χ^2 error values were acceptable as the observations were generally well described by the fitted curves and the residuals were randomly scattered around the zero line. The choice of the SFO model resulted in the best fit for these trials.

Table A 8: Calculated DT₅₀ values for pyraclostrobin in wheat plants and statistical indices of three different sites in Northern Europe (field trials from 2016)

Plant	Trial	Zone	Kinetic model	DT ₅₀ [d]	P (t-test)	χ^2 error[%]
wheat	L160146	North	SFO	2.22	p < 0.001	4.2
wheat	L160147	North	SFO	1.17	p < 0.001	14.9
wheat	L160148	North	SFO	2.11	p < 0.001	9.3

III. CONCLUSION

According to the recommendation of the FOCUS Kinetics guidance document reliable DT₅₀ values could be derived from the experimental data obtained for all three trials. The kinetic evaluation showed that the SFO model is appropriate to describe the residues of pyraclostrobin from the experimental data obtained in the field trials. The DT₅₀ values ranged between 1.17 – 2.22 d.

References

- Kramm, R. 2017. Study on the residue behaviour of pyraclostrobin (BAS 500 F) in young wheat plants after foliar application with BAS 500 06 F at growth stage 25/29 under field conditions in Northern Europe, in spring 2016. BASF DocID 2017/1029774.
- 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, 434pp

A 2.2.1.1.6 Study 6

Comments of zRMS:	Not evaluated by the zRMS. The study was considered as not essential for the risk assessment.
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Reference:	CP 10.1.2.2/6
Report	Study on the residue behaviour of Mefentrifluconazole (BAS 750 F) on wheat (young plants) after treatment with BAS 750 05 F under field conditions in North and South Europe, season 2018, Moreno, S., Galvez, O., 2019 Report No 849756,18/05/PF BASF DocID 2018/1205816 Authority registration No
Guideline(s):	OECD 509 Crop Field Trial (2009), EEC 7029/VI/95 rev. 5 (July 22 1997), EEC 7525/VI/95 rev. 10.1, International guidelines for distribution and pesticides application AEPLA FAO 1985
Deviations:	No
GLP:	Yes (certified by ENAC, Entidad Nacional de Acreditación, Madrid, Spain)
Acceptability:	Yes
Duplication (if vertebrate study)	--

The objective of the study was to determine the magnitude of residues of mefentrifluconazole (BAS 750 F) on young wheat plants (BBCH 11-13) after one application of BAS 750 05 F at the application rate of 1 x 2.0 L product/ha (corresponding to 0.15 kg mefentrifluconazole/ha).

I. MATERIAL AND METHODS

A. MATERIALS

Test item and application

Each trial consisted of a control plot (untreated) and one treated plot without replication. All applications were made as foliar sprays, using commercial ground equipment or equipment which simulated commercial applications. No product containing the test item was used on the test plots during the year 2018.

The test item mefentrifluconazole was applied as BAS 750 05 F separately on each plot once at a nominal application rate of 150 g active substance = 2.0 L product per ha with a spray volume of 200 L/ha according to Good Laboratory Practice and Good Agricultural Practice. The actual application rates were within 10% of the nominal values (0.143 – 0.156 kg a.s./ha).

B. STUDY DESIGN

Study site

During the 2018 growing season a total of eight trials were conducted in representative wheat growing areas in Germany, Belgium, the Netherlands, Spain and Italy.

Sampling information

Specimens of whole plant without roots were generally collected at DALA (days after last application) +0, +1, +2, +4, +7, +10±1, +14±1. Control (untreated) specimens were collected at DALA +0, +7 and +14±1, and were obtained prior to collection of the treated specimens to avoid contamination. All specimens were frozen within 6 hours of being collected and transferred to freezer storage on the day of sampling and were then stored frozen ($\leq -18^{\circ}\text{C}$).

The maximum storage interval from harvest until analysis was 196 days. Data indicate that residues were stable during the period of frozen storage prior to analysis.

Information on additional irrigation and precipitation

Additional irrigation

No irrigation has been conducted on any of the trials.

Precipitation

On the day of application, no rainfall occurred before the test item had fully dried on the crop. Precipitation was measured with a pluviometer set at the trial site. Precipitation data revealed that rainfall occurred at the following field trial sites: L170451 (1.0 mm at 6 DALA and 5.0 mm at 11 DALA), L170452 (0.5 mm at 4 DALA and 0.5 mm at 14 DALA), L170453 (4 mm at 13 DALA, 17.0 mm at 14 DALA), L170454 (26 mm at 4 DALA; during the first three days, from 26.04.2018 till 29.04.2018 afternoon, no rain at the trial site was measured. In the evening/night of 29.04.2018, there was a heavy rain of 26 mm), 3.0 mm at 5 DALA), L170455 (2 mm at 6 DALA), L170456 (4 mm at 6 DALA; the quantity of rainfall measured on 30.04.2018 corresponds to the amount accumulated from 29.04.2018 at 3pm onwards (after last measure)).

Residue analysis

All specimens were analysed for mefentrifluconazole using BASF method no. L0076/09. The method has a limit of quantitation of 0.01 mg/kg for the analyte.

C. Description of the analytical procedures

For analysis of plant materials, BASF method no. L0076/09 was used, which determines the analyte by means of HPLC-MS/MS. Validation of the analytical method was performed on plant matrices in a separate study (BASF DocID 2015/3001681). Mefentrifluconazole is extracted with a mixture of methanol, water and hydrochloric acid. An aliquot of the extract is centrifuged and partitioned at alkaline conditions against cyclohexane. The method has a limit of quantitation (LOQ) of 0.01 mg/kg. The maximum storage interval from harvest until analysis was 196 days. It was investigated earlier (BASF DocID 2016/1112644) that residues were stable during the period of frozen storage prior to analysis.

Procedural recoveries (overall mean) were 96.6% for mefentrifluconazole in young wheat plants using fortification levels between 0.01–30 mg/kg. The results are summarized in the following table.

Table A 9: Summary of wheat plant matrix recoveries for mefentrifluconazole

Test System	Matrix	Fortification Level [mg/kg]	Mean [%]	SD [±]	RSD [%]	n
			BAS 750 F			
Wheat	Whole plant no roots	0.01, 0.10 and 30	96.6	6.2	6.4	19

II. RESULTS AND DISCUSSION

The mefentrifluconazole residues in the wheat specimens taken 0 DALA (1-3 HALA (hours after last application)) ranged from 6.0 – 26 mg a.s./kg. They decreased to 5.5 - 20 mg a.s./kg in the specimens taken 1 DALA and further to 4.2 - 15 mg a.s./kg at 2 DALA. In the specimens taken at 4 DALA 2.9 - 10 mg/kg were determined. The residue level in the specimens taken was 0.97 - 8.2 mg a.s./kg at 7 DALA, 0.33 - 3.2 mg a.s./kg at 9-11 DALA, and 0.094 - 1.6 at 13-15 DALA. No residues of mefentrifluconazole at or above the LOQ of 0.010 mg/kg were present in control specimens.

Table A-10: Summary of residues of mefentrifluconazole in young wheat plants from trials conducted in Germany, The Netherlands, Belgium, Spain and Italy

Trial details	Sampling timing	Sampling no.	Date	Crop growth stage (BBCH)	Residues (mg a.s./kg)
<u>Trial no.</u> L170451 ¹⁾ <u>Study site:</u> Germany, Anterior Palatinate, Böhl- Iggelheim	1-3 HALA	1	17.04.2018	12-13	6.0
	1 DALA	2	18.04.2018	12-13	5.9
	2 DALA	3	19.04.2018	13	4.2
	4 DALA	4	21.04.2018	14	2.9
	7 DALA	5	24.04.2018	14	1.9
	10 DALA	6	27.04.2018	21	1.2
	13 DALA	7	30.04.2018	21	1.0
<u>Trial no.</u> L170452 ²⁾ <u>Study site:</u> Germany, Brandenburg, Lentzke	1-3 HALA	1	27.04.2018	12	9.0
	1 DALA	2	28.04.2018	12	11
	2 DALA	3	29.04.2018	12/21	9.8
	4 DALA	4	01.05.2018	12/21	6.9
	7 DALA	5	04.05.2018	13/21	4.6
	10 DALA	6	07.05.2018	13/21	3.2
	14 DALA	7	11.05.2018	13/22	1.6
<u>Trial no.</u> L170453 ³⁾ <u>Study site:</u> Belgium, Hainaut, Saint- Amand	1-3 HALA	1	16.04.2018	11	21
	1 DALA	2	17.04.2018	11	17
	2 DALA	3	18.04.2018	11	15
	4 DALA	4	20.04.2018	11-12	4.9
	7 DALA	5	23.04.2018	11-12	0.97
	10 DALA	6	26.04.2018	12-13	0.33
	14 DALA	7	30.04.2018	13	0.094
<u>Trial no.</u> L170454 ⁴⁾ <u>Study site:</u> The Netherlands, Limburg, Ven- Zelderheide	1-3 HALA	1	26.04.2018	13	17
	1 DALA	2	27.04.2018	13	15
	2 DALA	3	28.04.2018	13	15
	4 DALA	4	30.04.2018	14-15	4.4
	7 DALA	5	03.05.2018	19	2.0
	11 DALA	6	07.05.2018	23	1.3
	13 DALA	7	09.05.2018	23	0.55
<u>Trial no.</u> L170455 ⁵⁾ <u>Study site:</u> Spain, Seville, Alcalá	1-3 HALA	1	02.05.2018	11-12	21
	1 DALA	2	03.05.2018	11-13	15
	2 DALA	3	04.05.2018	11-13	10
	4 DALA	4	06.05.2018	12-13	7.6
	7 DALA	5	09.05.2018	13/21	2.4

Trial details	Sampling timing	Sampling no.	Date	Crop growth stage (BBCH)	Residues (mg a.s./kg)
del Río	9 DALA	6	11.05.2018	14/21	1.7
	14 DALA	7	16.05.2018	16/21	0.76
Trial no. L170456 ⁶⁾ Study site: Spain, Navarra, Fontellas	1-3 HALA	1	24.04.2018	11-12	26
	1 DALA	2	25.04.2018	11-12	20
	2 DALA	3	26.04.2018	12-13	15
	4 DALA	4	28.04.2018	13/21	10
	7 DALA	5	01.05.2018	13/21	8.2
	10 DALA	6	04.05.2018	20-21	2.6
	15 DALA	7	09.05.2018	22/23	0.96
Trial no. L170457 Study site: Italy, Cuneo, Castagnito d'Alba	1-3 HALA	1	16.04.2018	12	6.3
	1 DALA	2	17.04.2018	12	5.5
	2 DALA	3	18.04.2018	12	4.8
	4 DALA	4	20.04.2018	13	3.4
	7 DALA	5	23.04.2018	13	2.6
	10 DALA	6	26.04.2018	13	0.85
	14 DALA	7	30.04.2018	14	1.6
Trial no. L170458 Study site: Italy, Taranto, Massafra	1-3 HALA	1	19.04.2018	12-13	7.5
	1 DALA	2	20.04.2018	13-14	7.6
	2 DALA	3	21.04.2018	13-14	5.4
	4 DALA	4	23.04.2018	14	4.7
	7 DALA	5	26.04.2018	15	3.4
	10 DALA	6	29.04.2018	16	2.6
	13 DALA	7	02.05.2018	25	1.5

HALA: hours after last application; DALA: days after last application

- 1 Rainfall occurred at 6 DALA (1.0 mm) and 11 DALA (5.0 mm). Precipitation data from pluviometer set at trial site are given in Table 21 of the study report.
- 2 Rainfall occurred at 4 DALA (0.5 mm) and 14 DALA (0.5 mm). Precipitation data from pluviometer set at trial site are given in Table 21 of the study report.
- 3 Rainfall occurred at 13 DALA (4 mm) and 14 DALA (17.0 mm). Precipitation data from pluviometer set at trial site are given in Table 21 of the study report.
- 4 Rainfall occurred at 4 DALA (26 mm); during the first three days, from 26.04.2018 till 29.04.2018 afternoon, no rain at the trial site was measured. In the evening/night of 29.04.2018, there was a heavy rain of 26 mm) and 5 DALA (3.0 mm). Precipitation data from pluviometer set at trial site are given in Table 21 of the study report.
- 5 Rainfall occurred at 6 DALA (2 mm). Precipitation data from pluviometer set at trial site are given in Table 21 of the study report.
- 6 Rainfall occurred at 6 DALA (4 mm; the quantity of rainfall measured on 30.04.2018 corresponds to the amount accumulated from 29.04.2018 at 3pm onwards (after last measure)). Precipitation data from pluviometer set at trial site are given in Table 21 of the study report.

III. CONCLUSION

In whole plant samples collected directly after the application (BBCH 11-13) residues of mefentrifluconazole ranged between 6.0 – 26 mg a.s./kg. At the last sampling at 14 DALA (BBCH 13-25) residues decreased to a range of 0.094 - 1.6 mg/kg. No residues of mefentrifluconazole ≥ 0.010 mg/kg were present in control specimens.

A 2.2.1.1.7 Study 7

Comments of zRMS:	Not evaluated by the zRMS. The study was considered as not essential for the risk assessment.
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Reference:	CP 10.1.2.2/7
Report	Study on the residue behaviour of Mefentrifluconazole (BAS 750 F) on pea (young plants) after treatment with BAS 750 05 F under field conditions in North and South Europe, season 2018, Moreno, S., Galvez, O., 2019 Report No 849757 BASF DocID 2018/1205813 Authority registration No
Guideline(s):	EEC 79/117, EC 1107/2009 of the European Parliament and of the Council of 21 Oct 2009, OECD consensus document Number 13 (ENV/JM/MONO(2002)9), OECD-ENV/JM/MONO(99)22, Real Decreto 1369/2000, EEC 7029/VI/95 rev. 5 Appendix B (July 22 1997), OECD 509 Crop Field Trial (2009), OECD Principles of Good Laboratory Practice and Compliance Monitoring Number 4. Quality Assurance and GLP ENV/JM/MONO(99)20, ENV/MC/CHEM(98)17, EEC 7525/VI/95 rev. 10.1, 2004/10/EC (2004), International guidelines for distribution and pesticides application AEPLA FAO 1985, EEC 91/414
Deviations:	No
GLP:	Yes (certified by ENAC, Entidad Nacional de Acreditación, Madrid Spain)
Acceptability:	Yes
Duplication (if vertebrate study)	--

The objective of the study was to determine the magnitude of residues of mefentrifluconazole (BAS 750 F) on pea (young plants) after one application of BAS 750 05 F at the application rate of 1 x 2.0 L/ha product/ha (corresponding to 0.15 kg mefentrifluconazole/ha).

I. MATERIAL AND METHODS

A. MATERIALS

Test item and application

Each trial consisted of a control plot (untreated) and one treated plot. All applications were made as foliar sprays, using commercial ground equipment or equipment which simulated commercial applications. No product containing the test item was used on the test plots during the year 2018.

The test item mefentrifluconazole was applied as BAS 750 05 F separately on each plot once at a nominal application rate of 150 g active substance = 2.0 L product per ha with a spray volume of 200 L/ha according to Good Laboratory Practice and Good Agricultural Practice. The actual application rates were within 10% of the nominal values (0.145 – 0.153 kg a.s./ha).

B. STUDY DESIGN

Study site

During the 2018 growing season a total of seven trials were conducted in representative pea growing areas in Germany, The Netherlands, Spain and Italy.

Sampling information

Specimens of whole plant without roots were generally collected at DALA +0, +1, +2, +4, +7, +10±1, +14±1. Control (untreated) specimens were collected at DALA +0, +7 and +14±1, and were obtained prior to collection of the treated specimens to avoid contamination. Generally, the specimens were frozen within 6 hours of being taken and remained frozen at or below -18°C, including during transportation, until analysis.

The maximum storage interval from harvest until analysis was 170 days. Data indicate that residues were stable during the period of frozen storage prior to analysis.

Information on additional irrigation and precipitation

Additional irrigation

Irrigation has been conducted on trial L170450 (drip irrigation, 1 hour of duration, each on 20.4., 22.04., 25.04. and 30.04.2018).

Precipitation

On the day of application, no rainfall occurred before the test item had fully dried on the crop. Precipitation was measured with a pluviometer set at the trial site. Precipitation data revealed that rainfall occurred at following field trial sites: L180497 (0.5 mm at 7 DALA and 2.0 mm at 13 DALA), L170444 0.5 mm at 4 DALA and 0.5 mm at 14 DALA), L170446 (39 mm at 4 DALA (Due to heavy rain, plots were covered without touching the plants according to study plan during rainfall.), 3.0 mm at 5 DALA), L170447 (2 mm at 6 DALA), L170448 (4 mm at 6 DALA (the quantity of rainfall measured on 30.04.2018 corresponds to the amount accumulated from 29.04.2018 at 3pm onwards (after last measure)), L170449 (3 mm at 9 DALA, 1.0 mm at 12 DALA; after the last sampling on 13 DALA precipitation was measured on 13.06.18 of 4 mm).

Residue analysis

All specimens were analysed for mefentrifluconazole using BASF method no. L0076/09. The method has a limit of quantitation of 0.01 mg/kg for the analyte.

C. Description of the analytical procedures

For analysis of plant materials, BASF method no. L0076/09 was used, which determines the analyte by means of HPLC-MS/MS. Validation of the analytical method was performed on plant matrices in a separate study (BASF DocID 2015/3001681). Mefentrifluconazole is extracted with a mixture of methanol, water and hydrochloric acid. An aliquot of the extract is centrifuged and partitioned at alkaline conditions against cyclohexane. The method has a limit of quantitation (LOQ) of 0.01 mg/kg. The maximum storage interval from harvest until analysis was 170 days. It was investigated earlier (BASF DocID 2016/112644) that residues were stable during the period of frozen storage prior to analysis.

Procedural recoveries (overall mean) were 78.1% for mefentrifluconazole in young pea plants using fortification levels between 0.01–20 mg/kg. The results are summarized in the following table.

Table A 11: Summary of pea plant matrix recoveries for mefentrifluconazole

Test System	Matrix	Fortification Level [mg/kg]	Mean [%]	SD [±]	RSD [%]	n
			BAS 750 F			
Pea	Whole plant no roots	0.01, 0.10 and 20	78.1	4.0	5.2	11

II. RESULTS AND DISCUSSION

The mefentrifluconazole residues in the pea specimens taken 0 DALA (1-3 HALA) ranged from 7.5 - 12 mg a.s./kg. They decreased to 5.0 – 9.1 mg a.s./kg in the specimens taken 1 DALA. In the specimens taken 2 DALA 4.2 – 7.7 mg a.s./kg were determined. The residue level in the specimens taken 4 DALA was 1.1 – 4.0 mg/kg mg a.s./kg, those taken 7 DALA was 0.59 – 3.2 mg a.s./kg. The residue level in the specimens taken was 0.24 – 1.9 mg/kg at 9-11 DALA and 0.14 - 0.68 mg/kg at 13-15 DALA. No residues of mefentrifluconazole ≥ 0.010 mg/kg were present in control specimens.

Table A- 11: Summary of residues of mefentrifluconazole in young pea plants from trials conducted in Germany, The Netherlands, Spain and Italy

Trial details	Sampling timing	Sampling no.	Date	Crop growth stage (BBCH)	Residues (mg a.s./kg)
<u>Trial no.</u> L180497 ¹⁾ <u>Study site:</u> Germany, Anterior Palatinate, Hessheim	1-3 HALA	1	16.04.2018	13	7.5
	1 DALA	2	17.04.2018	13-14	7.1
	2 DALA	3	18.04.2018	14/30	5.5
	4 DALA	4	20.04.2018	14/32	3.5
	7 DALA	5	23.04.2018	15/34	1.7
	10 DALA	6	26.04.2018	16/36	1.0
	14 DALA	7	30.04.2018	16/36	0.68
<u>Trial no.</u> L170444 ²⁾ <u>Study site:</u> Germany, Brandenburg, Lentzke	1-3 HALA	1	27.04.2018	13	7.5
	1 DALA	2	28.04.2018	13	5.0
	2 DALA	3	29.04.2018	13	4.2
	4 DALA	4	01.05.2018	14/31	2.0
	7 DALA	5	04.05.2018	14/32	1.5
	10 DALA	6	07.05.2018	15-16/33	0.85
	14 DALA	7	11.05.2018	16-17/34	0.28
<u>Trial no.</u> L170446 ³⁾ <u>Study site:</u> The Netherlands, Limburg, Wellerloi	1-3 HALA	1	26.04.2018	13	10
	1 DALA	2	27.04.2018	13	6.0
	2 DALA	3	28.04.2018	13	5.5
	4 DALA	4	30.04.2018	14-15	1.1
	7 DALA	5	03.05.2018	15	0.59
	11 DALA	6	07.05.2018	16	0.24
	13 DALA	7	09.05.2018	17-18	0.14
<u>Trial no.</u> L170447 ⁴⁾ <u>Study site:</u> Spain, Seville, Alcalá del Río	1-3 HALA	1	02.05.2018	12	8.8
	1 DALA	2	03.05.2018	12	6.2
	2 DALA	3	04.05.2018	12-13	5.6
	4 DALA	4	06.05.2018	13-14	3.4
	7 DALA	5	09.05.2018	14-15	1.3
	9 DALA	6	11.05.2018	15	0.76
	14 DALA	7	16.05.2018	16	0.20

Trial details	Sampling timing	Sampling no.	Date	Crop growth stage (BBCH)	Residues (mg a.s./kg)
<u>Trial no.</u> L170448 ⁵⁾ <u>Study site:</u> Spain, Navarra, Fontellas	1-3 HALA	1	24.04.2018	11-12	11
	1 DALA	2	25.04.2018	11-12	9.1
	2 DALA	3	26.04.2018	11-12	7.7
	4 DALA	4	28.04.2018	11-13	4.0
	7 DALA	5	01.05.2018	14/30	3.2
	10 DALA	6	04.05.2018	14/31	1.9
	15 DALA	7	09.05.2018	14/34	0.65
<u>Trial no.</u> L170449 ⁶⁾ <u>Study site:</u> Italy, Cuneo, Castagnito d'Alba	1-3 HALA	1	30.05.2018	13	12
	1 DALA	2	31.05.2018	13	7.8
	2 DALA	3	01.06.2018	14	5.0
	4 DALA	4	03.06.2018	15	3.2
	7 DALA	5	06.06.2018	15	2.4
	9 DALA	6	08.06.2018	17	1.3
	13 DALA	7	12.06.2018	30	0.24
<u>Trial no.</u> L170450 <u>Study site:</u> Italy, Taranto, Palagiano	1-3 HALA	1	19.04.2018	12-13	9.6
	1 DALA	2	20.04.2018	13-14	7.3
	2 DALA	3	21.04.2018	13-14	5.8
	4 DALA	4	23.04.2018	14	3.4
	7 DALA	5	26.04.2018	15	1.9
	10 DALA	6	29.04.2018	16	1.5
	13 DALA	7	02.05.2018	35	0.65

HALA: hours after last application; DALA: days after last application

- 1 Rainfall occurred at 7 DALA (0.5 mm) and at 13 DALA (2.0 mm). Precipitation data from pluviometer set at trial site are given in Table 21 of the study report.
- 2 Rainfall occurred at 4 DALA (0.5 mm) and at 14 DALA (0.5 mm). Precipitation data from pluviometer set at trial site are given in Table 21 of the study report.
- 3 Rainfall occurred at 4 DALA (39 mm; due to heavy rain, plots were covered without touching the plants according to study plan during rainfall.) and at 5 DALA (3.0 mm). Precipitation data from pluviometer set at trial site are given in Table 21 of the study report.
- 4 Rainfall occurred at 6 DALA (2 mm). Precipitation data from pluviometer set at trial site are given in Table 21 of the study report.
- 5 Rainfall occurred at 6 DALA (4 mm; the quantity of rainfall measured on 30.04.2018 corresponds to the amount accumulated from 29.04.2018 at 3 pm onwards (after last measure)) and at 5 DALA (3.0 mm). Precipitation data from pluviometer set at trial site are given in Table 21 of the study report.
- 6 Rainfall occurred at 9 DALA (3 mm) and at 12 DALA (1.0 mm). Precipitation measured on 13.06.18 of 4 mm, is not relevant for the outcome of the study as it occurred after the last sampling, which was conducted on 12.06.). Precipitation data from pluviometer set at trial site are given in Table 21 of the study report.

III. CONCLUSION

In whole pea plant samples collected directly after the application (BBCH 11-13) residues of mefentrifluconazole ranged between 7.5 - 12 mg/kg. At the last sampling at 13-15 DALA (BBCH 14-36) residues decreased to a range of 0.14 - 0.68 mg/kg. No residues of mefentrifluconazole ≥ 0.010 mg/kg were present in control specimens.

A 2.2.1.1.8 Study 8

Comments of zRMS:	Not evaluated by the zRMS.
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Reference:	CP 10.1.2.2/8
Report	Calculation of DT ₅₀ dissipation times for BAS 750 F - Mefentrifluconazole on wheat plants under field conditions in North and South Europe, Szegedi, K., 2019 Report No CALC-2344 BASF DocID 2019/2034648 Authority registration No
Guideline(s):	FOCUS Degradation Kinetics (2006) SANCO/10058/2005 version 1.1 (December 2014)
Deviations:	No
GLP:	No. This report does not describe experimental studies related to the assessment of possible hazards for man or the environment and, therefore, is not subject to GLP regulations.
Acceptability:	Yes
Duplication (if vertebrate study)	--

This modeling report provides kinetic analysis and estimation of the dissipation times (DT₅₀ values) for mefentrifluconazole on young wheat plants. The residue decline of mefentrifluconazole after foliar application of the product BAS 750 05 F at growth stage BBCH 11-13 on young wheat plants has been investigated at a range of field trials at eight different sites in Germany, Belgium, the Netherlands, Spain and Italy, during the 2018 growing season (Moreno and Gálvez, 2018, BASF DocID 2018/1205816).

I. MATERIAL AND METHODS

Kinetic modeling strategy

Kinetic evaluation was performed in order to derive dissipation parameters for the residues of mefentrifluconazole in wheat plants. Since no specific recommendation is available how to carry out the kinetic evaluation for plant residue decline studies, guidance of the FOCUS workgroup on degradation kinetics (FOCUS 2006) was used in order to derive degradation parameters for modeling purposes. Thus, the selected DT₅₀ values are suitable input parameters for models that require single first order (SFO) DT₅₀ values or conservative substitutes.

The goodness-of-fit of the kinetic models was assessed by visual inspection and statistical measures, as recommended by the FOCUS guidance (FOCUS 2006) on degradation kinetics. The recommended kinetic models, *i.e.* the single first order kinetics (SFO) and the Gustafson-Holden model (FOMC) were applied. Testing of further models was not necessary in the current evaluation. For visual inspection, the recommended graphical representations of observed and modeled decline curves versus time and the residuals versus time are presented in the report. As goodness-of-fit measures, the χ^2 error is provided. The kinetic model was considered appropriate if the residuals are randomly distributed around zero, the χ^2 - error value ideally < 15 % and the estimated degradation parameters differed from zero at a 5% significance level (FOCUS, 2006).

According to FOCUS the χ^2 - error of 15 % should not be considered as an absolute cut-off criterion. For cases of a larger χ^2 - error (e.g. based on a large scatter in the data as it might be the case for field studies), the decision on the acceptability of the model was based on visual assessment, to evaluate if the fit still represents a reasonable description of the degradation behavior (see FOCUS 2006). If the overall pattern of decline in pesticide concentrations was represented adequately by the model and the distribution of the residuals was random (no systematic deviations), the half-life from the respective model was considered appropriate.

II. RESULTS AND DISCUSSION

The presented DT₅₀ values derived under consideration of guidance from the FOCUS kinetics working group are suitable endpoints for modeling purposes. The degradation rate constants of the different fits were estimated significantly different from zero as indicated by low P values. The visual assessment of the different fits showed that the χ^2 error values were acceptable as the observations were generally well described by the fitted curves and the residuals were randomly scattered around the zero line.

Table A- 14: Calculated DT₅₀ values for mefentrifluconazole in wheat plants and statistical indices

Plant	Trial	Country	Kinetic model	DT ₅₀ [d]	χ^2 error [%]	p (t-test)
Wheat	L170451	Germany	SFO	4.1	7.6	<0.001
Wheat	L170452	Germany	SFO	6.0	12.1	0.002
Wheat	L170453	Belgium	SFO	2.3	14.4	<0.001
Wheat	L170454	The Netherlands	SFO	2.8	18.7	0.004
Wheat	L170455	Spain	SFO	2.4	7.3	<0.001
Wheat	L170456	Spain	SFO	3.3	8.0	<0.001
Wheat	L170457	Italy	SFO	5.0	8.8	<0.001
Wheat	L170458	Italy	SFO	5.8	6.9	<0.001

III. CONCLUSION

The decline of mefentrifluconazole residues on young wheat plants was well described by single first order (SFO) kinetics and provided reliable DT₅₀ values for all trials. The DT₅₀ values of mefentrifluconazole in young wheat plants derived for the individual trials ranged between 2.3 and 6.0 days.

References

- FOCUS. 2006. Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration" Report of the FOCUS Work Group on Degradation Kinetics, EC Document Reference Sanco/10058/2005 version 1.1 (December 2014), 440pp.
- Moreno, S. and Gálvez, O. 2018. Study on the residue behaviour of mefentrifluconazole (BAS 750 F) on wheat (young plants) after treatment with BAS 750 05 F under field conditions in North and South Europe, season 2018. BASF DocID 2018/1205816.

A 2.2.1.1.9 Study 9

Comments of zRMS:	Not evaluated by the zRMS.
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Reference:	CP 10.1.2.2/9
Report	Calculation of DT ₅₀ dissipation times for BAS 750 F - Mefentrifluconazole on pea plants under field conditions in North and South Europe, Szegedi, K., 2019 Report No CALC-2345 BASF DocID 2019/2034650 Authority registration No
Guideline(s):	FOCUS Degradation Kinetics (2006) SANCO/10058/2005 version 1.1 (December 2014)
Deviations:	No
GLP:	No. This report does not describe experimental studies related to the assessment of possible hazards for man or the environment and, therefore, is not subject to GLP regulations.
Acceptability:	Yes
Duplication (if vertebrate study)	--

This modeling report provides kinetic analysis and estimation of the dissipation times (DT₅₀ values) for mefentrifluconazole on young pea plants. The residue decline of mefentrifluconazole after foliar application of the product BAS 750 05 F at growth stage BBCH 11-13 on young pea plants has been investigated at a range of field trials at seven different sites in Germany, the Netherlands, Spain and Italy, during the 2018 growing season (Moreno and Gálvez, 2018, BASF DocID 2018/1205813).

I. MATERIAL AND METHODS

Kinetic evaluation was performed in order to derive dissipation parameters for the residues of mefentrifluconazole in pea plants. Since no specific recommendation is available how to carry out the kinetic evaluation for plant residue decline studies, guidance of the FOCUS workgroup on degradation kinetics (FOCUS 2006) was used in order to derive degradation parameters for modeling purposes. Thus, the selected DT₅₀ values are suitable input parameters for models that require single first order (SFO) DT₅₀ values or conservative substitutes.

The goodness-of-fit of the kinetic models was assessed by visual inspection and statistical measures, as recommended by the FOCUS guidance (FOCUS 2006) on degradation kinetics. The recommended kinetic models, *i.e.* the single first order kinetics (SFO) and the Gustafson-Holden model (FOMC) were applied. Testing of further models was not necessary in the current evaluation. For visual inspection, the recommended graphical representations of observed and modeled decline curves versus time and the residuals versus time are presented in the report. As goodness-of-fit measures, the χ^2 error is provided. The kinetic model was considered appropriate if the residuals are randomly distributed around zero, the χ^2 - error value ideally < 15 % and the estimated degradation parameters differed from zero at a 5% significance level (FOCUS, 2006).

According to FOCUS the χ^2 - error of 15 % should not be considered as an absolute cut-off criterion. For cases of a larger χ^2 - error (e.g. based on a large scatter in the data as it might be the case for field studies), the decision on the acceptability of the model was based on visual assessment, to evaluate if the fit still represents a reasonable description of the degradation behavior (see FOCUS 2006). If the overall pattern of decline in pesticide concentrations was represented adequately by the model and the distribution of the residuals was random (no systematic deviations), the half-life from the respective model was considered appropriate.

II. RESULTS AND DISCUSSION

The presented DT₅₀ values derived under consideration of guidance from the FOCUS kinetics working group are suitable endpoints for modeling purposes. The degradation rate constants of the different fits were estimated significantly different from zero as indicated by low P values. The visual assessment of the different fits showed that the χ^2 error values were acceptable as the observations were generally well described by the fitted curves and the residuals were randomly scattered around the zero line.

Table A- 15: Calculated DT₅₀ values for mefentrifluconazole in pea plants and statistical indices

Plant	Trial	Country	Kinetic model	DT ₅₀ [d]	χ^2 error [%]	p (t-test)
Pea	L180497	Germany	SFO	3.5	6.5	<0.001
Pea	L170444	Germany	SFO	2.5	9.0	<0.001
Pea	L170446	The Netherlands	SFO	1.7	13.6	<0.001
Pea	L170447	Spain	SFO	2.8	5.9	<0.001
Pea	L170448	Spain	SFO	3.5	6.7	<0.001
Pea	L170449	Italy	SFO	2.1	12.2	<0.001
Pea	L170450	Italy	SFO	3.2	5.8	<0.001

III. CONCLUSION

The decline of mefentrifluconazole residues on young plants was well described by single first order (SFO) kinetics and provided reliable DT₅₀ values for all trials. The DT₅₀ values of mefentrifluconazole in young plants derived for the individual trials ranged between 1.7 and 3.5 days.

References

- FOCUS. 2006. Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration" Report of the FOCUS Work Group on Degradation Kinetics, EC Document Reference Sanco/10058/2005 version 1.1 (December 2014), 440pp.
- Moreno, S. and Gálvez, O. 2018. Study on the residue behaviour of mefentrifluconazole (BAS 750 F) on pea (young plants) after treatment with BAS 750 05 F under field conditions in North and South Europe, season 2018. BASF DocID 2018/1205813.

A 2.2.1.1.10 Study 10

The study is currently under evaluation in the course of the EU renewal process of pyraclostrobin (RAR of pyraclostrobin Rev. 1 – 10 January 2020 Vol. 3 – B9).

Comments of zRMS:	Study was evaluated and accepted for the regulatory use in RR for BAS 751 00 F. In the current evaluation, the study was considered as not essential for the risk assessment.
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Reference:	CP 10.1.2.2/10
Report	Field study on the acute and long-term effects of a Pyraclostrobin formulation (BAS 500 06 F) applied as foliar spray in spring to cereals on populations of small mammals (wood mice and common voles) in Central Europe (Germany) XXXXXXXXXX Report No EU-397737, EU-183, EU-P13035 BASF DocID 2014/1000041 Authority registration No
Guideline(s):	none “no guidelines available”
Deviations:	No
GLP:	Yes (certified by Landesamt fuer Umwelt, Messungen und Naturschutz Baden Wuerttemberg, Karlsruhe, Germany)
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive Summary

The study investigated the potential acute and long-term effects of pyraclostrobin (applied as BAS 500 06 F, as foliar spray to cereals), on wood mouse and common vole populations. Long-term effects were investigated by comparing species abundance and population development in pyraclostrobin applied as BAS 500 06 F – in treated winter cereal fields and untreated winter cereal fields over a period of six months.

This study showed no impacts of the fungicide pyraclostrobin applied as BAS 500 06 F to winter cereal under field conditions on resident small mammal populations, of which eight different parameters (captures/individuals in- and off-crop, trapping efficiency, minimum number alive, population growth rate, percentage of reproductively active individuals, percentage of juveniles, percentage of females, and adult body weight) were monitored for the whole growth period of winter cereal in Germany. This could clearly be demonstrated for the resident wood mouse (*Apodemus sylvaticus*) populations. Although sample sizes were low for the common vole (*Microtus arvalis*) populations, no impacts of the fungicide pyraclostrobin applied as BAS 500 06 F on a low-density population of common voles could be shown.

I. MATERIAL AND METHODS

Study site

The study was conducted between April 2013 and October 2013 in twelve winter cereal fields in the vicinity of the villages of Limburgerhof and Altrip in the federal state of Rhineland-Palatinate in Germany. The size of the study fields ranged between 2.0 ha and 5.5 ha.

Application

Six study fields were treated with the fungicide pyraclostrobin applied as BAS 500 06 F (treatment fields). The first application was carried out at the early plant growth stage around BBCH stage 25 to 30, and the second application was conducted 20 days after the first application which was according to the use pattern. The remaining six study fields received no treatment (control fields).

Test organisms

Test organisms were free-living, wild populations of the wood mouse (*Apodemus sylvaticus*) and the common vole (*Microtus arvalis*) naturally inhabiting the selected study fields and directly adjacent habitats.

Trapping: Live trapping (capture-mark-recapture) was carried out to compare the abundance and population dynamics of small mammal species in treated and untreated winter cereal fields and adjacent off-crop areas. Eighty Ugglan multiple-capture traps were set up in each study plot, with 60 traps placed inside the cereal field and 20 traps placed in the off-crop area adjacent to the study field. Study plots were defined as the area covered by the small mammal trapping grid. Captured individuals were marked with Passive Integrated Transponders (PIT) for individual identification. A total of ten trapping sessions, each trapping session consisting of three consecutive nights of trapping and one night of pre-baiting, were performed in each study fields. Trapping commenced prior to the first application of pyraclostrobin applied as BAS 500 06 F and continued until October 2013 in accordance with agricultural practice after harvest, crop rotation and the development of the following crop on the study fields. This monitoring method allowed assessing the trapping efficiency and Minimum Number Alive (MNA), the rate of population growth, the rate of reproduction, the proportion of juveniles, the sex ratio and the development of the body weight throughout the Field Phase. To assess whether the treatment with pyraclostrobin applied as BAS 500 06 F had any effects on mice and vole species, the results of the above mentioned parameters of the six treatment fields were compared with those of the six control fields.

II. RESULTS AND DISCUSSION

a) Wood mouse (*Apodemus sylvaticus*)

The trapping efficiencies of wood mice in the in-crop habitats were comparable in treatment and control plots over the course of the Field Phase. The trapping efficiencies in the in-crop habitats of the treatment plots experienced a noticeable increase after the second application of pyraclostrobin applied as BAS 500 06 F (after trapping session 3), while the trapping efficiencies in the in-crop habitats of the control plots increased only gradually. The relatively high trapping efficiencies in the in-crop habitats of treatment and control plots (trapping session 4 until trapping session 8) indicate that wood mice preferentially used the in-crop habitats during the times of advanced crop development. After the study fields were harvested there was a shift in habitat use from the in-crop habitat to the off-crop habitat.

The minimum number of individual wood mice alive (MNA) showed a similar pattern in control and treatment plots over the course of the Field Phase. However, MNAs in the treatment plots increased considerably until the population maximum was reached at trapping session 5 (mid-May). On the other hand, MNAs in the control plots increased only gradually and reached the population maximum at a later stage (trapping session 7; around end of June).

Altogether, this also indicates that there are no acute effects of pyraclostrobin applied as BAS 500 06 F on wood mouse populations, considering that MNAs increased even shortly after each application with pyraclostrobin applied as BAS 500 06 F. The MNAs in the control plots never reached the maximum of the MNAs in the treatment plots. The population growth rate showed a similar pattern to the MNA values in control and treatment plots.

At the beginning of the trapping period, almost all individuals captured in treatment and control plots were reproductively active. Over the course of the reproductive season of wood mice and after treatments with pyraclostrobin applied as BAS 500 06 F the percentage of reproductively active individuals in control and treatment plots followed an almost identical development from trapping session 3 until the end of the trapping period.

The mean percentage of juvenile wood mice over the course of the Field Phase followed a similar pattern in treatment and control plots. In treatment and control plots the percentage of juveniles was low during the first trapping sessions (early April), as expected at the beginning of the reproductive season of small mammals in Central Europe.

The percentage of female wood mice followed a similar pattern in control and treatment plots. The sex ratio remained below 50% in control and treatment plots in all trapping sessions except for the trapping session that was conducted after harvest (trapping session 9).

The development of the mean body weight of adult wood mice across all trapping sessions followed a similar pattern in treatment and control plots. Mean body weight decreased in the last two trapping sessions in both control and treatment plots, which reflects the normal development.

The mean recapture rate of marked wood mice was similar in treatment and control plots, indicating no difference in survival of wood mice. From trapping session 2 until 8 the rate of recapture was in parts very high with almost 80%, but decreased after harvesting of the study fields (trapping session 9 and 10).

b) Common vole (Microtus arvalis)

The number of common voles captured was too low to infer any confident conclusions about abundance and population parameters in the treatment and control plots.

The trapping efficiencies of common voles in the in-crop habitats followed a very similar pattern in the treatment and control plots. Common voles were first trapped in in-crop habitats in trapping session 4 (beginning of May) after the second application of pyraclostrobin applied as BAS 500 06 F. Thereafter numbers increased steadily until they reached a peak at trapping session 7 (around end of June) which was followed by a continuous decrease until the completion of the Field Phase. On the contrary, trapping efficiencies in the off-crop habitats of both treatment groups were very low, but showed a considerable increase during the last trapping session in October after the study fields were harvested. The low trapping efficiencies of common voles in the off-crop habitats reflect the low number of captured common voles in these habitats and may indicate the potential habitat preferences for this species.

The minimum number of common vole individuals alive (MNA) was similar in control and treatment plots throughout the trapping period. No common voles were captured in the first three trapping sessions in the treatment or in the control plots. The population maximum of common voles was reached at trapping session 7 (around end of June) at the time of high ground cover shortly before harvest. The population growth rate of common voles showed a similar pattern to the MNA values in control and treatment plots.

The mean percentage of reproductively active individuals fluctuated in control and treatment plots probably mainly due to the low number of individuals captured. In control and treatment plots the percentage of reproductively active common voles was highest in trapping session 8, coinciding with the time of highest ground cover. After harvest of the study fields the percentage of reproductively active individuals decreased again potentially reflecting the increased emigration from the study fields.

The mean percentage of juvenile common voles was low over the course of the Field Phase in control and treatment plots. In addition to the low number of juvenile captures, juveniles were not captured in all study fields. Thus, no clear understandings of the dynamics of this parameter can be achieved due to the low sample size.

Meaningful statements on the development of the sex ratio of common voles cannot be made due to the sex ratio being based on very small sample sizes in control and treatment plots likewise.

Mean body weight of adult common voles was similar in control and treatment plots across trapping sessions. The body weight decreased slightly towards the end of the trapping period potentially indicating the potential decline in the number of pregnant females.

Some common voles were recaptured despite the overall low number of captured common voles in treatment and control plots.

III. CONCLUSION

In conclusion, this study showed no impacts of the fungicide pyraclostrobin applied as BAS 500 06 F applied to winter cereal under field conditions on resident small mammal populations, of which eight different parameters (captures/individuals in- and off-crop, trapping efficiency, minimum number alive, population growth rate, percentage of reproductively active individuals, percentage of juveniles, percentage of females, and adult body weight) were monitored for the whole growth period of winter cereal in Germany. This could clearly be demonstrated for the resident wood mouse (*Apodemus sylvaticus*) populations. Although sample sizes were low for the common vole (*Microtus arvalis*) populations, no impacts of the fungicide pyraclostrobin applied as BAS 500 06 F on a low-density population of common voles could be shown.

A 2.2.1.1.11 Study 11

The study is currently under evaluation in the course of the EU renewal process of pyraclostrobin (RAR of pyraclostrobin Rev. 1 – 10 January 2020 Vol. 3 – B9).

Comments of zRMS:	Study was evaluated and accepted for the regulatory use in RR for BAS 751 00 F. In the current evaluation, the study was considered as not essential for the risk assessment.
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Reference:	CP 10.1.2.2/11
Report	Field study on the acute and long-term effects of a Pyraclostrobin formulation (BAS 500 06 F) applied as foliar spray on meadows to populations of common voles in Central Europe (Germany), XXXXXXXXXX Report No EU-765174 BASF DocID 2015/1126803 Authority registration No
Guideline(s):	OECD Principles of Good Laboratory Practice
Deviations:	No
GLP:	Yes (certified by Landesanstalt fuer Umwelt, Messungen und Naturschutz Baden-Wuerttemberg, Karlsruhe, Germany)
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive Summary

The present study aims to investigate potential acute and long-term effects of the fungicide pyraclostrobin applied as BAS 500 06 F (containing the active substance pyraclostrobin) on common voles (*Microtus arvalis*) in agriculturally managed meadows. Meadows were used as a surrogate for other crop types to ensure sufficient numbers of common voles being exposed to the test item pyraclostrobin applied as BAS 500 06 F. Acute and long-term effects were investigated by comparing common vole abundance and population parameters in pyraclostrobin applied as BAS 500 06 F – treated and untreated meadows over a period that covered the reproductive season of common voles.

When applying pyraclostrobin applied as BAS 500 06 F following a use pattern of 2 x 250 g pyraclostrobin/ha, no negative effects were detected in any of the many investigated parameters throughout the study, i.e. no adverse acute and long-term effects of the fungicide pyraclostrobin applied as BAS 500 06 F on common vole populations were found. This second field effect study therefore confirms the outcome of the first field effects study, and the result of the risk assessment presented in M-CP section 10.

I. MATERIAL AND METHODS

The study was conducted in agriculturally used meadows (study fields) in the vicinity of Dornburg in the Limburg-Weilburg district in Hessen, Germany, between May and October 2015. The size of the ten study fields ranged from 1.0 ha to 3.0 ha.

Five study fields were treated twice with pyraclostrobin applied as BAS 500 06 F at a nominal application rate of 1.25 L product/ha (250 g pyraclostrobin/ha) in a spray volume of 200 L/ha. The first application was carried out on 08-11 June 2015 once common vole populations were large enough to ensure a sufficient part of the population to be exposed to the test item. The second application was conducted approximately three weeks later on 29 June - 04 July 2015. The remaining five study fields served as untreated control fields.

Live trapping (capture-mark-recapture) was carried out to compare abundance, population dynamics, age structure and reproduction of common voles in treated and untreated study fields. A total of 10 trapping sessions, each session consisting of one night of pre-baiting and three consecutive nights of trapping were performed.

Ugglan multiple-capture traps were used to live-trap small mammals. Sixty Ugglan multiple live capture traps were set up in each study field. Captured individuals were marked with Passive Integrated Transponders (PIT) for individual identification. All study fields were mowed before the trapping of common voles commenced and again in late summer (after trapping session 8 at the end of August 2015). This monitoring method allowed assessing the trapping success (number of captured animals and the number of individuals), the minimum number alive (MNA), the recapture rate, the sex ratio, the rate of reproduction, the proportion of juveniles, and the development of the body weight throughout the Field Phase which covered the majority of the reproductive season of common voles. In order to assess whether the treatment with pyraclostrobin applied as BAS 500 06 F had any effects on common voles, the results of the above mentioned parameters of the five treatment fields were compared with those of the five control fields.

II. RESULTS

Overall, trapping success was high: 9161 captures of common voles were made in all study plots during the Field Phase of this study, including a total of 2495 individually marked animals.

Three abundance parameters were evaluated: number of captures and of individuals, minimum number of individuals alive (MNA) and recapture rate of marked individuals.

The number of individuals of common voles monitored was not statistically significantly different between control and treatment plots in any trapping session.

The minimum number of individual common voles alive (MNA) in the treatment and the control plots showed a similar pattern (Figure 1), and there were no statistically significant differences between the two groups.

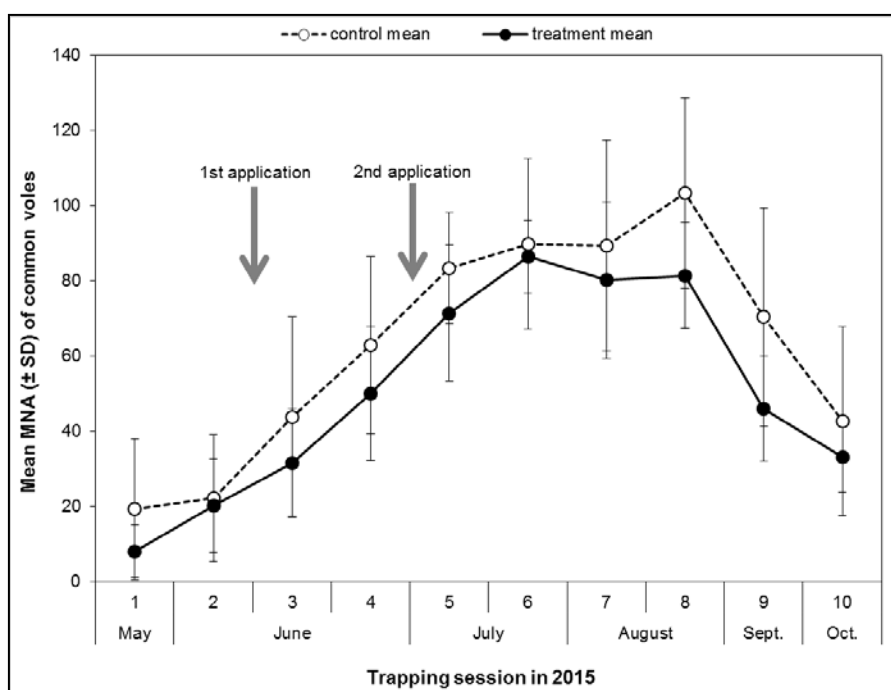


Figure A 1: Mean of the minimum number of common voles alive (MNA) in control and treatment plots (the applications of pyraclostrobin applied as BAS 500 06 F were conducted after trapping session 2 and 4. The error bars represent the standard deviation of the mean (\pm SD))

The mean recapture rate of marked common voles showed a similar development in treatment and control plots (Figure 2). The recapture rate of individuals exposed to pyraclostrobin applied as BAS 500 06 F in the treatment plots was not statistically significantly different than the recapture rate of common voles in the control plots.

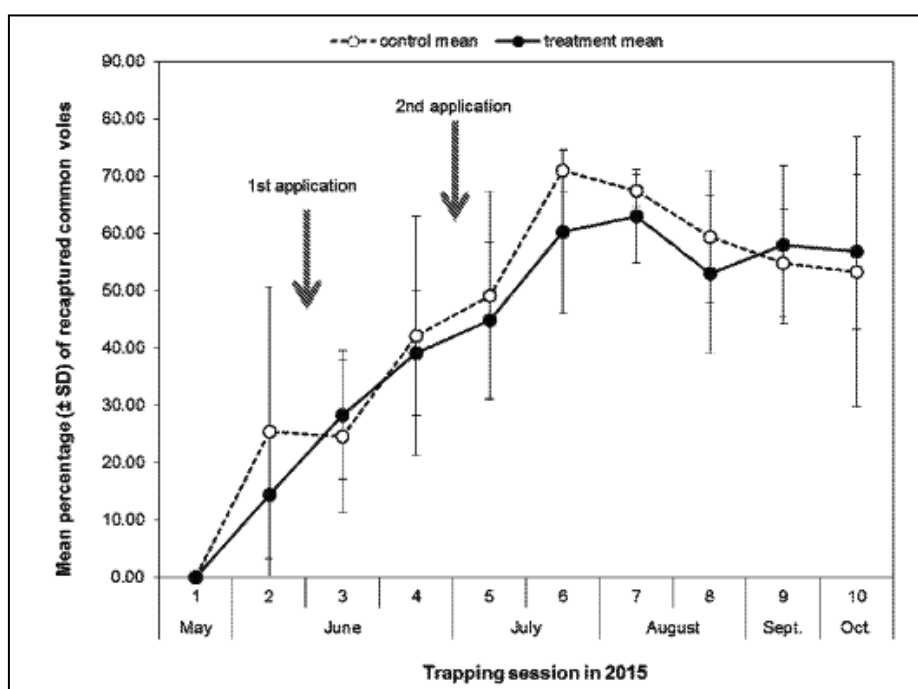


Figure A 2: Mean recapture rate (%) of common vole individuals in control and treatment plots (legend as in Figure 1)

Four population parameters were evaluated: sex ratio (proportion of females and males), reproductive activity (proportion of reproductively active animals), age structure (proportion of juveniles), and bodyweight development.

The sex ratio of common voles was similar in treatment and control plots, and the proportion of females fluctuated almost consistently between 50-60% throughout the trapping period. No statistically significant differences in the percentage of female common voles were detected between control and treatment plots.

The proportion of reproductively active common voles followed a similar pattern in the control and treatment plots over the course of the study (Figure 3), with most trapping sessions without statistically significant differences between the two groups. After the second application of pyraclostrobin applied as BAS 500 06 F, there was a marginal statistically significant difference ($p=0.05$) in trapping session no. 5, where the proportion of reproducing animals was even higher in the treatment plots than in the controls.

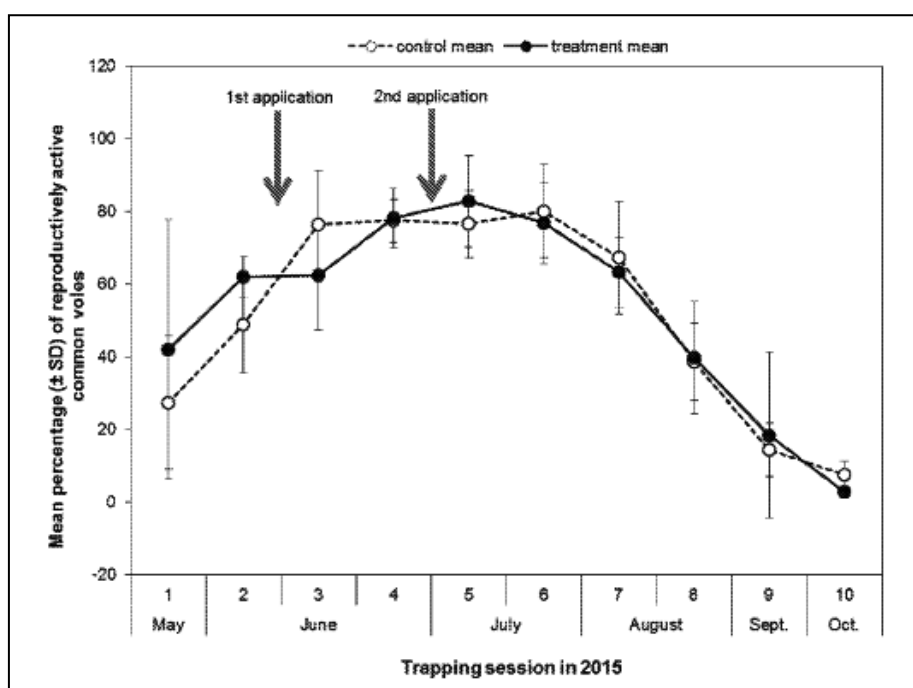


Figure A 3: Mean percentage (%) of reproductively active common voles in control and treatment plots (legend as in Figure 1)

The pattern of the proportion of juveniles during the trapping period was similar in the control and treatments plots (Figure 4). The differences observed in trapping session no. 1 (before the application) was unrelated to treatment and likely biased by the low numbers of juveniles present at the early phases of the reproduction season.

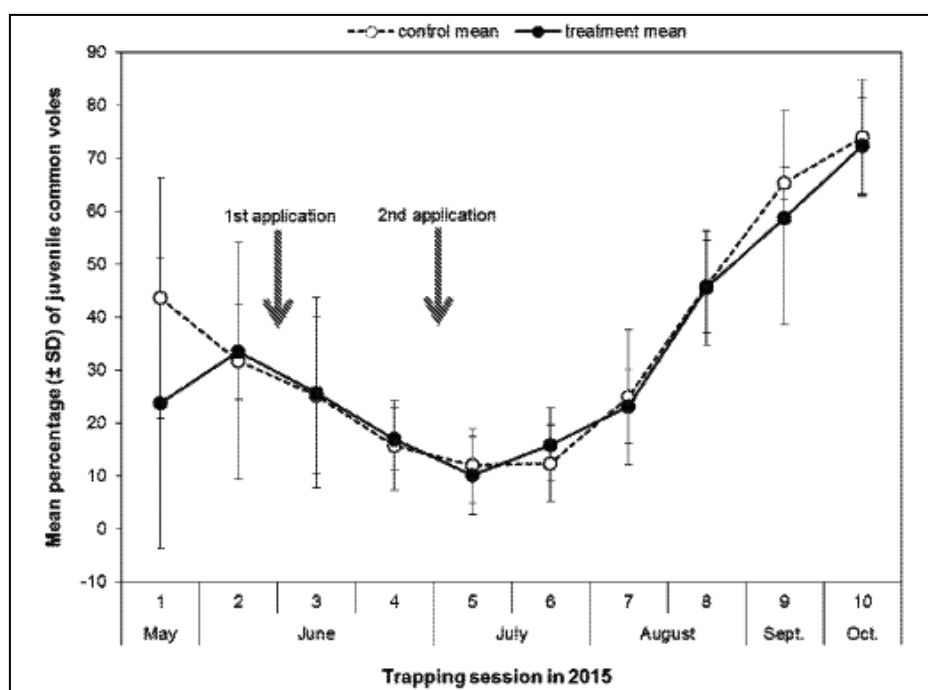


Figure A 4: Mean percentage (%) of juvenile common voles in control and treatment plots (legend as in Figure 1)

The development of the mean body weight of adults (females and males) over the course of the trapping period was similar in control and treatment plots (Figure 5). The difference in the mean body weight of females and males was not statistically significant between control and treatment plots throughout the trapping period. The mean body weight of juveniles (females and males) was similar in both experimental groups throughout the trapping period (Figure 6). A decline in the mean body weight of juveniles after the second application of pyraclostrobin applied as BAS 500 06 F occurred in both, control and treatment plots. Juvenile body weight was not statistically significantly different between control and treatment plots in any trapping session.

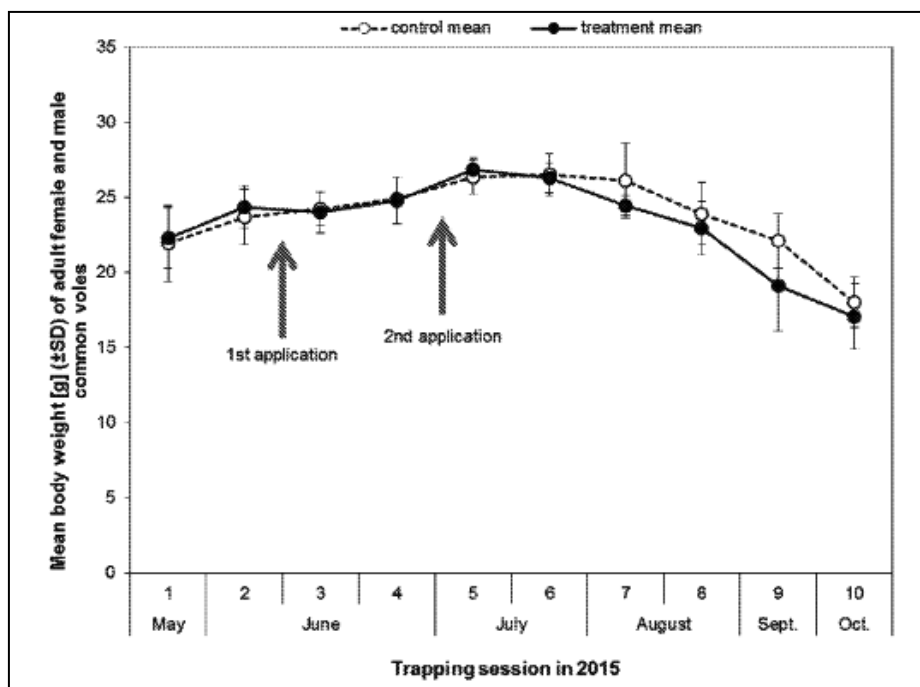


Figure A 5: Mean body weight (g) of adult common voles (females and males) in control and treatment plots (legend as in Figure 1)

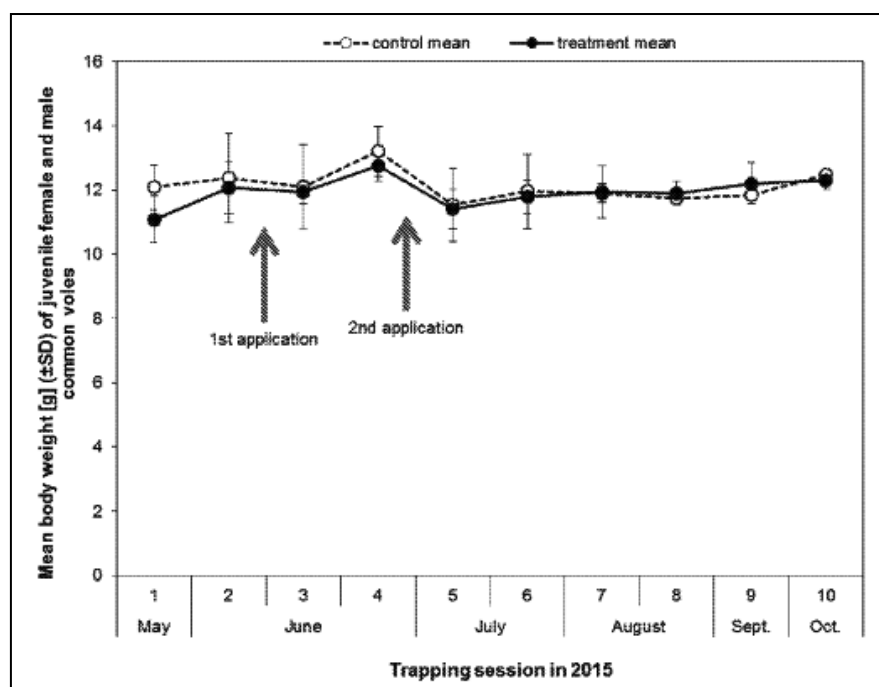


Figure A 6: Mean body weight (g) of juvenile common voles (females and males) in control and treatment plots (legend as in Figure 1)

III. CONCLUSION

The study was conducted under realistic worst-case field conditions, i.e. in a preferred common vole habitat with high abundances of common voles. Therefore, the study is well appropriate to assess potential effects of pyraclostrobin (applied as BAS 500 06 F) on common voles on the population level.

When applying pyraclostrobin applied as BAS 500 06 F following a use pattern of 2 x 250 g pyraclostrobin/ha, no negative effects were detected in any of the many investigated parameters throughout the study, i.e. no adverse acute and long-term effects of the fungicide pyraclostrobin applied as BAS 500 06 F on common vole populations were found. This second field effect study therefore confirms the outcome of the first field effects study (BASF DocID 2014/1000041 shown above).

Calculation of ‘maximum 21-day’ TWA factor using DT₅₀ foliage data

In the Tier 1 risk assessment, the default foliar half-life (DT₅₀) of 10 days according to EFSA/2009/1438 is used for pyraclostrobin and mefentrifluconazole, resulting in a time-weighted average factor (f_{twa}) of 0.53. However, data from substance-specific trials with pyraclostrobin and with mefentrifluconazole indicate a much faster dissipation of pyraclostrobin and of mefentrifluconazole in plant tissue. In total, BASF conducted 11 GLP field residue trials with pyraclostrobin and 15 GLP field trials with mefentrifluconazole in early growth stages of plants to obtain foliar residue decline data that are fully applicable for the Northern European residue zone.

According to Appendix F of the EFSA guidance document (EFSA/2009/1438), mammals have a clear preference for early growth stages of grasses (up to BBCH stage 30) and small leaves of dicotyledonous weeds. Hence, the field trials on the foliar residue decline of pyraclostrobin and mefentrifluconazole were specifically targeted to cover the early growth stages.

Foliage residue dissipation (DT₅₀) for pyraclostrobin

In total, BASF conducted 11 GLP field residue trials with pyraclostrobin in early growth stages of plants in the Northern European residue zone. Eight field trials conducted in the 2012 growing season in pea (BASF DocID 2013/1044539) and wheat (BASF DocID 2013/1045207) at BBCH 12-14, each trial comprising two treated plots, to obtain foliar residue decline data that are fully applicable for Northern Europe. Three additional trials, each comprising one treated plot, were conducted in wheat in the 2016 growing season at BBCH 25-29 (BASF DocID 2017/1029774).

Overall, seven field residue trials in wheat and four field residue trials in peas in Northern Europe were performed (for summaries see above). In this context, wheat and peas are considered suitable surrogate plants for monocotyledonous grasses and dicotyledonous weeds.

In the treated plots samples were frequently collected after application (0, 1, 2, 3, 4, 5, 7, 10, 12, 14 days after treatment) to allow a precise DT₅₀ calculation. During the sampling period (DAT 0 to DAT 14) the large majority of the test fields were not irrigated. Considering the observed rapid decline of pyraclostrobin (see Table A 15 below), a bias of the trial results by wash-off can be excluded.

The measured residue data in the plants were used to derive dissipation times of pyraclostrobin. The summaries of the field residue trials and the dissipation calculations are given above. For calculation details to obtain DT₅₀ values please refer to the reports BASF DocIDs 2013/1078114 and 2017/1037247.

The geometric mean DT₅₀ is calculated in accordance with recommendations of an EFSA guidance dealing with evaluation of laboratory and field dissipation studies to obtain DT₅₀ values of active substances (EFSA 2014/3662).

EFSA 2014/3662 considers the use of a geometric mean of DT₅₀ values (e.g., from soil dissipation studies) for exposure calculations justified, if values of 4 trials are available. For pyraclostrobin in total 11 DT₅₀ values out of independent trials are available, with eight of these comprising the results of two individual plots. The available trials cover different plant types. Based on this, the geometric mean of the DT₅₀ data for the respective plant types will be used in the refined risk assessment.

Table A 15 provides an overview of the DT₅₀ values obtained from the 11 GLP field residue trials in early growth stages in wheat and peas in Northern Europe.

Table A 15: Foliar residue decline trials with pyraclostrobin in Northern Europe: DT₅₀ in wheat and peas (BASF DocIDs 2013/1078114 and 2017/1037247)

wheat and peas (BASF DocIDs 2013/1076114 and 2017/1037247)							
BASF DocID of field trial data	Crop	BBCH	Location	Trial	Plot	DT ₅₀ [d]	Mean DT ₅₀ [d] of P2 and P3
2013/104520 7	Wheat	13/14	DE; Limburgerhof, Rheinland-Pfalz	L120103	P2	2.67	2.02 ¹⁾
	Wheat	13/14	DE; Limburgerhof, Rheinland-Pfalz		P3	1.37	
	Wheat	13/14	DE; Kleve, Nordrhein- Westfalen	L120104	P2	1.40	1.69 ¹⁾
	Wheat	13/14	DE; Kleve, Nordrhein- Westfalen		P3	1.97	
	Wheat	13/14	NL; Gennep, Limburg	L120105	P2	2.18	1.87 ¹⁾
	Wheat	13/14	NL; Gennep, Limburg		P3	1.56	
	Wheat	13/14	UK; Oxfordshire	L120106	P2	1.26	1.26 ¹⁾
	Wheat	13/14	UK; Oxfordshire		P3	1.25	
2017/102977 4	Wheat	25-29	DE; Limburgerhof, Rheinland-Pfalz	L160146	P2	2.22	2.22 ²⁾
	Wheat	25-29	DE; Stetten, Baden- Wuerttemberg	L160147	P2	1.17	1.17 ²⁾
	Wheat	25-29	DE; Mauchenheim, Rheinland-Pfalz	L160148	P2	2.11	2.11 ²⁾
Geometric mean (n = 7) DT ₅₀ [d] for monocotyledonous plants (food item ‘grasses/cereal shoots’)							1.72
2013/104453 9	Peas	12/13	DE; Limburgerhof, Rheinland-Pfalz	L120073	P2	1.28	1.39 ¹⁾
	Peas	12/13	DE; Limburgerhof, Rheinland-Pfalz		P3	1.50	
	Peas	12/13	DE; Kerken, Nordrhein- Westfalen	L120074	P2	1.91	1.73 ¹⁾
	Peas	12/13	DE; Kerken, Nordrhein- Westfalen		P3	1.55	
	Peas	12/13	FR (N); Loir et Cher	L120075	P2	3.76	3.13 ¹⁾
	Peas	12/13	FR (N); Loir et Cher		P3	2.50	
	Peas	12/13	UK; Essex	L120076	P2	2.10	2.08 ¹⁾
	Peas	12/13	UK; Essex		P3	2.06	
Geometric mean (n = 4) DT ₅₀ [d] for dicotyledonous plants (food item ‘non-grass herbs’)							1.99

¹⁾ Trial comprises two treated plots

²⁾ Trial comprises one treated plot

The geometric mean DT₅₀ values for pyraclostrobin are:

- DT₅₀ = 1.72 days for the food item ‘grasses/cereal shoots’
- DT₅₀ = 1.99 days for the food item ‘non-grass herbs’

Comments of zRMS:	The calculation is considered valid and acceptable for regulatory use.
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Foliage residue dissipation (DT₅₀) for mefentrifluconazole

In total, BASF conducted 15 GLP field residue trials in early growth stages of plants, to obtain foliar residue decline data that are fully applicable for the Central zone.

Overall, eight field residue trials in wheat (BASF DocID 2018/1205816) and seven field residue trials in peas (BASF DocID 2018/1205813) in Southern and Northern Europe were performed (for summaries see above). In this context, wheat and peas are considered suitable surrogate plants for monocotyledonous grasses and dicotyledonous weeds.

In the treated plot samples were frequently collected after application (0, 1, 2, 4, 7, 10±1, 14±1 days after treatment) to allow a precise DT₅₀ calculation. During the sampling period (DAT 0 to DAT 14) only one test field in pea was drip irrigated. Considering the observed rapid decline of mefentrifluconazole (see Table A 17 below) and the use of the drip method of irrigation, a bias of the trial results by wash-off can be excluded.

The measured residue data in the plants were used to derive dissipation times of mefentrifluconazole. The summaries of the field residue trials and the dissipation calculations are given above. For calculation details to obtain DT₅₀ values please refer to the reports by Szegedi, 2019a,b (BASF DocIDs 2019/2034648 and 2019/2034650).

The geometric mean DT₅₀ is calculated in accordance with recommendations of an EFSA guidance dealing with evaluation of laboratory and field dissipation studies to obtain DT₅₀ values of active substances (FOCUS 2006). FOCUS (2006) considers the use of a geometric mean of DT₅₀ values (*e.g.*, from soil dissipation studies) for exposure calculations justified, if values of 4 trials are available.

The available 8 field trials with wheat as representative of monocotyledonous plants cover the food item 'grasses/cereal shoots' and the available 7 field trials with pea as representative of dicotyledonous plants cover the food item 'non-grass herbs'. For each plant type DT₅₀ data are consistent between the Northern and Southern European residue zones (DT₅₀ for wheat 'North' vs. DT₅₀ for wheat 'South': two-sided $p = 0.78$, assuming equal variance; DT₅₀ for pea 'North' vs. DT₅₀ for pea 'South': two-sided $p = 0.62$, assuming equal variance). Therefore, for each plant type residue decline data from the two European residue zones can be merged. Based on this, the geometric mean of the DT₅₀ data for the respective plant types will be used in the refined risk assessment.

The table below provides an overview of the DT₅₀ values obtained from the 15 GLP field residue trials in early growth stages in wheat and peas in Northern and Southern Europe.

Table A-17: Foliar residue decline trials with mefentrifluconazole in Northern and Southern Europe: DT₅₀ in wheat and peas (BASF DocID 2019/2034648 and 2019/2034650)

Plant	Trial no.	Country	Zone	Kinetic model	DT ₅₀ [d]
Wheat	L170451	Germany	North	SFO	4.1
	L170452	Germany	North	SFO	6.0
	L170453	Belgium	North	SFO	2.3
	L170454	The Netherlands	North	SFO	2.8
	L170455	Spain	South	SFO	2.4
	L170456	Spain	South	SFO	3.3
	L170457	Italy	South	SFO	5.0
	L170458	Italy	South	SFO	5.8
Geometric mean (n = 8) DT₅₀ [d] for monocotyledonous plants (food item grasses/cereal shoots)					3.72
Pea	L180497	Germany	North	SFO	3.5
	L170444	Germany	North	SFO	2.5
	L170446	The Netherlands	North	SFO	1.7
	L170447	Spain	South	SFO	2.8
	L170448	Spain	South	SFO	3.5
	L170449	Italy	South	SFO	2.1
	L170450	Italy	South	SFO	3.2
Geometric mean (n = 7) DT₅₀ [d] for dicotyledonous plants (food item non-grass herbs)					2.67

The geometric mean DT₅₀ values for mefentrifluconazole are:

- DT₅₀ = 3.72 days for the food item grasses/cereal shoots
- DT₅₀ = 2.67 days for the food item non-grass herbs

Comments of zRMS:	Not evaluated by zRMS
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MAF x twa moving time window approach

The calculation of MAF and twa factor is conducted in accordance with the recommendations from EFSA/2009/1438 (Appendix H). An EXCEL spreadsheet was developed that describes the actual concentration in feed item from the days after first treatment (DAFT) up to 200 DAFT. Dissipation between the application events according to single first order kinetics (SFO) was introduced in the EXCEL spreadsheet as well as the build-up of residues through multiple applications. The geometric mean DT₅₀ values for peas and wheat for pyraclostrobin and mefentrifluconazole are used for the twa calculations.

The calculations follow the basic formula assuming single first order dissipation kinetic:

$$C_{act}(t) = C_0 * e^{-k * t}$$

C_{act(t)} actual concentration at time t
C₀ initial concentration
K degradation rate constant (= ln(2)/DT₅₀)
T time t

Furthermore, the established spreadsheet calculates - one after the other in a resolution of 0.1 d time steps - the average concentration factors for a 21 d time period, starting from the time of the first treatment (0 DAFT) up to 200 DAFT and scans for the maximum of the resulting twa values (moving time-frame approach) (EFSA 2009/1438 (Appendix H)). The high resolution of 0.1 d time steps leads to precise results even under consideration of short DT₅₀ values. The calculation of the twa, 21 d is described in the equation below.

Calculation of the twa over 21 d using a “moving time frame” approach:

$$twa, 21 d = \max \left[\frac{1}{21 * 10} \sum_{t=t_j, step 0.1}^{t_j+20.9} C_{act}(t) \right] \text{ for } j = 0.05, (200 - 21 - 0.05)$$

twa, 21 d (21) maximum average concentration in feed item for a 21 d interval
C_{act(t)} actual concentration at time t
t time
t_j start time point for integration
j time step running variable

For the use of BAS 758 00 F in cereals (2 applications, 14-day interval), the calculations result in the maximum 21-d twa factors for pyraclostrobin and mefentrifluconazole presented in Table A 16.

Table A 16: Maximum 21-d twa factor for pyraclostrobin and mefentrifluconazole considering two applications to cereals with a 14-day interval

Plant used for residue analyses	Food item	Pyraclostrobin		Mefentrifluconazole	
		DT ₅₀ [d]	Maximum 21-d twa factor	DT ₅₀ [d]	Maximum 21-d twa factor
Wheat	Grasses/cereal shoots	1.72	0.2293	3.72	0.4367
Pea	Non-grass herbs	1.99	0.2614	2.67	0.3363

Note that the MAF is set to 1 as this factor is already included in the calculation of the maximum 21-d twa factor.

Comments of zRMS: The calculation is considered valid and acceptable for regulatory use.

Calculation of food intake rate/body weight for the common vole

A refined FIR/bw is calculated for the common vole using a PD of 50% monocots and 50% dicots.

The FIR/bw values for the focal species mentioned above are calculated following EFSA/2009/1438 (Appendix G - Calculating exposure for the dietary intake approach). First, if the diet composition is given in dry weight, the specific energy content per gram food ($FE_{total,dry}$) is calculated according to the following formula:

$$FE_{total,dry} = \sum_i \left(PD_{i,dry} \times FE_i \times \frac{AE_i}{100} \right)$$

In which:

$FE_{total,dry}$ = Food energy of total mixed diet [kJ/g dry weight]
 $PD_{i,dry}$ = Fraction of food item [i] in mixed diet [related to dry weight]
 FE_i = Food energy of food item [i] in mixed diet [kJ/g dry weight]
 AE_i = Assimilation efficiency of food item [i] in mixed diet [%]

Second, the $PD_{i,dry}$ is transferred into $PD_{i,fresh}$ (Fraction of food item [i] in mixed diet [related to fresh weight]) as follows:

- The total dry weight of the diet needed to satisfy the DEE is calculated:

$$Total\ diet\ dry\ weight\ [g] = DEE / FE_{total,dry}$$

In which: DEE = Daily energy expenditure [kJ]

- Then, the dry weight of the respective food items contributing to the diet can be calculated:

$$Weight_{i,dry}\ [g] = Total\ diet\ dry\ weight\ [g] \times PD_{i,dry}$$

- Next, the $Weight_{i,dry}$ [g] is transferred into $Weight_{i,wet}$ [g]:

$$Weight_{i,wet}\ [g] = Weight_{i,dry}\ [g] \times (1 - MC_i / 100)$$

In which: MC_i = Moisture content of food item [i] in mixed diet [%]

- Finally, the $PD_{i,fresh}$ is calculated:

$$PD_{i,fresh} = Weight_{i,wet} / \sum Weight_{i,wet}$$

Third, the $FE_{total, fresh}$ is calculated according to the following formula:

$$FE_{total, fresh} = \sum_i \left[PD_{i, fresh} \times FE_i \times \left(1 - \frac{MC_i}{100} \right) \times \frac{AE_i}{100} \right]$$

In which:

$FE_{total, fresh}$ = Food energy of total mixed diet [kJ/g fresh weight]
 $PD_{i, fresh}$ = Fraction of food item [i] in mixed diet [related to fresh weight]
 FE_i = Food energy of food item [i] in mixed diet [kJ/g dry weight]
 MC_i = Moisture content of food item [i] in mixed diet [%]
 AE_i = Assimilation efficiency of food item [i] in mixed diet [%]

Fourth, the $FIR_{total, fresh}$ to reach the DEE of the indicator species is determined:

$$FIR_{total, fresh} = \frac{DEE}{FE_{total, fresh}}$$

In which: $FIR_{total, fresh}$ = Food intake rate of total mixed diet [g fresh weight]

Fifth, the FIR/bw value is calculated as the quotient of $FIR_{total, fresh}$ and the species' body weight.

$$FIR/bw = FIR_{total, fresh} / bw$$

In which: bw = body weight [g]

The FIR/bw value for the common vole is shown in Table A 17.

Table A 17: FIR/bw for the common vole

Food	PD _{i, wet} [%]	FE _i [kJ/g wet weight]	AE _i / 100	DEE [kJ]	Weight _{i, fresh} [g]	PD _{i, fresh}	FE _{total, fresh} [kJ/ g fresh weight]	FIR _{total, fresh} [g]	Body weight [g]	FIR/bw
Common vole										
Grasses (Monocots)	50	4.15	0.47	65.09	18.27	0.5	0.98			
Non-grass herbs (Dicots)	50	2.12	0.76		18.27	0.5	0.80			
Sum	100				36.55			36.55	25.0 ¹⁾	1.462

¹⁾ Body weight according to EFSA/2009/1438

Comments of zRMS:	Not evaluated by zRMS
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Relevance of voles

The common vole (*Microtus arvalis*) is considered as the representative species for the small herbivorous mammal “vole scenario” (EFSA/2009/1438) in the tier 1 risk assessment. At higher tier though, the notifier believes that the common vole is not a relevant species for the following reasons:

- Arable crops cannot be regarded as primary habitats for common voles

Common voles' primary habitats are characterized by undisturbed, dense vegetation which provides cover for shelter and food, such as grassland, alfalfa, meadows, fringes of shelterbelts, clearings, pastures uncultivated land, weed strips roadside verges, and wildflower strips. In contrast, secondary habitats are areas that offer sufficient conditions for voles to establish and reproduce only for a limited amount of time in the year. This is typically the case for arable crops, which are harvested in summer and plowed in autumn (in particular plowing dramatically reduces common vole survival). Furthermore, secondary habitats are colonized only when the populations cannot be sustained in the primary habitat anymore (*i.e.*, normally only during population outbreaks).

- Common vole populations naturally display strong cyclical changes, and a high ability to recover from decimation due to their very high reproductive potential

Common vole populations strongly fluctuate seasonally, mainly due to their seasonal breeding success. Typically, common vole populations increase dramatically in the spring, with renewal rates of approximately 80% per week. Notably, reproduction starts at an early age (approx. 14 days). Five to six pups per litter, produced after a 3 weeks gestation period, are commonly observed (on average, 4.5 litters are produced per breeding season). The highest population densities are usually observed in autumn, towards the end of the breeding period. In addition to the seasonal variation in vole abundance, vole population densities vary multi-annually, with population outbreak occurring in many parts of Europe approximately every 2-5 years. The typical population cycles observed in common vole populations strongly indicate that the populations are less likely influenced by plant protection products than by standard agricultural practices and natural conditions.

- Common voles are considered pests in many agricultural areas

At times of high abundance, common voles can cause significant crop damage especially in primary habitats such as perennial fodder crops like alfalfa and clover but also in secondary habitats, such as *e.g.*, cereals, rape, orchards, sugar beets and leafy and fruiting vegetables. Here, specific control measures such as the field application of rodenticides, habitat management/manipulation of vegetation, farming practices and biocontrol are necessary to avoid significant crop and economic damage. Due to the significant crop losses and management costs caused by the common vole at population outbreaks, this species belongs to the most serious vertebrate pest species in European agriculture.

For a comprehensive review of the habitat preferences, population dynamics, and the pest potential of the common vole, see Jacob et al. (2014).

In summary, ecological evidence indicates that common voles in arable crops are not essential to the maintenance of the population because arable crops constitute a secondary habitat for voles only and cannot sustain a vole population. Furthermore, the cyclical pattern of population densities of the common vole, which occasionally leads to mass occurrences with significant economic consequences for arable crops, indicates that in the long term, the local, management-related extinctions inside arable fields have negligible consequences on the overall population. In conclusion, the common vole is not a suitable focal species for higher tier risk assessments in arable crops.

Instead, the notifier suggests that the small herbivorous mammal scenario is covered by another rodent, *i.e.*, the omnivorous wood mouse (*Apodemus sylvaticus*).

The notifier is aware that there is currently no harmonization regarding the relevance of the vole in the EU. Whereas some MS require a refined risk assessment, other Member States have reduced trigger values for the vole or the refined RA is not required at all. Therefore, the notifier proposes that the decision on further refinement is considered at Member State level.

Reference

Jacob J, Manson P, Barfknecht R, Fredricks T (2013). Common vole (*Microtus arvalis*) ecology and management: implications of risk assessment of plant protection products. Pest Manag Sci, (2014). Volume 70(6), pages 869–878.

Comments of zRMS:	Not evaluated by zRMS
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**A 2.2.2 KCP 10.1.3 Effects on other terrestrial vertebrate wildlife (reptiles
and amphibians)**

No studies conducted.

A 2.3 KCP 10.2 Effects on aquatic organisms

A 2.3.1 KCP 10.2.1 Acute toxicity to fish, aquatic invertebrates, or effects on aquatic algae and macrophytes

A 2.3.1.1 Study 1

The following fish acute toxicity study performed with BAS 750 F is provided in support of the assessment and has not been previously evaluated on EU level.

Comments of zRMS:	Study was evaluated and accepted for the regulatory use in RR for BAS 751 00 F.
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Reference:	CP 10.2.1/1
Report	BAS 750 F - Acute toxicity study in the fathead minnow (<i>Pimephales promelas</i>), XXXXXXXXXX report No EU-805877, EU-18F0741/11E200 BASF DocID 2016/1155889 Authority registration No
Guideline(s):	EC 440/2008 C.1, OECD 203, EPA 72-1, EPA 850.1075
Deviations:	No
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive Summary

In a 96-hour static acute toxicity laboratory study, fathead minnows were exposed to a dilution water control and to nominal concentrations of 4.6, 10, 22, 46 and 100% of a saturated solution of BAS 750 F (corresponding to mean measured concentrations of 0.0916, 0.204, 0.462, 0.941 and 2.2 mg a.s./L) in groups of 10 animals in stainless steel aquaria containing 20 L water. Fish were observed for survival and symptoms of toxicity directly after start of exposure and 1, 6, 24, 48, 72 and 96 hours after start of exposure.

The biological results are based on mean measured concentrations of the test item. After 96 hours of exposure, no mortality was observed in the dilution water control and the test item concentrations of up to and including 0.462 mg a.s./L. At the two highest tested concentrations, all fish were dead after 96 hours of exposure. No sub-lethal effects were found at any of the test concentrations after 96 hours.

In a static acute toxicity study with fathead minnow the LC₅₀ (96 h) of BAS 750 F was determined to be 0.65 mg a.s./L based on mean measured concentrations. The NOEC (96 h) was determined to be 0.462 mg a.s./L (mean measured).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 750 F (Reg. no.: 583 437 8); batch no. COD-001740; purity: 98.8% (\pm 1.0%).

B. STUDY DESIGN

Test species: Fathead minnow (*Pimephales promelas*), approx. 4 month old; mean body length: 2.8 cm (2.4 cm – 3.4 cm); mean wet weight: 0.24 g (0.12 g – 0.40 g); supplied by in-house culture; no feeding from approx. 48 h befor test start.

Test design: Static (96 h); 5 test item concentrations plus a dilution water control, 2 replicates per treatment; 10 fish per aquarium (loading 0.1 g fish/L); assessment of mortality and sub-lethal effects within 1, 6, 24, 48, 72 and 96 hours after start of exposure.

Endpoints: LC₅₀, NOEC, mortality and sub-lethal effects.

Test concentrations: Control (dilution water), 4.6, 10, 22, 46 and 100% of a saturated solution of BAS 750 F (nominal), corresponding to mean measured concentrations of 0 (control), 0.0916, 0.204, 0.462, 0.941 and 2.20 mg a.s./L.

Test conditions: 20 L stainless steel aquaria, test volume: 20 L; dilution water: non-chlorinated charcoal filtered drinking water mixed with deionized water; hardness: 1.04 mmol CaCO₃/L; temperature: 24.1 – 24.6 °C; pH 8.1 – 8.4; oxygen content: 6.9 mg/L – 8.4 mg/L; conductivity: 248 µS/cm; photoperiod 16 h light : 8 h dark; light intensity: 114 – 431 Lux; no aeration; no feeding.

Analytics: Analytical verification of test item concentrations was conducted at start, 48 h and 96 h of exposure using a HPLC-method with MS detection.

Statistics: Descriptive statistics; probit method based on Finney for determination of LC50.

C. DESCRIPTION OF THE ANALYTICAL PROCEDURES

Concentrations of mefentrifluconazole (BAS 750 F) in test water were determined according to the analytical method APL0500/03. The validation of the analytical method is described in the study report. The analytical method APL0500/03 was slightly modified with respect to the chromatographic conditions to determine BAS 750 F in test water. Stock solutions were prepared by weighing about 50 mg test item into 100 mL acetonitrile. Calibration standards, ranging from 0.0002 mg/L to 0.004 mg/L, were prepared from intermediate solutions in test water/acetonitrile/formic acid mixture (80:20:0.1, v/v/v) by diluting with the same solvent mixture. The determination was performed by reversed phase UHPLC with MS detection. The limit of quantification (LOQ) was 0.001 mg/L and the limit of detection (LOD) was set to 0.002 mg/L. Details on measured fortification samples and obtained procedural recoveries for mefentrifluconazole are given in the table below.

Table A 18: Procedural recoveries for mefentrifluconazole

Matrix	Fortification level (mg/L)	n	Mean (%)	RSD (%)
Test water	0.001	5	106	4.3
Test water	5.0	5	103	1.8

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of BAS 750 F concentrations was conducted in each test item concentration at the beginning of the test, after 48 h and at the end of the exposure. The mean measured concentrations of the test item were < LoQ (Limit of quantification), 0.0916, 0.204, 0.462, 0.941 and 2.20 mg a.s./L. The analyzed contents of BAS 750 F ranged from 97% to 105% of overall mean measured concentrations at test initiation, from 93% to 103% after 48 h and from 95% to 103% of overall mean measured concentrations 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 and at test item concentrations of up to and including 0.462 mg a.s./L. At the two highest tested concentrations, all fish were dead after 96 hours of exposure. No sub-lethal effects were found at any of the test concentrations after 96 hours. The results are summarized in Table A 19.

Table A 19: Acute toxicity (96 h) of BAS 750 F to fathead minnow (*Pimephales promelas*)

Concentration [% saturated solution] (nominal)	Control	4.6	10	22	46	100
Concentration [mg a.s./L] (mean measured)	0	0.0916	0.204	0.462	0.941	2.2
Mortality [%] (96 h)	0	0	0	0	100	100
Symptoms (after 96 h)	none	none	none	none	n.d.	n.d.
Endpoints [mg BAS 750 F/L] (mean measured)						
LC ₅₀ (96 h)	0.65 (95% confidence limits: 0.577 – 0.731)					
NOEC (96 h)	0.462					

n.d. = not determined; all fish dead

Validity criteria according to OECD 203 (2019)	Obtained in this study
In the control(s) (dilution water control, solvent control), the mortality should not exceed 10% (or one fish, if fewer than 10 control fish are tested) at the end of the exposure	0%
The dissolved oxygen concentration must have been at least 60% of the air saturation value throughout the test	> 60% (6.9 – 8.4 mg/L)
Analytical measurement of test concentrations is compulsory (see § 24)	Analysis of each test concentrations. at 0, 48 and 96 hours after test start.

All validity criteria were met.

III. CONCLUSION

In a static acute toxicity study with fathead minnow the LC_{50} (96 h) of BAS 750 F was determined to be 0.65 mg a.s./L based on mean measured concentrations. The NOEC (96 h) was determined to be 0.462 mg a.s./L (mean measured).

A 2.3.1.2 Study 2

The following fish acute toxicity study performed with M750F005 (metabolite of BAS 750 F) is provided in support of the assessment and has not been previously evaluated on EU level.

Comments of zRMS:	The study was conducted to OECD guideline 203 and according to the principles of GLP. All validity criteria were met. The study is considered to be reliable and suitable for the risk assessment.
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Reference: CP 10.2.1/2

Report Reg.No. 6003433 (metabolite of BAS 750 F) - Acute toxicity study in the rainbow trout (*Oncorhynchus mykiss*),
XXXXXXXXXX
report No EU-12F0396/18E020, EU-867193
BASF DocID 2019/1022695
Authority registration No

Guideline(s): EC 440/2008 C.1 Acute Toxicity for Fish, OECD 203

Deviations: No

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

Acceptability: Yes

Duplication No
(if vertebrate study)

Executive Summary

In a 96-hour static acute toxicity laboratory study, rainbow trout (*Oncorhynchus mykiss*) were exposed to a water and solvent control and to a nominal concentration of 5 mg M750F005/L in groups of 10 animals in aquaria containing 20 L water. Fish were observed for survival and symptoms of toxicity 1, 6, 24, 48, 72 and 96 hours after start of exposure.

The biological results are based on nominal concentrations of the test item. No mortality occurred in the controls and in the test item. No additional adverse effects or abnormal behavior were observed in any of the test treatments.

In a 96-h static acute toxicity study with rainbow trout the LC₅₀ (96 h) for M750F005 was determined to be > 5 mg/L based on nominal concentration. The NOEC was determined to be ≥ 5.0 mg/L based on nominal concentration.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: M750F005, metabolite of BAS 750 F (Reg. No. 6003433), batch no. L87-34, purity: 96.9%;

B. STUDY DESIGN

Test species: Rainbow trout (*Oncorhynchus mykiss*), approx. 3.5 months old, mean body length 4.8 (4.3 – 5.5) cm, mean body weight 0.89 (0.5 – 1.49) g; supplied by 'Forellenzucht Trostadt GbR', Trostadt, Germany.

Test design: Static system (96 hours); 1 replicate per treatment; 10 fish per replicate (loading about 0.45 g fish/L); assessment of survival and symptoms of toxicity after 1, 6, 24, 48, 72 and 96 hours.

Endpoints: LCx and NOEC based on mortality and sublethal effects.

Test concentrations: Water control, solvent control (DMF), 5 mg M750F005/L (nominal).

Test conditions: ~24 L stainless steel aquaria (38.5x23.5x29 cm); test volume 20 L, dilution water: non-chlorinated charcoal-filtered municipal water mixed with deionized water; temperature: 11.9 – 12.3°C; pH 7.9 – 8.3; oxygen content: 7.9 – 10.4 mg/L; total hardness about 1 mmol/L (dilution water); acid capacity about 2.5 mmol/L (dilution water); photoperiod: 16 hours light : 8 hours dark; no aeration; no feeding.

Analytics: Analytical verification of the test item concentrations was performed using an LC-method with MS/MS detection.

Statistics: No statistical analysis was carried out since no lethality was observed up to the highest tested concentration.

C. DESCRIPTION OF THE ANALYTICAL PROCEDURES

Concentrations of M750F005 (metabolite of BAS 750 F) in test water were determined according to the analytical method L0359/01. The validation of the analytical method is described in another study (BASF Doc-ID: 2017/1066523). Fortification solutions for the high residue level (5 mg/L) were prepared by dilution of the stock solution with acetonitrile and solutions for the LOQ and 10 x LOQ fortifications were prepared by further dilution with acetonitrile/water (50/50, v/v) The determination was performed by HPLC-method with MS/MS detection.. The limit of quantification (LOQ) was 0.03 µg/L and the limit of detection (LOD) was set to 0.009 µg/L. To check on potential matrix effects quality control samples were prepared at LOQ measurement concentration level. The sample was prepared routinely with untreated test medium solution and compared to solvent standards. The recovery values of all replicates of the quality control sample were all in an acceptable range, therefore no significant matrix effect has been identified. Details on measured fortification samples and obtained procedural recoveries for M750F005 are given in the table below.

Table A 20: Procedural recoveries for mefentrifluconazole

Matrix	Fortification level (mg/L)	n	Mean (%)	RSD (%)
Test water	0.03	3	95.7	1.6
Test water	0.3	3	94.0	0.6
Test water	5000	3	98.3	1.1

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in the test item group at the beginning and at the end of the test. The analytically detected concentration was initially 88.4% of the nominal value and 88.0% at the end of the test. The biological results are based on nominal concentrations.

Biological results: No mortality occurred in the controls and in the treatment. No additional adverse effects or abnormal behavior were observed in the test treatment. The results are summarized in Table A 21.

Table A 21: Acute toxicity (96 h) of M750F005 to rainbow trout (*Oncorhynchus mykiss*)

Concentration [mg/L] (nominal)	Water Control	Solvent Control	5
Mortality [%] (96 h)	0	0	0
Symptoms (after 96 h) #	none	none	none
Endpoints [mg M750F005/L] (nominal)			
LC ₅₀ (96 h)	> 5 (confidence interval: n.d.)		
NOEC (96 h)	≥ 5		

n.d. not determined

Validity criteria according to OECD 203 (2019)	Obtained in this study
In the control(s) (dilution water control, solvent control), the mortality should not exceed 10% (or one fish, if fewer than 10 control fish are tested) at the end of the exposure	0%
The dissolved oxygen concentration must have been at least 60% of the air saturation value throughout the test	> 60% (7.9 – 10.4 mg/L)
Analytical measurement of test concentrations is compulsory (see § 24)	Analysis of each test concentrations. at 0 and 96 hours after test start.

All validity criteria were met.

III. CONCLUSION

In a 96-h static acute toxicity study with rainbow trout the LC₅₀ (96 h) for M750F005 was determined to be > 5 mg/L based on nominal concentration. The NOEC was determined to be ≥ 5.0 mg/L based on nominal concentration.

A 2.3.1.3 Study 3

The following fish acute toxicity study performed with BAS 560 F is provided in support of the assessment and was recently submitted under the Annex I renewal process for metrafenone (AIR 3). The study summary was provided in the revised Renewal Assessment Report of metrafenone (RAR, Vol. 3, B.9, December 2019).

<i>Comments of zRMS:</i>	The study was conducted to guidance and according to the principles of GLP. All validity criteria were met. The study is considered to be reliable and suitable for the risk assessment.
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Reference: CP 10.2.1/3

Report BAS 560 F: A 96-hour flow-through acute toxicity test with the sheepshead minnow (*Cyprinodon variegatus*),
XXXXXXXXXX
report No SubNo-200601-17-01,US-147A-206,US-136367
BASF DocID 2005/7003439
Authority registration No

Guideline(s): EPA 850.1075

Deviations: No

GLP: yes
(certified by United States Environmental Protection Agency)

Acceptability: Yes

Duplication No
(if vertebrate study)

Executive Summary

The acute toxicity of BAS 560 F (metrafenone) to the sheepshead minnow (*Cyprinodon variegatus*) was determined in a 96-hour flow-through test. BAS 560 F was tested at nominal concentrations of 0.063, 0.13, 0.25, 0.50 and 1.0 mg a.s./L, which was to the limit of solubility in the test system. A negative control (filtered saltwater) and a solvent control (0.1 mL/L dimethyl formamide) were tested in parallel. The fish were tested in groups of ten fish per replicate, with two replicates in each treatment and control group.

Results of analyses showed that concentrations generally remained stable over the 96-hour period. The mean measured concentrations were 0.072, 0.13, 0.24, 0.32 and 0.65 mg a.s./L. Since precipitate was visible in the two highest test concentrations (0.5 and 1.0 mg a.s./L (nominal)) these samples were also analyzed following centrifugation; the mean measured concentrations were 0.13 and 0.35 mg a.s./L, respectively. The results were based on the mean measured concentrations of both centrifuged and uncentrifuged samples.

The sheepshead minnows in the control groups and in all BAS 560 F treatment groups appeared normal throughout the test, with no mortalities or signs of toxicity noted.

The test was conducted to the limit of solubility in the test system. The no-mortality concentration and the no-observed-effect concentration (NOEC) were both 0.65 mg a.s./L (0.35 mg a.s./L based on centrifuged samples), the highest mean measured concentration tested. LC₅₀ values at 24, 48, 72 and 96 hours were estimated to be > 0.65 mg a.s./L (> 0.35 mg a.s./L based on centrifuged samples), the highest concentration tested.

I. MATERIAL AND METHODS

A. MATERIALS

- 1. Test material:** BAS 560 F (metrafenone; Reg. No. 4037710)
Batch number: AC12053-29
Purity: 94.2%
Description: Solid
- 2. Test concentrations:** 0 (negative and solvent control), 0.063, 0.13, 0.25, 0.50 and 1.0 mg a.s./L (mean measured: 0, 0.072, 0.13, 0.24, 0.32 (0.13 centrifuged) and 0.65 (0.35 centrifuged) mg a.s./L)
- 3. Reference item:** None
- 4. Dilution water:** Natural seawater collected at Indian River Inlet, Delaware filtered and diluted to a salinity of ~20‰ with well water
Vehicle: Dimethyl formamide (DMF)
- 5. Test organism:**
Species: Sheepshead minnow (*Cyprinodon variegatus*)
Age: Juveniles. All fish were from the same source and year class.
Weight: 0.23 g (0.10-0.31 g) average wet weight at the end of the test
Length: 2.2 cm (1.8-2.5 cm) average total length at the end of the test
Source: Aquatic BioSystems, Inc. Fort Collins, Colorado
Acclimation period: At least 14 days
Diet: Commercially-prepared diet and *Artemia nauplii*. Not fed at least two days prior to and during the test
Test vessels: 9 L glass aquaria filled with approximately 7 L of test water
Loading: 0.33 g fish/L

B. STUDY DESIGN

- 1. Environmental conditions:**
Temperature: 21.7-22.4 °C
Salinity: 20 – 21‰
pH: 8.2 – 8.3
Dissolved oxygen: ≥ 4.9 mg/L (≥ 63% of saturation)
Photoperiod: 16 h light: 8 h darkness (255 lux at test initiation)
Aeration: By Day 2 gentle aeration was added to maintain dissolved oxygen above 60% of saturation throughout the remainder of the test

2. Animal assignment and treatment:

Twenty impartially distributed fish per test group (two replicate chambers containing ten fish each) were exposed to the test substance at nominal concentrations of 0.063, 0.13, 0.25, 0.50 and 1.0 mg a.s./L. A negative (filtered saltwater) control and a solvent control (0.1 mL/L dimethyl formamide) were tested in parallel. The exposure period was 96 hours under flow-through conditions.

3. Dose preparation:

A primary stock solution was prepared by mixing a calculated amount of test substance into dimethyl formamide (DMF) at a nominal concentration of 10 mg a.s./L. Four additional solutions (at nominal concentrations of 0.63, 1.3, 2.5 and 5.0 mg a.s./mL) were prepared in DMF by proportional dilution of the primary stock. The five stock solutions were injected into the diluter mixing chambers where they were mixed with saltwater to achieve the desired test concentrations. The solvent control was prepared by injecting DMF into the mixing chamber for the solvent control. The concentration of DMF in the solvent control and all BAS 560 F treatment groups was 0.1 mL/L. The diluter was adjusted so that each test chamber received approximately 13 volume additions of test water every 24 hours.

4. Measurements and observations:

Observations were made 5.5, 24, 48, 72 and 96 hours after test initiation, to determine the number of mortalities in each treatment group. The number of individuals exhibiting signs of toxicity or abnormal behavior was also evaluated.

Samples were collected prior to test initiation from one test chamber of each treatment and control groups to confirm the operation of the diluter. Additional samples were collected from alternating replicate test chambers at 0, 48 and 96 hours to measure concentrations of the test substance. Samples were diluted with saltwater, as needed, and analyzed by HPLC using wavelength detection at 220 nm.

Temperature, dissolved oxygen and pH were measured every 24 hours during the test.

5. Statistics:

As no mortalities were observed during the test, LC₅₀ values could not be calculated but were estimated to be greater than the highest concentration tested. The no-mortality concentration and the NOEC were determined by visual interpretation of the mortality and observation data.

II. RESULTS AND DISCUSSION

A. BIOLOGICAL EFFECTS

The sheephead minnows in both control groups and in each BAS 560 F treatment groups appeared normal throughout the test, with no mortalities or signs of toxicity noted. The results are presented in the table below.

Table A 22: Mortality and effects of sheephead minnows (*Cyprinodon variegatus*) exposed to BAS 560 F

Mean measured concentration (mg a.s./L)	5.5 hours		24 hours		48 hours		72 hours		96 hours	
	No. Dead _a	Effects _b	No. Dead _a	Effects _b	No. Dead _a	Effects _b	No. Dead _a	Effects _b	No. Dead _a	Effects _b
Negative control	0	20 AN	0	20 AN	0	20 AN	0	20 AN	0	20 AN
Solvent control	0	20 AN	0	20 AN	0	20 AN	0	20 AN	0	20 AN
0.072	0	20 AN	0	20 AN	0	20 AN	0	20 AN	0	20 AN
0.13	0	20 AN	0	20 AN	0	20 AN	0	20 AN	0	20 AN
0.24	0	20 AN	0	20 AN	0	20 AN	0	20 AN	0	20 AN
0.32 ^c	0	20 AN	0	20 AN	0	20 AN	0	20 AN	0	20 AN
0.65 ^c	0	20 AN	0	20 AN	0	20 AN	0	20 AN	0	20 AN

^a Cumulative number of dead fish

^b Observed effects: AN = appear normal

^c Non-centrifuged mean measured concentration

B. ANALYSIS

At nominal concentrations ≤ 0.25 mg a.s./L, measured concentrations ranged from approximately 93% to 119% of nominal. At nominal concentrations of 0.5 and 1.0 mg a.s./L, results ranged from approximately 56% to 70% of nominal in samples analyzed without centrifugation. Since a precipitate was visible in the 0.50 and 1.0 mg a.s./L test chambers, samples were also analyzed following centrifugation (at 14000 rpm). The measured concentrations of the centrifuged samples ranged from approximately 20 to 37% of nominal. The lower recoveries and the presence of a precipitate in the 0.50 and 1.0 mg a.s./L test chambers indicate that the test was conducted to the limit of water solubility in the test system (0.3 mg a.s./L). The concentrations of BAS 560 F observed in the analysis are shown in the table below.

Table A 23: Measured concentrations of BAS 560 F in test samples

Nominal concentration (mg a.s./L)	Sampling time (hours)	Measured concentration (mg a.s./L)	Measured as % of nominal	Mean measured concentration (mg a.s./L)	Mean measured as % of nominal
0 (negative control)	0 48 96	< LOQ	-	-	-
0 (solvent control)	0 48 96	< LOQ	-	-	-
0.063	0 48 96	0.0712 0.0749 0.0707	113 119 112	0.072	114
0.13	0 48 96	0.137 0.133 0.127	105 102 97.7	0.13	100
0.25	0 48 96	0.242 0.233 0.242	96.8 93.0 97.0	0.24	96
0.50	0 48 96	0.320 0.338 0.296	64.1 67.6 59.1	0.32	64
1.0	0 48 96	0.558 0.697 0.694	55.8 69.7 69.4	0.65	65
0.50*	0 48 96	0.147 0.141 0.0998	29.4 28.1 20.0	0.13	26
1.0*	0 48 96	0.313 0.362 0.366	31.3 36.2 36.6	0.35	35

LOQ = Limit of quantification (0.040 mg a.s./L)

* Samples centrifuged at 14000 rpm

C. DEFICIENCIES

None.

III. CONCLUSION

The acute toxicity of BAS 560 F to sheepshead minnow (*Cyprinodon variegatus*) was assessed under flow-through conditions, to the limit of solubility in the test system. The 96-hour LC₅₀ value was > 0.65 mg a.s./L (mean measured; corresponding to > 0.35 mg a.s./L for centrifuged samples), the highest concentration tested. The no-mortality concentration and the no-observed-effect concentration (NOEC) were both 0.65 mg a.s./L (0.35 mg a.s./L when based on centrifuged samples).

A 2.3.1.4 Study 4

The following fish acute toxicity study performed with BAS 758 00 F is provided in support of the assessment and has not been previously evaluated on EU level.

Comments of zRMS:	The study was conducted to OECD guideline 203 and according to the principles of GLP. All validity criteria were met. The study is considered to be reliable and suitable for the risk assessment.
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Reference: CP 10.2.1/4

Report BAS 758 00 F – Acute Toxicity to Rainbow trout (*Oncorhynchus mykiss*)
in a static 96-Hour Test,
XXXXXXXXXX
report No 876354, 20200151
BASF DocID 2020/2033900
Authority registration No

Guideline(s): Sanco/3029/99 Rev.4 (2000), OECD 203 (2019)

Deviations: No

GLP: yes
(certified by Swiss Federal Office of Public Health, Berne, Switzerland)

Acceptability: Yes

Duplication No
(if vertebrate study)

Executive Summary

In a 96-hour static acute toxicity laboratory study, juvenile rainbow trout (*Oncorhynchus mykiss*) were exposed to a dilution water control and to nominal concentrations of 0.0156, 0.0313, 0.0625, 0.125 and 0.250 mg BAS 758 00 F/L in groups of 7 animals in glass aquaria containing 14 L test medium. Fish were observed for survival and symptoms of toxicity approximately 2 and 5 hours after start of exposure and two observations were performed in the morning and afternoon until test end on each of the following observation days.

The biological results are based on nominal concentrations of the test item. After 96 hours of exposure, no mortality or other adverse effects were observed in the control and up to and including the test item concentration of 0.0313 mg/L. In the 0.0625 mg/L group no mortality was observed, while 4 of 7 fish showed toxic symptoms (hypoactivity) and in the two highest concentration groups (0.125 and 0.250 mg/L) all fish were dead. In the two highest test item concentrations, mortality was statistically significantly different compared to the control at test end.

In a 96-h static acute toxicity study with rainbow trout, the LC_{50} (96 h) of BAS 758 00 F was determined to be 0.0884 mg/L based on nominal concentrations. The NOEC was determined to be 0.0625 mg/L (nominal).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 758 00 F, batch no. FD-200124-0004; content of a.s.: metrafenone (BAS 560 F, Reg. No. 4037710): 94.9 g/L (nominal: 100 g/L); mefentrifluconazole (BAS 750 F, Reg. No. 5834378): 66.7 g/L (nominal: 66.6 g/L); pyraclostrobin (BAS 500 F, Reg. No. 304428): 81.0 g/L (nominal: 80.0 g/L); density: 1.092 g/cm³.

B. STUDY DESIGN

Test species: Rainbow trout (*Oncorhynchus mykiss*), juveniles; mean body length: 4.35 cm (\pm 0.24 cm); mean wet weight: 0.74 g (\pm 0.13 g); supplied by fish breeding farm 'Fischzucht Lüscher', Mühlethal, Switzerland; no feeding from 2 d before test start.

Test design: Static system (96 h); 5 test item concentrations plus a dilution water control, 1 replicate per treatment; 7 fish per aquarium (loading: 0.37 g fish/L); assessment of mortality and symptoms of toxicity approximately 2 and 5 hours after start of exposure and on each of the following observation days, two observations were performed in the morning and afternoon until test end.

Endpoints: LC₅₀, NOEC, mortality and sub-lethal effects.

Test concentrations: Control (dilution water), 0.0156, 0.0313, 0.0625, 0.125 and 0.250 mg BAS 758 00 F/L (nominal).

Test conditions: 20-L glass aquaria; test volume: 14 L; dilution water: reconstituted test water; hardness: 1.25 mmol/L (nominal); temperature: 13°C; pH 7.2 – 7.3; oxygen content: 9.3 - 9.9 mg/L; photoperiod: 16 h light : 8 h dark (30-min transition period); light intensity: 910 - 980 lux; slight aeration; no feeding.

Analytics: Analytical verification of test item concentrations was conducted using an HPLC-method with MS/MS detection (analytical method for metrafenone: PTRL P 3309 G and for pyraclostrobin and mefentrifluconazole: L0361/01).

Statistics: Descriptive statistics; for determination of LC₅₀, Trimmed Spearman-Kärber procedure or binomial distribution for estimating, where appropriate; for determination of NOEC, Step-down Cochran-Armitage test procedure (one-sided greater, α = 0.05).

C. DESCRIPTION OF THE ANALYTICAL PROCEDURES

Concentrations of pyraclostrobin, mefentrifluconazole and metrafenone (contained in BAS 758 00 F) in test water were determined according to the analytical method L0361/01 with some adaptations (for pyraclostrobin and mefentrifluconazole) and PTRL P 3309 G with some adaptations (for metrafenone). The validation of the analytical methods is described in the study report.

L0361/01: The samples (10 mL) were completely diluted with 10 mL of acetonitrile / water / formic acid (400/600/2, v/v/v; F1) and shaken mechanically. If necessary, aliquots of the samples were further diluted into the calibration range with F1 / test water (1/1, v/v) and were analyzed by HPLC with MS/MS detection. The limit of quantification (LOQ) was 0.116 µg pyraclostrobin/L and 0.0954 µg mefentrifluconazole/L and the limit of detection (LOD) was set to 0.0206 µg pyraclostrobin/L and 0.0204 µg mefentrifluconazole/L.

PTRL P 3309 G: The samples (10 mL) were completely diluted with 10 mL of methanol and shaken mechanically. If necessary, aliquots of the samples were further diluted into the calibration range with test water / methanol (1/1, v/v) and were analyzed by HPLC with MS/MS detection. The LOQ was 0.136 µg metrafenone/L and the LOD was set to 0.0502 µg metrafenone/L.

For the assessment of potential matrix effects, matrix matched calibration standards were used. The storage time of the test samples was maximum 30 days. The investigations to demonstrate the storage stability of pyraclostrobin, mefentrifluconazole and pyraclostrobin in test samples was not necessary. Details on measured fortification samples and obtained procedural recoveries for mefentrifluconazole are given in Table A 24.

Table A 24: Procedural recoveries for BAS 758 00 F (determined via pyraclostrobin, mefentrifluconazole and metrafenone)

Analyte	Matrix	Fortification level (µg/L)	n	Mean (%)	RSD (%)
Pyraclostrobin	Dilution water	0.116	5	105	8.6
	Dilution water	22.7	5	97.7	5.5
Mefentrifluconazole	Dilution water	0.0954	5	90.6	5.6
	Dilution water	18.7	5	99.6	6.7
Metrafenone	Dilution water	0.136	5	95.2	8.4
	Dilution water	26.6	5	92.3	0.9

RSD = relative standard deviation

II. RESULTS AND DISCUSSION

Analytical measurements: Samples were taken from the control and the test item concentration of 0.0156 to 0.0625 mg/L at the start and at the end of the test (after 96 hours). From the test item concentration of 0.125 mg/L samples were taken at the start and after 48 hours. In this test concentration the last fish died after 48 hours. From the highest test item concentration of 0.250 mg/L samples were taken only at the start of the test, since all fish were dead after 5 hours. At the start of the test, the measured concentrations of metrafenone in the test media ranged between 90.3 and 100% of the nominal values and between 26.0 and 75.4% at the end of the test or if all fish were dead in a certain test concentration. For mefentrifluconazole, the measured concentrations in the test media ranged between 98.2 and 103% of the nominal values at the start and between 36.9 and 72.5% at the end of the test or if all fish were dead in a certain test concentration. The measured concentrations of pyraclostrobin in the test media ranged between 100 and 103% of the nominal values at the start and between 54.6 and 86.1% at the end of the test or if all fish were dead in a certain test concentration. As measured concentrations confirmed correct application of the test substance, the following biological results are based on nominal concentrations.

Biological results: After 96 hours of exposure, no mortality or other adverse effects were observed in the control and up to and including the test item concentration of 0.0313 mg/L. In the 0.0625 mg/L group no mortality was observed, while 4 of 7 fish showed toxic symptoms (hypoactivity) and in the two highest concentration groups (0.125 and 0.250 mg/L) all fish were dead. In the two highest test item concentrations, mortality was statistically significantly different compared to the control at test end (Step-down Cochran-Armitage Test Procedure; $\alpha = 0.05$). The results are summarized in Table A 25.

Table A 25: Acute toxicity (96 h) of BAS 758 00 F on rainbow trout (*Oncorhynchus mykiss*)

Concentration [mg/L] (nominal)	Control	0.0156	0.0313	0.0625	0.125	0.250
Mortality [%] (96 h)	0	0	0	0	100*	100*
Symptoms (after 96 h)	none	none	none	S	n.d.	n.d.
Endpoints [mg BAS 758 00 F/L] (nominal)						
LC ₅₀ (96 h)	0.0884 (95% confidence limits: 0.0625 - 0.125)					
NOEC (96 h)	0.0625					

* Statistically significantly different compared to the control (Step-down Cochran-Armitage Test Procedure; $\alpha = 0.05$)

n.d. = not determined; all fish dead

S = abnormal swimming behavior (hypoactivity)

Validity criteria:

Validity criteria according to OECD 203 (2019)	Obtained in this study
In the control(s) (dilution water control, solvent control), the mortality should not exceed 10% (or one fish, if fewer than 10 control fish are tested) at the end of the exposure	0%
The dissolved oxygen concentration must have been at least 60% of the air saturation value throughout the test	$\geq 93\%$ (9.3 - 9.9 mg/L)
Analytical measurement of test concentrations is compulsory (see § 24)	Samples were taken from the control and the test item concentration of 0.0156 to 0.0625 mg/L at the start and at the end of the test (after 96 hours). From the test item concentration of 0.125 mg/L samples were taken at the start and after 48 hours. In this test concentration the last fish died after 48 hours. From the highest test item concentration of 0.250 mg/L samples were taken only at the start of the test, since all fish were dead after 5 hours.

All validity criteria were met.

III. CONCLUSION

In a 96-h static acute toxicity study with rainbow trout, the LC₅₀ (96 h) of BAS 758 00 F was determined to be 0.0884 mg/L based on nominal concentrations. The NOEC was determined to be 0.0625 mg/L (nominal).

A 2.3.1.5 Study 5

The following acute toxicity study on *Crassostrea virginica* performed with BAS 560 F is provided in support of the assessment and was recently submitted under the Annex I renewal process for metrafenone (AIR 3). The study summary was provided in the revised Renewal Assessment Report of metrafenone (RAR, Vol. 3, B.9, December 2019).

<i>Comments of zRMS:</i>	The study was conducted to guidance and according to the principles of GLP. All validity criteria were met. The study is considered to be reliable and suitable for the risk assessment.
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Reference: CP 10.2.1/5

Report BAS 560 F: A 96-hour shell deposition test with the eastern oyster (*Crassostrea virginica*),
XXXXXXXXXX
report No SubNo-200601-18-01,US-147A-207,US-136373
BASF DocID 2005/7003442
Authority registration No

Guideline(s): EPA 850.1025

Deviations: No

GLP: yes
(certified by United States Environmental Protection Agency)

Acceptability: Yes

Duplication No
(if vertebrate study)

Executive Summary

The acute toxicity of BAS 560 F (metrafenone) to the eastern oyster (*Crassostrea virginica*) was determined under flow-through conditions. The oysters were exposed to nominal concentrations of 0.063, 0.13, 0.25, 0.50 and 1.0 mg a.s./L for 96 hours. A negative control (filtered saltwater) and solvent control (0.1 mL/L dimethyl formamide) were tested in parallel. The oysters were tested in groups of ten per test chamber, with one test chamber per test group.

Results of analyses showed mean measured concentrations between 80 and 85% of nominal concentrations for the three lowest test substance concentrations and mean measured concentrations of 56 and 57 % for the two highest test substance concentrations. This indicates that the test was conducted to the limit of solubility in the test system. In addition, a precipitate was observed for the highest test substance concentration (1.0 mg a.s./L (nominal)) and therefore these samples were also analyzed following centrifugation. The mean measured concentration after centrifugation was 33% of nominal concentration. The results were based on the mean measured concentrations of both centrifuged and uncentrifuged samples.

All the oysters in the control groups and in the BAS 560 F exposed groups appeared normal throughout the test, without any mortalities or signs of toxicity. Inhibition of shell growth for the oysters exposed to BAS 560 F was calculated relative to the pooled control and showed an increase in inhibition of 5.6% at the

lowest test substance concentration of 0.051 mg a.s./L (mean measured) to 100 % inhibition at the highest test substance concentration of 0.57 mg a.s./L (mean measured).

The EC₅₀ for *Crassostrea virginica* exposed to BAS 560 F for 96 hours was 0.22 mg a.s./L / 0.22 mg a.s./L, based on mean measured concentrations of uncentrifuged/centrifuged samples. The no-observed-effect concentration (NOEC) was determined to be 0.11 mg a.s./L (mean measured). The LC₅₀ was not estimated in the report, however, as no mortality was observed in the study, the LC₅₀ is > 0.57 mg a.s./L / > 0.33 mg a.s./L (based on mean measured concentrations of uncentrifuged/centrifuged samples).

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test material:** BAS 560 F (metrafenone; Reg. No. 4037710)
Batch number: AC12053-29
Purity: 94.2%
Description: Solid
2. **Test concentrations:** 0 (negative and solvent control), 0.063, 0.13, 0.25, 0.50 and 1.0 mg a.s./L; (mean measured: 0, 0.051, 0.11, 0.20, 0.28 and 0.57 mg a.s./L)
3. **Reference item:** None
4. **Dilution water:** Natural seawater collected at Indian River Inlet, Delaware, filtered and diluted to a salinity of 20‰ with well water
Vehicle: Dimethyl formamide (DMF)
5. **Test organism:**
Species: Eastern oyster (*Crassostrea virginica*)
Age/life stage: Not described
Length: 38.1 ± 5.1 mm
Source: Taylor Shellfish Farms, Shelton, USA
Acclimation period: 12 days
Diet: Suspension of marine microalgae, provided continuously during testing at a rate of 5.8 x 10⁹ cells/oyster/day
Test vessels: 54 L glass aquaria filled with 27 L of test water

B. STUDY DESIGN

1. **Environmental conditions:**
Temperature: 19.8 – 20.8 °C
Salinity: 19 - 20‰
pH: 8.1 – 8.3
Dissolved oxygen: 5.2 – 7.2 mg/L (4.8 mg/L represents 60% saturation at 20°C in saltwater with salinity of 20‰)
Photoperiod: 16 h light: 8 h darkness (192 lux, at test initiation)
2. **Animal assignment and treatment:**
At test initiation, oysters were randomly distributed to test chambers, until each group contained 20 oysters. The oysters were placed in the test chambers with flat valves facing up and umbos away from the flow of the water. The test included one test chamber per test group. The oysters were exposed for 96 hours under flow-through conditions.

3. Dose preparation:

A primary stock solution was prepared by mixing test substance into dimethyl formamide (DMF) at a nominal concentration of 10 mg a.s./L. Four additional solutions (at nominal concentrations of 0.63, 1.3, 2.5 and 5.0 mg a.s./mL) were prepared in DMF by proportional dilution of the primary stock. The five stock solutions were injected into the diluter mixing chambers, where they were mixed with saltwater to achieve the desired test concentrations of 0.063, 0.13, 0.25, 0.50 and 1.0 mg a.s./L. The solvent control was prepared by injecting DMF into the mixing chamber for the solvent control. The concentration of DMF in the solvent control and all BAS 560 F treatment groups was 0.1 mL/L. A test water only control was also included in the test. The diluter was adjusted so that each test chamber received 19 volume additions of test water every 24 hours.

4. Measurements and observations:

Observations were made at 6, 24, 48, 72 and 96 hours after test initiation to determine the number of mortalities, as well as the number of individuals with sublethal signs of toxicity. At test termination, the longest finger of new shell growth on each oyster was measured to the nearest 0.1 mm, to obtain the shell growth.

Samples were collected from each treatment and control group at 0, 48 and 96 hours to measure concentrations of the test substance. Samples were analyzed by HPLC using wavelength detection set at 220 nm.

5. Statistics:

The concentration of test substance that would reduce shell deposition by 50% relative to the (pooled) control (EC_{50}) was calculated using linear interpolation. Shell growth data in the treatment groups were compared to the (pooled) control data using analysis of variance (ANOVA) and Bonferroni's t-test to identify significant differences. The no-observed-effect concentration (NOEC) was determined from the statistical analysis and an assessment of the concentration-response pattern. The statistical analyses were conducted using TOXSTAT computer program (1996).

II. RESULTS AND DISCUSSION

A. MORTALITY AND APPEARANCE

No oysters died during the test, in the control groups and in all test substance groups. All oysters appeared normal throughout the test, without any signs of toxicity.

B. SHELL DEPOSITION

Shell deposition was not statistically significantly different between the seawater and the vehicle control, hence the two control groups were pooled. Inhibition of shell growth for the oysters exposed to BAS 560 F was calculated relative to the pooled control and the inhibition increased from 5.6% at the lowest test substance concentration of 0.051 mg a.s./L (mean measured) to 100 % inhibition at the highest test substance concentration of 0.57 mg a.s./L (mean measured). The results are presented in the table below. The 96-hour EC_{50} value was calculated to be 0.22 mg a.s./L, with a 95% confidence interval of 0.20 to 0.24 mg a.s./L, based on mean measured concentrations of both uncentrifuged and centrifuged samples. The NOEC was considered to be 0.11 mg a.s./L (mean measured).

Table A 26: Mean shell deposition and shell growth inhibition of eastern oyster (*Crassostrea virginica*) exposed to BAS 560 F

Nominal concentration (mg a.s./L)	Mean measured concentration (mg a.s./L)	Shell deposition Mean \pm sd ^a (mm)	Shell growth inhibition (%) ^b
Negative control	Negative control	3.16 \pm 1.41	-
Solvent control	Solvent control	2.61 \pm 0.97	-
Pooled control	Pooled control	2.88 \pm 1.23	-
0.063	0.051	2.72 \pm 0.96	5.6
0.13	0.11	2.33 \pm 0.99	19
0.25	0.20	1.76 \pm 0.76	39*
0.50	0.28	0.50 \pm 0.76	83*
1.0	0.57	0.00 \pm 0.00	100*

^a Mean and standard deviation for 20 oysters

^b Percent inhibition from the pooled control

* Statistically significantly different from the pooled control using Bonferroni's t-test ($p \leq 0.05$)

C. ANALYSIS

At nominal concentrations ≤ 0.25 mg a.s./L, mean measured concentrations ranged from 80 to 85% of nominal concentrations. At nominal concentrations of 0.5 and 1.0 mg a.s./L, mean measured concentration were 56 and 57% of nominal concentrations, respectively. Since a precipitate was visible in the highest test substance concentration of 1.0 mg a.s./L, samples for this test substance concentration were also analyzed following centrifugation at 14000 rpm for five minutes. The mean measured concentration of these centrifuged samples was 33% of the nominal concentration. The lower recoveries and the presence of a precipitate in the 0.50 and 1.0 mg a.s./L test chambers indicate that the water solubility in the test system was approximately 0.3 mg a.s./L. The results of the study were based on mean measured concentrations of both uncentrifuged and centrifuged samples. The concentrations of BAS 560 F observed in the analysis are shown in the table below.

Table A 27: Measured concentrations of BAS 560 F in test samples

Nominal concentration (mg a.s./L)	Sampling time (hours)	Measured concentration (mg a.s./L)	Mean measured concentration (mg a.s./L)	Mean measured as % of nominal
0 (negative control)	0 48 96	< LOQ	-	-
0 (solvent control)	0 48 96	< LOQ	-	-
0.063	0 48 96	0.0506 0.0560 0.0462	0.051	81
0.13	0 48 96	0.115 0.0929 0.117	0.11	85
0.25	0 48 96	0.200 0.202 0.207	0.20	80
0.50	0 48 96	0.256 0.306 0.280	0.28	56
1.0	0 48 96	0.570 0.588 0.546	0.57	57
1.0*	0 48 96	0.313 0.336 0.335	0.33	33

LOQ = Limit of quantification (0.040 mg a.s./L)

* Samples centrifuged at 14000 rpm for five minutes

D. DEFICIENCIES

None.

III. CONCLUSION

The EC₅₀ for eastern oyster (*Crassostrea virginica*) exposed to BAS 560 F (metrafenone) for 96 hours under flow-through conditions was 0.22 mg a.s./L (mean measured), based on the limit of solubility in the test system. The no-observed-effect concentration (NOEC) was determined to be 0.11 mg a.s./L, based on mean measured concentrations. The LC₅₀ was not estimated in the report, however, as no mortality was observed in the study, the LC₅₀ is ≥ 0.33 mg a.s./L (based on mean measured concentrations of centrifuged samples).

A 2.3.1.6 Study 6

The following acute toxicity study on *D. magna* performed with BAS 758 00 F is provided in support of the assessment and has not been previously evaluated on EU level.

Comments of zRMS:	The study was conducted to OECD guidance 202 and according to the principles of GLP. All validity criteria were met. The study is considered to be reliable and suitable for the risk assessment.
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Reference:	CP 10.2.1/6
Report	BAS 758 00 F - Effect on <i>Daphnia magna</i> in a static 48-Hour Immobilization Test, Eckenstein, H., 2021 report No 876353, 20200150 BASF DocID 2020/2033902 Authority registration No
Guideline(s):	OECD 202 (2004), SANCO/3029/99 Rev.4 (2000)
Deviations:	No
GLP:	yes (certified by Swiss Federal Office of Public Health, Berne, Switzerland)
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive Summary

In a 48-hour static acute toxicity laboratory study, water flea neonates were exposed to BAS 758 00 F at nominal concentrations of 0.044, 0.088, 0.175, 0.350 and 0.700 mg BAS 758 00 F/L and a control in 4 replicates per treatment, containing 5 daphnids each. Daphnids were observed for immobility and other non-lethal effects 24 hours and 48 hours after start of exposure.

The biological results are based on nominal concentrations of the test item. After 48 hours of exposure, no immobilized test organisms were determined in the control and up to and including the test item concentration of 0.175 mg/L. At 0.350 mg/L, 45% of the daphnids were immobile. At the highest concentration of 0.700 mg/L, 100% were immobilized, as well as 60% already after 24 hours of exposure. In addition, adverse effects, e.g. daphnids with reduced swimming activity compared to the control animals, were observed at these two highest test concentrations. At test end, immobility in the two highest test item concentrations, was statistically significantly different compared to the control.

In a 48-hour static acute toxicity study with *Daphnia magna*, the EC₅₀ of BAS 758 00 F was 0.362 mg/L based on nominal concentrations. The NOEC was determined to be 0.175 mg/L (nominal).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 758 00 F, batch no. FD-200124-0004; content of a.s.: metrafenone (BAS 560 F, Reg. No. 4037710): 94.9 g/L (nominal: 100 g/L); mefentrifluconazole (BAS 750 F, Reg. No. 5834378): 66.7 g/L (nominal: 66.6 g/L); pyraclostrobin (BAS 500 F, Reg. No. 304428): 81.0 g/L (nominal: 80.0 g/L); density: 1.092 g/cm³.

B. STUDY DESIGN

Test species: Water flea (*Daphnia magna* STRAUS); < 24 h old at exposure initiation; not first brood progeny; neonates collected from a laboratory culture (originally from the Daphnia Collection of the University of Basel/Switzerland in 2015).

Test design: Static system (48 hours); 5 test item concentrations plus control, 4 replicates with 5 daphnids each; assessment of immobility after 24 and 48 hours.

Endpoints: NOEC, EC₅₀ based on immobility of daphnids.

Test concentrations: Control (dilution water), 0.044, 0.088, 0.175, 0.350 and 0.700 mg BAS 758 00 F/L (nominal).

Test conditions: 100-mL glass beakers; test volume: 50 mL; dilution water: reconstituted test water (ISO test water); total hardness: 2.5 mmol/L (nominal); pH 7.8 - 8.1; oxygen content: 8.2 - 8.5 mg/L; water temperature: 21°C photoperiod: 16 h light: 8 h dark (30-min transition period); light intensity 1040 - 1050 lux; no feeding; no aeration.

Analytics: Analytical verification of test item concentrations was conducted using an HPLC-method with MS/MS detection (analytical method for metrafenone: PTRL P 3309 G and for pyraclostrobin and mefentrifluconazole: L0361/01).

Statistics: Descriptive statistics; for determination of EC₅₀, Trimmed Spearman-Kärber method; for determination of NOEC, Step-down Cochran-Armitage test procedure (one-sided greater, $\alpha = 0.05$).

C. DESCRIPTION OF THE ANALYTICAL PROCEDURES

Concentrations of pyraclostrobin, mefentrifluconazole and metrafenone (contained in BAS 758 00 F) in ISO test water were determined according to the analytical method L0361/01 with some adaptations (for pyraclostrobin and mefentrifluconazole) and PTRL P 3309 G with some adaptations (for metrafenone). The validation of the analytical methods is described in the study report.

L0361/01: The samples (10 mL) were completely diluted with 10 mL of acetonitrile / water / formic acid (400/600/2, v/v/v; D1) and shaken mechanically. If necessary, aliquots of the samples were further diluted into the calibration range with test water / D1 (1/1, v/v) and were analyzed by HPLC with MS/MS detection. The limit of quantification (LOQ) was 0.328 µg pyraclostrobin/L and 0.270 µg mefentrifluconazole/L and the limit of detection (LOD) was set to 0.102 µg pyraclostrobin/L and 0.0206 µg mefentrifluconazole/L.

PTRL P 3309 G: The samples (10 mL) were completely diluted with 10 mL of methanol and shaken mechanically. If necessary, aliquots of the samples were further diluted into the calibration range with test water / methanol (1/1, v/v) and were analyzed by HPLC with MS/MS detection. The LOQ was 0.384 µg metrafenone/L and the LOD was set to 0.254 µg metrafenone/L.

For the assessment of potential matrix effects, matrix matched calibration standards were used. The storage stability of the test samples was demonstrated within this study over a time covering the storage period. Details on measured fortification samples and obtained procedural recoveries for mefentrifluconazole are given in Table A 28.

Table A 28: Procedural recoveries for BAS 758 00 F (determined via pyraclostrobin, mefentrifluconazole and metrafenone)

Analyte	Matrix	Fortification level (µg/L)	n	Mean (%)	RSD (%)
Pyraclostrobin	ISO test water	0.328	5	109	2.1
	ISO test water	62.5	5	110	2.2
Mefentrifluconazole	ISO test water	0.270	5	100	2.3
	ISO test water	51.5	5	102	1.0
Metrafenone	ISO test water	0.384	5	94.1	0.9
	ISO test water	73.2	5	96.2	1.1

RSD = relative standard deviation

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. At the start of the test, the measured concentrations of metrafenone in the test media ranged between 89.6 and 92.5% of the nominal values and between 87.3 and 88.3% at the end of the test. For mefentrifluconazole, the measured concentrations in the test media ranged between 95.3 and 99.4% of the nominal values at the start and between 92.2 and 97.0% at the end of the test. The measured concentrations of pyraclostrobin in the test media ranged between 105 and 112% of the nominal values at the start and between 102 and 106% at the end of the test. As measured concentrations confirmed correct application of the test substance, the following biological results are based on nominal concentrations.

Biological results: After 48 hours of exposure, no immobilized test organisms were determined in the control and up to and including the test item concentration of 0.175 mg/L. At 0.350 mg/L, 45% of the daphnids were immobile. At the highest concentration of 0.700 mg/L, 100% were immobilized, as well as 60% already after 24 hours of exposure. In addition, adverse effects, e.g. daphnids with reduced swimming activity compared to the control animals, were observed at these two highest test concentrations. At test end, immobility in the two highest test item concentrations, was statistically significantly different compared to the control. (Step-down Cochran-Armitage Test Procedure; $\alpha = 0.05$). For results see Table A 29.

Table A 29: Effect of BAS 758 00 F on *Daphnia magna* mobility

Concentration [mg/L] (nominal)	Control	0.044	0.088	0.175	0.350	0.700
Immobility (24 h) [%]	0	0	0	0	0	60
Immobility (48 h) [%]	0	0	0	0	45*	100*
Endpoints [mg BAS 758 00 F/L] (nominal)						
EC ₅₀ (48 h)	0.362 (95% confidence limits: 0.311 - 0.423)					
NOEC (48 h)	0.175					

* Statistically significantly different compared to the control (Step-down Cochran-Armitage Test Procedure; $\alpha = 0.05$)

Validity criteria:

Validity criteria according to OECD 202 (2014)	Obtained in this study
In the control, including the control containing the solubilising agent, not more than 10% of the daphnids should have been immobilised. (Not more than 10% of the control daphnids should show immobilisation or other signs of disease or stress, for example, discoloration or unusual behaviour such as trapping at surface of water.)	0%
The dissolved oxygen concentration at the end of the test should be ≥ 3 mg/L in control and test vessels.	8.2 - 8.4 mg/L

All validity criteria were met.

III. CONCLUSION

In a 48-hour static acute toxicity study with *Daphnia magna*, the EC₅₀ of BAS 758 00 F was 0.362 mg/L based on nominal concentrations. The NOEC was determined to be 0.175 mg/L (nominal).

A 2.3.1.7 Study 7

The following toxicity study on green algae performed with BAS 560 F is provided in support of the assessment and was recently submitted under the Annex I renewal process for metrafenone (AIR 3). The study summary was provided in the revised Renewal Assessment Report of metrafenone (RAR, Vol. 3, B.9, December 2019).

Comments of zRMS:	Study not evaluated. The study was considered as not essential for the risk assessment.
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Reference:	CP 10.2.1/7
Report	Effect of BAS 560 F (Metrafenone, Reg.No. 4037710) on the growth of the green alga <i>Pseudokirchneriella subcapitata</i> , Hoffmann, F., 2012 report No 404715 BASF DocID 2011/1254828 Authority registration No
Guideline(s):	EPA 850.5400, OECD 201
Deviations:	No
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive Summary

The toxicity of BAS 560 F (metrafenone) to the green alga *Pseudokirchneriella subcapitata* was determined in a 96-hour laboratory study. The study was conducted under static conditions with nominal concentrations of 0.029, 0.058, 0.115, 0.230 and 0.460 mg a.s./L. Additionally, a negative control and solvent control (0.1 mL/L acetone) were tested. Five replicates were tested for each concentration level and the negative control, and ten for the solvent control.

Analytical verification of BAS 560 F was carried out in each test concentration at test initiation and at test termination. Average measured recoveries for BAS 560 F were 76.2% of nominal at test initiation and 74.1% of nominal at test termination. The test concentration of BAS 560 F in the centrifuged stock solution at test initiation was 0.342 mg/L which could be considered the functional solubility for these test conditions.

No morphological effects on the algae were observed at any concentration. The E_rC_{10} , E_rC_{50} (growth rate) and E_yC_{50} (yield) determined after 72 and 96 hours of exposure were all > 0.339 mg a.s./L (mean measured), the highest concentration tested. The E_yC_{10} was calculated to be 0.161 and 0.272 mg a.s./L (mean measured) after 72 and 96 hours of exposure, respectively. The no-observed-effect concentration (NOEC) was not reported in the study report, but was estimated to be 0.176 mg a.s./L (mean measured) for both growth rate and yield.

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test material:** BAS 560 F (metrafenone, Reg. No. 4037710)
Batch number: AC12053-29
Purity: 94.2%
Description: Solid
2. **Test concentrations:** 0 (negative and solvent control), 0.029, 0.058, 0.115, 0.230 and 0.460 mg a.s./L (mean measured: 0, 0.022, 0.044, 0.085, 0.176 and 0.339 mg a.s./L)
3. **Reference item:** None
4. **Test medium:** Standard algal medium according to OECD 201
Vehicle: Acetone
5. **Test organism:**
Species: Green alga *Pseudokirchneriella subcapitata*
Source: Stock cultures are cultivated in-house. Fresh strains are obtained from the “UTEX Culture collection of Algae, University of Texas at Austin, USA”
Initial cell density: 1×10^4 cells/mL
Test vessels: 100 mL Erlenmeyer dimple flask, containing 60 mL test medium

B. STUDY DESIGN

1. **Environmental conditions:**
Temperature: 22 ± 1 °C
pH: 7.49 – 7.60 (at test termination)
Photoperiod: Continuous illumination (~8000 lux)
Shaking: Constant at 135 rpm

2. **Assignment and treatment:**

A static system was used with test duration of 96 hours. The following nominal test concentrations were used: 0 (negative and solvent control (0.1 mL/L acetone)), 0.029, 0.058, 0.115, 0.230 and 0.460 mg a.s./L. Five replicates were tested for each concentration level and the negative control groups and ten for the solvent control group. Each test concentration and the control were inoculated to obtain initial algae density of 1×10^4 cells/mL. Each replicate consisted of 60 mL of test solution in a 100 mL flask.

3. **Dose preparation:**

A primary stock solvent solution was prepared by dissolving 4.82 mg of BAS 560 F in 1.0 mL acetone and then an aliquot (0.1 mL) of this solution was introduced to 1000 mL test medium. After centrifugation at 4000 rpm for 30 minutes to remove undissolved particles, the stock solution was colorless and clear. The different treatments were prepared by dilution of the stock solution with nutrient medium to reach the desired concentrations.

4. Measurements and observations:

Cell concentration in each flask was determined 24, 48, 72 and 96 hours after starting the experiment with a spectrophotometer at 623 nm, using 5 cm glass cuvettes (due to the high cell density a 1 cm glass cuvette was used for measurement at test termination). Algal medium without algae was used as a blank. The mean cell densities per treatment were used to calculate yield and growth rates. The percent inhibition values were calculated for each treatment group as the percent reduction in average yield and in average growth rate relative to the control replicates.

At the start and at the end of the test samples were taken out of pooled samples for verification of the test item concentrations. Samples were analyzed by an HPLC-method with MS-detection. At test termination, the pH of all individual samples (control as well as treated samples) was measured.

5. Statistics:

The mathematical determination of the EC_x was done by probit analysis. The calculations were conducted with a PC and the commercial software “TOXRAT Professional 2.10” (ToxRat Solutions GmbH, Alsdorf, Germany).

II. RESULTS AND DISCUSSION

A. BIOLOGICAL EFFECTS

The following results (based on geometric mean concentrations) with respect to yield and growth rate were determined from the concentration-response relationship.

72 hour $E_rC_{50} > 0.339$ mg a.s./L (95% confidence limits: not calculated)
72 hour $E_rC_{10} > 0.339$ mg a.s./L (95% confidence limits: not calculated)
72 hour $E_yC_{50} > 0.339$ mg a.s./L (95% confidence limits: not calculated)
72 hour $E_yC_{10} = 0.161$ mg a.s./L (95% confidence limits: 0.132 – 0.185 mg a.s./L)

96 hour $E_rC_{50} > 0.339$ mg a.s./L (95% confidence limits: not calculated)
96 hour $E_rC_{10} > 0.339$ mg a.s./L (95% confidence limits: not calculated)
96 hour $E_yC_{50} > 0.339$ mg a.s./L (95% confidence limits: not calculated)
96 hour $E_yC_{10} = 0.272$ mg a.s./L (95% confidence limits: 0.225 – 0.292 mg a.s./L)

The table below summarizes the percentage of inhibition (of both growth rate and yield) observed in each test group.

Table A 30: Percent inhibition of BAS 560 F on cell density, growth rate and yield of green algae *Pseudokirchneriella subcapitata*

Geometric mean measured concentration (mg a.s./L)	0.022	0.044	0.085	0.176	0.339
Inhibition at 96 h (yield) (%)	-3.1	-3.7	-0.8	-0.7	28.1
Inhibition at 96 h (growth rate) (%)	-0.5	-0.6	-0.1	-0.1	5.5

Negative values demonstrate an increase compared to the control

No morphological effects on the algae were observed at test termination.

B. ANALYSIS

Analytical verification of BAS 560 F was carried out in each tested concentration at the beginning and at the end of the test. Measured values for BAS 560 F at test initiation and at test termination are given in the table below.

Table A 31: Measured concentrations of BAS 560 F in the exposure solutions

Nominal concentration (mg a.s./L)	0.0 (negative control)		0.0 (solvent control)		0.029		0.058		0.115		0.23		0.46	
Sampling time (hours)	0	96	0	96	0	96	0	96	0	96	0	96	0	96
Measured concentration (mg a.s./L)	n.d.	n.d.	n.d.	n.d.	0.0220	0.0215	0.0454	0.0432	0.0866	0.0842	0.178	0.174	0.342	0.336
% nominal	--	--	--	--	75.9	74.0	78.2	74.4	75.3	73.2	77.2	75.6	74.4	73.1

n.d. = not detected

C. DEFICIENCIES

None.

III. CONCLUSION

The 96-hour exposure of BAS 560 F (metrafenone) to the freshwater green algae *Pseudokirchneriella subcapitata* under static conditions resulted in values for both E_rC_{50} (growth rate) and E_yC_{50} (yield) of > 0.339 mg a.s./L (mean measured), the highest test concentration. The E_rC_{10} was also > 0.339 mg a.s./L, based on mean measured concentrations. The E_yC_{10} was calculated to be 0.161 and 0.272 mg a.s./L (mean measured) after 72 and 96 hours of exposure, respectively. The no-observed-effect concentration (NOEC) was not presented in the study report, but was estimated to be 0.176 mg a.s./L (mean measured) for both growth rate and yield.

A 2.3.1.8 Study 8

The following toxicity study on green algae performed with BAS 758 00 F is provided in support of the assessment and has not been previously evaluated on EU level.

Comments of zRMS:	The study was conducted to OECD guidance 201 and according to the principles of GLP. All validity criteria were met. The study is considered to be reliable and suitable for the risk assessment.
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Reference:	CP 10.2.1/8
Report	BAS 758 00 F - Effect on <i>Pseudokirchneriella subcapitata</i> in a 72 Hour Algal Growth Inhibition Test, Eckenstein, H., 2021 report No 876352, 20200149 BASF DocID 2020/2033904 Authority registration No
Guideline(s):	OECD 201 (2011), SANCO/3029/99 rev. 4 (11 July 2000)
Deviations:	No
GLP:	yes (certified by Swiss Federal Office of Public Health, Berne, Switzerland)
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive Summary

In a 72-hour static toxicity laboratory study, the effect of BAS 758 00 F on the growth of the green alga *Pseudokirchneriella subcapitata* was investigated. Algae were exposed to nominal concentrations of 0.050, 0.16, 0.50, 1.6 and 5.0 mg BAS 758 00 F/L with 5 replicates per concentration and a control with 10 replicates. 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. The test item had a statistically significant inhibitory effect on the growth rate of the algae after the test period of 72-hours starting at the test item concentration of 0.50 mg/L. The yield of the algae after 72 hours of incubation was statistically significantly reduced starting at the test item concentration of 0.50 mg/L. No difference between the algae growing at the nominal test item concentration of 1.6 mg/L and the algal cells in the control was observed. The shape and size of the algal cells were obviously not affected by the test item up to at least this concentration.

In a 72-hour algae test with *Pseudokirchneriella subcapitata*, the E_rC_{50} (72 h) and the E_yC_{50} (72 h) of BAS 758 00 F were determined to be 3.82 mg/L and 1.43 mg/L, respectively, based on nominal concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 758 00 F, batch no. FD-200124-0004; content of a.s.: metrafenone (BAS 560 F, Reg. No. 4037710): 94.9 g/L (nominal: 100 g/L); mefentrifluconazole (BAS 750 F, Reg. No. 5834378): 66.7 g/L (nominal: 66.6 g/L); pyraclostrobin (BAS 500 F, Reg. No. 304428): 81.0 g/L (nominal: 80.0 g/L); density: 1.092 g/cm³.

B. STUDY DESIGN

Test species: Unicellular fresh water green alga, *Pseudokirchneriella subcapitata* (Reinsch) Korshikov (syn. *Selenastrum capricornutum* Prinz), SAG 61.81; stock obtained from the 'Collection of Algal Cultures (SAG, Institute for Plant Physiology, University of Goettingen)', Goettingen, Germany.

Test design: Static system (72 hours); 5 test item concentrations, each with 5 replicates per treatment plus a control with 10 replicates; daily assessment of growth via fluorescence measurement.

Endpoints: NOEC, EC₁₀, EC₂₀ and EC₅₀ with respect to growth rate and yield after exposure over 72 hours.

Test concentrations: Control (dilution water), 0.050, 0.16, 0.50, 1.6 and 5.0 mg BAS 758 00 F/L (nominal).

Test conditions: 75-mL Erlenmeyer flasks; test volume: 30 mL; test medium: AAP medium; initial cell density: 5000 cells/mL; pH 7.3 - 7.6; temperature: 20.9 - 21.0°C; continuous light at 4870 - 5010 lux; continuous shaking.

Analytics: Analytical verification of test item concentrations was conducted using an HPLC-method with MS/MS detection (analytical method for metrafenone: PTRL P 3309 G and for pyraclostrobin and mefentrifluconazole: L0361/01).

Statistics: Descriptive statistics; determination of EC_x via 3-Parameter Normal Cumulative Distribution Function (CDF); determination of NOEC via Williams or Welch t-test ($\alpha = 0.05$).

C. DESCRIPTION OF THE ANALYTICAL PROCEDURES

Concentrations of pyraclostrobin, mefentrifluconazole and metrafenone (contained in BAS 758 00 F) in AAP medium were determined according to the analytical method L0361/01 with some adaptations (for pyraclostrobin and mefentrifluconazole) and PTRL P 3309 G with some adaptations (for metrafenone). The validation of the analytical methods is described in the study report.

L0361/01: The samples (10 mL) were completely diluted with 10 mL of acetonitrile / water / formic acid (400/600/2, v/v/v; A1) and shaken mechanically. Test samples from day 3 were additionally centrifuged due to the presence of the algae (5 min, 2465g) in addition to an aliquot from one sample of each spike level. If necessary, aliquots of the samples were further diluted into the calibration range with test water / A1 (1/1, v/v) and were analyzed by HPLC with MS/MS detection. The limit of quantification (LOQ) was 0.370 µg pyraclostrobin/L and 0.305 µg mefentrifluconazole/L and the limit of detection (LOD) was set to 0.0549 µg pyraclostrobin/L and 0.0206 µg mefentrifluconazole/L.

PTRL P 3309 G: The samples (10 mL) were completely diluted with 10 mL of methanol and shaken mechanically. Test samples from day 3 were additionally centrifuged due to the presence of the algae (5 min, 2465g) in addition to an aliquot from one sample of each spike level. If necessary, aliquots of the samples were further diluted into the calibration range with test water / methanol (1/1, v/v) and were analyzed by HPLC with MS/MS detection. The LOQ was 0.433 µg metrafenone/L and the LOD was set to 0.0504 µg metrafenone/L.

For the assessment of potential matrix effects, matrix matched calibration standards were used. The storage stability of metrafenone in the test samples was demonstrated within this study over a time covering the storage period. Details on measured fortification samples and obtained procedural recoveries for mefentrifluconazole are given in Table A 32.

Table A 32: Procedural recoveries for BAS 758 00 F (determined via pyraclostrobin, mefentrifluconazole and metrafenone)

Analyte	Matrix	Fortification level (µg/L)	n	Mean (%)	RSD (%)
Pyraclostrobin	AAP medium	0.370	5	88.6	6.2
	AAP medium	451	5	95.4	2.0
Mefentrifluconazole	AAP medium	0.305	5	99.7	1.3
	AAP medium	371	5	103	2.3
Metrafenone	AAP medium	0.433	5	99.4	1.3
	AAP medium	528	5	113	2.5

RSD = relative standard deviation

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. At the start of the test, the measured concentrations of metrafenone in the test media ranged between 107 and 113% of the nominal values and between 84.7 and 111% at the end of the test. For mefentrifluconazole, the measured concentrations in the test media ranged between 89.2 and 98.7% of the nominal values at the start and between 82.3 and 94.0% at the end of the test. The measured concentrations of pyraclostrobin in the test media ranged between 86.1 and 95.8% of the nominal values at the start and between 75.1 and 90.3% at the end of the test. As the analytically measured values at test initiation confirmed the correct application of the test item, the following biological results are based on nominal concentrations of the test item.

Biological results: The test item had a statistically significant inhibitory effect on the growth rate of the algae after the test period of 72-hours starting at the test item concentration of 0.50 mg/L (Williams t-test, one-sided smaller, $\alpha = 0.05$). The yield of the algae after 72 hours of incubation was statistically significantly reduced starting at the test item concentration of 0.50 mg/L (Welch t-test, one-sided smaller, $\alpha = 0.05$). No difference between the algae growing at the nominal test item concentration of 1.6 mg/L and the algal cells in the control was observed. The shape and size of the algal cells were obviously not affected by the test item up to at least this concentration. The effects on algal growth are summarized in Table A 33.

Table A 33: Effect of BAS 758 00 F on the growth of the green alga *Pseudokirchneriella subcapitata*

Concentration [mg/L] (nominal)	Control	0.050	0.16	0.50	1.6	5.0
Inhibition in 72 h (growth rate) [%] #	--	-3.4	-2.3	2.5*	13.3*	62.7*
Inhibition in 72 h (yield) [%] #	--	-17.8	-11.4	11.5**	47.8**	95.8**
Endpoint [mg BAS 758 00 F/L] (nominal)						
ErC ₅₀ (72 h)	3.82 (95% confidence limits: 3.38 - 4.29)					
ErC ₂₀ (72 h)	1.91 (95% confidence limits: 1.74 - 2.11)					
ErC ₁₀ (72 h)	1.33 (95% confidence limits: 1.21 - 1.47)					
NOE _r C (72 h)	0.16					
EyC ₅₀ (72 h)	1.43 (95% confidence limits: 0.915 - 2.22)					
EyC ₂₀ (72 h)	0.664 (95% confidence limits: 0.464 - 0.959)					
EyC ₁₀ (72 h)	0.444 (95% confidence limits: 0.306 - 0.645)					
NOE _y C (72 h)	0.16					

Negative values indicate stimulated growth

* Statistically significantly different compared to the control (Williams Multiple Sequential t-test Procedure; $\alpha = 0.05$)

** Statistically significantly different compared to the control (Multiple Sequentially-rejective Welch-t-test After Bonferroni-Holm; $\alpha = 0.05$)

Validity criteria:

Validity criteria according to OECD 201 (2011)	Obtained in this study
The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period. This corresponds to a specific growth rate of 0.92/day (for species in Annex 2 of OECD 201)	123-fold
The mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3, for 72-hour tests) in the control cultures must not exceed 35%.	10%
The coefficient of variation of average specific growth rates during the whole test period in replicate control cultures must not exceed 7% in tests with <i>Pseudokirchneriella subcapitata</i> and <i>Desmodesmus subspicatus</i> . For other less frequently tested species, the value should not exceed 10%.	1.8%

All validity criteria were met.

III. CONCLUSION

In a 72-hour algae test with *Pseudokirchneriella subcapitata*, the E_rC_{50} (72 h) and the E_yC_{50} (72 h) of BAS 758 00 F were determined to be 3.82 mg/L and 1.43 mg/L, respectively, based on nominal concentrations.

A 2.3.1.9 Study 9

The following toxicity study on *Lemna gibba* performed with BAS 500 F is provided in support of the assessment and has not been previously evaluated on EU level.

Comments of zRMS:	Study not evaluated.
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Reference: CP 10.2.1/9

Report Growth and reproduction toxicity test with BAS 500 F and the duckweed, *Lemna gibba* G3,
Boeri R.L. et al., 2000
Report No SubNo-200002-44-01, US-1324-BA, MRID-45118715, US-97129
BASF DocID 2000/5037
Authority registration No

Guideline(s): EPA 123-2, EPA 850.4400

Deviations: No

GLP: yes
(certified by United States Environmental Protection Agency)

Acceptability: Yes

Duplication (if vertebrate study) Not relevant

Comments of zRMS:	Study not evaluated.
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Reference: CP 10.2.1/10

Report Addendum to study BASF DocID: 2000/5037 - Growth and Reproduction Toxicity with BAS 500 F and the Duckweed, *Lemna gibba* G3,
Anonymous, 2019
report No
BASF DocID 2019/2036269
Authority registration No

Guideline(s): None, not relevant for study amendment

Deviations: None, not relevant for study amendment

GLP: No, not relevant for study amendment

Acceptability: Yes

Duplication (if vertebrate study) Not relevant

Executive Summary

In a 14-day static toxicity laboratory study, the effect of pyraclostrobin on the growth of the duckweed *Lemna gibba* was investigated. The following nominal concentrations were applied: 0.13, 0.25, 0.50, 1.0 and 2.0 mg pyraclostrobin/L (corresponding to initial measured concentrations of 0.120, 0.202, 0.422, 0.896 and 1.72 mg a.s./L). Additionally, a solvent control (dimethylformamide) and a dilution water control were set up. Assessment of plant growth and other effects was conducted 1, 4, 6, 8, 11 and 14 days after test initiation. Percent growth inhibition relative to the control was calculated for each test concentration based upon biomass for the parameters frond number and dry weight.

The biological results are based on initial measured concentrations of the test item. The duckweed population in the control vessels showed sufficient growth. At the end of the test, chlorotic fronds were observed in the control, the solvent control and in all test item concentrations tested. Statistically significant effects on the number of normal, non-chlorotic fronds and the plant biomass compared to the pooled controls were observed at the highest tested concentration of 1.72 mg a.s./L.

In a 14-day aquatic-plant test with *Lemna gibba*, the E_bC_{50} values of pyraclostrobin based on frond number and dry weight were determined to be > 1.72 mg a.s./L and 1.72 mg a.s./L, respectively, based on initial measured concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Pyraclostrobin (BAS 500 F; Reg. no.: 304 428), batch no. 27882/191/C; purity: 97.09%.

B. STUDY DESIGN

Test species: Duckweed (*Lemna gibba* G3); inocula: 13 days old cultures; cultures maintained in-house; stock obtained from "Climate Stress Laboratory", USDA, Beltsville, Maryland, USA.

Test design: Static system; test duration 14 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; 3 plants with 3 - 4 fronds, total number of fronds at test initiation: 9 - 11 per replicate; assessment of growth and other effects on days 1, 4, 6, 8, 11 and 14.

Endpoints: EC_{50} and NOEC with respect to biomass development after exposure over 14 days.

Test concentrations: Control, solvent control (0.1 mL dimethylformamide/L), 0.13, 0.25, 0.50, 1.0 and 2.0 mg pyraclostrobin/L (nominal), corresponding to initial measured concentrations of 0.120, 0.202, 0.422, 0.896 and 1.72 mg a.s./L.

Test conditions: 500 mL glass flasks, test volume: 200 mL, M-Hoagland's media without sucrose or EDTA, pH 4.9 - 5.1 at test initiation and pH 5.5 - 5.7 at test termination; temperature: 24.7°C - 25.5°C, continuous light, light intensity: about 490 foot candles.

Analytics:	Analytical verification of the test item was conducted using an HPLC-method with UV-detection.
Statistics:	Descriptive statistics, t-test ($\alpha = 0.05$) for comparison of frond no. and dry weight in the control and solvent control, weighted least squares non-linear regression for determination of EC _x values based on frond no. and dry weight, Bonferroni's test for determination of the NOEC value ($\alpha = 0.05$).

C. DESCRIPTION OF THE ANALYTICAL PRODECDURES

Concentrations of BAS 500 F in dilution water were determined according to the analytical method described within the study report. An aliquot of each sample [10 mL aliquots for samples with nominal concentrations of 0 (control and solvent control) and 0.13 mg/L, 4.0 mL aliquots for samples with a nominal concentration of 0.25 mg/L, and 2.0 mL aliquots for samples with nominal concentrations of 0.50, 1.0, and 2.0 mg/L] was allowed to flow through a preconditioned C18 Bond—Elut cartridge at a rate of 1 to 2 drops per second, with the eluant going to waste. The cartridge was allowed to go to dryness. The cartridge was eluted twice with 5 mL acetonitrile into a 15 mL centrifuge tube. The extract was evaporated to dryness under a gentle stream of nitrogen in a water bath at approximately 40°C. The residue was reconstituted to a volume of 2 mL with 50:50 (v:v) acetonitrile/HPLC water and the samples were sonicated for approximately 2 minutes. A 1.25 mL aliquot of the extract for samples with a nominal concentration of 2.0 mg/L was diluted to a volume of 5.0 mL with 50:50 (v:v) acetonitrile/HPLC water. An aliquot of the extract was transferred to an autosampler vial for analysis. The determination was performed by HPLC-UV. The limit of quantification (LOQ) was 0.0005 mg/L and the limit of detection (LOD) was set to 0.0000000103 mg on column. Matrix spike samples and laboratory control samples were prepared with the test substance used to formulate the test concentrations (purity = 97.09%). Recovery of matrix spike was 73 - 87% and 78% of laboratory control spike.

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 pyraclostrobin ranged from 81 to 92% of nominal at test initiation and from 17% to 34% of nominal at test termination. The following biological results are based on initial measured concentrations.

Biological results: No statistically significant differences were determined between the control and the solvent control data (t-test; $\alpha = 0.05$). Thus, the data from both control groups were pooled and the pooled data set was used for statistical evaluation of treatment related effects. The duckweed population in the control vessels showed sufficient growth, increasing from an average of 11 fronds per vessel to an average of 198 fronds per vessel, corresponding to an 18 x multiplication. At the end of the test, chlorotic fronds were observed in the control, the solvent control and in all test item concentrations tested. Statistically significant effects on the number of normal, non-chlorotic fronds and the plant biomass compared to the pooled controls were observed at the highest tested concentration of 1.72 mg a.s./L (Bonferroni's test; $\alpha = 0.05$). Effects on biomass development are summarized in Table A 34.

Table A 34: Effects of pyraclostrobin on the biomass development of duckweed *Lemna gibba*

Concentration [mg a.s./L] (nominal)	Control	Solvent control	0.13	0.25	0.50	1.0	2.0
Concentration [mg a.s./L] (initial measured)	--	--	0.120	0.202	0.422	0.896	1.72
Number of non-chlorotic fronds (14 d) [% of pooled control]	--	--	100	84	83	91	55
Mean dry weight of fronds [mg]	25.1	21.9	24.9	22.1	21.2	23.1	15.4
Endpoints [mg pyraclostrobin/L] (initial measured)							
E _b C ₅₀ (14 d) based on frond no.	> 1.72						
E _b C ₅₀ (14 d) based on dry weight	1.72 (95% confidence limits: n.d.)						
NOEC (14 d)	0.896						

n.d. = not determined

Validity criteria according to OECD 221 (2006)	Obtained in this study
...the doubling time of frond number in the control must be less than 2.5 days (60 h), corresponding to approximately a seven-fold increase in seven days and an average specific growth rate of 0.275/day	> 7-fold increase (8 d) *

* Since no assessment of frond no. was done after 7 days, frond no. data after 8 day are considered for validity check.

All validity criteria were met.

III. CONCLUSION

In a 14-day aquatic-plant test with *Lemna gibba*, the E_bC₅₀ values of pyraclostrobin based on frond number and dry weight were determined to be > 1.72 mg a.s./L and 1.72 mg a.s./L, respectively, based on initial measured concentrations.

A 2.3.1.10 Study 10

The following toxicity study on *Lemna gibba* performed with BAS 560 F is provided in support of the assessment and was recently submitted under the Annex I renewal process for metrafenone (AIR 3). The study summary was provided in the revised Renewal Assessment Report of metrafenone (RAR, Vol. 3, B.9, December 2019).

<i>Comments of zRMS:</i>	Study not evaluated.
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<i>Reference:</i>	<i>CP 10.2.1/11</i>
<i>Report</i>	<i>Effect of BAS 560 F (Metrafenone, Reg.No. 4037710) on the growth of Lemna gibba, Hoffmann F., 2012 Report No 404714 BASF DocID 2011/1254832 Authority registration No</i>
<i>Guideline(s):</i>	<i>ASTM E 1415-91, EPA 850.4400, OECD 221</i>
<i>Deviations:</i>	<i>No</i>
<i>GLP:</i>	<i>yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)</i>
<i>Acceptability:</i>	<i>Yes</i>
<i>Duplication (if vertebrate study)</i>	<i>No</i>

Executive Summary

In a 7-day static toxicity study, the effect of metrafenone on the growth of duckweed *Lemna gibba* was investigated. *Lemna gibba* was exposed to nominal concentrations of 0.029, 0.058, 0.115, 0.230, and 0.460 mg a.s./L. Additionally, a negative control and a solvent control (0.1 mL/L acetone) were tested. Three replicates were tested for each test substance concentration and the negative control group, and six replicates for the solvent control group. Assessment of growth and other effects was conducted on days three, five and seven after test initiation. The percentage growth inhibition, relative to the solvent control was calculated for each test concentration based upon growth rates and final yield for the parameters frond number and plant dry weight (biomass).

Measured concentrations of metrafenone ranged from 80.3% to 93.5% of nominal concentrations at test initiation and from 57.2% to 68.4% of nominal concentrations at test termination. The biological results were based on geometric mean measured concentrations.

No morphological effects on *Lemna gibba* were observed in the control groups and at any of the test item concentrations tested.

In this 7-day toxicity test with *Lemna gibba*, the E_rC_{50} (growth rate) and E_yC_{50} (yield) of metrafenone were determined to be both > 0.327 mg a.s./L based on frond number and dry weight (geometric mean measured). The E_rC_{10} based on frond number and dry weight was also > 0.327 mg a.s./L (geometric mean measured). The E_yC_{10} based on frond number was calculated to be 0.280 mg a.s./L (geometric mean measured) and the E_yC_{10} based on dry weight was 0.240 mg a.s./L (geometric mean measured). The no-observed-effect concentration (NOEC) was not presented in the study report, but was estimated to be 0.159 mg a.s./L for all endpoints.

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test material:** BAS 560 F (metrafenone; Reg. No. 40.37710)
Batch number: AC12053-29
Purity: 94.2%
Description: Solid
2. **Test concentrations:** 0 (negative and solvent control), 0.029, 0.058, 0.115, 0.230 and 0.460 mg a.s./L; (geometric mean measured concentrations: 0, 0, 0.021, 0.046, 0.089, 0.159 and 0.357 mg a.s./L)
3. **Reference item:** None
4. **Test medium:** 20x AAP medium
Vehicle: Acetone
5. **Test organism:**
Species: Duckweed *Lemna gibba* G3
Source: Cultures maintained in-house, stock obtained from "ÖkoTox Moser & Pickl GbR", Stuttgart, Germany
Initial frond numbers: Eleven fronds (on three plants)
Test vessels: 400 mL glass beakers, containing 160 mL test or control medium

B. STUDY DESIGN

1. **Environmental conditions:**
Temperature: 24.2 °C
pH: 7.5 at test initiation; 8.4 – 8.5 at test termination
Photoperiod: Continuous illumination (8100 lux)

2. **Assignment and treatment:**

At test initiation, three plants from a 10-day old culture were added to each vessel randomly; two plants with four fronds and one plant with three fronds. Three replicate test vessels were tested in each treatment group and the negative control group, and the solvent control included six replicates. The duckweed was exposed for seven days under static conditions.

3. Dose preparation:

A primary stock solvent solution was prepared by dissolving BAS 560 F in acetone, at a nominal concentration of 4.6 mg a.s./mL. This solution was diluted in test medium, at a concentration of 0.1 mL/L, and was then centrifuged for 30 minutes at 4000 rpm to remove undissolved particles. Secondary stock solutions were prepared by further diluting the primary stock solution in the test medium, to obtain nominal concentrations of 0.029, 0.058, 0.115, 0.230 and 0.460 mg a.s./L. The negative control group was exposed to test medium only and the solvent control group was exposed to acetone diluted in test medium, at a concentration of 0.1 mL/L.

4. Measurements and observations:

Frond production and appearance (necrosis, chlorosis, changes in plant size or shape and root growth) were recorded on days three, five and seven. In addition, biomass was determined at test initiation with three samples of the inoculum culture and at test termination with the plant material from each replicate from each test concentration and the controls.

Samples were taken for verification of the test item concentrations at test initiation and termination. These samples were analyzed by HPLC with MS-detection.

Temperature was recorded continuously. The pH was measured at test initiation and test termination.

5. Statistics:

Calculations of growth rate and yield, based on frond number and dry weight, were made and analyzed by probit analysis to obtain EC_x values. These analyses were performed using TOXSTAT Professional 2.10.

II. RESULTS AND DISCUSSION

A. BIOLOGICAL EFFECTS

The duckweed population in the control vessels showed exponential growth, increasing from eleven fronds per vessel to an average of 115 fronds per vessel in the control after 7 days (corresponding to a 10.4 x multiplication). The dry weight increased from 2.0 mg to an average of 22.5 mg per vessel in the control at test termination. No morphological effects on *Lemna gibba* were observed in the control groups and at any of the test item concentrations tested. The results are summarized in the table below.

Table A 35: Effects of metrafenone on the growth of duckweed *Lemna gibba*

Concentration (mg a.s./L) (nominal)	Solvent control	Control	0.029	0.058	0.115	0.230	0.460
Concentration (mg a.s./L) (geometric mean measured)	--	--	0.021	0.046	0.089	0.159	0.327
Inhibition in 7 d (%) ^a (growth rate based on frond no.)	--	-0.3	-1.5	-0.7	0.3	-0.8	6.0
Inhibition in 7 d (%) ^a (yield based on frond no.)	--	0.9	-4.0	-1.8	0.8	-2.1	14.6
Inhibition in 7 d (%) (growth rate based on dry weight)	--	0.3	0.4	1.1	1.9	1.7	5.6
Inhibition in 7 d (%) (yield based on dry weight)	--	-1.5	1.2	3.0	4.9	4.5	14.0
Endpoints (mg a.s./L) (geometric mean measured (95% confidence limits))							
E _r C ₅₀ / E _y C ₅₀ (7 d) based on frond no.	> 0.327 (nd)						
E _r C ₅₀ / E _y C ₅₀ (7 d) based on dry weight	> 0.327 (nd)						
E _r C ₁₀ (7 d) based on frond no. / dry weight	> 0.327 (nd)						
E _y C ₁₀ (7 d) based on frond no.	0.280 (0.250 – 0.300)						
E _y C ₁₀ (7 d) based on dry weight	0.240 (0.150 – 0.390)						

^a Inhibition compared to the solvent control; negative values indicate stimulated growth
nd = not determined

B. ANALYSIS

Measured concentrations of metrafenone ranged from 80.3% to 93.5% of nominal at test initiation and from 57.2% to 68.4% of nominal at test termination, as shown in the table below. The biological results are based on geometric mean measured concentrations.

Table A 36: Measured concentrations of BAS 560 F in the exposure solutions

Nominal concentration (mg a.s./L)	Sampling time (days)	Mean measured concentration (mg a.s./L)	% of nominal concentration	Geometric mean measured concentrations (mg a.s./L)
0.0 (negative control)	0 7	< LOQ	-	-
0.0 (solvent control)	0 7	< LOQ	-	-
0.029	0 7	0.0271 0.0166	93.3 57.2	0.021
0.058	0 7	0.0531 0.0397	91.6 68.4	0.046
0.115	0 7	0.107 0.0740	93.5 64.3	0.089
0.230	0 7	0.185 0.136	80.3 59.3	0.159
0.460	0 7	0.384 0.279	83.6 60.6	0.327

LOQ = limit of quantification (0.001 mg a.s./L)

C. DEFICIENCIES

None.

III. CONCLUSION

In this 7-day static toxicity test with the duckweed *Lemna gibba*, the ErC_{50} (growth rate) and EyC_{50} (yield) of metrafenone based on frond number and dry weight were determined to be both > 0.327 mg a.s./L (geometric mean measured), the highest concentration tested. The ErC_{10} based on frond number and dry weight was also > 0.327 mg a.s./L (geometric mean measured). The EyC_{10} based on frond number was calculated to be 0.280 mg a.s./L and the EyC_{10} based on dry weight was 0.240 mg a.s./L, both based on geometric mean measured. The no-observed-effect concentration (NOEC) was not presented in the study report, but was estimated to be 0.159 mg a.s./L for all endpoints.

A 2.3.2 KCP 10.2.2 Additional long-term and chronic toxicity studies on fish, aquatic invertebrates and sediment dwelling organisms

A 2.3.2.1 Study 1

The following study was already submitted in the Annex I inclusion process of pyraclostrobin. It was noted that the report to the following modified ELS study was not sufficiently clear concerning the control mortality. Therefore, the report has been re-evaluated based on the original raw data and clarifying information is provided in an amendment to the report (BASF DocID 2018/1123384); also see remarks in the summary below.

Comments of zRMS:	Study not evaluated by zRMS.
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Reference:	CP 10.2.2/1
Report	<p>BAS 500 F - Early life-stage toxicity test on the rainbow trout (<i>Oncorhynchus mykiss</i> WALBAUM 1792) in a flow through system with variable concentrations,</p> <p>XXXXXXXXXX</p> <p>Report No 52F0494/965189</p> <p>BASF DocID 1999/11537</p> <p>Authority registration No</p>
Guideline(s):	EPA 72-4 (a), OECD 210, EPA 540/9-86-138
Deviations:	No
GLP:	<p>Yes</p> <p>(certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)</p> <p>The standard method was modified with regard to the concentrations which varied according to the described scheme. Since the study was carried out with eggs produced outside of the normal breeding season a lower viability was expected and the study was started with the double number of eggs per replicate as would have been used in a standard study (50 instead of 25). Hatch rate and survival of the control group in this study are decreased in comparison to values from historical studies with eggs obtained during the normal breeding season.</p>
Acceptability:	Yes
Duplication (if vertebrate study)	No

Reference: CP 10.2.2/2

Report Amendment 1: BAS 500 F - Early life-stage toxicity test on the rainbow trout (*Oncorhynchus mykiss* WALBAUM 1792) in a flow through system with variable concentrations,
XXXXXXXXXX
Report No EU-52F0494/965189
BASF DocID 2018/1123384
Authority registration No

Guideline(s): OECD 210, EPA 72-4 (a), EPA 540/9-86-138

Deviations: No

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

Acceptability: Yes

Duplication No
(if vertebrate study)

Executive Summary

The chronic toxicity of pyraclostrobin to rainbow trout (*Oncorhynchus mykiss*) embryos, larvae and young fish was investigated in an extended 97-day early life-stage test under flow-through conditions. Embryos were exposed to a dilution water control and to pyraclostrobin at nominal concentrations of 0.0025, 0.005, 0.01 and 0.02 mg a.s./L. Hatchability, survival rate, time to hatch and swim-up, and behavior of rainbow trout embryos, larvae and young fish were assessed throughout the study. Individual fish lengths and weights were measured at test termination.

The biological results are based on nominal concentrations. In the control at the end of hatch (day 37) the survival was 23.5% of day 0. At the termination of swim-up day 58 the survival in the control was 25% and at the end of the study (day 97) there were only 18% survival compared to day 0. Thus, the number of surviving individuals in the control is rather low. Compared to the control group the survival up to the start of hatch was statistically significantly decreased in the 0.01 mg a.s./L and in the 0.02 mg a.s./L dose groups to 72.2 and 62% of the control survival, respectively. Statistically significant decrease in survival was also observed in the 0.0025 mg a.s./L dose group but not in the 0.005 mg a.s./L group. In comparison to the control, survival from start of the hatch to termination of the hatch was not impaired in any of the dose groups. Survival from the end of the hatch to the end of swim-up was significantly decreased in the 0.02 mg a.s./L dose group, the highest concentration tested as all larvae died until day 48. From the end of swim-up to the end of the study no substance-related statistically significant effect was observed in the surviving dose groups. Over the whole study period the survival in the 0.0025, 0.01 and the 0.02 mg a.s./L dose groups were statistically significantly decreased in comparison to the control group, however in the 0.0025 and the 0.01 mg a.s./L the survival was 97.2% of the control group. As there was no significant decrease in survival in the 0.005 mg a.s./L dose group the decrease in the 0.0025 mg a.s./L dose group was considered to be not treatment-related and that in the 0.01 mg a.s./L dose group was considered to be questionably attributed to the test compound.

In a modified early life stage study with rainbow trout (*Oncorhynchus mykiss*) the overall NOEC (97 d) for pyraclostrobin was determined to be 0.0050 mg a.s./L based on nominal concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Pyraclostrobin (BAS 500 F, Reg. No. 304 428); batch no. CP 029053; purity: 99.0 %.

B. STUDY DESIGN

Test species: Rainbow trout (*Oncorhynchus mykiss* WALBAUM 1792), embryos (appr. 60 min after fertilization)

Test design: Flow-through system, study was terminated on day 97, 60 days after completion of hatch (day 37); 4 replicates of 50 embryos per test vessel and per concentration; mortality was determined daily, time to hatch and swim up were determined, toxic signs and abnormalities were determined at least workdays, body weights and lengths were determined at the end of the study.

Endpoints: NOEC values based on mortality and sublethal effects. Due to the steep dose response curve in this study, no reliable EC₁₀ and EC₂₀ value could be derived from this study.

Test concentrations: Control, 0.0025, 0.0050, 0.010 and 0.020 mg a.s./L (nominal). Concentrations followed a realistic model of expected concentrations in the field on the basis of analytical results of a microcosm study with BAS 500 00 F applied in 14-day intervals. The 14-day concentration maxima were increased during the study according to increasing application volumes during spring and summer.

Test conditions: Aquaria (29 x 21 x 22 cm) with a test volume of 9 L water; temperature: 11 +/- 1 °C; pH 7.6 – 8.2; oxygen content 8.9 – 11.6 mg/L; total hardness 2.2 – 2.5 mmol/L; photoperiod: until swim-up aquaria were kept in the dark after swim-up dim light with a cycle of 16 hours light and 8 hours dark; flow rate: 10 L/hour/test group.

Analytics: Analytical verification of test item concentrations was conducted using an RP-HPLC-method (Method No. CP 314).

Statistics: Descriptive statistics; Dunnett's test (two-sided) for body weight and length, log-rank test (two-sided) for survival.

C. DESCRIPTION OF THE ANALYTICAL PRODECDURES

Concentrations of BAS 500 F in tap water were determined according to the analytical method CP 314. Samples containing > 0.0025 mg a.s./L were diluted to a concentration range within the calibration series. In general, an aliquot of 7 mL of the samples was transferred into a 10 mL volumetric flask and diluted with 3 mL acetonitrile. An aliquot of 5.0 mL of the final dilution was injected into the HPLC system. Each sample was extracted and injected twice. The determination was performed by HPLC-UV. The limit of quantification (LOQ) was 0.05 µg/L. No interference from the matrix with the a.s. could be observed under the conditions used in this study as indicated by a blank sample.

Fortified samples were analyzed (see table below). The average procedural recovery for BAS 500 F was 105%.

Table A 37: Procedural recoveries for BAS 500 F

Matrix	Fortification level (µg/L)	n	mean (%)	±SD	RSD (%)
tap water	0.05465	3	107.0	0.00033	0.6
tap water	0.1093	3	103.1	0.00048	0.4

II. RESULTS AND DISCUSSION

Analytical measurements: Generally, the analytically determined concentration values of the test substance were in good agreement with the desired concentration of the saw-tooth shaped concentration scheme and were in the range of ±20% of the theoretical concentration. Occasionally during the study short deviations of the concentration values occurred due to technical problems with the dilution system. The biological results are based on nominal concentrations.

Biological results: In the control at the end of hatch (day 37) the survival was 23.5% of day 0. At the termination of swim-up day 58 the survival in the control was 25% and at the end of the study (day 97) there were only 18% survival compared to day 0. Thus, the number of surviving individuals in the control is rather low. Compared to the control group the survival up to the start of hatch was statistically significantly decreased in the 0.01 mg a.s./L and in the 0.02 mg a.s./L dose groups to 72.2 and 62% of the control survival, respectively. Statistically significant decrease in survival was also observed in the 0.0025 mg a.s./L dose group but not in the 0.005 mg a.s./L group. In comparison to the control, survival from start of the hatch to termination of the hatch was not impaired in any of the dose groups. Survival from the end of the hatch to the end of swim-up was significantly decreased in the 0.02 mg a.s./L dose group, the highest concentration tested as all larvae died until day 48. From the end of swim-up to the end of the study no substance-related statistically significant effect was observed in the surviving dose groups. Over the whole study period the survival in the 0.0025, 0.01 and the 0.02 mg a.s./L dose groups were statistically significantly decreased in comparison to the control group, however in the 0.0025 and the 0.01 mg a.s./L the survival was 97.2% of the control group. As there was no significant decrease in survival in the 0.005 mg a.s./L dose group the decrease in the 0.0025 mg a.s./L dose group was considered to be not treatment-related and that in the 0.01 mg a.s./L dose group was considered to be questionably attributed to the test compound. The results are summarized in the table below.

Table A 38: Early life stage test (97 d) of BAS 500 F on rainbow trout (*Oncorhynchus mykiss*)

Concentration [mg a.s./L]	Control	0.0025	0.005	0.01	0.02
Start of hatch [day]	31	31	30	30	30
End of hatch [day]	37	37	37	37	37
Start of swim-up [day]	48	52	48	52	--
Survival of embryos days 0-30 related to 200 start individuals [%]	54	41*	54.5	39*	33.5*
Survival of embryos/larvae at day 37 related to surviving embryos at day 30 [%]	43.5	52.4	41.3	53.8	56.7
Embryo/larvae survival at the termination of hatch (day 37) related to 200 start individuals [%]	23.5	21.5*	22.5	21.0*	19.0*
Survival of larvae at termination of hatch (day 37) related to fertilized eggs [%]	83.9	76.8	80.4	75	67.9
Survival of larvae days 37 – 58 related to number of hatched larvae [%]	87.2	95.3	91.1	88.1	0*
Larvae survival at the termination of swim-up (days 58) related to 200 start individuals [%]	20.5	20.5	20.5	18.5*	0*
Survival of young fish at day 98 related to day 56 survivors [%] 1	87.8	85.4	92.7	94.6	--
Young fish mean survival rate (0-97 d) related to 200 start individuals [%]	18	17.5*	19	17.5*	0*
Mean wet weight [% of control]	100	94.1	94	92.1	--
Mean body length [% of control]	100	98.2	99.7	98.6	--
Symptoms #	none	none	none	none	A, N
	Endpoint [mg pyraclostrobin/L] (nominal)				
NOECoverall (97 d)	0.0050				

* = statistically significant decrease from control Symptoms: A = apathy; N = Narcotic state

Symptoms: A = apathy; N = Narcotic state

Validity criteria according to OECD 210 (2013)	Obtained in this study
The dissolved oxygen concentration should be >60% of the air saturation value throughout the test	> 60% (8.9 – 11.6 mg/L)
The water temperature should not differ by more than + 1.5 °C between test chambers or between successive days at any time during the test, and should be within the temperature ranges specified for the test species; <i>i.e.</i> Annex 2: for <i>P. promelas</i> : 25 ±1.5 °C	Temp. range: 10 – 12 °C Differences between test chambers / between successive days < 1.5 °C
The analytical measure of the test concentrations is compulsory	± 20 of nominal throughout the test *
Overall survival of fertilized eggs and post hatch success in the controls and, where relevant, in the solvent controls should be greater than or equal to the limits defined in Annex 2 <i>i.e.</i> for <i>P. promelas</i> : > 70% hatching success and > 75% post hatch survival	83.9% hatching success [#] and 87.8% post-hatch success ^{##} in control

The minor and short-lived deviation from the recommendations for the temperature are of no relevance for the overall outcome of the study. All other validity criteria were met. The study is valid.

Remarks: The report specifies the fertilization rate in the viability control group as only 28%. This was determined via keeping 2 x 50 individuals during the first 14 days in parallel to the test groups. This information is not reflected appropriately by the data provided in the Appendix to the report, especially not by the survival data for the viability control (Table 2 in report, page 46ff). To recheck the validity of the statement on the viability control. in the report, the raw data from the study were carefully re-examined. This revealed that detailed information from the examination of the viability control after termination on day 14 are available in the raw data but were not included properly in the report. The respective data is now provided in the amendment to the report.

The mean number of survivors over the test groups at all developmental stages (Table 1 of the report, page 45) is very similar over the test groups with a very clear treatment-related effect in the highest concentration group only indicating a steep concentration-effect relationship – as is generally the case for this test substance in fish studies - with marginal effects in group 3 (concentration maximum 10.0 µg a.s./L) and no survival at all in group 4 (concentration maximum 20.0 µg a.s./L).

The authors of the original report had considered the reduced fertilization rate and lower numbers of viable individuals and applied a conservative approach during the result evaluation. Despite inconclusive findings in test group 3 (concentration maximum 10.0 µg a.s./L) they were regarded as potentially treatment-related effects. The survival data in test group 2, however, is very similar to the control group over all developmental stages and partly higher, clearly indicating no impact at this level.

Overall, the study report had some deficiencies in correctly reflecting all information needed to assess and utilize the viability control. This information is now added by the amendment to the report following a careful re-examination of the raw data. This information demonstrates that the original author statements regarding the validity of the study was appropriate. Even with a slightly lower number of individuals than required in a standard Early Life Stage Study at the time when the study was conducted, the results of this study provide a clear NOEC for test group 2 (concentration maximum 5.0 µg a.s./L).

III. CONCLUSION

In a modified early life stage study with rainbow trout (*Oncorhynchus mykiss*) the overall NOEC (97 d) for pyraclostrobin was determined to be 0.0050 mg a.s./L based on nominal concentrations.

Due to the steep dose response curve in this study and the low number of tested concentrations, no reliable EC₁₀ and EC₂₀ value could be derived from this study. Overall, effects $\geq 10\%$ (compared to control) only occurred at test concentrations greater than the NOEC. Thus, the NOEC is the more conservative endpoint.

A 2.3.2.2 Study 2

The following fish early life-stage test on sheepshead minnow (Cyprinodon variegatus) was performed with the active substance pyraclostrobin. The study was recently submitted under the Annex I renewal process for pyraclostrobin (AIR 3) and is provided in support of the chronic assessment for fish.

<i>Comments of zRMS:</i>	Study was evaluated and accepted for the regulatory use in RR for BAS 751 00 F. In the current evaluation, the study was considered as not essential for the risk assessment.
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Reference: CP 10.2.2/3

Report Early life stage toxicity of BAS 500 F to the sheepshead minnow, *Cyprinodon variegatus*,
XXXXXXXXXX
Report No: 2126-BA
BASF DocID 2000/5247
Authority registration No

Guideline(s): EPA 72-4

Deviations: No

GLP: yes
(certified by United States Environmental Protection Agency)

Acceptability: Yes

Duplication No
(if vertebrate study)

Executive Summary

The chronic toxicity of pyraclostrobin to sheepshead minnows (*Cyprinodon variegatus*) was evaluated in a 36-day early life-stage test under flow-through conditions. Embryos were exposed to a dilution water control, a solvent control and to nominal concentrations of 0.0034, 0.0065, 0.013, 0.025 and 0.050 mg pyraclostrobin/L (corresponding to mean measured concentrations of 0.00291, 0.00557, 0.0108, 0.0240 and 0.0445 mg a.s./L). Hatchability, survival rate and behavior of sheepshead minnow embryos and fry were assessed throughout the study. Individual fish lengths and weights were measured at test termination.

The results are based on mean measured concentrations. Egg hatch was complete on day 4 in the control groups and the three lowest test item treatments. At 0.0240 mg a.s./L, egg hatch was completed on day 5. In the highest test item concentration of 0.0445 mg a.s./L, all animals were dead before hatch (day 4). Mean survival rates at hatch (day 4) and at test end (32 days post-hatch) were $\geq 95\%$ in both the dilution water control and the solvent control and in the test item concentrations of up to and including 0.0108 mg a.s./L. At 0.0240 mg a.s./L mean survival was 29% at hatch on day 4 and 55% on day 32 post-hatch. No statistically significant effects on survival, time to hatch, time to first feeding, total length, wet weight or dry weight compared to the pooled control were observed at concentrations of up to and including 0.0108 mg a.s./L.

In an early life stage study with sheepshead minnows (*Cyprinodon variegatus*) the overall NOEC (36 d) for pyraclostrobin was determined to be 0.0108 mg a.s./L based on mean measured concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Pyraclostrobin (BAS 500 F, Reg. No. 304 428), lot no. N68, purity: 93.5%, dissolved in dimethylformamide (lot no. BW660).

B. STUDY DESIGN

Test species: Sheepshead minnow (*Cyprinodon variegatus*); eggs less than 24 hours before test initiation, source: "Aquatic BioSystems, Inc.", Fort Collins Colorado, USA.

Test design: Flow-through system (36 d); 5 test item concentrations plus a dilution water control and a solvent control; 4 replicate test chambers per treatment with 20 fertilized eggs in each; a proportional diluter system was used for intermittent introduction of the solutions to the test chambers. During the embryo stage, the developing embryos were incubated in glass cups. At the end of hatch (day 4 and 5), fish were released into the test chamber and randomly thinned to 10 fish per vessel. Daily assessment of hatch, swim-up, survival, signs of toxicity and abnormal behavior. On day 36 surviving animals were sacrificed and measured for length and weight.

Endpoints: NOEC values based on hatch rate, post-hatch survival, sublethal effects, growth and time spans to hatch. Due to the steep dose response curve in this study, no reliable EC₁₀ and EC₂₀ value could be derived from this study.

Test concentrations: Control (dilution water), solvent control (0.1 mL dimethylformamide/L) and 0.0034, 0.0065, 0.013, 0.025 and 0.050 mg pyraclostrobin/L (nominal); corresponding to mean measured concentrations of 0.00291, 0.00557, 0.0108, 0.0240 and 0.0445 mg a.s./L.

Test conditions: Test vessels: 9 L glass aquaria (15 x 30 x 20 cm) with a test volume of approx. 7.0 L; 4 replicate test chambers; glass incubation cups (used during embryo stage) with 8.5 cm diameter closed on one end with Nitex® screen; two incubation cups per test chamber; dilution water: filtered natural seawater diluted with deionized water; water temperature: 29.0 °C - 30.8 °C; pH 7.5 - 8.1; oxygen content: 5.4 mg/L - 7.9 mg/L; salinity: 15 - 16 ppt; light intensity: approx. 42 foot candles; photoperiod: 16 hours light : 8 hours dark; flow rate: approx. 6.7 volume additions per 24 hours per vessel; feeding: fish were fed 2-3 times daily *ad libitum* freshly hatched *Artemia salina* nauplii from day 6 onwards until 1 day before study termination; no aeration.

Analytics: Analytical verification of test item concentrations was conducted using an HPLC-method with UV-detection.

Statistics: Descriptive statistics; t-test for comparison of the dilution water control and solvent data ($\alpha = 0.05$); ANOVA followed by Bonferroni's test, William's test or Kruskal & Wallis's test to calculate NOEC values.

C. DESCRIPTION OF THE ANALYTICAL PRODECDURES

Concentrations of BAS 500 F in tap water were determined according to the analytical method number 415 (modified). An aliquot of each sample was allowed to flow through a pre-conditioned (2 mL methanol followed by twice with 2 mL HPLC water) C¹⁸ Bond-Elut cartridge at a rate of 1 to 2 drops per second. The sample was then eluted twice with 5 mL ethyl acetate into a 15 mL centrifuge tube. The extract was evaporated to dryness. A 2 mL volume of acetonitrile was added to each tube to remove any residual ethyl acetate and the extract was again evaporated to dryness. The residue was reconstituted to a volume of 2 mL with 50:50 (v:v) acetonitrile/HPLC water and sonicated. An aliquot of the extract was transferred to its respective autosampler vial for analysis. The determination was performed by HPLC-UV.

The limit of detection (LOD) was determined to be 0.234 ng on column when analysis was performed using the HP 1050 and 1.15 ng on column when performed using the HP 1100. The LOD was determined as three times the mean peak height for all control samples collected during the definitive toxicity test converted to nanograms on column using the amount divided by the peak height of the lowest concentration calibration standard. The LOQ was established at 0.50 µg/L, determined as the lowest concentration successfully analyzed during method validation.

The analytical method was validated (MV-975) by preparing samples of BAS 500 F in dilution water at nominal concentrations of 0.0005 mg/L, 1.0 mg/L, and 5.0 mg/L. Samples were prepared in triplicate and analyzed both with and without centrifugation. The average procedural recovery for BAS 500 F was 75.6%. Details on measured fortification samples are given in the table below.

Table A 39: Fortification samples for BAS 500 F

Matrix	Fortification level (mg/L)	n	mean (%)	±SD	RSD (%)
filtered natural seawater (uncentrifuged)	0.0005	3	84.2	0.01473	3.5
filtered natural seawater (uncentrifuged)	1	3	88.4	0.03732	4.2
filtered natural seawater (uncentrifuged)	5	3	86.0	0.08	1.90
filtered natural seawater (centrifuged)	0.0005	3	72.0	0.02	6.61
filtered natural seawater (centrifuged)	1	3	78.60	0.01572	2.00
filtered natural seawater (centrifuged)	5	3	44.40	0.12490	5.63

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of the test item concentrations was conducted in all concentrations at test initiation, at regular intervals during the study and at test end, except for the highest test item concentration, where analytical measurements were only conducted on days 0 and 7. Recoveries of pyraclostrobin ranged from 75.3 to 103.1% of nominal concentrations at test initiation and from 79.4 to 84.0% of nominal at test termination. The following biological results are based on mean measured concentrations.

Biological results: No statistically significant differences were determined between the control and the solvent control data (t-test; $\alpha = 0.05$). Thus, the data from both control groups were pooled and the pooled data set was used for statistical evaluation of treatment related effects. Egg hatch was complete on day 4 in the control groups and the three lowest test item treatments. At 0.0240 mg a.s./L egg hatch was completed on day 5. In the highest test item concentration of 0.0445 mg a.s./L, all animals were dead before hatch (day 4).

Mean survival rates at hatch (day 4) and at test end (32 days post-hatch) were $\geq 95\%$ in both the dilution water control and the solvent control and in test item concentrations of up to and including 0.0108 mg a.s./L. At 0.0240 mg a.s./L, mean survival was 29% at hatch and 55% on day 32 post-hatch, respectively. No statistically significant effects on survival, time to hatch, time to first feeding, total length, wet weight or dry weight compared to the pooled control were observed at concentrations of up to and including 0.0108 mg a.s./L (Bonferroni's test / William's test / Kruskal & Wallis's test). Sublethal effects (i.e., lethargy and/or erratic swimming) were noted at 0.0108 mg a.s./L on day 4 (at hatch) and at 0.0240 mg a.s./L on days 4 and 5. These effects were not observed at any other time during the test. No other sublethal effects (other than delayed hatch) were observed at any test concentration at any time during the test. The results are summarized in the table below.

Table A 40: Chronic toxicity of pyraclostrobin to sheepshead minnow (*Cyprinodon variegatus*) in a fish early life stage test (36 d)

Concentration [mg a.s./L] (nominal)	Control	Solvent control	0.0034	0.0065	0.013	0.025	0.050
Concentration [mg a.s./L] (mean measured)	--	--	0.00291	0.00557	0.0108	0.0240	0.0445
Embryo survival at hatch on day 4 ¹⁾ [%]	100	95	98	99	99	29	0
Survival of larvae 32 days post hatch [%]	95	100	95	95	98	55	0
Percent live normal at hatch on day 4 [%]	100	95	98	99	98	9	0
Percent live normal 32 days post hatch [%]	95	100	95	95	98	55	0
Mean total length [mm]	17.7	18.1	18.5	19.0	18.6	18.7	-- ²⁾
Mean wet weight [mg]	85.0	85.5	97.5	98.6	98.3	108.7	-- ²⁾
Mean dry weight [mg]	20.4	20.8	21.8	23.8	22.3	23.2	-- ²⁾
Endpoint [mg pyraclostrobin/L] (mean measured)							
NOEC_{overall} (36 d)	0.0108						

¹⁾ at 0.0240 and 0.0445 mg a.s./L hatch was complete on day 5.

²⁾ not determined; no fish survived at the concentration above 0.0240 mg a.s./L.

Validity criteria according to OECD 210 (2013)	Obtained in this study
The dissolved oxygen concentration should be >60% of the air saturation value throughout the test	> 60% (5.4 – 7.9 mg/L)
The water temperature should not differ by more than + 1.5 °C between test chambers or between successive days at any time during the test, and should be within the temperature ranges specified for the test species; i.e. Annex 2: for <i>C. variegatus</i> : 25 ± 1.5 °C	Temp. range: 29.0 – 30.8 °C * Differences between test chambers / between successive days < 1.5 °C
The analytical measure of the test concentrations is compulsory	75.3 – 103.1% of nominal throughout the test; therefore, results are based on mean measured concentrations
Overall survival of fertilized eggs and post hatch success in the controls and, where relevant, in the solvent controls should be greater than or equal to the limits defined in Annex 2 i.e. for <i>P. promelas</i> : > 70% hatching success and > 75% post hatch survival	100% hatching success and 95% post-hatch success in control; 95% hatching success and 100% post-hatch success in solvent control

The study is considered valid.

* The temperature in this study were higher than the recommended range for this fish species in the recent OECD TG 210. However, it should be noted that the study was conducted following the US EPA Guideline (EPA 72-4). According to OTS Guidance 40 CFR § 797.1600 the recommended temperature for all life stage of sheepshead minnow is 30 °C. In addition, the ASTM (American Society for Testing and Materials) Standard Guide for Conducting Early Life-Stage Toxicity Tests with Fishes (1241–92, p. 180–207, Philadelphia, PA (1992)) recommends a test temperature of 25 - 30 °C. In any case, the individually recorded daily temperatures were always within the specified range (< 1.5 °C differences) and the temperature range was similar in all treatments during the whole study conduct. Overall, the deviations are of no relevance for the overall outcome of the study.

III. CONCLUSION

In an early life stage study with sheepshead minnows (*Cyprinodon variegatus*) the overall NOEC (36 d) for pyraclostrobin was determined to be 0.0108 mg a.s./L based on mean measured concentrations.

Due to the steep dose-response curve in this study no reliable EC₁₀ and EC₂₀ value could be derived from this study. Overall, effects ≥ 10% only occurred at test item concentrations greater than the NOEC. Thus, the NOEC is the more conservative endpoint.

A 2.3.2.3 Study 3

*The following fish early life-stage test on fathead minnow (*Pimephales promelas*) was performed with the active substance pyraclostrobin. The study was recently submitted under the Annex I renewal process for pyraclostrobin (AIR 3) and is provided in support of the chronic assessment for fish.*

<i>Comments of zRMS:</i>	Study was evaluated and accepted for the regulatory use in RR for BAS 751 00 F. In the current evaluation, the study was considered as not essential for the risk assessment.
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Reference: CP 10.2.2/4

Report Early life stage toxicity of BAS 500 F to the fathead minnow, *Pimephales promelas*,
XXXXXXXXXX
Report No: 1948-BA
BASF DocID 2000/5053
Authority registration No

Guideline(s): EPA 72-4(a), EPA 850.1400

Deviations: No

GLP: yes
(certified by United States Environmental Protection Agency)

Acceptability: Yes

Duplication No
(if vertebrate study)

Executive Summary

The chronic toxicity of pyraclostrobin to fathead minnow (*Pimephales promelas*) was evaluated in a 36-day early life-stage test under flow-through conditions. Embryos were exposed to a dilution water control, a solvent control and to nominal concentrations of 0.0014, 0.0026, 0.0049, 0.010 and 0.020 mg pyraclostrobin/L (corresponding to mean measured concentrations of 0.000944, 0.00218, 0.00414, 0.00837 and 0.0161 mg a.s./L). Hatchability, survival rate and behavior of fathead minnow embryos and fry were assessed throughout the study. Individual fish lengths and weights were measured at test termination.

The results are based on mean measured concentrations. Egg hatch was complete on day 4 in the control groups and all test item treatments. Mean survival rates at hatch (day 4) and at test end (32 days post-hatch) were $\geq 90\%$ in both the dilution water control, the solvent control and at test item concentrations up to and including 0.00837 mg a.s./L, whereas all fish had died before hatch (day 4) at the highest tested concentration of 0.0161 mg a.s./L. No statistically significant effects on survival, time to hatch, time to first feeding, total length, wet weight or dry weight compared to the pooled control were observed at concentrations of up to and including 0.00414 mg a.s./L. A slight sublethal effect on live, normal fathead minnow at 0.00414 mg/L was apparent on only 4% of the fish on one day (day 4), and although statistically significant, the effect was not considered to be of biological relevance.

In an early life-stage study with fathead minnow (*Pimephales promelas*) the overall NOEC (36 d) for pyraclostrobin was determined to be 0.00414 mg a.s./L based on mean measured concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Pyraclostrobin (BAS 500 F, Reg. No. 304 428), lot no. 27882/191/C, purity: 97.09%, dissolved in dimethylformamide (lot no. BV921).

B. STUDY DESIGN

Test species: Fathead minnow (*Pimephales promelas*), eggs less than 24 hours at test initiation, source: "Aquatic BioSystems, Inc.", Fort Collins, Colorado, USA.

Test design: Flow-through system (36 d); 5 test item concentrations plus a dilution water control and a solvent control; 4 replicate test chambers per treatment with 20 fertilized eggs in each; a proportional diluter system was used for intermittent introduction of the solutions to the test chambers. During the embryo stage, the developing embryos were incubated in glass cups. At the end of hatch (day 4), fish were released into the test chamber and randomly thinned to 10 fish per vessel. Daily assessment of hatch, survival, signs of toxicity and abnormal behavior. At test termination surviving animals were sacrificed and measured for length and weight.

Endpoints: NOEC values based on hatchability, survival, toxic signs and growth rates. Due to the steep dose response curve in this study, no reliable EC₁₀ and EC₂₀ value could be derived from this study.

Test concentrations: Control (dilution water), solvent control (0.1 mL dimethylformamide/L), 0.0014, 0.0026, 0.0049, 0.010 and 0.020 mg a.s./L (nominal), corresponding to mean measured concentrations of 0.000944, 0.00218, 0.00414, 0.00837 and 0.0161 mg a.s./L.

Test conditions: Test vessels: 9 L glass aquaria (approx. 15 x 30 x 20 cm) with a test volume of approx. 8.0 L; 4 replicate test chambers; glass incubation cups (used during embryo stage) closed on one end with Nitex[®] screen; two incubation cups per test chamber; dilution water: filtered deionized water sterilized with UV and aerated; temperature: 23.0 °C - 26.4 °C; pH 7.4 - 7.8; oxygen content: 8.0 mg/L - 9.4 mg/L; total hardness: 40 - 44 mg CaCO₃/L; conductivity: 130 - 180 µmhos/cm; light intensity: approx. 45 foot candles; photoperiod: 16 hours light : 8 hours dark; flow rate: approx. 6.6 volume additions per 24 hours per vessel; feeding: fish were fed 2-3 times daily *ad libitum* freshly hatched *Artemia salina* nauplii from day 5 onwards until 1 day before study termination; no aeration.

Analytics: Analytical verification of test item concentrations was conducted using an HPLC-method with UV-detection.

Statistics: Descriptive statistics; t-test for comparison of the dilution water control and solvent control data; ANOVA followed by Bonferroni's test or William's test to calculate NOEC values.

C. DESCRIPTION OF THE ANALYTICAL PRODECDURES

Concentrations of BAS 500 F in filtered deionized water were determined according to the analytical method 415 (modified). An aliquot of each sample was allowed to flow through a pre-conditioned (2 mL methanol followed by twice with 2 mL HPLC water) C¹⁸ Bond-Elut cartridge at a rate of 1 to 2 drops per second. The sample was then eluted twice with 5 mL ethyl acetate into a 15 mL centrifuge tube. The extract was evaporated to dryness. A 2 mL volume of acetonitrile was added to each tube to remove any residual ethyl acetate and the extract was again evaporated to dryness. The residue was reconstituted to a volume of 2 mL with 50:50 (v:v) acetonitrile/HPLC water and sonicated. An aliquot of the extract was transferred to its respective autosampler vial for analysis. The determination was performed by HPLC-UV.

The limit of detection (LOD) was determined to be 0.0476 ng on column, determined as three times the mean area count for all control samples collected during the definitive toxicity test converted to nanograms on column using the amount divided by the area of the lowest concentration calibration standard, The LOQ was established at 0.50 µg/L determined as the lowest concentration successfully analyzed during method validation.

The analytical method was validated (MV-975) by preparing samples of BAS 500 F in dilution water at nominal concentrations of 0.0005 mg/L, 1.0 mg/L, and 5.0 mg/L. Samples were prepared in triplicate and were analyzed both with and without centrifugation. The average procedural recovery for BAS 500 F was 75.6%. Details on measured fortification samples are given in the table below.

Table A 41: Fortification samples for BAS 500 F

Matrix	Fortification level (mg/L)	n	mean (%)	±SD	RSD (%)
filtered natural seawater (uncentrifuged)	0.0005	3	84.2	0.01473	3.50
filtered natural seawater (uncentrifuged)	1	3	88.4	0.03732	4.22
filtered natural seawater (uncentrifuged)	5	3	86.0	0.08185	1.90
filtered natural seawater (centrifuged)	0.5	3	72.0	0.02381	6.61
filtered natural seawater (centrifuged)	1	3	78.6	0.01572	2.00
filtered natural seawater (centrifuged)	5	3	44.4	0.12490	5.63

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of the test item concentrations was conducted in all concentrations at test initiation, at regular intervals during the study and test end, except for the highest test item concentration, where analytical measurements were only conducted on days 0 and 7. Recoveries of pyraclostrobin ranged from 74.5 to 90.0% of nominal concentrations in all treatments at test initiation and from 80.9 to 88.5% of nominal in the 0.0026, 0.0049 and 0.010 mg/L (nominal) treatments at test termination. Measured contents in samples from the lowest test item treatment were below the limit of quantification (LOQ = 0.50 µg/L) on days 35 and 36; this is believed to be due to an unobserved diluter malfunction on test days 35 and 36. All analysis at this concentration prior to that time resulted in recoveries ranging from 75 to 84% of nominal. The following biological results are based on mean measured concentrations.

Biological results: No statistically significant differences were determined between the control and the solvent control data (t-test; $\alpha = 0.05$). Thus, the data from both control groups were pooled and the pooled data set was used for statistical evaluation of treatment related effects. Egg hatch was complete on day 4 in the control and all test item treatments. Mean survival rates at hatch (day 4) and at test end (32 days post-hatch) were $\geq 90\%$ in both the dilution water control and solvent control and at test item concentrations up to and including 0.00837 mg a.s./L, whereas all fish had died before hatch (day 4) in the highest tested concentration of 0.0161 mg a.s./L. No statistically significant effects on survival, time to hatch, time to first feeding, total length, wet weight or dry weight compared to the pooled control were observed at concentrations of up to and including 0.00414 mg a.s./L (Bonferroni's test / William's test). Sublethal effects, observed as fish exhibiting lethargy and/or a loss of equilibrium or change in coloration, were noted at 0.0161 mg a.s./L on day 3 (complete mortality occurred on day 4 at this concentration), at 0.00837 mg a.s./L on days 4 - 6, 8 - 12, and on day 34, and at 0.00414 mg a.s./L on day 4. These effects were not observed at any other time during the test. As the effect on live, normal fathead minnow at 0.00414 mg/L was apparent on only 4% of the fish on one day (day 4), and although statistically significant, the effect was not considered to be of biological relevance. The results are summarized in the table below.

Table A 42: Chronic toxicity of pyraclostrobin to fathead minnow (*Pimephales promelas*) in a fish early life-stage test (36 d)

Concentration [mg a.s./L] (nominal)	Control	Solvent control	0.0014	0.0026	0.0049	0.010	0.020
Concentration [mg a.s./L] (mean measured)	--	--	0.000944	0.00218	0.00414	0.00837	0.0161
Embryo survival at hatch on day 4 [%]	98	99	99	99	94	90	0
Survival of larvae 32 days post hatch [%]	100	98	95	95	100	90	0
Percent live normal at hatch on day 4 [%]	98	99	99	99	90 [#]	80	0
Percent live normal 32 days post hatch [%]	100	98	95	95	100	90	0
Mean total length [mm]	23.3	23.0	23.3	22.8	23.0	22.3	n.d.
Mean wet weight [mg]	110.5	108.8	108.5	106.9	107.0	96.8	n.d.
Mean dry weight [mg]	23.6	23.0	23.2	22.7	23.3	22.4	n.d.
	Endpoints [mg pyraclostrobin/L] (mean measured)						
NOEC_{overall} (36 d)	0.00414						

n.d. = not determined; no fish survived at the concentration above 0.00837 mg a.s./L.

[#] Because the effect on live, normal fathead minnow at 0.00414 mg a.s./L was apparent on only 4% of the fish on one day (day 4), the effect was not considered to be of biological significance.

Validity criteria according to OECD TG 210 (2013)	Obtained in this study
The dissolved oxygen concentration should be >60% of the air saturation value throughout the test.	> 60% (8.0 – 9.4 mg/L)
The water temperature should not differ by more than ± 1.5 °C between test chambers or between successive days at any time during the test and should be within the temperature ranges specified for the test species (for fathead minnow: 25 ± 1.5 °C).	Temp. range: 23.0 – 26.4 °C Differences between test chambers / between successive days > 1.5 °C *
The analytical measure of the test concentrations is compulsory. When the measured concentrations do not remain within 80-120% of the nominal concentration, the effect concentrations should be determined and expressed relative to the arithmetic mean concentration for flow-through tests.	75 – 84% of nominal throughout the test; therefore, results are based on mean measured concentrations
Overall survival of fertilized eggs and post-hatch success in the controls and, where relevant, in the solvent controls should be greater than or equal to the limits defined in Annex 2 (for fathead minnow: 70% hatching success, 75% post-hatch success).	98% hatching success and 100% post-hatch success in control; 98% hatching success and 98% post-hatch success in solvent control

The study is considered valid.

* The temperatures measured during the toxicity test were slightly below the recommended minimum temperature of 23.5 °C (in the controls during week 2) and differences between test chambers or between successive days were > 1.5 °C on some occasions. However, the deviations were only minor and were observed in all treatments during the whole study conduct. Besides, the US EPA guideline OPPTS 850.1400 states a temperature range of for fathead minnow of 25 ± 2 °C. Overall, these slight temperature deviations are of no relevance for the overall outcome of the study.

III. CONCLUSION

In an early life-stage study with fathead minnow (*Pimephales promelas*) the overall NOEC (36 d) for pyraclostrobin was determined to be 0.00414 mg a.s./L based on mean-measured concentrations.

Due to the steep concentration-response curve in this study no reliable EC₁₀ and EC₂₀ value could be derived from this study. Overall, effects $\geq 10\%$ only occurred at test item concentrations greater than the NOEC. Thus, the NOEC is the more conservative endpoint.

A 2.3.2.4 Study 4

The following fish early life-stage test on fathead minnow (*Pimephales promelas*) performed with BAS 560 F is provided in support of the assessment and was recently submitted under the Annex I renewal process for metrafenone (AIR 3). The study summary was provided in the revised Renewal Assessment Report of metrafenone (RAR, Vol. 3, B.9, December 2019).

<i>Comments of zRMS:</i>	The study was conducted to OECD guidance 210 and according to the principles of GLP. All validity criteria were met. The study is considered to be reliable and suitable for the risk assessment.
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Reference: CP 10.2.2/5

Report BAS 560 F (Metrafenone) - Early life-stage toxicity test on the fathead minnow (*Pimephales promelas*) in a flow through system,
XXXXXXXXXX
report No 404710
BASF DocID 2012/1009601
Authority registration No

Guideline(s): EPA 540/9-86-138, EPA 72-4 (a), EPA 850.1400, OECD 210

Deviations: No

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

Acceptability: Yes

Duplication No
(if vertebrate study)

<i>Reference:</i>	<i>CP 10.2.2/6</i>
<i>Report</i>	<i>Concentration control analysis of BAS 560 F (Metrafenone) in mixing-water, GV/T project-no. 50F0437/01E002,</i> <i>Obermann, M., 2012</i> <i>report No 404710, 50F0437/01E002</i> <i>BASF DocID 2012/1016030</i> <i>Authority registration No</i>
<i>Guideline(s):</i>	<i>none</i>
<i>Deviations:</i>	<i>No</i>
<i>GLP:</i>	<i>yes</i> <i>(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)</i>
<i>Acceptability:</i>	<i>Yes</i>
<i>Duplication</i> <i>(if vertebrate study)</i>	<i>No</i>

Executive Summary

The chronic toxicity of metrafenone to fathead minnow (*Pimephales promelas*) was evaluated in a 33-day early life-stage (ELS) test under flow-through conditions. Embryos were exposed to 16, 25, 40, 63 and 100 % of a saturated solution of the active ingredient as nominal concentrations (corresponding to mean measured concentrations of 0.095, 0.150, 0.204, 0.364 and 0.535 mg a.s./L). A dilution water control was tested in parallel. Hatchability, pre- and post-hatch survival rate, time to hatch and swim-up, signs of toxicity and growth parameters of fathead minnow embryos were assessed throughout the study.

The measured concentrations in the analyzed samples were within the range of $\pm 20\%$ of the overall mean measured concentration, except for samples in the two highest test groups with deviations of 77% and 79% of the mean measured concentration on the last day of the exposure. The biological results were based on mean measured concentrations.

No test substance-related effect was observed on the time to start or end of hatching and the time to swim-up. The survival from hatch to the end of swim-up and from the end of swim-up to the end of exposure (day 6 – 33) as well as the overall survival (day 0 - 33) was not statistically significantly reduced in the treatment group in comparison to the control group. There were no observable test substance related signs of toxicity or abnormalities (sublethal effects) in any of the tested concentration groups. In comparison to the control group, the mean wet weights and the total body lengths of the surviving fish at the end of the exposure period were statistically significantly reduced in the two highest test concentrations of 0.364 and 0.535 mg a.s./L.

In a 33-day early life stage study with fathead minnow (*Pimephales promelas*), the overall no-observed-effect concentration (NOEC) for metrafenone was determined to be 0.204 mg a.s./L and the lowest-observable-effect concentration (LOEC) was 0.364 mg a.s./L, based on mean measured concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test material:** Metrafenone (BAS 560; Reg. No. 4 037 710)
Batch number: AC12053-29
Purity: 94.2%
Description: Beige solid
2. **Test concentrations:** 0 (control), 16%, 25%, 40%, 63% and 100% of a saturated solution of the a.s. (nominal), (mean measured: 0, 0.095, 0.150, 0.204, 0.364 and 0.535 mg a.s./L)
3. **Reference item:** None
4. **Dilution water:** Non-chlorinated, filtered drinking water (diluted with deionized water)
Vehicle: Dilution water
5. **Test organism:**
Species: Fathead minnow (*Pimephales promelas*)
Age: Embryos (fertilized, less than four hours old)
Source: Parents were obtained from Osage Catfisheries Inc., USA
Diet: Live brine shrimp nauplii (*Artemia* sp.) and fine milled commercial fish diet ("Tetramin") from day 6 on, twice daily
Test vessels: Cylindrical glass vessels, water volume: 1.7 L (until day 15); stainless steel aquaria (29 x 21 x 22 cm), water volume: 9.0 L (from day 15 until test termination)

B. STUDY DESIGN

1. **Environmental conditions:**
Temperature: 24.2 °C – 25.6 °C
pH: 7.9 – 8.2
Dissolved oxygen: 5.7 – 8.4 mg/L
Hardness: 1.02 – 1.05 mmol CaCO₃/L
Photoperiod: 16 h light: 8 h darkness (80 – 196 lux)
Aeration: Slight aeration via glass tubes

2. Animal assignment and treatment

Fertilized eggs (embryos) and larvae were exposed in cylindrical glass vessels and were transferred into stainless steel aquaria on day fifteen. The test solution flowed continuously from the mixing tank into an "udder", which divided the test solution into four equal parts for the four replicate test aquaria (containing 25 embryos each). Flow rates were 150 mL/minute/treatment group and 2.25 L/hour/test vessel (providing a 6-fold exchange rate of the water volume in each larger test vessel (9 L) every 24 hours). The fish were exposed to test concentrations of 16%, 25%, 40%, 63% and 100% of a saturated nominal solution of the active substance, corresponding to 0.095, 0.150, 0.204, 0.364 and 0.535 mg a.s./L (mean measured). A control group (dilution water only) was tested in parallel. The fish were exposed for 33 days under flow-through conditions.

3. Dose preparation:

As the test substance is poorly soluble in water, a saturated solution of the test substance was prepared using a saturation column. The saturation column was prepared by dissolving 10 g of test substance in 200 mL acetone. This solution was then poured over glass wool and the solvent acetone was completely evaporated, leaving the test substance adhered to the glass wool. The treated glass wool was placed in a glass column. The outflow of the column was collected in a stock solution tank. A metering pump delivered the stock solution to each mixing tank, where it was continuously diluted with aerated dilution water to generate the nominal test concentration for each test material concentration group. From there the solution was distributed equally among the four test vessels per treatment group. A similarly prepared column, without test substance, was used for the control group.

4. Measurements and observations

Throughout the exposure period, hatching, swim-up, mortality, signs of toxicity and abnormal behavior were assessed daily. After 33 days, the fish were sacrificed and the body length and weight of surviving individuals were determined.

On days 0, 8, 15, 22, 29 and 33 (test termination) analytical verification of the test item BAS 560 F in Mixing-Water was conducted by reversed-phase HPLC with MS-detection and external calibration according to method APL0500/03. The study Palmer et al (2005b) provides a method description and validation details for the determination of BAS 560 F in aqueous solutions. Refer to Document M-CA, Section 4, CA 4.1.2/15 for a detailed summary of the method. The test item and reference item, were identified as BAS 560 F, Batch No.: AC12053-29 and had a purity of 94.2%. In order to get information on potential unsolved particles of the test item in the test samples, centrifuged as well as not centrifuged samples were measured.

Temperature was recorded daily. Dissolved oxygen and pH were measured every three days.

5. Statistics

Dunnett's test (two-sided) was carried out for statistical evaluation of the weight and length data. For the survival data, Fisher's exact test (one-sided) was used to compare the exposed groups to the control group. Wilcoxon-test (one-sided) was performed to examine variability between replicates.

II. RESULTS AND DISCUSSION

A. BIOLOGICAL EFFECTS

No test substance-related effect was observed on the time to start or end of hatching and the time to swim-up. The survival from hatch to the end of swim-up and from the end of swim-up to the end of exposure (day 6 – 33) as well as the overall survival (day 0 - 33) was not statistically significantly decreased in the treatment group in comparison to the control group. There were no observable test substance related signs of toxicity or abnormalities (sublethal effects) in any of the tested concentration groups. In comparison to the control group the mean wet weights and the total body lengths of the surviving fish at the end of the exposure period were statistically significantly decreased in the two highest test concentrations of 0.364 and 0.535 mg a.s./L. The results are summarized in the table below.

Table A 43: Chronic toxicity of metrafenone to fathead minnow (*Pimephales promelas*) in a fish early life stage test (33 d)

Concentration (mean measured) (mg a.s./L)	Control	0.095	0.150	0.204	0.364	0.535
Hatching success (%)	96	96	94	93	95	92
Survival of larvae from hatch until end of swim-up (day 6) (%)	97	98	99	100	96	99
Post-hatch survival of young fish (day 6 to 33) (%)	94	94	97	91	96 ^a	94
Survival from day 0 to test termination (day 33) (%)	87	88	90	85	87	85
Start of hatch (day)	3	3	3	3	3	3
End of hatch (day)	5	5	5	5	5	5
Start of swim-up	5	5	5	5	5	5
End of swim-up	6	6	6	6	6	6
Symptoms	none	none	none ^b	none	none	none
Mean weight (33 d) (mg)	197	201	211	202	182	168 **
% of control	100	102	107	103	93	85
Mean length (33 d) (cm)	2.8	2.8	2.8	2.7	2.6 **	2.5 **
% of control	100	100	101	99	95	92
	Endpoints (mg a.s./L) (mean measured)					
NOEC _{overall} (33 d)	0.204					

^a One fish killed by handling was subtracted from the number of swim-up larvae at risk.

^b One fish with a vertebral deformation was seen; however, this cannot be related to the test substance as no abnormalities were observed in higher concentrations.

** Statistically significantly different compared to the control (Dunnett's test; $p \leq 0.01$)

B. ANALYSIS

The individually measured concentrations in uncentrifuged samples were within the range of 80 to 120% of the overall mean measured concentration, as shown in the table below.

Samples were also analyzed after centrifugation (17700 G, 20 minutes), to confirm the absence of undissolved test substance in the test solution. The mean measured concentrations of centrifuged samples were 4.3 to 9.7% lower than the corresponding mean measured concentrations of uncentrifuged samples. The minimal loss after centrifugation and the high degree of consistency of measured concentrations among test vessel replicates over the exposure period support the conclusion that no undissolved material was present in the test solution.

The biological results were based on mean measured concentrations of the uncentrifuged samples.

Table A 44: Measured concentrations of BAS 560 F in test samples

Nominal concentration (%)	Sampling time (days)	Measured concentration (mg a.s./L)	Mean measured concentration (mg a.s./L)	Mean measured as % of mean measured stock solution	Measured concentration as % of nominal concentration
Stock solution	0	0.627	0.577	-	-
	8	0.541			
	15	0.627			
	22	0.512			
	29	0.642			
	33	0.511			
0 (negative control)	0	< LOD	-	-	-
	8				
	15				
	22				
	29				
	33				
16	0	0.112	0.095	16.4	102.5
	8	0.092			
	15	0.104			
	22	0.125			
	29	0.154			
	33	0.136			
25	0	0.180	0.150	26.0	104
	8	0.139			
	15	0.166			
	22	0.125			
	29	0.154			
	33	0.136			
40	0	0.241	0.204	35.3	88.25
	8	0.202			
	15	0.232			
	22	0.165			
	29	0.196			
	33	0.185			
63	0	0.423	0.364	63.2	100.3
	8	0.341			
	15	0.414			
	22	0.336			
	29	0.391			
	33	0.281			
100	0	0.598	0.535	92.7	92.7
	8	0.541			
	15	0.637			
	22	0.500			
	29	0.511			
	33	0.423			
Stock solution	0	0.627	0.577	-	-
	8	0.541			
	15	0.627			
	22	0.512			
	29	0.642			
	33	0.511			

LOD = Limit of detection (0.001 mg/L)

C. DEFICIENCIES

None.

III. CONCLUSION

In an early life stage study, the overall no-observed-effect concentration (NOEC) for fathead minnow (*Pimephales promelas*) exposed to metrafenone under flow-through conditions was determined to be 0.204 mg a.s./L, based on mean measured concentrations. The lowest-observable-effect concentration (LOEC) was 0.364 mg a.s./L (mean measured). The EC₁₀ value is not derived from the study. Besides control, 5 concentrations were tested. The NOEC is based on a statistically significant decrease in mean length compared to control in the two highest test groups. However, effects were < 10%. Although statistically significant effects on mean weight were observed in the highest test group and effects were 15%, the calculation of an EC₁₀ value is not considered applicable, as it would be based on a single concentration showing an effect.

A 2.3.2.5 Study 5

The following chronic toxicity study on *Americamysis bahia* performed with BAS 560 F is provided in support of the assessment and was recently submitted under the Annex I renewal process for metrafenone (AIR 3). The study summary was provided in the revised Renewal Assessment Report of metrafenone (RAR, Vol. 3, B.9, December 2019).

Comments of zRMS:	The study was conducted to guidance and according to the principles of GLP. All validity criteria were met. The study is considered to be reliable and suitable for the risk assessment.
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Reference: CP 10.2.2/7

Report BAS 560 F - Life-cycle toxicity test with mysids (*Americamysis bahia*),
Cafarella, M., 2007
report No SubNo-200710-15-01,US-986.6171,US-136370
BASF DocID 2007/7009454
Authority registration No

Guideline(s): EPA 850.1350, FIFRA 72-4

Deviations: No

GLP: yes
(certified by United States Environmental Protection Agency)

Acceptability: Yes

Duplication No
(if vertebrate study)

Executive Summary

In a life-cycle toxicity study mysids (*Americamysis bahia*) were exposed to BAS 560 F (metrafenone). The test was conducted under flow-through conditions over a period of 28 days with the following nominal test item concentrations: 3.1, 6.3, 13, 25, and 50 µg a.s./L. A control and solvent control (14 µL/L triethylene glycol) were tested in parallel. Initially 60 mysids (30 per replicate test vessel) were exposed to each test concentration and the controls. After reaching sexual maturity (day 14) mysids were redistributed within the test aquaria and mature male/female pairs were transferred to one of ten pairing chambers (one pair per chamber).

Analysis of the test solutions demonstrated that the expected concentration-gradient was maintained during the 28-day exposure. Mean measured concentrations ranged from 88% to 99% of nominal and defined the treatment levels tested as 2.9, 6.2, 12, 22 and 45 µg a.s./L.

Statistical analysis determined no significant difference in survival (male, female and combined) in any treatment levels tested as compared to the control (100%). A statistically significant difference in number of offspring per female and offspring per female per reproductive day was demonstrated for organisms exposed to the 45 µg a.s./L (mean measured) when compared to the control. Statistical analysis determined no significant difference in body length and weight (for both male and female mysids) in any treatment levels tested as compared to the control.

Based on statistical analysis of mysid reproduction the lowest-observable-effect concentration (LOEC) was determined to be 45 µg a.s./L and the no-observed-effect concentration (NOEC) was 22 µg a.s./L, based on mean measured concentrations. Therefore, the geometric mean Maximum-Acceptable-Toxicant (MATC) was estimated to be 31 µg a.s./L (mean measured). Since no concentration tested resulted in $\geq 50\%$ reduction in survival, the 28-d LC₅₀ value was empirically estimated to be > 45 µg a.s./L (mean measured), the highest concentration tested.

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test material:** BAS 560 F (Metrafenone; Reg.No. 4037710)
Batch number: AC12053-29
Purity: 94.2%
Description: Not reported
2. **Test concentrations:** 0 (negative and solvent control), 0.0031, 0.0063, 0.013, 0.025, 0.050 mg a.s./L (mean measured: 0.0029, 0.0062, 0.012, 0.022 and 0.045 mg a.s./L)
3. **Reference item:** None
4. **Dilution water:** Artificial seawater (salinity $20 \pm 3\text{‰}$)
Vehicle: Triethylene glycol
5. **Test organism:**
Species: *Americamysis bahia*
Age: ≤ 23 hours old
Source: In-house culture. Brood stock was originally obtained from Aquatic BioSystems, Inc., Fort Collins, Colorado.
Diet: During the test, mysids were fed twice daily with brine shrimp (*Artemia salina*) nauplii ≤ 24 hours old. Prior to pairing at least one of the two feedings was with brine shrimp enriched with Selco®, a supplemental substance high in saturated fatty acids. During the period subsequent to pairing the Selco® enriched brine shrimp was fed to the test organisms every other day.
Test vessels: Glass test aquaria each measured 39 x 20 x 25 cm equipped with a glass self-starting siphon drain which allowed the solution volume within the aquarium to fluctuate between approximately 3.9 and 7.0 L. Each exposure aquarium contained two non-paired mysid retention chambers (glass petri dishes, 10 cm in diameter, 2 cm deep). Pairing chambers were 6-cm diameter petri dishes. Solution volume fluctuated from 390 to 710 mL and 100 to 180 mL in the non-paired mysid and pairing retention chambers, respectively.

B. STUDY DESIGN

1. Environmental conditions:

Temperature:	25 – 27 °C
Salinity:	18 – 21‰
pH:	8.1 – 8.3
Dissolved oxygen:	5.5 – 7.3 mg/L
Photoperiod:	16 hours light: 8 hours darkness (550 - 1100 lux)

2. Animal assignment and treatment:

Mysids (≤ 23 hours old) were distributed among 28 beakers (15 mysids in each) containing culture water. Each group of 15 mysids was transferred to one of the 28 labeled retention chambers. The test was initiated when the retention chambers were placed in their respective test aquaria. Each aquarium contained two retention chambers yielding 30 mysids per replicate vessel and 60 organisms for each treatment and the controls. When the mysids reached sexual maturity (day 14) they were redistributed within the test aquaria. Mature male/female pairs within each exposure aquarium were transferred from the retention chambers to one of ten pairing chambers (one pair per chamber). The remaining mysids (after isolation of male-female pairs) were pooled and placed in one of the initial retention chambers within each aquarium where they were maintained for the duration of the chronic test. Male mysids from this pool were used to replace dead males from the paired (male/female) groups. Females which died in the pairing jars were not replaced.

3. Dose preparation:

To obtain the test concentrations, a 3.5 mg a.s./mL stock solution was prepared by adding 0.1861 g (taking the 94.2% purity into account) of BAS 560 F to 50 mL triethylene glycol. Appropriate amounts of the stock solution and dilution water were delivered in cycles into a diluter's chemical mixing chamber. The solution contained in the mixing chamber constituted the highest nominal test concentration (50 μg a.s./L) and was subsequently diluted (50%) to provide the remaining nominal exposure concentrations. During each cycle of the diluter system, approximately 500 mL of exposure solution was delivered to each replicate test vessel. During the study, the diluter provided the exposure solutions to each test vessel at a rate of approximately 15 aquarium volume additions per day. In a similar way, the solvent was mixed and delivered to the test vessels and the solvent concentration in the solvent control and each treatment level was 14 $\mu\text{L/L}$.

4. Measurements and observations:

After males and females had been paired (day 14), the number of dead males and females, the number of offspring produced by each individual female and any abnormal appearance or behavior was recorded daily throughout the study. At test termination, the individual body length to the nearest 0.1 mm and the total dry body weight to the nearest 0.01 mg of all mysids were determined and recorded separately for each replicate of each concentration and the controls. Reproduction was calculated for each replicate aquarium as the total number of offspring produced to the total number of females contained within each chamber. In addition, the number of reproductive females in each replicate of each treatment and the control was determined.

Water samples were taken from alternate replicate test solutions of each treatment level and the control on test days 0, 8, 14, 22 and 28 for analysis. All exposure solutions and QC samples were analyzed for BAS 560 F using high performance liquid chromatography with ultraviolet detection (HPLC/UV) based on validated methodology.

Temperature, dissolved oxygen concentration, pH and salinity were measured daily.

5. Statistics:

All statistical analyses were performed against the dilution water (negative) control data. Student's t-Test established no statistical differences between negative control and solvent control. Significant differences in the percent survival were determined following arcsine transformation. Shapiro-Wilk's Test and Bartlett's Test were used for normality and homogeneity of data, respectively. Williams' Test was used to determine treatment level effects. TOXSTAT® Version 3.5 was used to perform the statistical computations.

During this study, no concentration tested caused a reduction of 50% survival, therefore, the LC₅₀ values were empirically estimated to be greater than the highest mean measured concentration tested and no statistical analyses were performed.

II. RESULTS AND DISCUSSION

A. BIOLOGICAL EFFECTS

At termination of the test, the mysids in the control and solvent control met the performance criteria of the OPPTS 850.1350 guideline (> 70% survival of F₀ mysids between pairing and test termination, > 75% of the females in the control released young and the controls produced > 3 offspring per female).

The biological results of the study i.e. male, female and combined mysid survival, a summary of first generation (F₀) reproductive success data and measurements of growth, as average total body length and average dry body weight, for all surviving adult mysids (F₀) at test termination are summarized in the table below.

Table A 45: Summary of effects on adult survival, reproduction and growth of *Americamysis bahia* exposed to BAS 560 F

Mean measured concentration (µg/L BAS 560 F)	Mean % survival			% of females producing young	Mean number of offspring per female	Mean number of offspring per female per reproductive day	Mean body length of mysids (mm)		Mean dry weight of mysids (mg)	
	♂	♀	♂/♀				♂	♀	♂	♀
Control	100	100	100	100	5.8	0.44	7.4	7.6	0.87	1.06
Solvent control	95	96	96	100	4.9	0.36	7.8	7.8	0.90	0.99
2.9	84	83	85	89	5.8	0.43	8.0	8.2	0.90	1.14
6.2	100	100	100	95	6.5	0.47	7.4	7.4	0.90	1.05
12	96	100	98	85	3.1	0.22	7.3	7.2	0.85	1.00
22	96	89	93	85	3.4	0.27	8.1	7.9	0.90	1.06
45	95	93	95	25	1.4*	0.10*	7.5	7.5	0.90	1.05

*Statistically significant as compared to the negative (solvent-free) control based on William's test

B. ANALYSIS

The diluter system which prepared and delivered the test solutions to the exposure aquaria functioned properly throughout the 28-day study. Analysis of the stock used to prepare test solutions during the definitive test resulted in recoveries ranging from 94 to 120% of nominal concentration (3.5 mg a.s./mL) from artificial seawater. These analytical results and records of toxicant pump stock usage indicate that the appropriate amount of BAS 560 F was delivered to the exposure system during this study. The results of the analysis of the exposure solutions for BAS 560 F concentration during the in-life portion of the definitive test are presented in the following table. Analysis of the test solutions demonstrated that the expected concentration-gradient was generally maintained during the 28-day exposure.

Table A 46: Measured concentrations of BAS 560 F in the exposure solutions

Nominal concentration (µg a.s./L)	Measured concentration (µg a.s./L) ^a						% Nominal ^b
	Day 0	Day 8	Day 14	Day 22	Day 28	Mean (SD) ^b	
Control	< 1.1	< 1.4	< 0.95	< 1.3	< 1.2	n.a.	n.a.
Solvent control	< 1.1	< 1.4	< 0.95	< 1.3	< 1.2	n.a.	n.a.
3.1	2.7	2.6	2.9	2.7	3.4	2.9 (0.33)	92
6.3	6.0	6.1	5.9	6.2	6.9	6.2 (0.42)	99
13	11	11	12	12	13	12 (0.94)	91
25	22	21	21	22	24	22 (1.1)	88
50	44	45	43	43	50	45 (2.9)	90

^aSamples were alternated between replicate A and B. Day 0, 14 and 28 samples were taken from replicate A/ Day 8 and 22 samples were taken from replicate B.

^b Mean measured values, standard deviations and % of nominal were calculated using the actual analytical results and not the rounded values (two significant figures) presented in this table.

SD = standard deviation

n.a. = not applicable

C. DEFICIENCIES

None.

III. CONCLUSION

The Lowest-Observed-Effect Concentration (LOEC) and the No-Observed-Effect Concentration (NOEC) for mysids (*Americamysis bahia*) exposed to metrafenone under flow-through conditions were determined to be 45 µg a.s./L and 22 µg a.s./L (mean measured), respectively, based on reproduction. Therefore, the geometric mean Maximum-Acceptable-Toxicant (MATC) was estimated to be 31 µg a.s./L (mean measured) and the 28-day LC₅₀ value was determined to be > 45 µg a.s./L (mean measured), the highest concentration tested. NOEC at second highest test concentration of 5 concentrations. The EC_{10/20} calculation not deemed feasible as only 1 concentration above NOEC.

A 2.3.2.6 Study 6

A spiked water toxicity study with *Chironomus riparius* was already evaluated during the previous Annex I inclusion process. The following additional spiked sediment toxicity study with *C. riparius* was also performed with the active substance pyraclostrobin. The study was recently submitted under the Annex I renewal process for pyraclostrobin (AIR 3) and is provided in support of the assessment covering the exposure *via* sediment.

Comments of zRMS:	Study was evaluated and accepted for the regulatory use in RR for BAS 751 00 F.
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Reference:	CP 10.2.2/8
Report	Effects of BAS 500 F (Pyraclostrobin) on the development of sediment dwelling larvae of <i>Chironomus riparius</i> in a sediment-water system - Exposed via spiked sediment, Kuhl, R., Wydra V., 2013 report No 407435 BASF DocID 2012/1185699 Authority registration No
Guideline(s):	OECD 218 (2004)
Deviations:	No
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive Summary

In a 28-day static spiked sediment study, non-biting midge larvae (*Chironomus riparius*) were exposed to pyraclostrobin at nominal concentrations of 0.3, 0.6, 1.2, 2.4 and 4.8 mg a.s./kg dry sediment. Additionally, a solvent (acetone) control and a water control were set up. All test item concentrations and the water control had 4 replicates, whereas 6 replicates were tested for the solvent control. 20 larvae were added to each test vessel.

The biological results are based on nominal concentrations of the test item. Additionally, biological endpoints based on mean measured values are given. In the control and the solvent control mean emergence rates of 78.8 and 71.7% and mean development rates of 0.057 and 0.055 were observed, respectively. In the test item concentrations of up to and including 4.8 mg a.s./kg dry sediment, between 60 and 76% of the test animals emerged until day 28. Statistically significant differences compared to the pooled control were found for the emergence rates at the highest test item concentration. No statistically significant effect on the development was observed in any treatment group.

In a 28-day static sediment test with *Chironomus riparius* the NOEC values of pyraclostrobin were determined to be 2.4 mg a.s./kg dry sediment (nominal; equivalent to the mean measured concentration of 1.37 mg a.s./kg dry sediment) based on emergence rate and ≥ 4.8 mg a.s./kg dry sediment (nominal; equivalent to the mean measured concentration of ≥ 2.83 mg a.s./kg dry sediment) based on development rate.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Pyraclostrobin (BAS 500 F, Reg. No. 304 428), batch no. 10-510009, purity: 100% (analytical).

B. STUDY DESIGN

Test species: Non-biting midge (*Chironomus riparius*), first instar larvae, 3 days old at test initiation; source: in-house culture.

Test design: Static system (28 days); 5 test concentrations plus a solvent (acetone) control and a water control, 4 replicates per test item concentration and for the water control, 6 replicates for the solvent control; 20 larvae were added to each test vessel; assessment of emergence rate and development rate.

Endpoints: NOEC and EC₅₀ (regarding emergence rate and development rate).

Test concentrations: Solvent (acetone) control, water control, 0.3, 0.6, 1.2, 2.4 and 4.8 mg a.s./kg dry sediment (nominal), corresponding to mean measured concentrations of 0.66, 1.37 and 2.83 mg a.s./kg dry sediment in the 1.2, 2.4 and 4.8 a.s./kg dry sediment treatments.

Test conditions: 600 mL glass vessels with 100 g spiked wet artificial sediment (according to OECD 218), 400 mL M4 water (Elendt medium) corresponding to a water layer of about 6.4 cm; pH 7.6 - 8.6; oxygen 62% to 100%; total hardness: 284.8 - 320.4 mg CaCO₃/L at test initiation and 311.5 - 329.3 mg CaCO₃/L at test termination; conductivity: 588 µS/cm; ammonia: 1.2 mg/L at test initiation and 2.0 mg/L at test termination; water temperature: 20°C - 21°C; light intensity: 620 - 720 lux; photoperiod: 16 h light : 8 h dark; continuous gentle aeration; food: TetraMin (days 0-10: 0.5 mg food/larva/day, days 11-27: 0.5 - 1.0 mg food/larva/day).

Analytics: Analytical verification of test item concentrations was conducted using a LC-MS/MS-method (Method no. L0166/01).

Statistics: Descriptive statistics, Student-t-test ($p < 0.05$) for comparison of the emergence and development rates in the control groups; ANOVA followed by one-sided Williams' t-test for determination of the NOEC based on emergence and development rate ($\alpha = 0.05$).

C. DESCRIPTION OF THE ANALYTICAL PRODECURES

Concentrations of BAS 500 F in overlying water, pore water and sediment were determined according to the analytical method L0166/01. The validation of the analytical method is described in the study report. The overlying water and the pore water were separated from the sediment by decanting and centrifugation, respectively. An aliquot of the overlying water and pore water was diluted with acetonitrile 1+4 (v+v; factor 5). The diluted samples were filtrated by means of a membrane filter (pore size 0.45µm; polytetrafluoroethylene) and transferred in an HPLC vial. For sediment extraction, 25 mL acetone were added to approximately 5 g sediment, from which the pore water was removed. After 30 min horizontal shaking the overlying extract was removed. It was centrifuged (5 min; 4000 rpm) and filtered by means of a membrane filter (0.45 µm polytetrafluoroethylene). An aliquot of 5 mL extract was evaporated to dryness by means of a rotatory evaporator and re-dissolved in 5 mL of a mixture of acetonitrile/test water 80/20 (v/v). Finally, the samples were diluted with acetonitrile / test water 80/20 (v/v) to match the calibration range. The determination was performed by LC with MS/MS detection. The limit of quantification (LOQ) was 1.83 µg/L for water and 0.57 mg/kg for sediment and the limit of detection (LOD) was set to 0.012 µg/L. The average procedural recovery for BAS 500 F was 97.1% for water and 93.8% for sediment. Details on measured fortification samples are given in the tables below.

Table A 47: Fortification samples for BAS 500 F in water

Matrix	Fortification level (µg/L)	n	mean (%)	±SD	RSD (%)
water	1	5	91.6	0.06725	7.26
water	1.25	5	104.9	0.03935	2.99
water	25	5	97.8	1.80768	7.37
water	125	5	94.0	1.93856	1.65

*SD is based on recovery

Table A 48: Fortification samples for BAS 500 F in sediment

Matrix	Fortification level (mg/kg)	n	Calculated concentration found (mg/kg)	% of nominal
sediment	7.151	1	6.504	91.0
sediment	7.293	1	6.894	94.5
sediment	0.573	1	0.613	107.0
sediment	0.584	1	0.615	105.3
sediment	7.105	1	6.116	86.1
sediment	7.069	1	6.276	88.8
sediment	7.237	1	5.536	76.5
sediment	0.563	1	0.577	102.5
sediment	0.557	1	0.45	80.8
sediment	0.585	1	0.619	105.8
			Mean	94
			SD	11
			RSD	12

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations in the overlying water, the pore water and the sediment were conducted in the test concentrations of 1.2 and 4.8 mg a.s./kg dry sediment at the beginning and the end of the test. Recoveries in the sediment were in a range between 86 and 101% of the nominal concentrations at test initiation, and < LOQ (limit of quantification = 0.57 mg a.s./kg dry sediment) and 17% of nominal at test termination. Pyraclostrobin concentrations found in the overlying water ranged from < LOQ (LOQ = 0.001 mg/L) to 0.4% of nominal concentrations on day 0 and from < LOQ to 0.03% of nominal on day 28. Measured pore water concentrations were between 0.2% and 0.9% of nominal on test start and < LOQ (LOQ = 0.001 mg/L) in both test treatments on test end. The following biological results are based on nominal sediment concentrations. Additionally, biological endpoints based on mean measured values are given.

Biological results: In the control and the solvent control mean emergence rates of 78.8% and 71.7% and mean development rates of 0.057 and 0.055 were observed, respectively. In the test item concentrations of up to and including 4.8 mg a.s./kg dry sediment, between 60% and 76% of the test animals emerged until day 28. No statistically significant difference was observed between the controls (Student-t-test, $p < 0.05$). Hence, the controls were pooled and used as the reference in all evaluations. Statistically significant differences compared to the pooled control were found for the emergence rates at the highest test item concentration (Williams Multiple t-test, $\alpha = 0.05$). No statistically significant effect on the development was observed in any treatment group (Williams Multiple t-test, $\alpha = 0.05$). The results are summarized in Table A 49.

Table A 49: Effects of pyraclostrobin on emergence and development of *Chironomus riparius*

Concentration [mg a.s./kg dry sediment] (nominal)	Control	Solvent control	0.3	0.6	1.2	2.4	4.8
Emergence rate (ER) [% emerged midges] #	78.8 ± 19.7	71.7 ± 11.7	66.3 ± 13.1	75.0 ± 4.1	73.8 ± 12.5	76.3 ± 9.5	60.0 ± 10.8 *
Development rate per day (DR) #	0.057 ± 0.001	0.055 ± 0.001	0.057 ± 0.003	0.056 ± 0.001	0.056 ± 0.002	0.059 ± 0.003	0.058 ± 0.002
Endpoints [mg pyraclostrobin/kg dry sediment] (nominal)							
EC ₅₀ emergence rate (28 d)	> 4.8 (mean measured: > 2.83)						
NOEC _{emergence rate} (28 d)	2.4 (mean measured: 1.37)						
NOEC _{development rate} (28 d)	≥ 4.8 (mean measured: ≥ 2.83)						

Values represent mean and standard deviation from all replicates, each with 20 larvae.

* Statistically significant difference compared to the pooled control (Williams Multiple t-test, $\alpha = 0.05$).

Validity criteria according to OECD TG 218 (2004)	Obtained in this study
The emergence in the controls must be at least 70% at the end of test.	78.8 and 71.7% (control and solvent control, respectively)
<i>C. riparius</i> emergence to adults from control vessels should occur between 12 and 23 days after their insertion into the vessels.	between day 16 and 20 after insertion
At the end of test, pH and dissolved oxygen concentration should be measured in each vessel: oxygen concentration ≥60% of air saturation value at the temperature used, the pH of overlying water should be in the 6-9 range in all test vessels.	O ₂ : 62 – 100% pH: 8.3 – 8.6
The water temperature should not differ by more than ±1.0°C	20 - 21°C

All validity criteria were met.

III. CONCLUSION

In a 28-day static sediment test with *Chironomus riparius* the NOEC values of pyraclostrobin were determined to be 2.4 mg a.s./kg dry sediment (nominal; equivalent to the mean measured concentration of 1.37 mg a.s./kg dry sediment) based on emergence rate and ≥ 4.8 mg a.s./kg dry sediment (nominal; equivalent to the mean measured concentration of ≥ 2.83 mg a.s./kg dry sediment) based on development rate.

A 2.3.2.7 Study 7

The following spiked sediment *Chironomus* study was performed with the soil (sediment) metabolite BF-500-3. The study was submitted for the Annex I renewal process of pyraclostrobin and is currently in the evaluation phase on EU level.

<i>Comments of zRMS:</i>	Study was evaluated and accepted for the regulatory use in RR for BAS 751 00 F
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<i>Reference:</i>	<i>CP 10.2.2/9</i>
<i>Report</i>	<i>Effects of Reg.No. 340266 (metabolite of BAS 500 F (Pyraclostrobin), synonymous: 500M07, BF 500-3) on the development of sediment dwelling larvae of Chironomus riparius in a sediment-water system - exposed via spiked sediment,</i> <i>Kuhl R., Wydra V., 2013</i> <i>Report No: 423857</i> <i>BASF DocID 2013/1237446</i> <i>Authority registration No</i>
<i>Guideline(s):</i>	<i>OECD 218 (2004)</i>
<i>Deviations:</i>	<i>No</i>
<i>GLP:</i>	<i>yes</i> <i>(certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden),</i>
<i>Acceptability:</i>	<i>Yes</i>
<i>Duplication</i> <i>(if vertebrate study)</i>	<i>No</i>

Executive Summary

In a 28-day static spiked sediment study, non-biting midge larvae (*Chironomus riparius*) were exposed to BF 500-3 (metabolite of pyraclostrobin) at nominal test item concentrations of 1.0, 2.0, 4.0, 8.0 and 16.0 mg/kg dry sediment. Additionally, a solvent (acetone) control and a water control were set up. All test item concentrations and the water control had 4 replicates, whereas 6 replicates were tested for the solvent control. 20 larvae were added to each test vessel.

The biological results are based on nominal concentrations of the test item. In the control and the solvent control mean emergence rates of 90.0 and 88.3% were observed, respectively. The mean development rate was 0.059 in both control groups. In test item concentrations of up to and including 16.0 mg/kg dry sediment, between 87.5 and 95.0% of the test animals emerged until day 28. No statistically significant differences were found for the emergence and development rates at any test item concentration when compared to the pooled control.

In a 28-day static sediment test with *Chironomus riparius* the NOEC of BF 500-3 (metabolite of pyraclostrobin) was determined to be ≥ 16.0 mg a.s./kg dry sediment based on emergence and development rate (nominal).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BF 500-3 (Reg. No. 340 266; synonym: 500M07; metabolite of pyraclostrobin), batch no. L74-118, purity: 99.9%.

B. STUDY DESIGN

Test species: Non-biting midge (*Chironomus riparius*), first instar larvae, 3 days old at test initiation; source: in-house culture.

Test design: Static system (28 days); 5 test concentrations plus a solvent (acetone) control and a water control, 4 replicates per test item concentration and for the water control, 6 replicates for the solvent control; 20 larvae were added to each vessel; assessment of emergence rate and development rate.

Endpoints: NOEC and EC₅₀ (regarding emergence rate and development rate).

Test concentrations: Solvent control, water control, 1.0, 2.0, 4.0, 8.0 and 16.0 mg/kg dry sediment (nominal).

Test conditions: 600 mL glass vessels with 100 g spiked wet artificial sediment (according to OECD 218), 280 mL M4 water (Elendt medium) corresponding to a water layer of about 6.0 cm; pH 8.1 - 8.7; oxygen saturation 86% - 102%; total hardness: 302.6 - 311.5 mg CaCO₃/L at test initiation and 302.6 - 329.3 mg CaCO₃/L at test termination; conductivity: 620 µS/cm; ammonia: 0.8 mg/L at test initiation and 0.6 - 0.8 mg/L at test termination; water temperature: 19°C - 21°C; light intensity: 670 - 870 lux; photoperiod: 16 h light : 8 h dark; continuous gentle aeration; food: TetraMin on workdays, 0-10 days: 0.5 mg food/larva/day, 11-27 days: 0.5 - 1.0 mg food/larva/day.

Analytics: Analytical verification of test item concentrations was conducted using a LC-MS/MS-method.

Statistics: Descriptive statistics, Student's t-test ($p < 0.05$) for comparison of the emergence and development rates in the control groups; ANOVA followed by Williams' Multiple Sequential t-test procedure for determination of the NOEC based on emergence and development rate ($\alpha = 0.05$).

C. DESCRIPTION OF THE ANALYTICAL PRODECDURES

Concentrations of BF 500-3 in overlying water, pore water and sediment were determined according to the analytical method given in the report. The validation of the analytical method is described in the study report. The overlying water and the pore water were separated from the sediment by decanting and centrifugation, respectively. An aliquot of the overlying water and pore water was diluted with acetonitrile 1+4 (v+v; factor 5). The diluted samples were filtrated by means of a membrane filter (pore size 0.45µm; polytetrafluoroethylene) and transferred in an HPLC vial. For sediment extraction, 25 mL acetone were added to approximately 5 g sediment, from which the pore water was removed. After 30 min horizontal shaking the overlying extract was removed. It was centrifuged (5 min; 3000 rpm) and filtered by means of a membrane filter (0.45 µm polytetrafluoroethylene). An aliquot of 5 mL extract was evaporated to dryness by means of a rotatory evaporator and re-dissolved in 5 mL of a mixture of acetonitrile/test water 80/20 (v/v). Finally, the samples were diluted with acetonitrile / test water 80/20 (v/v) to match the calibration range. The determination was performed by LC with MS/MS detection. The limit of quantification (LOQ) was 2 µg/L for water and 1.3 mg/kg for sediment and the limit of detection (LOD) was set to 0.09 µg/L. The average procedural recovery for BF 500-3F was 93% for the water and 89% for sediment. Details on measured fortification samples are given in the tables below.

Table A 50: Fortification samples for BF 500-3 in water

Matrix	Fortification level (µg/L)	n	mean (%)	±SD	RSD (%)
water	2	4	100.1	0.22061	10.91
water	10	4	90.5	0.21452	2.36
water	50	5	90.5	1.30924	2.87

Table A 51: Fortification samples for BF 500-3 in sediment

Matrix	Fortification level (mg/kg)	n	Calculated concentration found (mg/kg)	% of nominal
sediment	19.560	1	18.289	86
sediment	19.373	1	19.320	91
sediment	1.322	1	1.278	89
sediment	1.307	1	1.192	84
sediment	19.915	1	17.533	81
sediment	19.525	1	19.051	89
sediment	19.606	1	15.680	73
sediment	1.315	1	1.323	92
sediment	1.305	1	1.522	107
sediment	1.311	1	1.346	94
			Mean	89
			RSD	10

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations in the overlying water, the pore water and the sediment was conducted in the test concentrations of 2.0 and 16.0 mg/kg dry sediment at the beginning and the end of the test. Mean recoveries in the sediment were in a range between 96 and 101% of the nominal concentrations at test initiation and between 75 and 81% of nominal at test termination. Overlying water concentrations ranged from 2.18 to 11.23 µg/L at test start (= insertion of larvae) and < LoQ (limit of quantification) to 2.13 µg/L at test end. The pore water concentrations ranged from 8.62 to 66.58 µg/L at test start and from 2.51 to 13.62 µg/L at test termination. Since the analytical measurements of initial sediment concentrations confirmed the correct application of the test item, the following biological results are based on nominal sediment concentrations.

Biological results: In the control and the solvent control mean emergence rates of 90.0 and 88.3% were observed, respectively. The mean development rate was 0.059 in both control groups. In the test item concentrations of up to and including 16.0 mg/kg dry sediment between 87.5 and 95.0% of the test animals emerged until day 28. No statistically significant differences were observed between the control groups (Student's t-test, $p < 0.05$). Hence, the controls were pooled and used as the reference in all evaluations. No statistically significant differences were found for the emergence and development rates at any test item concentration when compared to the pooled control (Williams Multiple Sequential t-test procedure, $\alpha = 0.05$). The results are summarized in the table below.

Table A 52: Effects of BF 500-3 (metabolite of pyraclostrobin) on emergence and development of *Chironomus riparius*

Concentration [mg BF 500-3/kg dry sediment] (nominal)	Control	Solvent control	1.0	2.0	4.0	8.0	16.0
Emergence rate (ER) [% emerged midges] (28 d) #	90.0 ± 7.1	88.3 ± 9.8	87.5 ± 6.5	95.0 ± 4.1	90.0 ± 9.1	87.5 ± 2.9	91.3 ± 7.5
Development rate per day (DR) (28 d) #	0.059 ± 0.001	0.059 ± 0.003	0.060 ± 0.001	0.060 ± 0.001	0.060 ± 0.002	0.061 ± 0.002	0.059 ± 0.002
Endpoints [mg BF 500-3/kg dry sediment] (nominal)							
EC ₅₀ emergence rate (28 d)	> 16.0						
NOEC _{emergence & development rate} (28 d)	≥ 16.0						

Values represent mean and standard deviation from all replicates, each with 20 larvae.

Validity criteria according to OECD TG 218 (2004)	Obtained in this study
The emergence in the controls must be at least 70% at the end of test.	90.0 and 88.3% (control and solvent control)
<i>C. riparius</i> emergence to adults from control vessels should occur between 12 and 23 days after their insertion into the vessels.	between day 14 and 21 after insertion
At the end of test, pH and dissolved oxygen concentration should be measured in each vessel: oxygen concentration ≥ 60% of air saturation value at the temperature used, the pH of overlying water should be in the 6-9 range in all test vessels.	O ₂ : 62 – 100% pH: 8.1 – 8.7
The water temperature should not differ by more than ±1.0°C	19 - 21°C

All validity criteria were met.

III. CONCLUSION

In a 28-day static sediment test with *Chironomus riparius* the NOEC of BF 500-3 (metabolite of pyraclostrobin) was determined to be ≥ 16.0 mg a.s./kg dry sediment based on emergence and development rate (nominal).

A 2.3.2.8 Study 8

The following spiked sediment *Chironomus* study was performed with the soil (sediment) metabolite BF-500-6. The study was submitted for the Annex I renewal process of pyraclostrobin and is currently in the evaluation phase on EU level.

<i>Comments of zRMS:</i>	Study not evaluated. The study was considered as not essential for the risk assessment.
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<i>Reference:</i>	<i>CP 10.2.2/10</i>
<i>Report</i>	<i>Chronic toxicity of Reg. No. 364380 (BF 500-6; metabolite of Pyraclostrobin) to the non-biting midge Chironomus riparius - A spiked sediment study,</i> <i>Backfisch K., 2014</i> <i>Report No: 439917</i> <i>BASF DocID 2014/1001481</i> <i>Authority registration No</i>
<i>Guideline(s):</i>	<i>OECD 218 (2004)</i>
<i>Deviations:</i>	<i>No</i>
<i>GLP:</i>	<i>yes</i> <i>(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)</i>
<i>Acceptability:</i>	<i>Yes</i>
<i>Duplication</i> <i>(if vertebrate study)</i>	<i>No</i>

Executive Summary

In a 28-day static spiked sediment study, non-biting midge larvae (*Chironomus riparius*) were exposed to BF 500-6 (metabolite of pyraclostrobin) at nominal test item concentrations of 2.0, 6.0, 18.0, 54.0 and 162.0 mg/kg dry sediment (corresponding to mean measured concentrations of 2.0, 6.5, 18.3, 48.7 and 151.5 mg/kg dry sediment). Even the highest test concentration did not cause 50% effect on emergence or development rate. However, no clear NOEC was reached in the first experiment; therefore, a second test was conducted with lower nominal concentrations of 0.5, 1.0 and 2.0 mg/kg dry sediment (corresponding to mean measured concentrations of 0.6, 1.2 and 2.8 mg/kg dry sediment). Additionally, for each trial a solvent (acetone) control and a water control were set up. In the first experiment all test item concentrations and the water control had 4 replicates, whereas 6 replicates were tested for the solvent control. For the second experiment 4 replicates for each test item concentration and three replicates for each control were used. 20 larvae were added to each test vessel.

The biological results are based on mean measured concentrations of the test item. In the control and the solvent control of the first trial mean emergence rates of 0.9375 and 0.9583 were observed, respectively. The mean development rate was 0.0625 in the water control and 0.0617 in the solvent control. No statistically significant differences were found for the development rates at test item concentrations of up to and including the highest tested concentration when compared to the pooled control. The emergence rate was slightly, but statistically significantly reduced in all tested concentration groups. Therefore, a second test was added including some lower concentrations in order to derive a clear NOEC. Mean emergence rate in the second experiment was 0.8333 and 0.9333 in the control and the solvent control, respectively. The mean development rate was 0.0638 in the water control and 0.0626 in the solvent control. As expected, no statistically significant effect on the development rates was observed in any of the lower treatments whereas the emergence rate was significantly reduced at the highest test concentration of 2.8 mg/kg dry sediment.

In a 28-day spiked sediment test with *Chironomus riparius* the NOEC of BF 500-6 (metabolite of pyraclostrobin) was determined to be 1.2 mg/kg dry sediment based on emergence rate and ≥ 151.5 mg/kg dry sediment based on development rate (mean measured).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BF 500-6 (Reg. No. 364 380; synonym: 500M01, metabolite of pyraclostrobin); batch no. 01311-142; purity: 99.2%.

B. STUDY DESIGN

Test species: Non-biting midge (*Chironomus riparius*), first instar larvae from in-house culture (non-GLP), originally obtained from "the Zoological Institute of the J.W. Goethe University", Frankfurt, Germany.

Test design: Static system (28 days); first experiment: 5 test concentrations plus a solvent (acetone) control and a water control, 4 replicates per test item concentration and for the water control, 6 replicates for the solvent control; a second test was conducted with lower concentrations since no clear NOEC was reached in the first experiment: 3 test concentrations with four replicates plus a solvent (acetone) control and a water control with three replicates. 20 larvae were added to each vessel; assessment of emergence rate and development rate.

Endpoints: NOEC, EC₅₀ values (regarding emergence rate and development rate).

Test concentrations: First experiment: solvent control, water control, 2.0, 6.0, 18.0, 54.0 and 162.0 mg/kg dry sediment (nominal); corresponding to mean measured concentrations of 2.0, 6.5, 18.3, 48.7 and 151.5 mg/kg dry sediment; Second experiment: solvent control, water control, 0.5, 1.0 and 2.0 mg/kg dry sediment (nominal); corresponding to mean measured concentrations of 0.6, 1.2 and 2.8 mg/kg dry sediment.

Test conditions: 600 mL glass vessels with 100 g spiked wet artificial sediment (according to OECD 218), 400 mL M4 water (Elendt medium) corresponding to a water layer of about 8.0 cm; pH 7.52 - 8.17; oxygen content: 6.99 - 8.60 mg/L; total hardness: 2.48 mmol/L (1st trial) / 2.47 mmol/L (2nd trial); conductivity: 634 / 657 μ S/cm; ammonia: 0.2 / 0.8 mg/L at test initiation and 0.5 / 2.0 mg/L at test termination; water temperature: 19.7°C - 20.6°C; light intensity: 361 - 461 lux; photoperiod: 16 h light : 8 h dark; continuous gentle aeration; food: finely ground and suspended TetraMin, 0.25 - 1.0 mg food/larva/day (i.e. 5 - 20 mg/vessel/day).

Analytics: Analytical verification of test item concentrations was conducted using an

HPLC-method with MS-detection (Method no. APL0500/03).

Statistics:

Descriptive statistics, Probit analysis using linear maximum likelihood regression for determination of the EC_x values; ANOVA followed by Chi-square test with Bonferroni correction or Dunnett's Multiple t-test for determination of the NOEC based on emergence and development rate ($\alpha = 0.05$).

C. DESCRIPTION OF THE ANALYTICAL PRODECDURES

Concentrations of BF 500-6 in M4 medium and sediment were determined according to the analytical method APL0500/03. The validation of the analytical method is described in the study report. For the analytical investigations of the water samples the test samples were concentrated on a C18 SPE cartridge, after reconstitution with an acetonitrile/water-mixture analysis was conducted by HPLC/MS system. Separation was done by reversed phase HPLC using MS-detection. For analyzing of the sediment samples an additional work-up step, based on BASF Method L0166, was implemented for sample preparation. This step includes an extraction with acetonitrile following a dilution step after separation before measurement. The determination was performed by HPLC with MS detection. The limit of quantification (LOQ) was 1 µg/L for water and 0.25 mg/kg for sediment. The limit of detection (LOD) was not given in the report. Mean recovery rates of 89% up to 97% were found for the aqueous samples and 93% and 96% for the sediment samples. Details on measured fortification samples are given in the tables below.

Table A 53: Fortification samples for BF 500-6 in water

Matrix	Fortification level (mg/L)	Date of sampling	n	mean (%)	±SD	RSD (%)
M4 water	0.1	19.02.2014	3	93.67	0.00473	5.05
M4 water	0.1	25.02.2014	3	101.33	0.00847	8.62
M4 water	0.1	03.04.2014	1	87.00	-	-
M4 water	0.001	19.02.2014	3	81.67	0.00006	6.74
M4 water	0.001	25.02.2014	3	98.00	0.00005	5.10
M4 water	0.001	03.04.2014	1	80.00	-	-

Table A 54: Fortification samples for BF 500-6 in sediment

Matrix	Fortification level (mg/kg dry sediment)	Date of sampling	n	mean (%)	±SD	RSD (%)
Sediment	175	08.04.2014	3	88.00	10.58301	6.87
Sediment	175	14.04.2014	3	97.33	15.27525	8.97
Sediment	0.25	08.04.2014	3	86.00	0.00346	1.61
Sediment	0.25	14.04.2014	3	106.80	0.00600	2.25

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations in the overlying water, the pore water and the sediment was conducted in all test concentrations at the beginning and the end of the test for both trials. Mean recoveries in the sediment in the first experiment were in a range between 89% and 114% of the nominal concentrations at test initiation and between 92% and 109% of nominal at test termination. Overlying water concentrations ranged from < LoQ (limit of quantification = 1 µg/L) to 163.0 µg/L at test start (= insertion of larvae) and < LoQ to 2.42 µg/L at test end. The very high measurement in the overlying water in the highest treatment group at start of the test is likely due to some contamination of the sample (with sediment during sampling). The pore water concentrations ranged from < LoQ to 4.49 µg/L at test start and from < LoQ to 8.59 µg/L at test termination. Mean recoveries in the sediment during the second trial were between 120% and 141% of the nominal concentrations at test start and between 112% and 137% of nominal at test end. Mean measured test item concentrations in the overlying water and the pore water were < LoQ in all treatment groups at test start and at test end. The following biological results are based on mean measured sediment concentrations.

Biological results: In the control and the solvent control of the first trial mean emergence rates of 0.9375 and 0.9583 were observed, respectively. The mean development rate was 0.0625 in the water control and 0.0617 in the solvent control. No statistically significant differences were observed between the control groups (Chi-square test with Bonferroni correction, Dunnett's Multiple t-test procedure, $\alpha = 0.05$). Hence, the controls were pooled and used as the reference in all evaluations. No statistically significant differences were found for the development rates at test item concentrations of up to and including the highest tested concentration when compared to the pooled control (Chi-square test with Bonferroni correction, Dunnett's Multiple t-test procedure, $\alpha = 0.05$). The emergence rate was slightly, but statistically significantly reduced in all tested concentration groups ($\alpha = 0.05$). Therefore, a second test was added including some lower concentrations in order to derive a clear NOEC. Mean emergence rate in the second experiment was 0.8333 and 0.9333 in the control and the solvent control, respectively. The mean development rate was 0.0638 in the water control and 0.0626 in the solvent control. Since there were no statistically significant differences observed between the control groups ($\alpha = 0.05$), the control data were pooled and used as the reference in all evaluations. As expected, no statistically significant effect on the development rates was observed in any of the lower treatments whereas the emergence rate was significantly reduced at the highest test concentration of 2.8 mg/kg dry sediment ($\alpha = 0.05$). The results are summarized in the table below.

Table A 55: Effects of BF 500-6 (metabolite of pyraclostrobin) on emergence and development of *Chironomus riparius* (trial 1 and 2)

development of <i>Chironomus riparius</i> (trial 1 and 2)								
Trial 1								
Concentration [mg/kg dry sediment] (nominal)	Control	Solvent control	2.0	6.0	18.0	54.0	162.0	
Concentration [mg/kg dry sediment] (mean measured)	Control	Solvent control	2.0	6.5	18.3	48.7	151.5	
Emergence rate (ER) (28 d) #	0.9375 ± 0.025	0.9583 ± 0.0376	0.8250 ± 0.0289 *	0.7875 ± 0.025 *	0.8500 ± 0.0913 *	0.7625 ± 0.1315 *	0.7125 ± 0.1436 *	
Development rate (DR) (28 d) #	0.0625 ± 0.0013	0.0617 ± 0.0012	0.0634 ± 0.0011	0.0617 ± 0.0022	0.0623 ± 0.0023	0.0625 ± 0.0017	0.0643 ± 0.0025	
Trial 2								
Concentration [mg/kg dry sediment] (nominal)	Control	Solvent control	0.5		1.0		2.0	
Concentration [mg/kg dry sediment] (mean measured)	Control	Solvent control	0.6		1.2		2.8	
Emergence rate (ER) (28 d) #	0.8333 ± 0.0289	0.9333 ± 0.0764	0.8375 ± 0.0629		0.8500 ± 0.0707		0.7000 ± 0.1155 *	
Development rate (DR) (28 d) #	0.0638 ± 0.0001	0.0626 ± 0.0012	0.0625 ± 0.0012		0.0650 ± 0.0024		0.0646 ± 0.0019	
	Overall endpoints [mg BF 500-6/kg dry sediment] (mean measured)							
EC ₅₀ emergence & development rate (28 d)	> 151.5							
NOEC emergence rate (28 d)	1.2							
NOEC development rate (28 d)	≥ 151.5							

Values represent mean and standard deviation from all replicates, each with 20 larvae.

* Statistically significant difference compared to the pooled control (Chi-square test with Bonferroni correction, Dunnett's Multiple t-test procedure, $\alpha = 0.05$)

Validity criteria according to OECD TG 218 (2004)	Obtained in this study
The emergence in the controls must be at least 70% at the end of test.	93.4 and 95.8% (control and solvent control)
<i>C. riparius</i> emergence to adults from control vessels should occur between 12 and 23 days after their insertion into the vessels.	between day 14 and 23 after insertion
At the end of test, pH and dissolved oxygen concentration should be measured in each vessel: oxygen concentration ≥60% of air saturation value at the temperature used, the pH of overlying water should be in the 6-9 range in all test vessels.	O ₂ : ≥ 62 (6.99 – 8.60 mg/L) pH: 7.52 – 8.17
The water temperature should not differ by more than ±1.0°C	19.7 – 20.6°C

All validity criteria were met.

III. CONCLUSION

In a 28-day spiked sediment test with *Chironomus riparius* the NOEC of BF 500-6 (metabolite of pyraclostrobin) was determined to be 1.2 mg/kg dry sediment based on emergence rate and ≥ 151.5 mg/kg dry sediment based on development rate (mean measured).

A 2.3.2.9 Study 9

The following spiked sediment *Chironomus* study was performed with the soil (sediment) metabolite BF-500-7. The study was submitted for the Annex I renewal process of pyraclostrobin and is currently in the evaluation phase on EU level.

<i>Comments of zRMS:</i>	Study not evaluated. The study was considered as not essential for the risk assessment.
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<i>Reference:</i>	<i>CP 10.2.2/11</i>
<i>Report</i>	<i>Chronic toxicity of Reg.No. 369315 (BF 500-7; Metabolite of Pyraclostrobin) to the non-biting midge Chironomus riparius - A spiked sediment study,</i> <i>Backfisch K., 2014</i> <i>Report No:439918</i> <i>BASF DocID 2014/1001482</i> <i>Authority registration No</i>
<i>Guideline(s):</i>	<i>OECD 218 (2004)</i>
<i>Deviations:</i>	<i>No</i>
<i>GLP:</i>	<i>yes</i> <i>(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)</i>
<i>Acceptability:</i>	<i>Yes</i>
<i>Duplication</i> <i>(if vertebrate study)</i>	<i>No</i>

Executive Summary

In a 28-day static spiked sediment study, non-biting midge larvae (*Chironomus riparius*) were exposed to BF 500-7 (metabolite of pyraclostrobin) at nominal test item concentrations of 2.0, 6.0, 18.0, 54.0 and 162.0 mg/kg dry sediment (corresponding to mean measured concentrations of 0.96, 4.55, 13.83, 47.15 and 123.5 mg/kg dry sediment). Additionally, a solvent (acetone) control and a water control were set up. All test item concentrations and the water control had 4 replicates, whereas 6 replicates were tested for the solvent control. 20 larvae were added to each test vessel.

The biological results are based on mean measured concentrations of the test item. Mean emergence rates in both controls were above 90%. The mean development rates were 0.0650 and 0.0644 for the water control and the solvent control, respectively. No statistically significant differences were found for the emergence and development rates at any test item concentration when compared to the water control and the solvent control.

In a 28-day static sediment test with *Chironomus riparius* the NOEC of BF 500-7 (metabolite of pyraclostrobin) was determined to be ≥ 123.5 mg a.s./kg dry sediment based on emergence and development rate (mean measured).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BF 500-7 (Reg. No. 369 315; metabolite of pyraclostrobin), batch no. L83-168, purity: 96.2%.

B. STUDY DESIGN

Test species: Non-biting midge (*Chironomus riparius*), first instar larvae from in-house culture, originally obtained from "the Zoological Institute of the J.W. Goethe University" Frankfurt, Germany.

Test design: Static system (28 days); 5 test concentrations plus a solvent (acetone) control and a water control, 4 replicates per test item concentration and for the water control, 6 replicates for the solvent control; 20 larvae were added to each vessel; assessment of emergence rate and development rate.

Endpoints: NOEC and EC₅₀ (regarding emergence rate and development rate).

Test concentrations: Solvent control, water control, 2.0, 6.0, 18.0, 54.0 and 162.0 mg BF 500-7/kg dry sediment (nominal), corresponding to mean measured concentrations of 0.96, 4.55, 13.83, 47.15 and 123.5 mg/kg dry sediment.

Test conditions: 600 mL glass vessels with 100 g spiked wet artificial sediment (according to OECD 218), 400 mL M4 water (Elendt medium) corresponding to a water layer of about 8.0 cm; pH 7.41 - 8.11; oxygen content: 7.35 - 8.45 mg/L (saturation > 60%); total hardness: 2.53 mmol/L at test initiation; conductivity: 617 µS/cm at test initiation; ammonia: 0.8 mg/L; water temperature: 19.3°C - 20.1°C; light intensity: 397 - 592 lux; photoperiod: 16 h light: 8 h dark; continuous gentle aeration except during addition of the larvae and about 24 hours afterwards; food: TetraMin, until DAI 23: 0.25 - 1.0 mg food/larva/day.

Analytics: Analytical verification of test item concentrations was conducted using an HPLC-method with MS-detection (Method No. APL0500/03).

Statistics: Descriptive statistics, Dunnett's Multiple t-test procedure ($p < 0.05$) for comparison of the water control, the solvent control and the treatment groups; ANOVA followed by Chi-square test with Bonferroni correction for determination of the NOEC based on emergence and development rate ($\alpha = 0.05$), Probit and Weibull analysis using linear max. likelihood regression for determination of EC_x values.

C. DESCRIPTION OF THE ANALYTICAL PRODECDURES

Concentrations of BF 500-7 in M4 medium and sediment were determined according to the analytical method APL0500/03. The validation of the analytical method is described in the study report. For the analytical investigations of the water samples the test samples were concentrated on a C18 SPE cartridge, after reconstitution with an acetonitrile/water-mixture analysis was conducted by HPLC/MS system. Separation was done by reversed phase HPLC using MS-detection. For analyzing of the sediment samples an additional work-up step, based on BASF Method L0166, was implemented for sample preparation. This step includes an extraction with acetonitrile following a dilution step after separation before measurement. The determination was performed by HPLC with MS detection. The limit of quantification (LOQ) was 1 µg/L for water and 1 mg/kg for sediment. The limit of detection (LOD) was not given in the report. Mean recovery rates of 80% up to 95% were found for the aqueous samples and 88% and 103% for the sediment samples. Details on measured fortification samples are given in the tables below.

Table A 56: Fortification samples for BF 500-7 in water

Matrix	Fortification level (mg/L)	Date of sampling	n	mean (%)	±SD	RSD (%)
M4 water	0.1	13.03.2014	3	93.70	0.00424	4.5
M4 water	0.1	17.03.2014	3	82.77	0.00258	3.1
M4 water	0.001	13.03.2014	3	95.17	0.00004	4.0
M4 water	0.001	17.03.2014	3	80.23	0.00003	3.4

Table A 57: Fortification samples for BF 500-7 in sediment

Matrix	Fortification level (mg/L)	Date of sampling	n	mean (%)	±SD	RSD (%)
Sediment	1.0	19.03.2014	3	104.00	0.01000	1.0
Sediment	175	21.03.2014	3	87.24	0.57735	0.4

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations in the overlying water, the pore water and the sediment was conducted in the controls and in each concentration at the beginning and the end of the test. Recoveries in the sediment were in the range between 66 and 87% of the nominal concentrations at test initiation and between 46 and 86% of nominal at test termination. Overlying water concentrations ranged from < LoQ (limit of quantification) to 5.32 µg/L at test start (= insertion of larvae) and from < LoQ to 2.7 µg/L at test end. The pore water concentrations ranged from < LoQ to 1.95 µg/L at test start and from < LoQ to 2.31 µg/L at test termination. The following biological results are based on the mean measured sediment concentrations.

Biological results: Mean emergence rates in both controls were above 90%. The mean development rates were 0.0650 and 0.0644 for the water control and the solvent control, respectively. No statistically significant differences were found for the emergence and development rates at any test item concentration when compared to the water control and the solvent control (Chi-square test with Bonferroni correction, $\alpha = 0.05$). The results are summarized in the table below.

Table A 58: Effects of BF 500-7 (metabolite of pyraclostrobin) on emergence and development of *Chironomus riparius*

Concentration [mg BF 500-7/kg sediment] (nominal)	dry	Control	Solvent control	2.0	6.0	18.0	54.0	162.0
Concentration [mg BF 500-7/kg sediment] (mean measured)	dry	--	--	0.96	4.55	13.83	47.15	123.5
Emergence rate (ER) (28 d) #		0.9375 ± 0.0479	0.9333 ± 0.0516	0.9250 ± 0.0500	0.9000 ± 0.0816	0.9000 ± 0.1225	0.9000 ± 0.1080	0.8500 ± 0.1080
Development rate per day (DR) (28 d) #		0.0650 ± 0.0008	0.0644 ± 0.0007	0.0635 ± 0.0014	0.0639 ± 0.0012	0.0633 ± 0.0005	0.0636 ± 0.0034	0.0633 ± 0.0034
Endpoints [mg BF 500-7/kg dry sediment] (mean measured)								
EC ₅₀ emergence rate (28 d)		> 123.5						
NOEC _{emergence & development rate} (28 d)		≥ 123.5						

Values represent mean and standard deviation from all replicates, each with 20 larvae.

Validity criteria according to OECD TG 218 (2004)	Obtained in this study
The emergence in the controls must be at least 70% at the end of test.	93.8 and 93.2% (control and solvent control)
<i>C. riparius</i> emergence to adults from control vessels should occur between 12 and 23 days after their insertion into the vessels.	between day 14 and 23 after insertion
At the end of test, pH and dissolved oxygen concentration should be measured in each vessel: oxygen concentration ≥60% of air saturation value at the temperature used, the pH of overlying water should be in the 6-9 range in all test vessels.	O ₂ : ≥ 60 (7.35 – 8.45 mg/L) pH: 7.41 – 8.11
The water temperature should not differ by more than ±1.0°C	19.3 – 20.1°C

All validity criteria were met.

III. CONCLUSION

In a 28-day static sediment test with *Chironomus riparius* the NOEC of BF 500-7 (metabolite of pyraclostrobin) was determined to be ≥ 123.5 mg a.s./kg dry sediment based on emergence and development rate (mean measured).

A 2.3.2.10 Study 10

The following spiked sediment study on *Chironomus riparius* performed with BAS 560 F is provided in support of the assessment and was recently submitted under the Annex I renewal process for metrafenone (AIR 3). The study summary was provided in the revised Renewal Assessment Report of metrafenone (RAR, Vol. 3, B.9, December 2019).

<i>Comments of zRMS:</i>	The study was conducted to OECD guidance 218 and according to the principles of GLP. All validity criteria were met. The study is considered to be reliable and suitable for the risk assessment.
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<i>Reference:</i>	CP 10.2.2/12
<i>Report</i>	<i>Chronic toxicity of Reg.No. 4037710 (BAS 560 F; Metrafenone) to the non-biting midge Chironomus riparius - A spiked sediment study,</i> <i>Backfisch, K., Weltje, L., 2011</i> <i>report No 376973</i> <i>BASF DocID 2010/1145509</i> <i>Authority registration No</i>
<i>Guideline(s):</i>	OECD 218 (2004)
<i>Deviations:</i>	No
<i>GLP:</i>	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)
<i>Acceptability:</i>	Yes
<i>Duplication</i> (if vertebrate study)	No

Executive Summary

In a spiked sediment study, non-biting midge larvae (*Chironomus riparius*) were exposed to BAS 560 F (metrafenone). The midges were exposed for 28 days to nominal test concentrations of 20, 40, 80, 160 and 320 mg a.s./kg sediment dry weight (dw), under static conditions. Additionally, a solvent (acetone) control and a water control were tested. All test item concentrations and the water control had four replicates, whereas six replicates were tested for the solvent control.

Mean recoveries of metrafenone in sediment were in the range of 80.3% - 102.3% of nominal concentrations. The biological results were based on initial measured concentrations. First emerged midges were observed on day thirteen. No statistically significant differences were found for the emergence rates and the development rates at any test item concentration when compared to the water and solvent control.

In this 28-day spiked sediment test with *Chironomus riparius* exposed to metrafenone, the no-observed-effect concentration (NOEC) for development rate and emergence rate was determined to be 296.0 mg a.s./kg sediment dw (initial mean measured), the highest concentration tested. The lowest-observable-effect concentration (LOEC) and EC₅₀ were both > 296.0 mg a.s./kg sediment dw.

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test material:** BAS 560 F (metrafenone; Reg. No. 4037710)
Batch number: AC12053-29
Purity: 94.2%
Description: Solid
2. **Test concentrations:** 0 (negative and solvent control), 20, 40, 80, 160 and 320 mg a.s./kg sediment dry weight (dw)
3. **Reference item:** None
4. **Dilution water:** Reconstituted water, M4 according to Elendt
Vehicle: Acetone
Artificial substrate: According to OECD 218; containing 5% sphagnum peat, 20% kaolin clay, 0.75% CaCO₃ and 75% quartz sand, and with a pH of 7.02
5. **Test organism:**
Species: Non-biting midge (*Chironomus riparius*)
Age/life stage: First instar larvae, < 48 hours old
Source: In-house culture
Diet: Commercially available fish food TetraMin, 0.25 to 1.0 mg was added per larva each day
Test vessels: 600 mL glass vessels with 100 g wet artificial sediment and 400 mL dilution water

B. STUDY DESIGN

1. **Environmental conditions:**
Temperature: 20.1 – 21.5 °C
pH: 7.45 – 8.36
Dissolved oxygen: 7.74 – 8.93 mg/L
Hardness: 2.6 mmol/L
Photoperiod: 16 hours light: 8 hours darkness (535 - 850 lux)
Aeration: Constant aeration

2. **Animal assignment and treatment:**

Three days prior to initiation of the exposure, fresh egg masses were collected from the culture and transferred to petri dishes with dilution water. About two days later the first larvae started hatching. At initiation of the exposure, the first instar larvae (< 48 hours old) were collected and added to test vessels randomly, until each test vessel contained twenty larvae. During addition of the larvae and for 24 hours afterwards, the aeration was stopped to give the larvae the opportunity to settle into the sediment. The midges were exposed for 28 days, under static conditions, to nominal test item concentrations of 20, 40, 80, 160 and 320 mg a.s./kg sediment dry weight (dw). A water control and solvent control (acetone) were tested in parallel. Each test item group and the water control group consisted of four replicates, and the solvent control contained six replicates.

3. Dose preparation:

First a primary stock solution was prepared by mixing BAS 560 F in acetone, at a nominal concentrations of 200 mg a.s./mL. The five different dosing stock solutions were obtained by further dilution of the primary stock solution in acetone. The dosing stock solutions were each mixed through an aliquot of the artificial sediment and these premixtures were placed in a fume hood for 1.5 hours for the acetone to partially evaporate. The premixtures were mixed through the rest of the artificial sediment. Each replicate received 100 g of sediment and then 400 mL of the dilution water was slowly added. The sediment/water mixtures were allowed to acclimate for two days prior to introduction of the test organisms.

4. Measurements and observations:

Before emergence of the first midge, behavior and mortality were recorded at least three times a week. From the onset of emergence, behavior and mortality were determined daily, as well as the gender of the emerged adults.

Analytical verification of test item concentrations in sediment, overlaying water and pore water samples, taken at exposure initiation and termination, was conducted using a HPLC-method with MS detection.

Temperature, dissolved oxygen concentration and pH were measured once a week.

5. Statistics:

Emergence rate and development rate were calculated using the appropriate equations and these data were statistically analyzed using ANOVA followed by Williams Multiple Sequential t-test Procedure ($\alpha = 0.05$). Statistical analysis was performed using ToxRatPro Version 2.10.

II. RESULTS AND DISCUSSION

A. BIOLOGICAL EFFECTS

First emerged midges were observed on day thirteen. No statistically significant differences were found for the emergence rates and the development rates at any test item concentration when compared to the solvent control. The results are summarized in the table below.

Table A 59: Effects of BAS 560 F (metrafenone) spiked sediment exposure on emergence and development of *Chironomus riparius*

Concentration (initial measured) (mg a.s./kg dry sediment)	Control	Solvent control (acetone)	17.7	34.7	80.5	164.0	296.0
Mean emergence rate, ER (SD)	0.8500 (0.0707)	0.8333 (0.0606)	0.8125 (0.0479)	0.8000 (0.0913)	0.7750 (0.0866)	0.7375 (0.1109)	0.7250 (0.1555)
Mean development rate per day, DR (SD)	0.0705 (0.0013)	0.0709 (0.0027)	0.0739 (0.0019)	0.0742 (0.0003)	0.0729 (0.0009)	0.0723 (0.0016)	0.0723 (0.0018)
	Endpoints (mg a.s./kg dry sediment) (initial mean measured)						
EC ₅₀ emergence	> 296.0						
NOEC _{emergence rate}	296.0						
NOEC _{development rate}	296.0						

SD = standard deviation

B. ANALYSIS

Mean recoveries of metrafenone in sediment were in the range of 80.3% - 102.3% of nominal concentrations. As recommended in OECD 218, the biological results were based on mean measured sediment concentrations at test initiation. Measured concentrations in overlaying water and pore water were quite similar and for nominal test concentrations of 80 mg a.s./kg sediment dw and higher the measured concentrations were close to the water solubility limit of metrafenone. In the overlaying water, the concentrations ranged from 0.056 to 0.624 mg a.s./L at test initiation and from 0.068 to 0.677 mg a.s./L at test termination. The pore water concentrations in the spiked sediment samples were between 0.039 and 0.901 mg a.s./L at test initiation and between 0.047 and 0.470 mg a.s./L at test termination.

Table A 60: Measured concentrations of BAS 560 F in the sediment in mg a.s./kg dry sediment

Nominal concentration (mg a.s./kg sediment dw)	Sampling time (days)	Measured concentration (mg a.s./kg sediment dw)	Mean measured as % of nominal
0 (negative control)	0 28	< LOQ	-
0 (solvent control)	0 28	< LOQ	-
20	0 28	17.7 16.1	88.5 80.5
40	0 28	34.7 33.5	86.8 83.8
80	0 28	80.5 72.7	100.6 90.9
160	0 28	164 129	102.5 80.6
320	0 28	296 276	92.5 86.3

LOQ = Limit of quantification (0.01 mg a.s./kg)

C. DEFICIENCIES

None.

III. CONCLUSION

The no-observed-effect concentration (NOEC) and lowest-observable-effect concentration (LOEC) for development rate and emergence rate of non-biting midges (*Chironomus riparius*) exposed to metrafenone for 28 days under static conditions were determined to be 296.0 and > 296.0 mg a.s./kg sediment dw, respectively, the highest mean measured test concentration in the spiked sediment. The EC₅₀ for emergence was > 296.0 mg a.s./kg sediment dw (initial mean measured). The EC_{10/20} calculation not applicable as NOEC at highest concentration tested. Although an EC₁₀ for the emergence endpoint could be calculated in principle, the data for each treatment level are variable and therefore, the confidence interval around an EC₁₀ estimate would likely be quite large.

A 2.3.3 KCP 10.2.3 Further testing on aquatic organisms

Not relevant.

A 2.4 KCP 10.3 Effects on arthropods

A 2.4.1 KCP 10.3.1 Effects on bees

A 2.4.1.1 KCP 10.3.1.1 Acute toxicity to bees

A 2.4.1.1.1 KCP 10.3.1.1.1 Acute oral toxicity to bees

A 2.4.1.1.1.1 Study 1

The following acute oral toxicity study with honey bees performed with pyraclostrobin is provided in support of the assessment and was recently submitted under the Annex I renewal process for pyraclostrobin (AIR 3). The study summary was provided in the revised Renewal Assessment Report of pyraclostrobin (RAR, Vol. 3, B.9, September 2021).

Comments of zRMS:	Study not evaluated by zRMS.
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Reference:	CP 10.3.1.1.1/1
Report	Effects of BAS 500 F (acute contact and oral) on honey bees (<i>Apis mellifera</i> L.) in the laboratory Sekine T., 2013 Report No 80831035 BASF DocID 2013/1003210 Authority registration No
Guideline(s):	OECD 213 (1998), OECD 214 (1998)
Deviations:	No
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive Summary

In a limit test, young adult worker bees (*Apis mellifera carnica*) were exposed orally to pyraclostrobin (BAS 500 F). Therefore, a nominal dose of 100.0 µg a.s./bee was tested, resulting in an actual uptake of 110.0 µg a.s./bee. Additionally, honey bees were treated with dimethoate as reference item at 0.05 to 0.32 µg/bee (nominal) or with water and solvent as control treatments. The test was conducted with 5 replicates per treatment group; each replicate contained 10 bees. Assessment of mortality was done after 4, 24 and 48 hours.

No mortality was observed after 48 hours in the control and the test item treatments. The LD₅₀ was determined to be > 110.0 µg a.s./bee. No behavioural abnormalities of the bees could be observed.

The oral LD₅₀ value (48 h) for pyraclostrobin was > 110.0 µg a.s./bee.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 500 F, batch no. 10-510009, content: pyraclostrobin (BAS 500 F, Reg. No. 304 428): 100% (± 1.0%).

Test species: Honey bee *Apis mellifera carnica*; young adult worker bees; derived from a healthy and queen-right colony, source: in-house culture; collected on the morning of use.

B. STUDY DESIGN

Test design: Limit test for oral toxicity; duration 48 h; 3 treatment groups (water control, solvent control, test item and 4 concentrations of the reference item) with 5 replicates per treatment group, each replicate consisting of 10 bees per cage; assessment of mortality after 4, 24 and 48 hours.

Endpoints: Mortality, resulting in an LD₅₀ value; behavioural abnormalities.

Reference item: Dimethoate (nominal 400 g/L).

Test doses: Water control, solvent control and 100.0 µg a.s./bee (nominal) resulting in an actual uptake of 110.0 µg a.s./bee.

Test conditions: Temperature: 24 – 25 °C; relative humidity: 56% – 81%; photoperiod: 24 h darkness.

Analytics: No analytical verification of the test item is required according to the current test guideline.

Statistics: Descriptive statistics.

II. RESULTS AND DISCUSSION

No mortality was observed after 48 hours in the control and the test item treatments. The LD₅₀ was determined to be > 110.0 µg a.s./bee. No behavioural abnormalities of the bees could be observed. The results are summarized in Table A 61.

Table A 61: Toxicity of pyraclostrobin to honey bees (*Apis mellifera*) in an oral toxicity test

Consumed dosage [µg a.s./bee]	Mortality [%]		
	4 h	24 h	48 h
Water control	0.0	0.0	0.0
Solvent control	0.0	0.0	0.0
110.0	0.0	0.0	0.0
Endpoint [µg a.s./bee]			
LD ₅₀ (48 h)	> 110.0		

The LD₅₀ value (24 h and 48 h) for the reference item in the oral toxicity test was determined to be 0.23 µg a.s./bee.

Validity criteria:

Validity criteria according to OECD 213 (1998)	Obtained in this study
Control mortality ≤ 10%	0% (water and solvent control)
LD ₅₀ (24 h) of the reference item should be in the specified range 0.10 – 0.35 µg a.s./bee	0.23 µg a.s./bee

All validity criteria were met.

III. CONCLUSION

The oral LD₅₀ value (48 h) for pyraclostrobin was > 110.0 µg a.s./bee.

A 2.4.1.1.1.2 Study 2

The following acute oral toxicity study with bumble bees performed with pyraclostrobin is provided in support of the assessment and was recently submitted under the Annex I renewal process for pyraclostrobin (AIR 3). The study summary was provided in the revised Renewal Assessment Report of pyraclostrobin (RAR, Vol. 3, B.9, September 2021).

Comments of zRMS:	Study not evaluated by zRMS.
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Reference:	CP 10.3.1.1.1/2
Report	Acute toxicity of BAS 500 F to the bumblebee <i>Bombus terrestris</i> L. under laboratory conditions Amsel K., 2016 Report No EU-773881, 16 10 48 031 B BASF DocID 2016/1000530 Authority registration No
Guideline(s):	Hanewald et al. (2013), OECD 213 (1998), OECD 214 (1998), Van der Steen (1996), Van der Steen (2001)
Deviations:	No
GLP:	yes (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive Summary

In an acute oral limit test, young adult worker bumble bees (*Bombus terrestris*) were exposed to BAS 500 F. The toxicity of the test item was determined at a dose rate of 100.0 µg a.s./bumble bee; the resulting oral uptake was 97.2 µg a.s./bumble bee (based on analyzed purity). Additionally, bumble bees were treated with Dimethoate EC 400 (a.s. dimethoate) as a reference item at dose rates ranging from 0.25 to 1.47 µg consumed dimethoate/bumble bee (analyzed), and furthermore with a 50% (w/v) sucrose solution and 50% (w/v) sucrose solution including 5% acetone as controls.

After 96 hours of oral exposure, no mortality occurred in the control group fed with 50% (w/v) sucrose solution or with the sucrose solution including 5% acetone. In the test item treatment, slight mortality of 1.7% was observed after oral consumption of 97.2 µg a.s./bumble bee, after 96 hours.

No behavioural effects of bumble bees were observed at all tested dose rates in the oral toxicity test.

In the acute oral toxicity test with BAS 500 F, the resulting LD₅₀ after 96 hours was estimated to be > 97.2 µg consumed a.s./bumble bee.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 500 F, batch no. COD-001236, content: pyraclostrobin (BAS 500 F, Reg. No. 304 428); purity: 99.02% analyzed.

B. STUDY DESIGN

Test species: *Bombus terrestris* L. (bumble bee), young adult worker bumble bees derived from healthy and queen-right hives; source: Biobest Belgium N.V., Westerlo, Belgium; collected on the morning prior to use.

Test design: In a 96-hour test, adults of *Bombus terrestris* were exposed to 1 dose of BAS 500 F in treated food (50% (w/v) sucrose solution including 5% acetone). In total, 4 treatment groups were set up: 1 dose rate of the test item, 2 control groups and 4 dose rates of the reference item with 60 replicates for the test item and sucrose control including 5% acetone and 30 replicates for the sucrose control and the reference item and 1 bumble bee per replicate, respectively. Assessments of bumble bee mortality and behavioural effects were done after 4, 24, 48, 72 and 96 hours.

Endpoints: Mortality, behavioural impairments.

Reference item: Dimethoate EC 400 (dimethoate, 400 g/L nominal).

Test doses: Control: 50% (w/v) sucrose solution, acetone control: 50% (w/v) sucrose solution including 5% acetone; BAS 500 F: 100.0 µg/bumble bee (resulting in an actual uptake of 97.2 µg BAS 500 F/bumble bee), reference item: 0.25, 0.45, 0.80 and 1.47 µg dimethoate/bumble bee.

Test conditions: Temperature: 24.9 °C – 25.1 °C, relative humidity: 59.6% – 60.5%, photoperiod: 24 h darkness; food: 50% (w/v) sucrose solution.

Analytics: No analytical verification of the test item was conducted.

Statistics: Descriptive statistics; Fisher's Exact Binomial Test with Bonferroni Correction for mortality data (one-sided greater, $\alpha = 0.05$).

II. RESULTS AND DISCUSSION

After 96 hours of oral exposure, no mortality occurred in the control group fed with 50% (w/v) sucrose solution or with the sucrose solution including 5% acetone. In the test item treatment, slight mortality of 1.7% was observed after oral consumption of 97.2 µg a.s./bumble bee, after 96 hours. The results are summarized below.

Table A 62 Toxicity of BAS 500 F to *Bombus terrestris* (bumble bee) in an oral toxicity test

Treatment	Dosage	Mortality [%]			
		24 h	48 h	72 h	96 h
Control	Sucrose	0.0	0.0	0.0	0.0
	Sucrose + 5% acetone	0.0	0.0	0.0	0.0
BAS 500 F [µg consumed a.s./bumble bee]	97.2	1.7	1.7	1.7	1.7
Endpoint [µg consumed a.s./bumble bee]					
LD ₅₀ (96 h)	> 97.2				

Validity criteria:

Validity criteria according to OECD 247 (2017)	Obtained in this study
Control mortality ≤ 10%	0% (water and solvent control)
Reference item mortality ≥ 50%	100% (at 1.47 µg a.s./bumble bee)

All validity criteria were met.

III. CONCLUSION

In the acute oral toxicity test with BAS 500 F, the resulting LD₅₀ after 96 hours was estimated to be > 97.2 µg consumed a.s./bumble bee.

A 2.4.1.1.1.3 Study 3

Comments of zRMS:	The study was conducted to OECD guidance 213 and according to the principles of GLP. All validity criteria were met. The study is considered to be reliable and suitable for the risk assessment.
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Reference:	CP 10.3.1.1.1/3
Report	Acute toxicity of BAS 758 00 F to the honeybee <i>Apis mellifera</i> L. under laboratory conditions, Franke, M., 2020 Report No 876356, 2048BAA0109, BASF DocID 2020/2037657 Authority registration No
Guideline(s):	OECD 213 (1998), OECD 214 (1998)
Deviations:	No
GLP:	yes (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany),
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive Summary

In an oral toxicity test, adult worker bees (*Apis mellifera* L. ssp. Buckfast) were exposed to BAS 758 00 F. The toxicity of the test item was determined at nominal dose rates of 60.0, 120, 240, 480 and 960 µg BAS 758 00 F/bee that is corresponding to 13.5, 27.1, 54.2, 108 and 21713.5, 27.1, 54.2, 108 and 217 µg total a.s./bee (based on sum of nominal content of a.s.), respectively. The resulting oral uptake was 60.0, 120, 240, 480 and 952 µg product/bee that is corresponding to 13.5, 27.1, 54.2, 108 and 215 µg total a.s./bee, respectively. Additionally, honeybees were treated with Dimethoate EC 400 as reference item at dose rates ranging from 0.086 to 0.250 µg dimethoate/bee (based on analyzed content of a.s.), and furthermore with a 50% (w/v) sucrose solution as control. Assessments of mortality and behavioral abnormalities were made after 4, 24 and 48 hours.

After 48 hours of oral exposure, no mortality occurred in the untreated control group fed with pure sucrose solution. In the test item treatment, statistically significant mortality was observed after oral consumption above 60.0 µg BAS 758 00 F/bee, after 48 hours. No behavioural effects were observed 24 and 48 hours after oral consumption of ≤ 952 µg BAS 763 00 F/bee when compared to the control group.

In an acute oral toxicity test with BAS 758 00 F on honey bees, the LD₅₀ (48 h) was determined to be 249 µg BAS 758 00 F/bee.

I. MATERIALS AND METHODS

A. MATERIALS

Test item: BAS 758 00 F; batch no. FD-200124-0004; content of a.s.: metrafenone (BAS 560 F, Reg. No. 4 037 710): 94.9 g/L analyzed (100.0 g/L nominal), mefentrifluconazole (BAS 750 F, Reg. No. 5 834 378): 66.7 g/L analyzed (66.6 g/L nominal), pyraclostrobin (BAS 500 F, Reg. No. 304 428): 81.0 g/L analyzed (80.0 g/L nominal); density: 1.092 g/cm³.

B. STUDY DESIGN

Test species: Honey bee (*Apis mellifera* L. Buckfast) adult worker bees, 3 - 5 weeks old; derived from a healthy and queen-right colony; collected in the morning of use from the top of the bee hive and directly transferred into each test cage without anesthesia; source: BioChem agrar GmbH, Leipzig, Germany.

Test design: In a 48-hour test, adult worker bees of *Apis mellifera* L. were exposed orally to BAS 758 00 F via treated food (50% (w/v) sucrose solution). The following treatment groups were set up: 5 concentration of the test item, 1 untreated control and 4 concentrations of the reference item with 3 replicates per treatment group and 10 bees per replicate, respectively. Assessments of honey bee mortality and behavioral effects were done after 4, 24 and 48 hours.

Endpoints: Mortality (LD₅₀), behavioral impairments.

Reference item: Dimethoate EC 400 (a.s.: dimethoate, analyzed content: 411.2 g/L, nominal: 400 g/L).

Test concentrations: Control: untreated sucrose solution (50% w/v); test item at dose rates of 60.0, 120, 240, 480 and 960 µg BAS 758 00 F/bee; reference item at dose rates of 0.086, 0.123, 0.175 and 0.250 µg a.s./bee.

Nominal doses		Consumed doses	
BAS 758 00 F [µg/bee]	Total active substances [µg a.s./bee]	BAS 758 00 F [µg/bee]	Total active substances [µg a.s./bee]
60.0	13.5	60.0	13.5
120	27.1	120	27.1
240	54.2	240	54.2
480	108	480	108
960	217	952	215

Test conditions: Temperature: 24.1 °C – 25.2 °C; relative humidity: 49% - 66%; photoperiod: 24 h darkness (with exception of diffuse artificial light during assessments and handling, only); food: 50% (w/v) sucrose solution.

Analytics: No analytical verification of the test item is required according to the current test guideline. Hence, no analytical verification was conducted.

Statistics: Descriptive statistics; Fisher's Exact Binominal Test with Bonferroni-Holm Correction for mortality data (one-sided greater, $\alpha = 0.05$).

II. RESULTS AND DISCUSSION

After 48 hours of oral exposure, no mortality occurred in the untreated control group fed with pure sucrose solution. In the test item treatment, statistically significant mortality was observed after oral consumption above 60.0 µg BAS 758 00 F/bee, after 48 hours (Fisher's Exact Binomial Test with Bonferroni Correction, one-sided greater, $\alpha = 0.05$). No behavioural effects were observed 24 and 48 hours after oral consumption of ≤ 952 µg BAS 763 00 F/bee when compared to the control group. The results are summarized in Table A 63.

Table A 63: Effects of BAS 758 00 F to honey bees (*Apis mellifera* L.) in an oral toxicity test

Treatment	Dosage [consumed]	After 24 hours		After 48 hours	
		Mortality [%]		Mortality [%]	
		total	corr.	total	corr.
Control	Sucrose solution	0.0	--	0.0	--
BAS 758 00 F [µg/bee]	60.0	3.3	--	10.0	--
	120	26.7 *	--	33.3 *	--
	240	46.7 *	--	50.0 *	--
	480	66.7 *	--	73.3 *	--
	952	80.0 *	--	80.0 *	--
Endpoint [µg consumed BAS 758 00 F/bee]					
249 (95% confidence limit: 183 - 340)					
LD ₅₀ (48 h)					

* Mortality statistically significant different compared to the control (Fisher's Exact Binomial Test with Bonferroni Correction, one-sided greater, $\alpha = 0.05$).

The LD₅₀ value (24 h) for the reference item in the oral toxicity test was determined to be 0.120 µg a.s./bee (95% confidence limits: 0.105 – 0.133 µg a.s./bee) - based on actual consumption.

Validity criteria:

Validity criteria according to OECD 213 (1998)	Obtained in this study
Control mortality $\leq 10\%$	0.0%
LD ₅₀ (24 h) of the reference item should be in the specified range 0.10 – 0.35 µg a.s./bee	0.120 µg a.s./bee

All validity criteria were met.

III. CONCLUSION

In an acute oral toxicity test with BAS 758 00 F on honey bees, the LD₅₀ (48 h) was determined to be > 249 µg BAS 758 00 F/bee.

A 2.4.1.1.2 KCP 10.3.1.1.2 Acute contact toxicity to bees

A 2.4.1.1.2.1 Study 1

The following acute contact toxicity study with honey bees performed with pyraclostrobin is provided in support of the assessment and was recently submitted under the Annex I renewal process for pyraclostrobin (AIR 3). The study summary was provided in the revised Renewal Assessment Report of pyraclostrobin (RAR, Vol. 3, B.9, September 2021).

Comments of zRMS:	Study not evaluated by zRMS.
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Reference:	CP 10.3.1.1.2/1
Report	Effects of BAS 500 F (acute contact and oral) on honey bees (<i>Apis mellifera</i> L.) in the laboratory, Sekine T., 2013 Report No 80831035 BASF DocID 2013/1003210 Authority registration No
Guideline(s):	OECD 213 (1998), OECD 214 (1998)
Deviations:	No
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive Summary

In a limit test, young adult worker bees (*Apis mellifera carnica*) were exposed to pyraclostrobin (BAS 500 F). The toxicity was determined in a contact limit test at a nominal dose of 100.0 µg a.s./bee. Additionally, honey bees were treated with dimethoate as reference item at 0.10 to 0.30 µg/bee or with water and solvent as a control groups. The test was conducted with 5 replicates per treatment group, each replicate contained 10 bees. Assessment of mortality was done after 4, 24 and 48 hours.

No mortality was observed in the control and test item treatments. The LD₅₀ was determined to be >100.0 µg a.s./bee. One single bee showed a moving coordination problem during the 48 hours assessment. This was the only occurrence of test item related behavioural abnormalities during the entire time of the contact test.

The contact LD₅₀ value (48 h) for pyraclostrobin was > 100.0 µg a.s./bee.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 500 F, batch no. 10-510009, content: pyraclostrobin (BAS 500 F, Reg. No. 304 428): 100% (\pm 1.0%).

Test species: Honey bees *Apis mellifera carnica*; young adult worker bees; derived from a healthy and queen-right colony, source: in-house culture; collected on the morning of use.

B. STUDY DESIGN

Test design: Limit test for contact toxicity; duration 48 h; 4 treatment groups (water control, solvent control, test item and 4 concentrations of the reference item) with 5 replicates per treatment group, each replicate consisting of 10 bees per cage; assessment of mortality after 4, 24 and 48 hours.

Endpoints: Mortality, resulting in a LD₅₀ value; behavioural abnormalities.

Reference item: Dimethoate (nominal 400 g/L).

Test doses: Water control, solvent control and 100.0 µg a.s./bee.

Test conditions: Temperature: 24 – 25 °C; relative humidity: 56% – 81%; photoperiod: 24 h darkness.

Analytics: No analytical verification of the test item is required according to the current test guideline.

Statistics: Descriptive statistics.

II. RESULTS AND DISCUSSION

No mortality was observed in the control and test item treatments. The LD₅₀ was determined to be >100.0 µg a.s./bee. One single bee showed a moving coordination problem during the 48 hours assessment. This was the only occurrence of test item related behavioural abnormalities during the entire time of the contact test. The results are summarized in Table A 64.

Table A 64: Toxicity of pyraclostrobin to honey bees (*Apis mellifera*) in a contact toxicity test

Treatment [µg a.s./bee]	Mortality [%]		
	4 h	24 h	48 h
Water control	0.0	0.0	0.0
Solvent control	0.0	0.0	0.0
100.0	0.0	0.0	0.0
Endpoint [µg a.s./bee]			
LD ₅₀ (48 h)	> 100.0		

The LD₅₀ value (24 h) for the reference item in the contact toxicity test was determined to be 0.27 µg a.s./bee.

Validity criteria:

Validity criteria according to OECD 214 (1998)	Obtained in this study
Control mortality $\leq 10\%$	0.0%
LD ₅₀ (24 h) of the reference item should be in the specified range 0.10 – 0.30 µg a.s./bee	0.27 µg a.s./bee

All validity criteria were met.

III. CONCLUSION

The contact LD₅₀ value (48 h) for pyraclostrobin was > 100.0 µg a.s./bee.

A 2.4.1.1.2.2 Study 2

The following acute oral toxicity study with bumble bees performed with pyraclostrobin is provided in support of the assessment and was recently submitted under the Annex I renewal process for pyraclostrobin (AIR 3). The study summary was provided in the revised Renewal Assessment Report of pyraclostrobin (RAR, Vol. 3, B.9, September 2021).

Comments of zRMS:	Study not evaluated by zRMS.
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Reference:	CP 10.3.1.1.2/2
Report	Acute toxicity of BAS 500 F to the bumblebee <i>Bombus terrestris</i> L. under laboratory conditions Amsel K., 2016 Report No EU-773881, 16 10 48 031 B BASF DocID 2016/1000530 Authority registration No
Guideline(s):	Hanewald et al. (2013), OECD 213 (1998), OECD 214 (1998), Van der Steen (1996), Van der Steen (2001)
Deviations:	No
GLP:	yes (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive Summary

In an acute contact limit test, young adult worker bumble bees (*Bombus terrestris*) were exposed to BAS 500 F. The toxicity of the test item was determined at a dose rate of 100.0 µg a.s./bumble bee (based on analyzed purity). Additionally, bumble bees were treated with Dimethoate EC 400 (a.s. dimethoate) as a reference item at dose rates ranging from 2.5 to 10.1 µg dimethoate/bumble bee (analyzed), and furthermore with deionized water, TritonX solution and acetone as controls.

After 96 hours, 3.3% mortality occurred in the control group treated with deionized water and no mortality occurred in the control groups treated with TritonX solution and acetone. In the test item treatment, no mortality occurred after thoracic application of 100.0 µg a.s./bumble bee, after 96 hours. No behavioural effects of bumble bees were observed at all tested dose rates in the contact toxicity test.

In the acute contact toxicity test with BAS 500 F, the resulting LD₅₀ after 96 hours was estimated to be > 100.0 µg a.s./bumble bee.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 500 F, batch no. COD-001236, content: pyraclostrobin (BAS 500 F, Reg. No. 304 428); purity: 99.02% analyzed.

B. STUDY DESIGN

Test species: *Bombus terrestris* L. (bumble bee), young adult worker bumble bees derived from healthy and queen-right hives; source: Biobest Belgium N.V., Westerlo, Belgium; collected on the morning prior to use.

Test design: In a 96 hour test, adults of *Bombus terrestris* were exposed to 1 dose rate of BAS 500 F in an appropriate carrier (acetone) placed on the dorsal bumble bee thorax. In total, 5 treatment groups were set up: 1 dose rate of the test item, 3 control groups and 4 dose rates of the reference item. For the test item and acetone control 60 replicates and for the reference item, water and TritonX control 30 replicates and 1 bumble bee per replicate were used, respectively. Assessments of bumble bee mortality and behavioural effects were done after 4, 24, 48, 72 and 96 hours.

Endpoints: Mortality, behavioural impairments.

Reference item: Dimethoate EC 400 (dimethoate, 400 g/L nominal).

Test doses: Control: deionized water, acetone control: pure acetone, TritonX control: 0.5% (v/v) TritonX solution; BAS 500 F: 100.0 µg/bumble bee (the test item was solved in acetone), reference item: 2.5, 4.0, 6.4 and 10.1 µg dimethoate/bumble bee.

Test conditions: Temperature: 24.9 – 25.1 °C, relative humidity: 59.6% – 60.5%, photoperiod: 24 h darkness; food: 50% (w/v) sucrose solution.

Analytics: No analytical verification of the test item was conducted.

Statistics: Descriptive statistics; Fisher's Exact Binomial Test with Bonferroni Correction for mortality data (one-sided greater, $\alpha = 0.05$).

II. RESULTS AND DISCUSSION

After 96 hours, 3.3% mortality occurred in the control group treated with deionized water and no mortality occurred in the control groups treated with TritonX solution and acetone. In the test item treatment, no mortality occurred after thoracic application of 100.0 µg a.s./bumble bee, after 96 hours. The results are summarized below.

Table A 65 Toxicity of BAS 500 F to *Bombus terrestris* (bumble bee) in a contact toxicity test

Treatment	Dosage	Mortality [%]			
		24 h	48 h	72 h	96 h
Control	deionized water	0.0	0.0	3.3	3.3
	0.5% Triton X	0.0	0.0	0.0	0.0
	acetone	0.0	0.0	0.0	0.0
BAS 500 F [µg a.s./bumble bee]	100	0.0	0.0	0.0	0.0
Endpoint [µg a.s./bumble bee]					
LD ₅₀ (96 h)	> 100.0				

Validity criteria:

Validity criteria according to OECD 246 (2017)	Obtained in this study
Control mortality ≤ 10%	0% (both solvent controls) 3.3% (water control)
Reference item mortality ≥ 50%	100% (at 6.4 µg a.s./bumble bee)

All validity criteria were met.

III. CONCLUSION

In the acute contact toxicity test with BAS 500 F, the resulting LD₅₀ after 96 hours was estimated to be > 100.0 µg a.s./bumble bee.

A 2.4.1.1.2.3 Study 3

Comments of zRMS:	The study was conducted to OECD guidance 214 and according to the principles of GLP. All validity criteria were met. The study is considered to be reliable and suitable for the risk assessment.
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Reference:	CP 10.3.1.1.2/3
Report	Acute toxicity of BAS 758 00 F to the honeybee <i>Apis mellifera</i> L. under laboratory conditions, Franke, M., 2020 Report No 876356, 2048BAA0109, BASF DocID 2020/2037657 Authority registration No
Guideline(s):	OECD 213 (1998), OECD 214 (1998)
Deviations:	No
GLP:	yes (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany),
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive Summary

In a contact toxicity test, adult worker bees (*Apis mellifera* L. ssp. Buckfast) were exposed to BAS 758 00 F. The toxicity of the test item was determined at nominal dose rates of 60.0, 120, 240, 480 and 960 µg BAS 758 00 F/bee that is corresponding to 13.5, 27.1, 54.2, 108 and 217 µg total a.s./bee (based on sum of nominal content of a.s.), respectively. Additionally, honey bees were treated with Dimethoate EC 400 as reference item at dose rates ranging from 0.105 to 0.250 µg a.s./bee (based on analyzed content of a.s.: dimethoate), and furthermore with deionized water and tween solution (deionized water + wetting agent) as controls. Assessments of mortality and behavioral abnormalities were made after 4, 24, 48, 72 and 96 hours.

After 48 hours of contact exposure, no mortality occurred in the control groups either treated with deionized water or tween solution. In the test item treatment, statistically significant mortality of 20.0 and 76.7% was observed after thoracic application of 480 and 960 µg BAS 758 00 F/bee, after 48 hours. Due to a significant increase of the be mortality by more than 10% between the 24 h and 48 h assessments, the contact test was extended up to 96 hours. After 96 hours, no mortality was observed in the control groups either treated with deionized water or tween solution. In the test item treatment, statistically significant mortality of 23.3 and 76.7% was observed after thoracic application of 480 and 960 µg BAS 758 00 F/bee, respectively, after 96 hours. Effects on behaviour were predominantly observed at the higher dose rates of ≥ 480.0 µg BAS 758 00 F/bee and at the 4 h and 24 h assessments.

In an acute contact toxicity test with BAS 758 00 F on honey bees, the LD₅₀ (96 h) was determined to be 685 µg BAS 758 00 F/bee.

I. MATERIALS AND METHODS

A. MATERIALS

Test item: BAS 758 00 F; batch no. FD-200124-0004; content of a.s.: metrafenone (BAS 560 F, Reg. No. 4 037 710): 94.9 g/L analyzed (100.0 g/L nominal), mefentrifluconazole (BAS 750 F, Reg. No. 5 834 378): 66.7 g/L analyzed (66.6 g/L nominal), pyraclostrobin (BAS 500 F, Reg. No. 304 428): 81.0 g/L analyzed (80.0 g/L nominal); density: 1.092 g/cm³.

B. STUDY DESIGN

Test species: Honey bee (*Apis mellifera* L. ssp. Buckfast) adult worker bees, 3 - 5 weeks old; derived from a healthy and queen-right colony; collected in the morning of use from the top of the bee hive and directly transferred into each test cage without anesthesia; source: BioChem agrar GmbH, Leipzig, Germany.

Test design: In a 96-hour test, adult worker bees of *Apis mellifera* L. were exposed to BAS 758 00 F in an appropriate carrier (Tween®80 as wetting agent) placed on the dorsal bee thorax. The following treatment groups were set up: 5 concentrations of the test item, 2 untreated control and 4 concentrations of the reference item with 3 replicates per treatment group and 10 bees per replicate, respectively. Application volume of 2 µL/bee. Assessments of honey bee mortality and behavioral effects were done after 4, 24, 48, 72 and 96 hours.

Endpoints: Mortality (LD₅₀), behavioral impairments.

Reference item: Dimethoate EC 400 (a.s.: dimethoate, analyzed content: 411.2 g/L, nominal: 400 g/L).

Test concentrations: Untreated control: deionized water; Solvent control: deionized water + 1% (v/v) Tween®80; Test item at dose rates of 60.0, 120, 240, 480 and 960 µg BAS 758 00 F/bee; reference item at dose rates of 0.105, 0.141, 0.188 and 0.250 µg a.s./bee.

Nominal doses	
BAS 758 00 F [µg/bee]	Total active substances [µg a.s./bee]
60.0	13.5
120	27.1
240	54.2
480	108
960	217

Test conditions: Temperature: 24.1 °C – 25.2 °C; relative humidity: 49% - 66%; photoperiod: 24 h darkness (with exception of diffuse artificial light during assessments and handling, only); food: 50% (w/v) sucrose solution.

Analytics: No analytical verification of the test item is required according to the current test guideline. Hence, no analytical verification was conducted.

Statistics: Descriptive statistics; Fisher's Exact Binominal Test with Bonferroni-Holm Correction for mortality data (one-sided greater, $\alpha = 0.05$).

II. RESULTS AND DISCUSSION

After 48 hours of contact exposure, no mortality occurred in the control groups either treated with deionized water or tween solution. In the test item treatment, statistically significant mortality of 20.0 and 76.7% was observed after thoracic application of 480 and 960 µg BAS 758 00 F/bee, after 48 hours (Fisher's Exact Binomial Test with Bonferroni Correction, one-sided greater, $\alpha = 0.05$). Due to a significant increase of the be mortality by more than 10% between the 24 h and 48 h assessments, the contact test was extended up to 96 hours. After 96 hours, no mortality was observed in the control groups either treated with deionized water or tween solution. In the test item treatment, statistically significant mortality of 23.3 and 76.7% was observed after thoracic application of 480 and 960 µg BAS 758 00 F/bee, respectively, after 96 hours. Effects on behaviour were predominantly observed at the higher dose rates of ≥ 480.0 µg BAS 758 00 F/bee and at the 4 h and 24 h assessments. The results are summarized in Table A 66.

Table A 66: Effects of BAS 758 00 F to honey bees (*Apis mellifera* L.) in a contact toxicity test

Treatment	Dosage [applied]	After 24 hours		After 48 hours		After 72 hours		After 96 hours	
		Mortality [%]		Mortality [%]		Mortality [%]		Mortality [%]	
		total	corr.	total		total		total	corr.
Control	Water	0.0	--	0.0		0.0		0.0	--
	Tween	0.0	--	0.0		0.0		0.0	--
BAS 758 00 F [µg/bee]	60.0	0.0	--	0.0		0.0		0.0	--
	120	0.0	--	0.0		0.0		0.0	--
	240	0.0	--	0.0		0.0		0.0	--
	480	16.7 *	--	20.0 *		20.0 *		23.3 *	--
	960	56.7 *	--	76.7 *		76.7 *		76.7 *	--
	Endpoint [µg/bee]								
LD ₅₀ (96 h)	685 (95% confidence limit: 587 - 816)								

* Mortality statistically significant different compared to the control (Fisher's Exact Binomial Test with Bonferroni Correction, one-sided greater, $\alpha = 0.05$).

The LD₅₀ value (24 h) for the reference item in the contact toxicity test was determined to be 0.150 µg a.s./bee (95% confidence limits: 0.136 – 0.164 µg a.s./bee).

Validity criteria:

Validity criteria according to OECD 214 (1998)	Obtained in this study
Control mortality $\leq 10\%$	0.0% water control 0.0% tween control
LD ₅₀ (24 h) of the reference item should be in the specified range 0.10 – 0.30 µg a.s./bee	0.150 µg a.s./bee

All validity criteria were met.

III. CONCLUSION

In an acute contact toxicity test with BAS 758 00 F on honey bees, the LD₅₀ (96 h) was determined to be 685 µg BAS 758 00 F/bee.

A 2.4.1.2 KCP 10.3.1.2. Chronic toxicity to bees

A 2.4.1.2.1 Study 1

The following chronic toxicity study with adult honey bees performed with pyraclostrobin is provided in support of the assessment and was recently submitted under the Annex I renewal process for pyraclostrobin (AIR 3). The study summary was provided in the revised Renewal Assessment Report of pyraclostrobin (RAR, Vol. 3, B.9, September 2021).

Comments of zRMS:	Study not evaluated by zRMS.
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Reference:	CP 10.3.1.2/1
Report	Honey bee (<i>Apis mellifera</i>), chronic oral toxicity test with BAS 500 F (Pyraclostrobin) under laboratory conditions, Altenburg M., Obermann M., 2017 Report No EU-761022 BASF DocID 2017/1142796 Authority registration No
Guideline(s):	OECD proposal for a new guideline <i>Apis mellifera</i> L. chronic 2016, OECD-ENV/JM/MONO/(2007)17, SANCO/3029/99 rev. 4 (11 July 2000)
Deviations:	No
GLP:	yes (certified by Landesamt fuer Umwelt, Mainz, Germany)
Acceptability:	Yes
Duplication (if vertebrate study)	No

Reference:	CP 10.3.1.2/2
Report	Amendment 1: Honey bee (<i>Apis mellifera</i>), chronic oral toxicity test with BAS 500 F (Pyraclostrobin) under laboratory conditions, Altenburg, M., Obermann, M., 2019 Report No 761022 BASF DocID 2019/1024628 Authority registration No
Guideline(s):	OECD proposal for a new guideline <i>Apis mellifera</i> L. chronic 2016, OECD-ENV/JM/MONO/(2007)17, SANCO/3029/99 rev. 4 (11 July 2000)
Deviations:	No
GLP:	yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive Summary

In a 10-day chronic oral toxicity test, max. 2-day old worker honey bees (*Apis mellifera*) were exposed to 50% (w/v) aqueous sugar (sucrose) solution containing the test item BAS 500 F by continuous and *ad libitum* feeding. The chronic toxicity of the test item was determined at nominal doses of 1, 2, 4, 8 and 16 µg a.s./bee/day (effective doses were 0.75, 1.03, 1.96, 3.77 and 6.45 µg a.s./bee/day), corresponding to nominal concentrations of 40.7, 81.3, 162.6, 325.2 and 650.4 mg a.s./kg food, respectively. Additionally, honey bees were treated with Dimethoate EC 400 (BAS 152 11 I) as a reference item at a nominal dose of 46.2 ng BAS 152 11 I/bee/day (corresponding to 17.2 ng dimethoate/bee/day). Untreated diet served as control, and untreated diet with acetone served as a solvent control.

After 10 days of continuous exposure, no mortality was observed in the control, the solvent control as well as in the two lowest test item treatments (i.e., at nominal doses of 1 and 2 µg a.s./bee/day corresponding to nominal concentrations of 40.7 and 81.3 mg a.s./kg food). However, statistically significantly increased mortalities (compared to the solvent control) of 20.0%, 73.3% and 73.3% were observed in test item treatments of 4, 8 and 16 µg a.s./bee/day (corresponding to nominal concentrations of 162.6, 325.2 and 650.4 mg a.s./kg food).

During the study, minor sublethal effects were observed in the test item treatment of 8 µg a.s./bee/day, where 3 moribund bees were observed and in the test item treatment of 16 µg a.s./bee/day where up to 7 bees were affected.

Due to low analytical recovery rates of BAS 500 F in the feeding solution, the nominal dose and concentration of the test item fed in the study was calculated according to the analyzed recovery. Therefore, endpoints are based on corrected doses and concentrations, respectively.

In a 10-day chronic toxicity feeding test with BAS 500 F (Reg. No. 304 428), the NOED was determined to be 1.0 µg a.s./bee/day, and the NOEC to be 57.6 mg a.s./kg food. The LD₅₀ and the LC₅₀ were determined to be 3.4 µg a.s./bee/day and 208.5 mg a.s./kg food, respectively.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 500 F (Reg. No. 304 428); batch no.: L81-2; analyzed purity: 99.9% (tolerance $\pm 1.0\%$).

B. STUDY DESIGN

Test species: *Apis mellifera carnica* (honey bee); max. 2 day old bees; derived from healthy and queen right colonies; source: in-house colonies.

Test design: In a 10-day test, young adults of *Apis mellifera* were exposed daily to 5 doses of BAS 500 F dissolved with acetone in treated food (50% (w/v) aqueous sucrose solution). In total, 8 treatment groups were set up: control (1 untreated control and 1 solvent control), test item (5 doses) and reference item (1 dose) with 3 replicates per dose and 10 bees per replicate. Assessments of bee mortality and behavioural effects were done daily during the study.

Endpoints: Mortality, behavioural impairments.

Reference item: BAS 152 11 I (dimethoate, analyzed content of a.s.: 405.9 g/L).

Test doses: Control: untreated diet (50% (w/v) aqueous sucrose solution)
Solvent control: untreated diet (50% (w/v) aqueous sucrose solution with acetone)

Test item treatments:

Nominal dose [µg a.s./bee/day]	Effective corrected dose [µg a.s./bee/day] ¹⁾	Nominal concentration [mg a.s./kg food]	Corrected concentration [mg a.s./kg food] ²⁾
1	0.8	40.7	31.2
2	1.0	81.3	57.6
4	2.0	162.6	115.2
8	3.8	325.2	230.3
16	6.5	650.4	422.5

¹⁾ Based on the effective uptake of food during the study and corrected by reduced analytical recovery.

²⁾ Corrected test concentration due to a reduced recovery of the test item in the feeding solutions.

Reference item: 17.2 ng dimethoate/bee corresponding to 0.7 mg dimethoate/kg food.

Test conditions: Temperature: 29.3 °C - 33.4 °C; relative humidity: 32.5% - 65.3%, photoperiod: 24 h darkness; food: 50% (w/v) aqueous sucrose solution.

Analytics: Analytical verification of test item concentrations was conducted using LC-MS/MS.

Statistics: Descriptive statistics; Step-down Cochran-Armitage Test Procedure for mortality; calculation of NOEC and NOEDD of the test item ($\alpha = 0.05$; one-sided greater) with Qualitative Trend Analysis by Contrasts (Monotonicity of Concentration/Response) and Tarone's Test Procedure ($\alpha = 0.010$). Probit analysis using linear max. likelihood regression for calculation of $EC_{(x)}$ values.

C. DESCRIPTION OF THE ANALYTICAL PROCEDURES

Concentrations of BAS 500 F in feeding solution were determined according to the analytical method L0357/01. The validation of the analytical method is described in the study report. 0.5 g of honey bee feeding solution is weighed into a culture tube with screw cap and spiked with fortification solution (prepared in methanol). The sample is extracted with 10 mL of acetonitrile/water (50/50, v/v) and sonicated for 5 min. Further dilutions with methanol/water (50/50, v/v) in the range of the calibration curve were prepared. The determination was performed by LC-MS/MS. The limit of quantification (LOQ) was 20 mg/kg and the limit of detection (LOD) was set to 4 mg/kg. The influence of the matrix-load was investigated by using quality control samples. The recovery values of all quality control samples were always in an acceptable range with a mean recovery of 101%, therefore no significant matrix effect has been identified. The investigations on the stability of the analyte in the feeding solution showed no significant decrease within the tested period of 7 days, under refrigerated conditions. Details on measured fortification samples and obtained procedural recoveries for BAS 500 F are given in the tables below.

Table A 67: Recovery Data of BAS 500 F in honey bee feeding solutions

Mass Transition	Fortification Level [mg/L]	Number of Replicates	Mean Recovery [%]	RSD [%]	Mean Recovery [%]	RSD [%]
388 → 194	20	5	88	3.9	93	6.5
	650	5	98	2.9		
388 → 163	20	5	90	4.0	94	6.0
	650	5	98	4.7		

II. RESULTS AND DISCUSSION

After 10 days of continuous exposure, no mortality was observed in the control, the solvent control as well as in the two lowest test item treatments (i.e., at nominal doses of 1 and 2 µg a.s./bee/day corresponding to nominal concentrations of 40.7 and 81.3 mg a.s./kg food). However, statistically significantly increased (compared to the solvent control; Step-down Cochran-Armitage Test Procedure; $\alpha = 0.05$; one-sided greater) mortalities of 20.0%, 73.3% and 73.3% were observed in test item treatments of 4, 8 and 16 µg a.s./bee/day (corresponding to nominal concentrations of 162.6, 325.2 and 650.4 mg a.s./kg food). During the study, minor sublethal effects were observed in the test item treatment of 8 µg a.s./bee/day, where 3 moribund bees were observed and in the test item treatment of 16 µg a.s./bee/day where up to 7 bees were affected. The results are summarized in the table below:

Table A 68: Cumulative mortality and toxicity endpoints of honey bees (*Apis mellifera* L.) exposed to BAS 500 F in a chronic oral toxicity test

Nominal mean doses [µg a.s./bee/day]	Effective corrected dose ¹⁾ [µg a.s./bee/day]	Corrected concentration ²⁾ [g a.s./kg food]	Mortality after 10 days
			Cumulative mortality [%]
Control	--	--	0.0
Solvent control	--	--	0.0
1	0.8	31.2	0.0
2	1.0	57.6	0.0
4	2.0	115.2	20.0*
8	3.8	230.3	73.3*
16	6.5	422.5	73.3*
Endpoints		10 days	
Test item doses ¹⁾ [µg consumed a.s./bee/day]	NOEDD ³⁾	1.0	
	LDD ₁₀ ⁴⁾	1.5 (95% CL: 0.2 – 2.4)	
	LDD ₂₀ ⁴⁾	2.0 (95% CL: 0.6 – 3.1)	
	LDD ₅₀ ⁴⁾	3.4 (95% CL: 2.0 – 7.3)	
Test item concentrations ²⁾ [g a.s./kg food]	NOEC ³⁾	57.6	
	LC ₁₀ ⁴⁾	85.2 (95% CL: 7.1 – 144.3)	
	LC ₂₀ ⁴⁾	115.8 (95% CL: 21.5 – 189.3)	
	LC ₅₀ ⁴⁾	208.5 (95% CL: 112.0 – 511.7)	

95% CL = 95 %-confidence limits

* Statistically significantly different compared to the solvent control (Step-down Cochran-Armitage Test Procedure; $\alpha = 0.05$; one-sided greater).

¹⁾ Based on the effective uptake of food during the study and corrected by reduced analytical recovery.

²⁾ Corrected test concentration due to a reduced recovery of the test item in the feeding solutions.

³⁾ Step-down Cochran-Armitage Test Procedure; $\alpha = 0.05$; one-sided greater.

⁴⁾ Probit analysis using linear max. likelihood regression.

In the reference item treatment, the mortality was 100% after 10 days of exposure.

Validity criteria:

Validity criteria according to OECD 245 (2017)	Obtained in this study
Control mortality from $\leq 15\%$ at D10 across all replicates	0% untreated control 0% solvent control
Reference item mortality $\geq 50\%$ on D10	100%

All validity criteria were met.

III. CONCLUSION

In a 10-day chronic toxicity feeding test with BAS 500 F (Reg. No. 304 428), the NOED was determined to be 1.0 µg a.s./bee/day, and the NOEC to be 57.6 mg a.s./kg food. The LD₅₀ and the LC₅₀ were determined to be 3.4 µg a.s./bee/day and 208.5 mg a.s./kg food, respectively.

A 2.4.1.2.2 Study 2

The following chronic toxicity study with adult honey bees performed with BAS 560 02 F and BAS 560 AA F (formulations of BAS 560 F, metrafenone) is provided in support of the assessment and was recently submitted under the Annex I renewal process for metrafenone (AIR 3). The study summary was provided in the revised Renewal Assessment Report of metrafenone (RAR, Vol. 3, B.9, December 2019).

Comments of zRMS:	Study not evaluated by zRMS.
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Reference:	CP 10.3.1.2/3
Report	BAS 560 02 F and BAS 560 AA F blank formulation - Assessment of effects on the adult honey bee, <i>Apis mellifera</i> L., in 10 days chronic feeding test under laboratory conditions, Verge, E., 2014 Report No EU-S14-00029, EU-427151,S14-00029 BASF DocID 2014/1093920 Authority registration No
Guideline(s):	None. No guidelines available: based on OECD Guideline Proposal (2013) (If none, give justification, e.g., “ no guidelines available” or “ methods used comparable to guideline(s) xxx”)
Deviations:	No
GLP:	yes (certified by Landesanstalt fuer Umwelt, Messungen und Naturschutz Baden-Wuerttemberg, Karlsruhe, Germany),
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive Summary

The toxicity of BAS 560 02 F to the honey bee *Apis mellifera* was determined in a chronic feeding study in the laboratory. Young adult worker bees were exposed for ten days to exposure solutions, which were replaced and weighted daily, with nominal concentrations of 357.1, 714.3, 1428.5, 2857 and 5714 mg a.s./kg food. The blank formulation BAS 560 AA F (at a nominal concentration of 7730 mg product/kg food), a negative control (feeding solution only) and a reference item (Perfekthion, at a nominal concentration of 0.9 mg dimethoate/kg food) were tested in parallel. Four replicates were tested for each test substance concentration, the blank formulation, the negative control and the reference item.

With the food intake measurements, the consumed concentrations were calculated. For the nominal concentrations of 357.1, 714.3, 1428.5, 2857 and 5714 mg a.s./kg food for BAS 560 02 F the measured uptake was 15.0, 32.6, 62.3, 128 and 291 µg a.s./bee/day. For BAS 560 AA F the measured uptake was 372 µg product/bee/day.

Mortality in the groups exposed to BAS 560 02 F ranged from 0 to 10% and was not statistically significantly different from the mortality in the control group (5%). No mortality was observed in the group exposed to the blank formulation BAS 560 AA F, indicating that the observed effects for BAS 560 02 F were caused by the active substance and not the formulation. Some bees exposed to BAS 560 02 F at concentrations of 714.3 mg a.s./kg food and higher showed sub-lethal effects such as reduced coordination.

The 10-day LC_{50} value for *Apis mellifera* exposed to BAS 560 02 F was determined to be > 5714 mg a.s./kg food (nominal), which corresponds to a 10-day LDD_{50} > 291 µg a.s./bee/day (measured). The no-observed-effect concentration (NOEC) was determined to be 5714 mg a.s./kg food, which corresponds to a no-observed-effect daily dose (NOEDD) of 291 µg a.s./bee/day.

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test material:** BAS 560 02 F
Batch number: 0008024566
Purity: 501.7 g/L metrafenone (analyzed)
Description: Beige liquid

Blank formulation: BAS 560 AA F
Batch number: FD-140630-0026
Purity: Without metrafenone
Description: Beige liquid
2. **Test concentrations:** BAS 560 02 F: 357.1, 714.3, 1428.5, 2857 and 5714 mg a.s./kg food (nominal; effective mean uptake: 15.0, 32.6, 62.3, 128 and 291 µg a.s./bee/day)
BAS 560 AA F: 7730 mg product/kg food (nominal; effective mean uptake: 372 µg product/bee/day)
3. **Reference item:** Perfekthion (BAS 152 11 I, containing 400.9 g dimethoate/L), tested at a concentration of 0.9 mg a.s./kg food (nominal; effective mean uptake: 0.02 µg a.s./bee/day)
4. **Vehicle:** 50% (w/v) aqueous sucrose solution
5. **Test organism:**
Species: Honey bee *Apis mellifera* L.
Age/life stage: Young adult worker bees
Source: Healthy colonies, descended from a breeding line of a beekeeper in Baden-Württemberg, Germany
Diet: Treated or untreated 50% (w/v) aqueous sucrose solution, *ad libitum*
Test units: Stainless steel cages (8 x 4 x 6 cm), with a transparent front side to enable observations and a perforated board at the bottom to guarantee sufficient air supply. The feeding solutions were offered in syringes

B. STUDY DESIGN

1. **Environmental conditions:**
Temperature: 32.3 – 33.4°C
Relative humidity: 53.2 – 62.0%
Photoperiod: Continuous darkness, except when the feeding solutions were replaced and the assessments were made
2. **Assignment and treatment:**

At test initiation, ten bees were added to each test unit. Four replicate test units were tested in each treatment, reference item and control group. The bees were continuously exposed to freshly prepared feeding solutions for ten days with treated (BAS 560 02 F, BAS 560 AA F or reference item) or untreated (control) 50% (w/w) aqueous sucrose solution.

3. Dose preparation:

A stock solvent solution was prepared (fresh daily) by dissolving BAS 560 02 F or BAS 560 AA F in tap water, at a concentration of 533 mg product/mL. This solution was diluted with 50% (w/v) aqueous sucrose solution to reach the nominal test concentrations of 357.1, 714.3, 1428.5, 2857 and 5714 mg a.s./kg food for BAS 560 02 F and 7730 mg product/kg food for BAS 560 AA F. The negative control group was exposed to untreated 50% (w/v) aqueous sucrose solution and the reference item group received a 50% (w/v) aqueous sucrose solution containing the reference item Perfekthion at a concentration of 0.9 mg a.s./kg food.

4. Measurements and observations:

Mortality and sub-lethal effects (moribund, affected, apathy, cramps or vomiting) were recorded daily during the exposure period of ten days. In addition, the weight of the syringes containing the feeding solutions was determined daily before and after feeding.

Temperature and relative humidity were recorded continuously throughout the test period of ten days.

5. Statistics:

Fisher's test (Bonferroni-Holms corrected) was used to evaluate differences between mortality data of bees treated with BAS 560 02 F and bees treated with BAS 560 AA F, and between these treated groups and the control group. These calculations were performed using ToxRat Professional 2.10.

II. RESULTS AND DISCUSSION

A. MORTALITY AND BEHAVIOUR

Apis mellifera in the control group showed a cumulative mortality of 5% after ten days (see table below) and no sub-lethal effects were observed for these bees during the test period. Mortality in the groups exposed to BAS 560 02 F and BAS 560 AA F was not statistically significantly different from the mortality in the control group. In the group exposed to the reference item Perfekthion 97.4% (corrected) of the bees died.

In the BAS 560 02 F treatment group, no behavioral abnormalities were observed at the lowest concentration. At the concentrations of 714.3 mg a.s./kg food one moribund bee (showing only very feeble movements) was observed at the assessment Day 9. Affected bees (reduced coordination) were recorded at the assessments Day 5 to Day 7 at the concentration of 1428.5 mg a.s./kg food, at the assessments Day 5 and Day 6 at the concentration of 2857 mg a.s./kg food and at the assessments Day 2 and Day 5 to Day 10 at the concentration of 5714 mg a.s./kg food. In the BAS 560 AA F treatment group, no behavioral abnormalities were observed during the entire 10-day test period.

The table below presents the percent mortality and sub-lethal effects.

Table A 69: Mortality and sub-lethal effects for *Apis mellifera* exposed to BAS 560 02 F and the blank formulation BAS 560 AA F for ten days

Treatment	Concentration	Mean mortality		Cumulative number of affected honey bees ³
	Nominal (mg/kg food)	(%)	Corrected (%) ^{1,2}	
Control	0.0	5.0	-	0
BAS 560 02 F	357.1	7.5	2.6	0
	714.3	10.0	5.3	1m
	1428.5	0.0	-5.3	5a
	2857	2.5	-2.6	3a
	5714	5.0	0.0	8a
BAS 560 AA F	7730	0.0	-5.3	0
Reference item Perfekthion	0.9	97.5	97.4	n.d.

¹ Corrected mortality according to Schneider-Orelli (1947)

² Negative values demonstrate an increase compared to the control

³ Total number of observations of bees showing a sub-lethal effect, with the observations for replicate units and days cumulated; m = moribund and a = affected

n.d. = Not determined

B. FOOD CONSUMPTION

The mean measured food intake in the control group was 42.8 mg/bee/day. The table below shows the results from the food intake measurements for all test groups. With these food intake measurements, the mean uptake of BAS 560 02 F, BAS 560 AA F and Perfekthion was calculated.

Table A 70: Consumption rates for *Apis mellifera* exposed to BAS 560 02 F and the blank formulation BAS 560 AA F for ten days

Treatment	Concentration		
	Nominal (mg/kg food)	Mean consumption of feeding solution (mg/bee/day)	Mean daily uptake of test item (µg/bee/day)
Control	0.0	42.8	-
BAS 560 02 F	357.1	42.1	15.0
	714.3	45.7	32.6
	1428.5	43.6	62.3
	2857	44.8	128
	5714	51.0	291
BAS 560 AA F	7730	48.1	372
Reference item Perfekthion	0.9	27.5	0.02

C. DEFICIENCIES

None.

III. CONCLUSION

The 10-day LC₅₀ value for honey bees (*Apis mellifera*) exposed to BAS 560 02 F was determined to be > 5714 mg a.s./kg food, the highest nominal concentration tested, which corresponds to a 10-day LDD₅₀ > 291 µg a.s./bee/day (measured). The no-observed-effect concentration (NOEC) was determined to be 5714 mg a.s./kg food, which corresponds to a no-observed-effect daily dose (NOEDD) of 291 µg a.s./bee/day.

A 2.4.1.2.3 Study 3

Comments of zRMS:	The study was conducted to OECD guidance TG 245 and according to the principles of GLP. All validity criteria were met. The study is considered to be reliable and suitable for the risk assessment.
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Reference:	CP 10.3.1.2/4
Report	Chronic toxicity of BAS 758 00 F to the honey bee <i>Apis mellifera</i> L. under laboratory conditions, Dressler, K., 2021 Report No 892147, 2148BAC0053 BASF DocID 2021/2008152 Authority registration No
Guideline(s):	OECD TG 245 (2017)
Deviations:	No
GLP:	yes (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany),
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive Summary

In a 10-day chronic toxicity feeding test, max. two days old worker honey bees (*Apis mellifera* L. ssp. Buckfast) were exposed to a daily application of BAS 758 00 F diluted in the bee food (50% (w/v) aqueous sucrose solution). The chronic oral toxicity of the test item was determined at nominal doses of 8.00, 16.0, 32.0, 64.0 and 128 µg product/bee/day (effective doses were 7.80, 12.7, 20.9, 25.8 and 35.0 µg product/bee/day), corresponding to concentrations of 204, 408, 815, 1630 and 3261 mg product/kg food. Additionally, honey bees were treated with Danadim® Progress (dimethoate) as reference item at a nominal dose of 27.3 ng a.s./bee/day. Untreated diet was served as a control. Assessments of bee mortality, food consumption and behavioural abnormalities were conducted daily.

After 10 days of continuous exposure, no mortality was observed in the control group. In the test item group, bees effectively consumed doses of 7.80, 12.7, 20.9, 25.8 and 35.0 µg BAS 758 00 F/bee/day which resulted in mortalities of 0.0, 3.3, 43.3, 90.0 and 100%, respectively. The obtained mortalities in the three highest test item doses, 20.9, 25.8 and 35.0 µg consumed product/bee/day were statistically significantly increased compared to the control group. Behavioural abnormalities were observed in test item doses of 64.0 and 128 µg BAS 758 00 F/bee/day (effectively consumed dose of 25.8 and 35.0 µg BAS 758 00 F/bee/day). In the highest test item dose, 2 bee out of 22 bees was observed as being affected (uncoordinated movements) on day 1. On day 4, all bees of this test item treatment group were dead. In test item dose 64.0 µg BAS 758 00 F/bee/day (effectively consumed dose of 25.8 µg BAS 758 00 F/bee/day), 1 bee out of 23 bees and 1 bee out of 11 bees was observed as being moribund or affected (uncoordinated movements) on day 5 and 7, respectively. No other behavioural abnormalities were observed in any test item treatment group on any other assessment day.

In a 10-day chronic toxicity feeding test with BAS 758 00 F the NOEDD and NOEC were determined to be 12.7 µg consumed product/bee/day and 408 mg product/kg food, respectively. The LDD₅₀ and LC₅₀ were determined to be 21.4 µg consumed product/bee/day and 896 mg product/kg food, respectively.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 758 00 F; batch no. FD-200124-0004; content of a.s.: metrafenone (BAS 560 F, Reg. No. 4 037 710): 94.9 g/L analysed (100.0 g/L nominal), mefentrifluconazole (BAS 750 F, Reg. No. 5 834 378): 66.7 g/L analysed (66.6 g/L nominal), pyraclostrobin (BAS 500 F, Reg. No. 304 428): 81.0 g/L analysed (80.0 g/L nominal); density: 1.092 g/cm³.

B. STUDY DESIGN

Test species: Honey bee (*Apis mellifera* L. ssp. Buckfast) adult worker bees, max. two days old; derived from healthy and queen-right colonies; source: BioChem agrar GmbH, Leipzig, Germany.

Test design: In a 10-day chronic test, young adults of *Apis mellifera* L. were daily exposed to 5 doses of BAS 758 00 F in treated food (50% (w/v) aqueous sucrose solution). The following treatment groups were set up: 5 doses of the test item, one untreated control group fed with 50% (w/v) aqueous sucrose solution and one dose of the reference item; with 3 replicates per dose and 10 bees per replicate. Assessments of bee mortality, food consumption and behavioural effects were done daily. Concentration of both active substances in the highest and lowest test item feeding concentration were determined analytically on the first and last day of application.

Endpoint: Mortality (LDD_{10/20/50}, LC_{10/20/50}, NOEDD, NOEC), behavioural abnormalities.

Reference item: Danadim® Progress (a.s.: dimethoate, analysed content: 411.2 g dimethoate/L, nominal: 400 g/L).

Test concentrations: Control: 50% (w/v) aqueous sucrose solution;
Test item: applied in 50% (w/v) sucrose solution:

	Nominal dose [µg/bee/day]			Consumed dose [µg/bee/day]			
BAS 758 00 F	BAS 560 F	BAS 750 F	BAS 500 F	BAS 758 00 F	BAS 560 F	BAS 750 F	BAS 500 F
8.00	0.733	0.488	0.586	7.80	0.714	0.475	0.571
16.0	1.47	0.976	1.17	12.7	1.16	0.773	0.929
32.0	2.93	1.95	2.35	20.9	1.92	1.28	1.53
64.0	5.86	3.90	4.69	25.8	2.36	1.57	1.89
128	11.7	7.81	9.38	35.0	3.21	2.14	2.57

The doses correspond to concentrations of 204, 408, 815, 1630 and 3261 mg product/kg food (equivalent to 18.7, 37.3, 74.6, 149 and 299 mg BAS 560 F/kg food; 12.4, 24.9, 49.7, 99.4 and 199 mg BAS 750 F/kg food; and 14.9, 29.9, 59.7, 119 and 239 mg BAS 500 F/kg food).

Reference item (applied in 50% sucrose solution): treated diet at a nominal dose of 27.3 ng dimethoate/bee/day (corresponding to a concentration of 0.694 mg dimethoate/kg food).

Test conditions:	Temperature: 32.5 – 37.8 °C, relative humidity: 50.5 – 80.7%, photoperiod: constant darkness (diffuse artificial light of only during assessments and exchange of feeders), food: 50% (w/v) aqueous sucrose solution.
Analytics:	Analytical verification of the test item was conducted by BASF method L0452/02 using high performance liquid chromatography (HPLC) with mass-spectrometric (MS-MS) detection.
Statistics:	Descriptive statistics; Step-down Cochran-Armitage Test Procedure for mortality data and determination of NOEDD and NOEC (one-sided greater, $\alpha = 0.05$). Weibull analysis and Probit analysis using linear maximum likelihood regression for the calculation of LDD _x and LC _x values along with their 95% confidence limits, respectively.

C. DESCRIPTION OF THE ANALYTICAL PROCEDURES

Concentrations of BAS 750 F, BAS 560 F and BAS 500 F (contained in BAS 758 00 F) in honey bee feeding solution were determined according to the analytical method L0452/02. The validation of the analytical method is described in the study report. Feeding solution samples of 0.25 g each were extracted with acetonitrile/water/formic acid 50/50/1 (v/v/v). After shaking the samples on the vertical shaker for about 15 min, one portion of the BEKOlut-Citrate-Kit-1/5 (QuEChERS-Salt) was added followed by shaking immediately by hand following centrifugation for phase separation. A 1 mL aliquot of the acetonitrile-phase was taken and diluted with acetonitrile/water/formic acid 50/50/1 (v/v/v) to a final volume of 10 mL. For measurement, final volumes were further diluted into the calibration range using extracts from untreated control samples. The acetonitrile phase of each sample extract was injected into the HPLC system. The determination was performed by HPLC-MS/MS. The limit of quantification (LOQ) was 0.01 mg/kg and the limit of detection (LOD) was set to $\leq 30\%$ of LOQ or 0.008 mg/kg, both on lab sample level before extraction (based on active ingredient). Matrix effects were compensated by using matrix-matched calibration standards with solvent standards (= standards in acetonitrile/water/formic acid (50/50/1, v/v/v)) at identical nominal concentrations, for quantification of BAS 750 F, BAS 560 F and BAS 500 F because matrix effect was deemed to be significant. The effects were assessed by comparing the standard's peak areas. The storage stability of the analytes in bee feeding solution was not determined as all samples were analysed within 30 days of sampling. Details on measured fortification samples and obtained procedural recoveries for BAS 750 F, BAS 560 F and BAS 500 F are given in Table A 71.

Table A 71: Procedural recoveries for BAS 750 F, BAS 560 F and BAS 500 F in honey bee diet

Substance	Matrix	Fortification level (mg/kg)	n	Mean (%)	RSD (%)
BAS 750 F	Honey bee diet	0.10	5	94.5	8.4
		400	5	86.9	6.8
BAS 560 F	Honey bee diet	0.10	5	96.6	6.0
		400	5	85.9	6.2
BAS 500 F	Honey bee diet	0.10	5	74.3	6.1
		400	5	88.0	5.9

II. RESULTS AND DISCUSSION

After 10 days of continuous exposure, no mortality was observed in the control group. In the test item groups, bees effectively consumed doses of 7.80, 12.7, 20.9, 25.8 and 35.0 µg BAS 758 00 F/bee/day which resulted in mortalities of 0.0, 3.3, 43.3, 90.0 and 100%, respectively. The obtained mortalities in the three highest test item doses, 20.9, 25.8 and 35.0 µg consumed product/bee/day were statistically significantly increased compared to the control group (Step-down Cochran-Armitage Test Procedure, $\alpha = 0.05$, one-sided greater).

Behavioural abnormalities were observed in test item doses of 64.0 and 128 µg BAS 758 00 F/bee/day (effectively consumed dose of 25.8 and 35.0 µg BAS 758 00 F/bee/day). In the highest test item dose, 2 bee out of 22 bees was observed as being affected (uncoordinated movements) on day 1. On day 4, all bees of this test item treatment group were dead. In test item dose 64.0 µg BAS 758 00 F/bee/day (effectively consumed dose of 25.8 µg BAS 758 00 F/bee/day), 1 bee out of 23 bees and 1 bee out of 11 bees was observed as being moribund or affected (uncoordinated movements) on day 5 and 7, respectively. No other behavioural abnormalities were observed in any test item treatment group on any other assessment day.

The results are summarised in Table A 72.

Table A 72: Toxicity of BAS 758 00 F to *Apis mellifera* L. in a chronic toxicity feeding test after 10 days

Treatment			Mortality after 10 days		
Nominal dosage [µg product/bee/day]	Consumed dosage [µg product/bee/day] ¹⁾	Concentration [mg product/kg food]	Mean mortality [%]	Corrected mortality [%]	Number of bees with behavioural abnormalities ²⁾
Sucrose control	--	--	0.0	--	0 (30)
8.00	7.80	204	0.0	--	0 (30)
16.0	12.7	408	3.3	--	0 (29)
32.0	20.9	815	43.3 *	--	0 (17)
64.0	25.8	1630	90.0 *	--	0 (3)
128	35.0	3261	100 *	--	--
Endpoints [10 days]					
Test item doses	LDD ₁₀ [µg consumed product/bee/day] ^{1) 3)} (95% CL lower – upper)		15.9 (12.4 - 17.9)		
	LDD ₂₀ [µg consumed product/bee/day] ^{1) 3)} (95% CL lower – upper)		17.9 (15.0 - 19.5)		
	LDD ₅₀ [µg consumed product/bee/day] ^{1) 3)} (95% CL lower – upper)		21.4 (19.7 - 22.6)		
	NOEDD [µg consumed product/bee/day] ^{1) 4)}		12.7		
Test item concentrations	LC ₁₀ [mg product/kg food] ⁵⁾ (95% CL lower – upper)		508 (376 - 613)		
	LC ₂₀ [mg product/kg food] ⁵⁾ (95% CL lower – upper)		617 (488 - 725)		
	LC ₅₀ [mg product/kg food] ⁵⁾ (95% CL lower – upper)		896 (768 - 1046)		
	NOEC [mg product/kg food] ⁴⁾		408		

CL: Confidence limits

* Statistically significant difference in pairwise comparison between treatment and untreated control group (Step-down Cochran-Armitage Test Procedure, $\alpha = 0.05$, one-sided greater).

¹⁾ Taking into account the actual food uptake and evaporation.

²⁾ Behavioural abnormalities at the end of the test referring to the number of remaining bees, given in parentheses.

³⁾ Lethal dietary doses/concentrations were calculated using Weibull analysis (linear max. likelihood regression).

⁴⁾ No observed effect dietary dose/concentration were calculated using Step-down Cochran-Armitage Test Procedure ($\alpha = 0.05$; one-sided greater).

⁵⁾ Lethal dietary doses/concentrations were calculated using Probit analysis (linear max. likelihood regression).

The reference item, applied at 27.3 ng a.s./bee/day (equivalent to 0.694 mg a.s./kg food), caused 96.7% mortality of the exposed honey bees after 10 days.

Validity criteria:

Validity criteria according to OECD 245 (2017)	Obtained in this study
Control mortality from $\leq 15\%$ at D10 across all replicates	0.0% control
Reference item mortality $\geq 50\%$ on D10	96.7%

All validity criteria were met.

III. CONCLUSION

In a 10-day chronic toxicity feeding test with BAS 758 00 F the NOEDD and NOEC were determined to be 12.7 µg consumed product/bee/day and 408 mg product/kg food, respectively. The LDD₅₀ and LC₅₀ were determined to be 21.4 µg consumed product/bee/day and 896 mg product/kg food, respectively.

A 2.4.1.3 KCP 10.3.1.3 Effects on honey bee development and other honey bee life stages

A 2.4.1.3.1 Study 1

The following chronic toxicity study with honey bee larvae performed with pyraclostrobin is provided in support of the assessment and was recently submitted under the Annex I renewal process for pyraclostrobin (AIR 3). The study summary was provided in the revised Renewal Assessment Report of pyraclostrobin (RAR, Vol. 3, B.9, September 2021).

Comments of zRMS:	Study not evaluated by zRMS.
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Reference:	CP 10.3.1.3/1
Report	Repeated exposure of BAS 500 F (Pyraclostrobin) to honey bee (<i>Apis mellifera</i>) larvae under laboratory conditions (in vitro), Royer S., 2017 Report No EU-761023 BASF DocID 2017/1142794 Authority registration No
Guideline(s):	OECD 237 (2013) Honey bee (<i>Apis mellifera</i>) larval toxicity test single exposure, OECD Draft Guideline on Honey bee (<i>Apis mellifera</i>) larval toxicity test (2015)
Deviations:	No
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)
Acceptability:	Yes
Duplication (if vertebrate study)	No

Reference:	CP 10.3.1.3/2
Report	Amendment No. 1: Repeated exposure of BAS 500 F (Pyraclostrobin) to honey bee (<i>Apis mellifera</i>) larvae under laboratory conditions (in vitro), Royer S., 2017 Report No EU-761023 BASF DocID 2017/1142798 Authority registration No
Guideline(s):	OECD 237 (2013) Honey bee (<i>Apis mellifera</i>) larval toxicity test single exposure, OECD Draft Guideline on Honey bee (<i>Apis mellifera</i>) larval toxicity test (2015)
Deviations:	No
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive Summary

The effects of the test item BAS 500 F (Reg. No. 304 428) on survival and successful adult emergence of the honey bee larvae *Apis mellifera* were investigated in a laboratory test with repeated exposure over a period of 22 days. Honey bee larvae were exposed to BAS 500 F diluted in the larvae food on days 3, 4, 5 and 6. The toxicity of the test item was determined at dose rates of 2.2, 4.0, 7.1, 12.8 and 23.1 µg a.s./larva (corresponding to 14.3, 25.7, 46.3, 83.3 and 150 mg a.s./kg food). Additionally, honey bee larvae were treated with dimethoate as reference item at a dose of 7.1 µg a.s./larva (concentration: 46 mg a.s./kg food). Untreated diet and untreated diet mixed with 0.35% (w/w) acetone served as control groups. All treatment groups consisted of 3 replicates, each with 16 larvae. For each replicate the 16 larvae originated from one of three different bee hives. Assessments of survival of the larvae and pupae, and any sublethal effects, were conducted on rearing days 4, 5, 6, 7, 8, 15 and 22. Successful adult emergence was recorded on rearing day 22.

After 8 days, larval mortalities of 2.1 and 6.3% were observed in the untreated and solvent control, respectively. Feeding BAS 500 F in concentrations of 14.3, 25.7, 46.3, 83.3 and 150 mg a.s./kg food (corresponding to total doses of 2.2, 4.0, 7.1, 12.8 and 23.1 µg a.s./larva) caused mean mortalities after 8 days of 6.3, 8.3, 0.0, 16.7 and 58.3%, respectively (corresponding to corrected mortalities of 0.0, 2.1, -6.7, 11.1 and 55.5%, respectively). Statistically significant differences compared to the solvent control were only observed in the highest concentration of 150 mg a.s./kg food (corresponding to a total dose of 23.1 µg a.s./larva).

After 22 days, mortalities of 8.3 % and 16.7 % were recorded in the control and solvent control, respectively. Feeding BAS 500 F in concentrations of 14.3, 25.7, 46.3, 83.3 and 150 mg a.s./kg food (corresponding to total doses of 2.2, 4.0, 7.1, 12.8 and 23.1 µg a.s./larva) caused mean mortalities after 22 days of 12.5, 16.7, 2.1, 18.8 and 87.5%, respectively (corresponding to corrected mortalities of -5.0, 0.0, -17.5, 2.5 and 85.0%, respectively). Statistically significant differences compared to the solvent control were only observed in the highest concentration of 150 mg a.s./kg food (corresponding to a total dose of 23.1 µg a.s./larva). No sublethal effects in any treatment could be recorded.

In a chronic oral toxicity study with repeated exposure to BAS 500 F on honey bee larvae, the 8-day LC₅₀ is estimated to be 143.8 mg a.s./kg food (corresponding to an LD₅₀ of 22.1 µg a.s./larva). The 8-day NOEC was determined to be 83.3 mg a.s./kg food (corresponding to a NOED of 12.8 µg a.s./larva).

After 22 days, the EC₅₀ was determined to be 130.9 mg a.s./kg food (corresponding to an ED₅₀ of 20.1 µg a.s./larva). The 22-day NOEC value was determined to be 83.3 mg a.s./kg food (corresponding to a NOED of 12.8 µg a.s./larvae).

I. MATERIALS AND METHODS

A. MATERIALS

Test item: BAS 500 F (pyraclostrobin, Reg. No. 304 428); batch no.: COD-001236; analyzed purity: 99.02% (tolerance ±1%).

B. STUDY DESIGN

Test species: Larvae of *Apis mellifera carnica* (honey bee); synchronized first larval stage (L1); derived from three healthy and queen-right colonies; source: in-house colonies.

Test design: 22-day chronic feeding test with repeated exposure according to according to the OECD Draft Guidance Document “Honey Bee (*Apis mellifera*) Larval Toxicity Test, Repeated Exposure” (July 2015) and OECD Guidelines for the testing of chemicals No. 237: “Honey bee (*Apis mellifera*) larval toxicity test, single exposure” (adopted: 26 July 2013). The larvae in the 1st larval stage were transferred individually from the combs into plastic queen cups that were placed in 48-well cellular culture plates (one plate per treatment group) in an incubator. Larvae were fed with an artificial food (aqueous yeast/sugar solution mixed with 50% royal jelly (w/w)) for 6 days. The amount and the composition of the food was adopted according to the requirements of the larvae that change over time. On days 3, 4, 5 and 6, larvae were fed with food containing five different concentrations of BAS 500 F. In total, 8 treatment groups were set up: 5 doses of the test item, 1 untreated control and 1 solvent control as well as a 1 reference item treatment, each with 3 replicates per treatment and 16 larvae per replicate. Survival of the larvae and pupae, and any sublethal effects, were recorded on rearing days 4, 5, 6, 7, 8, 15 and 22. Successful adult emergence was recorded on rearing day 22.

Endpoints: NOEC/NOED (days 8 and 22), LC₅₀/LD₅₀ (day 8) and EC₅₀/ED₅₀ (day 22).

Reference item: Dimethoate (analyzed purity: 98.8%). The effects of the reference item were investigated in this study at a concentration of 46 mg a.s./kg food, corresponding to a total dose of 7.1 µg a.s./larva.

Test doses: Control: untreated diet (aqueous yeast/sugar solution mixed with 50% royal jelly (w/w))
Solvent control: untreated diet with acetone (0.35% w/w)
Test item treatments:

Nominal dose/concentration of BAS 500 F	
Doses [µg a.s./larva]	Concentrations [mg a.s./kg food]
2.2	14.3
4.0	25.7
7.1	46.3
12.8	83.3
23.1	150

Test conditions: Mean measured temperature: 34.5 °C on days 1 – 8, 34.2 °C on days 8 – 15 and 34.7 °C on days 15 – 22. Mean relative humidity: 93.3% on days 1 – 8, 76.8% on days 8 – 15 and 52.2% on days 15 – 21.

Analytics: Analytical verification of test item concentrations was conducted using LC-MS/MS.

Statistics: Descriptive statistics; Fisher's Exact Binomial Test with Bonferroni Correction, Weibull Analysis (one-sided greater, $\alpha = 0.05$).

C. DESCRIPTION OF THE ANALYTICAL PROCEDURES

Concentrations of BAS 500 F in honey bee larvae diet were determined according to the analytical method L0357/01. The validation of the analytical method is described in the study report. 0.5 g of larvae diet is weighed into a 15 mL culture tube with screw cap and spiked with fortification solution (prepared in methanol). The sample is extracted with 10 mL of acetonitrile/water (50/50, v/v) and sonicated for 5 min. An aliquot of 0.1 mL of the extract was transferred into a culture tube and diluted with 9.9 mL of methanol/water (50/50, v/v). The determination was performed by LC-MS/MS. The limit of quantification (LOQ) was 0.5 mg/kg and the limit of detection (LOD) was set to 0.1 mg/kg. Solvent based and matrix-matched standards (quality control samples) were analyzed to assess potential matrix effects on the analysis of BAS 500 F. The findings demonstrate that the matrix load in the tested quality control samples had negligible influence ($< \pm 20\%$) on the analysis of BAS 500 F. Stability was confirmed for BAS 500 F in final extracts for a duration of at least 6 days, when stored at a maximum temperature of 15°C. Details on measured fortification samples and obtained procedural recoveries for BAS 500 F are given in the tables below.

Table A 73: Recovery Data of BAS 500 F in honey bee larvae diet

Mass Transition	Fortification Level [mg/kg]	Number of Replicates	Mean Recovery [%]	RSD [%]	Mean Recovery [%]	RSD [%]
388 → 194	0.5	5	104	4.8	100	5.3
	200	5	97	2.3		
388 → 163	0.5	5	105	4.3	102	4.1
	200	5	99	1.0		

II. RESULTS AND DISCUSSION

After 8 days, larval mortalities of 2.1% and 6.3% were observed in the untreated and solvent control, respectively. Feeding BAS 500 F in concentrations of 14.3, 25.7, 46.3, 83.3 and 150 mg a.s./kg food (corresponding to total doses of 2.2, 4.0, 7.1, 12.8 and 23.1 µg a.s./larva) caused mean mortalities after 8 days of 6.3, 8.3, 0.0, 16.7 and 58.3%, respectively (corresponding to corrected mortalities of 0.0, 2.1, -6.7, 11.1 and 55.5%, respectively). Statistically significant differences compared to the solvent control were only observed in the highest concentration of 150 mg a.s./kg food (corresponding to a total dose of 23.1 µg a.s./larva; Fisher's Exact Binomial Test with Bonferroni Correction, one-sided greater, $\alpha = 0.05$).

After 22 days, mortalities of 8.3% and 16.7% were recorded in the control and solvent control, respectively. Feeding BAS 500 F in concentrations of 14.3, 25.7, 46.3, 83.3 and 150 mg a.s./kg food (corresponding to total doses of 2.2, 4.0, 7.1, 12.8 and 23.1 µg a.s./larva) caused mean mortalities after 22 days of 12.5, 16.7, 2.1, 18.8 and 87.5%, respectively (corresponding to corrected mortalities of -5.0, 0.0, -17.5, 2.5 and 85.0%, respectively). Statistically significant differences compared to the solvent control were only observed in the highest concentration of 150 mg a.s./kg food (corresponding to a total dose of 23.1 µg a.s./larva; Fisher's Exact Binomial Test with Bonferroni Correction, one-sided greater, $\alpha = 0.05$). No sublethal effects in any treatment could be recorded. The results are summarized in Table A 74.

Table A 74: Toxicity of BAS 500 F to larvae of *Apis mellifera* (honey bee) in a repeated exposure oral toxicity test after 22 days

Dosage [µg a.s./larva]	Concentration [mg a.s./kg food]	Mortality [%]				Adult emergence (day 22) [%]
		Larvae (day 8)		Overall (day 22)		
		Absolute	Corrected ¹⁾	Absolute	Corrected ¹⁾	
Control	Control	2.1	--	8.3	--	91.7
Acetone solvent control	Acetone solvent control	6.3	--	16.7	--	83.3
2.2	14.3	6.3	0.0	12.5	-5.0	87.5
4.0	25.7	8.3	2.1	16.7	0.0	83.3
7.1	46.3	0.0	-6.7	2.1	-17.5	97.9
12.8	83.3	16.7	11.1	18.8	2.5	81.2
23.1	150	58.3	55.5 *	87.5	85.0 *	12.5
Endpoints (larval mortality, day 8)						
LD ₁₀ [µg a.s./larva]		12.0 (95% CL: 8.8 – 14.1)				
LD ₂₀ [µg a.s./larva]		15.3 (95% CL: 12.6 – 17.3)				
LD ₅₀ [µg a.s./larva]		22.1 (95% CL: 19.8 – 25.6)				
NOED [µg a.s./larva]		12.8				
LC ₁₀ [mg a.s./kg food]		77.9 (95% CL: 57.5 – 91.7)				
LC ₂₀ [mg a.s./kg food]		99.4 (95% CL: 81.9 – 112.3)				
LC ₅₀ [mg a.s./kg food]		143.8 (95% CL: 128.4 – 166.3)				
NOEC [mg a.s./kg food]		83.3				
Endpoints (adult emergence, day 22)						
ED ₁₀ [µg a.s./larva]		15.6 (95% CL: 11.9 – 17.5)				
ED ₂₀ [µg a.s./larva]		17.3 (95% CL: 14.1 – 18.9)				
ED ₅₀ [µg a.s./larva]		20.1 (95% CL: 18.2 – 21.3)				
NOED [µg a.s./larva]		12.8				
EC ₁₀ [mg a.s./kg food]		101.4 (95% CL: 77.5 – 113.8)				
EC ₂₀ [mg a.s./kg food]		112.2 (95% CL: 92.0 – 122.6)				
EC ₅₀ [mg a.s./kg food]		130.9 (95% CL: 118.3 – 138.1)				
NOEC [mg a.s./kg food]		83.3				

¹⁾ According to Schneider-Orelli (1947).

* Statistically significant difference when compared to the solvent control (Fisher's Exact Binomial Test with Bonferroni Correction, alpha = 0.05, one-sided greater).

95% CL = 95% Confidence limits

Validity criteria:

Validity criteria according to OECD 239 (2016)	Obtained in this study
Control mortality from D3 to D8 \leq 15% across all replicates	2.1% untreated control 6.3% solvent control
Adult emergence in the control group \geq 70% at D22 across all replicates	91.7% untreated control 83.3% solvent control
Effects of the reference item: Dimethoate: larval mortality \geq 50% on D8 across all replicates	Dimethoate: 97.9% at D8

All validity criteria were met.

III. CONCLUSION

In a chronic oral toxicity study with repeated exposure to BAS 500 F on honey bee larvae, the 8-day LC_{50} is estimated to be 143.8 mg a.s./kg food (corresponding to an LD_{50} of 22.1 μ g a.s./larva). The 8-day NOEC was determined to be 83.3 mg a.s./kg food (corresponding to a NOED of 12.8 μ g a.s./larva).

After 22 days, the EC_{50} was determined to be 130.9 mg a.s./kg food (corresponding to an ED_{50} of 20.1 μ g a.s./larva). The 22-day NOEC value was determined to be 83.3 mg a.s./kg food (corresponding to a NOED of 12.8 μ g a.s./larvae).

A 2.4.1.3.2 Study 2

The following chronic toxicity study with honey bee larvae performed with BAS 560 02 F and BAS 560 AA F (formulations of BAS 560 F, metrafenone) is provided in support of the assessment and was recently submitted under the Annex I renewal process for metrafenone (AIR 3). The study summary was provided in the revised Renewal Assessment Report of metrafenone (RAR, Vol. 3, B.9, December 2019).

Comments of zRMS:	Study not evaluated by zRMS.
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Reference:	CP 10.3.1.3/3
Report	BAS 560 02 F and BAS 560 AA F blank formulation - Honey bee (<i>Apis mellifera</i> L.) larval toxicity test (repeated feeding exposure), Eckert, J., 2015 Report No EU-S14-00372, S14-00372 BASF DocID 2014/1093921 Authority registration No
Guideline(s):	OECD 237 (2013) Honey bee (<i>Apis mellifera</i>) larval toxicity test single exposure, OECD Draft Test Guideline on Honey bee (<i>Apis mellifera</i>) Larval Toxicity Test Repeated Exposure (February 2014)
Deviations:	No
GLP:	yes (certified by Landesamt fuer Umwelt, Messungen und Naturschutz Baden Wuerttemberg, Karlsruhe, Germany),
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive Summary

The toxicity of BAS 560 02 F to honey bee *Apis mellifera* larvae was determined in a repeated feeding study in the laboratory. First instar larvae were fed daily with diet containing BAS 560 02 F at nominal concentrations of 81.125, 162.25, 324.5, 649 and 1298 mg a.s./kg food (equivalent to a cumulative dose of 12.50, 24.99, 49.98, 99.95 and 199.89 µg a.s./larva), from exposure day three to day six. The blank formulation BAS 560 AA F (at a nominal concentration of 1758 mg product/kg food, corresponding to a cumulative dose of 270.73 µg product/larva), a negative control (feeding solution only) and the reference item dimethoate (at nominal concentration of 40 mg dimethoate/kg diet, equivalent to a cumulative dose of 6.16 µg a.s./larva) were tested in parallel. Bee larvae were obtained from three different colonies, and each colony represented a replicate in each test group, with twelve bee larvae per replicate. Mortality and food rejection were assessed after eight days (120 hours after the first feeding with treated diet).

Chemical analysis demonstrated that the measured concentrations of the BAS 560 02 F stock solutions from day three to day six were between 100 and 102% of the nominal concentrations.

The mortality of the bee larvae exposed to the two highest concentrations of BAS 560 02 F of 649 and 1298 mg a.s./kg diet was statistically significantly increased compared to the control. Hence, the 120-hour no-observed-effect concentration (NOEC) was determined to be 324.50 mg a.s./kg diet, which corresponds to a 120-hour no-observed-effect dose (NOED) of 49.98 µg a.s./larva. The 120-hour LC₅₀ value for *Apis mellifera* larvae exposed to BAS 560 02 F was calculated to be 750.74 mg a.s./kg diet, which corresponds to a 120-hour LD₅₀ value of 115.61 µg a.s./larva. The blank formulation BAS 560 AA F did not cause mortality, indicating that the observed effects for BAS 560 02 F were caused by the active substance and not the formulation.

I. MATERIAL AND METHODS

A. MATERIALS

- 1. Test material:** BAS 560 02 F
Batch number: 0008024566
Purity: 501.7 g/L metrafenone (analyzed)
Description: Beige liquid

Blank formulation: BAS 560 AA F
Batch number: FD-140630-0026
Purity: Without metrafenone
Description: Beige liquid
- 2. Test concentrations:** BAS 560 02 F: 81.125, 162.25, 324.5, 649 and 1298 mg a.s./kg diet (nominal; equivalent to a cumulative dose of 12.50, 24.99, 49.98, 99.95 and 199.89 µg a.s./larva)
BAS 560 AA F: 1758 mg product/kg diet (nominal; equivalent to a cumulative dose of 270.73 µg product/larva)
- 3. Reference item:** Dimethoate (BAS 152 I; purity: 99.8%), tested at 40 mg dimethoate/kg diet (equivalent to a cumulative dose of 6.16 µg a.s./larva)
- 4. Vehicle:** Aqueous sugar and yeast solution mixed with fresh organic royal jelly (without antibiotics, pesticides and heavy metals (analyzed))
- 5. Test organism:**
Species: Honey bee *Apis mellifera* L.
Age/life stage: First instar larvae (L1)
Source: Three healthy, adequately fed, disease-free and queen-right colonies, obtained from Eurofins Agrosience Services, EcoChem GmbH, Niefern-Öschelbronn, Germany
Diet: Daily feeding, except on day 2. Three different artificial diets (A, B and C), adapted to the needs of each larval stage, were prepared during the test. The cumulative feeding volume of treated diet was 140 µL diet/larva.
 - Diet A (20 µL/larva, on day 1): 50% weight of fresh royal jelly + 50% weight of aqueous solution containing 2% weight of yeast extract, 12% weight of glucose and 12% weight of fructose

- Diet B (20 µL/larva, on day 3): 50% weight of fresh royal jelly + 50% weight of aqueous solution containing 3% weight of yeast extract, 15% weight of glucose and 15% weight of fructose
- Diet C (30 µL on day 4, 40 µL on day 5 and 50 µL on day 6): 50% weight of fresh royal jelly + 50% weight of aqueous solution containing 4% weight of yeast extract, 18% weight of glucose and 18% weight of fructose

Test units:

Crystal polystyrene grafting cells with a diameter of 9 mm, which were sterilised by immersion in ethanol (70%) and then dried. Hereafter, the cells were placed into a 48-well plate, which was previously filled with a piece of dental roll wetted with 15% (w/v) glycerol. The plates were placed into a hermetically sealed Plexiglass desiccator, which was placed into an incubator with forced air circulation

B. STUDY DESIGN

1. Environmental conditions:

Temperature: 33.3 – 34.4°C

Relative humidity: 51.4 – 100.0%

Photoperiod: Continuous darkness, except during feeding and assessments

2. Assignment and treatment:

First instar (L1) honey bee larvae were selected from three different colonies and individually placed into cellular well-plates, using a grafting tool. The larvae were obtained from the three different colonies, with twelve larvae from the same colony in one replicate and hence three replicates per treatment group. At this day of grafting, the larvae were fed untreated diet A. At day three, four, five and six, the larvae were fed with a defined quantity of diet B or C, which contained the nominal BAS 560 02 F concentrations of 81.125, 162.25, 324.5, 649 and 1298 mg a.s./kg diet (nominal; equivalent to a cumulative dose of 12.50, 24.99, 49.98, 99.95 and 199.89 µg a.s./larva). Feeding occurred separately for each larva, using a sterile pipette. The food drop was placed next to the larvae. The blank formulation BAS 560 AA F (1758 mg product/kg diet (nominal; equivalent to a cumulative dose of 270.73 µg product/larva)), a control (diet only), and a reference product (dimethoate; at 40 mg dimethoate/kg diet (nominal), equivalent to a cumulative dose of 6.16 µg a.s./larva) were concurrently tested.

3. Dose preparation:

The stock solution of aqueous sugar solution was prepared daily. The treated solutions for BAS 560 02 F and BAS 560 AA F were prepared at each application day (day three, four, five and six), by dilution of the test items in deionised and autoclaved water. These solutions were mixed through the sugar solution. Thereafter, the royal jelly was added to each stock solution, to reach the final test concentrations. The control was exposed to the sugar solution only.

4. Measurements and observations:

Mortality was assessed daily from day four to day eight, which was 24 to 120 hours after the first feeding with treated diet. On day eight, the presence of uneaten food was also qualitatively assessed.

Samples of the stock solutions of BAS 560 02 F and the control solution were taken at each day of application and analyzed for the concentration of the test item, using HPLC-PDA.

Temperature and humidity were recorded continuously.

5. Statistics:

Mortality data were corrected according to Abbott (1925). Fisher's exact test with Bonferroni correction (one-sided greater, $\alpha = 0.05$) was used to assess whether there was a significant difference between mortality data of the treated groups and the control group. The LC_{50} value with 95% confidence limits was calculated using Probit analyses with linear maximum likelihood regression. All statistics were performed with ToxRat Professional 2.10.

II. RESULTS AND DISCUSSION

A. MORTALITY AND FOOD UPTAKE

No mortality occurred in the control group during the test period. Statistically significant difference from the control group was observed for honey bee larvae exposed to the two highest BAS 560 02 F concentrations of 649 and 1298 mg a.s./kg diet. No mortality was observed in the groups exposed to BAS 560 AA F and 97.2% bees died in the group exposed to the reference item dimethoate.

The measured concentrations of metrafenone ranged between 100 and 102% of the nominal concentrations.

The table below shows the mortality and food rejection for all the test groups at day eight (120 hours after the first feeding with treated diet).

Table A 75: Mortality and food rejection of *Apis mellifera* larvae exposed to BAS 560 02 F and the blank formulation BAS 560 AA F

Treatment	Concentration (mg/kg diet)	Corrected cumulative mortality (%) ^{1,2}					Alive larvae with uneaten food ²
		D4	D5	D6	D7	D8	
Control	0.0	0.0	0.0	0.0	0.0	0.0	0
BAS 560 02 F	81.125	0.0	0.0	0.0	0.0	0.0	0
	162.25	0.0	0.0	0.0	0.0	0.0	0
	324.5	0.0	0.0	5.6	5.6	5.6	2
	649	0.0	11.1	30.6*	38.9*	41.7*	15
	1298	0.0	47.2*	80.6*	83.3*	83.3*	6
BAS 560 AA F	1758	0.0	0.0	0.0	0.0	0.0	1
Reference item Perfekthion	40	25.0**	58.3**	91.7**	97.2**	97.2**	1

¹ Corrected mortality compared to the control group, according to Abbott (1925)

² With a total number of 36 larvae per group

* Significantly increased compared to the control (Fisher's Exact test with Bonferroni Correction)

** Significantly increased compared to the control (Fisher's Exact test)

The table below shows Assessment of Mortality on Day 4 to Day 8 including Presence of Uneaten Food on Day 8.

Table A 76: Assessment of Mortality on Day 4 to Day 8 including Presence of Uneaten Food on Day 8

Treatment group	Concentration ¹⁾	No. of larvae						
		D3	D4	D5	D6	D7	D8	
		introduced	dead	dead	dead	dead	dead	alive with uneaten food
Control group								
Replicate 1	0.0	12	0	0	0	0	0	0
Replicate 2		12	0	0	0	0	0	0
Replicate 3		12	0	0	0	0	0	0
Total		36	0	0	0	0	0	0
Test item group 1	[mg a.i./kg diet]							
Replicate 1	81.125	12	0	0	0	0	0	0
Replicate 2		12	0	0	0	0	0	0
Replicate 3		12	0	0	0	0	0	0
Total		36	0	0	0	0	0	0
Test item group 2	[mg a.i./kg diet]							
Replicate 1	162.25	12	0	0	0	0	0	0
Replicate 2		12	0	0	0	0	0	0
Replicate 3		12	0	0	0	0	0	0
Total		36	0	0	0	0	0	0
Test item group 3	[mg a.i./kg diet]							
Replicate 1	324.5	12	0	0	1	1	1	0
Replicate 2		12	0	0	0	0	0	1
Replicate 3		12	0	0	1	1	1	1
Total		36	0	0	2	2	2	2
Test item group 4	[mg a.i./kg diet]							
Replicate 1	649	12	0	1	3	4	5	4
Replicate 2		12	0	3	4	4	4	5
Replicate 3		12	0	0	4	6	6	6
Total		36	0	4	11*	14*	15*	15
Test item group 5	[mg a.i./kg diet]							
Replicate 1	1298	12	0	4	11	12	12	0
Replicate 2		12	0	7	7	7	7	5
Replicate 3		12	0	6	11	11	11	1
Total		36	0	17*	29*	30*	30*	6
Test item group 6	[mg product/kg diet]							
Replicate 1	1758	12	0	0	0	0	0	0
Replicate 2		12	0	0	0	0	0	0
Replicate 3		12	0	0	0	0	0	1
Total		36	0	0	0	0	0	1
Reference group	[mg dimethoate/kg diet]							
Replicate 1	40	12	5	8	12	12	12	0
Replicate 2		12	3	6	9	11	11	1
Replicate 3		12	1	7	12	12	12	0

Total		36	9**	21**	33**	35**	35**	1
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¹⁾ purity considered

D: Day of the test

* Significantly increased compared to the control (Fisher's Exact Test with Bonferroni Correction, one-sided greater, $\alpha = 0.05$)

** Significantly increased compared to the control (Fisher's Exact Test, one-sided greater, $\alpha = 0.05$)

B. DEFICIENCIES

None.

III. CONCLUSION

The 120-hour LC_{50} value for *Apis mellifera* larvae exposed to BAS 560 02 F was calculated to be 750.74 mg a.s./kg diet, which corresponds to a 120-hour LD_{50} value of 115.61 μ g a.s./larva. The 120-hour no-observed-effect concentration (NOEC) was determined to be 324.50 mg a.s./kg diet, which corresponds to a 120-hour no-observed-effect dose (NOED) of 49.98 μ g a.s./larva. The blank formulation BAS 560 AA F did not cause mortality, which indicates that the observed effects for BAS 560 02 F were caused by the active substance and not the formulation.

An $EC_{10/20}$ calculation is not considered feasible as effects at the NOEC were 5.6% and 0 % at the 3 concentrations below the NOEC. At the next highest test concentration above the NOEC (spacing factor 2) effects were already 41.7%. Thus, the $EC_{10/20}$ is expected to be in between the NOEC and the next highest concentration but would be just estimated from 2 data points which would not provide statistically sound data.

A 2.4.1.3.3 Study 3

Comments of zRMS:	The study was conducted to OECD guidance TG 239 and according to the principles of GLP. All validity criteria were met. The study is considered to be reliable and suitable for the risk assessment.
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Reference:	CP 10.3.1.3/4
Report	Repeated exposure of honey bee (<i>Apis mellifera</i> L.) larvae - to BAS 758 00 F under laboratory conditions, Schmidt, K., 2022 Report No 892148, 2148BLC0039 BASF DocID 2020/2037661 Authority registration No
Guideline(s):	OECD 239 (2016)
Deviations:	No
GLP:	yes (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany),
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive Summary

The effects of the test item BAS 758 00 F on survival and adult emergence of honey bee larvae (*Apis mellifera*) were investigated in a laboratory test with repeated exposure over a time period of 22 days. Synchronised 1st stage (L1) honey bee larvae (*Apis mellifera*) were fed with artificial diet for 5 days (day 1, 3, 4, 5 and 6). On days 3, 4, 5 and 6, larvae were fed with diet at concentrations of 15.8, 39.5, 98.8, 247.0 and 617.4 mg product/kg food, corresponding to total doses of 2.5, 6.3, 15.6, 39.1 and 97.7 µg product/larva. Untreated diet served as a control and dimethoate at a dose rate of 7.4 µg/larva served as reference item treatment. All treatment groups and the control contained larvae from three different bee colonies. Assessments of larval mortality were done 24, 48, 72, 96 and 120 hours after start of the treatment (respectively D4, D5, D6, D7, D8). Additionally, other observations such as small body size or large quantities of unconsumed food after 120 hours (D8) were noted. Pupal mortality was assessed at D15 and emergence of adults was evaluated at D22.

After 120 hours of repeated oral exposure (on D8), no larval mortality was observed in the control. Pupal mortality (from D8 to D15) was 8.3% in the control. The control group showed a pupal mortality of 13.9% at D22. In the test item treated groups, cumulated larval mortalities at D8 ranged from 0.0 to 5.6%. Pupal mortalities (from D8 to D15) ranged from 3.0 to 14.4%. Pupal mortalities from D8 to D22 ranged from 6.1 to 19.9% in the test item treatment groups. Total mortalities at D22 ranged from 8.3 to 22.2%. No statistically significant difference was found at any test item concentration compared to the control. On D8 uneaten food was not observed in any test item groups and the control group. In the final assessment at D22, an adult emergence rate of 86.1% was determined for the honey bees in the control group. In the test item group, adult honey bees emerged at rates ranging from 77.8 to 91.7%. No statistically significant difference was found at any test item concentrations compared to the control.

In a repeated exposure larval toxicity study with BAS 758 00 F, the LD₅₀ (larval mortality on D8) was estimated to be > 97.7 µg product/larva, which is equivalent to a LC₅₀ of > 617.4 mg product/kg food. The respective NOED was ≥ 97.7 µg product/larva and the corresponding NOEC was ≥ 617.4 mg product/kg food. The ED₅₀ (successful adult emergence up to D22) was estimated to be > 97.7 µg product/larva, which is equivalent to an EC₅₀ of > 617.4 mg product/kg food. The respective NOED was ≥ 97.7 µg product/larva and the corresponding NOEC was ≥ 617.4 mg product/kg food.

I. MATERIALS AND METHODS

A. MATERIALS

Test item: BAS 758 00 F; batch no. FD-200124-0004; content of a.s.: mefentrifluconazole (BAS 750 F, Reg. No. 5 834 378): 66.7 g/L analyzed (66.6 g/L nominal); metrafenone (BAS 560 F, Reg. No. 4 037 710): 94.9 g/L analyzed (100.0 g/L nominal), pyraclostrobin (BAS 500 F, Reg. No. 304 428): 81.0 g/L analyzed (80.0 g/L nominal); density: 1.092 g/cm³.

B. STUDY DESIGN

Test species: Larvae of honey bee (*Apis mellifera* L. ssp. Buckfast); synchronised first larval stage instar (L1); derived from at least three healthy and queen-right colonies adequately fed and healthy; source: test facility own stock.

Test design: 22-day repeated exposure larval toxicity test according to OECD 239 (2016). L1 honey bee larvae (*Apis mellifera*) were transferred from brood combs to polystyrene grafting cells in 48-well cell culture plates 2 days before start of the treatment (on D1). After this, the larvae were fed during larval development with artificial diet, containing the test item on rearing days 3, 4, 5 and 6. In total, 5 treatment groups were set up: 5 doses of the test item, 1 untreated control group and 1 dose of the reference item; each with 3 replicates and 12 larvae per replicate. Assessments of larval mortality were done 24, 48, 72, 96 and 120 hours after start of the treatment (respectively D4, D5, D6, D7, D8). Additionally, other observations such as small body size or large quantities of unconsumed food after 120 hours (D8) were noted. Pupal mortality was assessed at D15 and emergence of adults was evaluated at D22.

Endpoints: Successful adult emergence (dose-response relationship, EC/D₅₀, NOEC/D), mortality (LC/D₅₀), qualitative observations: body size, remaining food.

Reference item: Dimethoate (analyzed purity: 99.32%, w/w).

Test doses: Control: untreated diet (50% aqueous yeast/sugar solution with 50% royal jelly).

Test item treatments:

Dose [µg/larva]				
BAS 758 00 F	Total a.s.	BAS 560 F	BAS 750 F	BAS 700 F
2.5	0.6	0.2	0.2	0.2
6.3	1.4	0.6	0.4	0.5
15.6	3.5	1.4	1.0	1.1
39.1	8.8	3.6	2.4	2.9
97.7	22.1	8.9	6.0	7.2
Concentration [mg/kg food]				
BAS 758 00 F	Total a.s.	BAS 560 F	BAS 750 F	BAS 700 F
15.8	3.6	1.4	1.0	1.2
39.5	8.9	3.6	2.4	2.9
98.8	22.3	9.0	6.0	7.2
247.0	55.8	22.6	15.1	18.1
617.4	139.4	56.5	37.7	45.2

Content of the active substances is based on their nominal content in BAS 758 00 F.

Reference item: treated diet with a dose of 7.4 µg a.s./larva (corresponding concentration: 46.8 mg a.s./kg food).

Test conditions: Temperature: Day 1 to day 22: 34.1 - 34.8°C
Relative humidity:
Day 1 to day 8: 97.7 - 97.8%
Day 8 to day 15: 80.1 - 84.3%
Day 15 to day 22: 60.4 - 63.1%
Photoperiod: darkness (except during assessments)
Food: 50% aqueous yeast/sugar solution with 50% royal jelly.

Analytics: Analytical verification of the test item concentrations in honey bee larvae diet was conducted by BASF method L0452/02 using liquid chromatography with mass-spectrometric (HPLC-MS/MS) detection.

Statistics: Descriptive statistics; Multiple Sequentially-rejective Fisher Test After Bonferroni-Holm (D8) and Chi² 2x2 Table Test with Bonferroni Correction (D22) for the determination of NOED/NOEC; Logit analysis using linear max. likelihood regression for adult emergence determination.

C. DESCRIPTION OF THE ANALYTICAL PROCEDURES

Concentrations of BAS 560 F, BAS 750 F and BAS 700 F (contained in BAS 758 00 F) in honey bee feeding solution were determined according to the analytical method L0452/02. The validation of the analytical method is described in the study report. Feeding solution samples of 0.25 g each were extracted with 4 mL acetonitrile/water/formic acid 50/50/1 (v/v/v). After shaking the samples on the vertical shaker for about 15 min at 300 rpm, a portion of the BEKOlut-Citrate-Kit-1/5 (QuEChERS-Salt) was added followed by shaking immediately by hand for at least 30 s and following centrifugation for 5 min at 4000 rpm. A 1 mL aliquot of the acetonitrile-phase was taken and diluted with acetonitrile/water/formic acid 50/50/1 (v/v/v) to a final volume of 10 mL. Of this, 1 mL was taken and further diluted with acetonitrile/water/formic acid 50/50/1 (v/v/v) to a final volume of 10 mL. For measurement, final volumes were further diluted into the calibration range using extracts from untreated control samples. The acetonitrile phase of each sample extract was injected into the HPLC system.

The determination was performed by HPLC-MS/MS. The limit of quantification (LOQ) was 0.1 mg/kg and the limit of detection (LOD) was set to $\leq 30\%$ of LOQ or 0.02 mg/kg, both on lab sample level before extraction (based on active substance). Potential matrix effects were compensated by using matrix-matched calibration standards with solvent standards (= standards in acetonitrile/water/formic acid (50/50/1, v/v/v)) at identical nominal concentrations, for quantification of BAS 560 F, BAS 750 F and BAS 700 F. The effects were assessed by comparing the standard's peak areas. The analytes in bee feeding solution were determined to be stable over 60 days of storage at -18°C in the dark. Details on measured fortification samples and obtained procedural recoveries for BAS 560 F, BAS 750 F and BAS 700 F in BAS 758 00 F are given in Table A 77.

Table A 77: Procedural recoveries for BAS 560 F, BAS 750 F and BAS 700 F in honey bee food

Substance	Matrix	Fortification level (mg/kg)	n	Mean (%)	RSD (%)
BAS 750 F	Honey bee diet	0.10	6	95.2	5.9
		100	6	91.2	5.3
BAS 560 F	Honey bee diet	0.10	6	91.1	4.3
		100	6	87.9	4.5
BAS 700 F	Honey bee diet	0.10	6	94.6	4.9
		100	6	91.6	4.6

RSD = relative standard deviation

II. RESULTS AND DISCUSSION

After 120 hours of repeated oral exposure (on D8), no larval mortality was observed in the control. Pupal mortality (from D8 to D15) was 8.3% in the control. The control group showed a pupal mortality of 13.9% at D22. In the test item treated groups, cumulated larval mortalities at D8 ranged from 0.0 to 5.6%. Pupal mortalities (from D8 to D15) ranged from 3.0 to 14.4%. Pupal mortalities from D8 to D22 ranged from 6.1 to 19.9% in the test item treatment groups. Total mortalities at D22 ranged from 8.3 to 22.2%. No statistically significant difference was found at any test item concentration compared to the control (Multiple Sequentially-rejective Fisher Test After Bonferroni-Holm, $\alpha = 0.05$, one-sided greater). On D8 uneaten food was not observed in any test item groups and the control group.

In the final assessment at D22, an adult emergence rate of 86.1% was determined for the honey bees in the control group. In the test item group, adult honey bees emerged at rates ranging from 77.8 to 91.7%. No statistically significant difference was found at any test item concentrations compared to the control (Chi² 2x2 Table Test with Bonferroni Correction, $\alpha = 0.05$, one-sided greater). The results are summarised in Table A 78.

Table A 78: Toxicity of BAS 758 00 F to *Apis mellifera* (honey bee) in a repeated exposure larval toxicity test after 22 days

Dosage [µg product/l arva]	Concentratio n [mg product/ kg food]	D8 mortality [%]		D15 pupal mortality [%]		D22 pupal mortality [%]		D22 total mortality [%]		D22 adult emergence [%]
		abs.	corr.	abs.	corr.	abs.	corr.	abs.	corr.	
Control	Control	0.0	--	8.3	0.0	13.9	--	13.9	--	86.1
2.5	15.8	0.0	--	5.6	0.0	8.3	0.0	8.3	0.0	91.7
6.3	39.5	0.0	--	5.6	0.0	13.9	0.0	13.9	0.0	86.1
15.6	98.8	2.8	--	3.0	0.0	6.1	0.0	8.3	0.0	91.7
39.1	247.0	5.6	--	11.6	3.6	11.6	0.0	16.7	3.2	83.3
97.7	617.4	2.8	--	14.4	6.6	19.9	7.0	22.2	9.7	77.8
Endpoints [D8]										
LD ₅₀ [µg product/larva] ¹⁾		> 97.7								
NOED [µg product/larva] ²⁾		≥ 97.7								
LC ₅₀ [mg product/kg food] ¹⁾		> 617.4								
NOEC [mg product/kg food] ²⁾		≥ 617.4								
Endpoints [D22]										
ED ₅₀ [µg product/larva] ¹⁾		> 97.7								
ED ₂₀ [µg product/larva] ¹⁾		> 97.7								
ED ₁₀ [µg product/larva] ³⁾		96.0 (95% lower - upper CL = 54.2 - 170.1)								
NOED [µg product/larva] ⁴⁾		≥ 97.7								
EC ₅₀ [mg product/kg food] ¹⁾		> 617.4								
EC ₂₀ [mg product/kg food] ¹⁾		> 617.4								
EC ₁₀ [mg product/kg food] ³⁾		606.9 (95% lower - upper CL = 342.5 - 1075.4)								
NOEC [mg product/kg food] ⁴⁾		≥ 617.4								

Negative values are set to 0; abs.: absolute; corr.: corrected cumulative mortality according to the formula of Abbott (1925), modified by Schneider-Orelli (1947).; CL: confidence limit

¹⁾ Due to effects < 10%/< 20%, the values were estimated to be higher than the highest dose/concentration.

²⁾ Multiple Sequentially-rejective Fisher Test After Bonferroni-Holm ($\alpha = 0.05$, one-sided greater).

³⁾ Based on the Logit analysis using linear max. likelihood regression.

⁴⁾ Chi² 2x2 Table Test with Bonferroni Correction ($\alpha = 0.05$, one-sided greater).

On D8, the mortality in the reference item treatment was 86.1%.

Validity criteria:

Validity criteria according to OECD 239 (2021)	Obtained in this study
Average control mortality from D3 to D8 ≤ 15%	0% control
Average adult emergence in the control group ≥ 70% at D22	86.1% control
Effects of the reference item: Dimethoate: average larval mortality ≥ 50% on D8	86.1% at D8

All validity criteria were met.

III. CONCLUSION

In a repeated exposure larval toxicity study with BAS 758 00 F, the LD₅₀ (larval mortality on D8) was estimated to be > 97.7 µg product/larva, which is equivalent to a LC₅₀ of > 617.4 mg product/kg food. The respective NOED was ≥ 97.7 µg product/larva and the corresponding NOEC was ≥ 617.4 mg product/kg food. The ED₅₀ (successful adult emergence up to D22) was estimated to be > 97.7 µg product/larva, which is equivalent to an EC₅₀ of > 617.4 mg product/kg food. The respective NOED was ≥ 97.7 µg product/larva and the corresponding NOEC was ≥ 617.4 mg product/kg food.

A 2.4.1.4 KCP 10.3.1.4 Sub-lethal effects

BAS 758 00 F poses no unacceptable risk to bees. Further studies are not necessary

A 2.4.1.5 KCP 10.3.1.5 Cage and tunnel tests

A 2.4.1.5.1 Study 1

Comments of zRMS:	<p>The purpose of this study was to determine potential effects of BAS 758 00 on the honeybee (<i>Apis mellifera</i> L.) after one foliar application of 1650 mL/ha on full-flowering Phacelia (BBCH 65) under semi-field conditions during bee flight. Main endpoints were mortality, foraging activity, bee behavior and colony development. Additionally, the levels of residue of active substances were determined in spray solution, flowers and bee food items, namely pollen and nectar.</p> <p>No significant differences on adult and pupal bee mortality, foraging activity, behavior of bees, colony strength and brood development were observed between the test item and control over the entire course of the study.</p> <p>Further details are given in the study summary.</p> <p>The study is considered to be reliable and suitable for the risk assessment.</p>
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Reference:	CP 10.3.1.5/1
Report	<p>Effects of BAS 758 00 F on the honeybee <i>Apis mellifera</i> L. under semi-field conditions (tunnel test) with additional assessments on colony and brood development,</p> <p>Schnurr, A., 2022</p> <p>Report No 876357, 2148BTB0002</p> <p>BASF DocID 2021/2047630</p> <p>Authority registration No</p>
Guideline(s):	Pistorius et al. (2012), EPA 850.3040, EPPO PP 1/170 (4) (2010), OECD Guidance document No. 75 (2007), SANTE/2020/12830 rev. 1
Deviations:	No
GLP:	<p>yes</p> <p>(certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany),</p>
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive summary

A tunnel test was carried out to determine the effects of BAS 758 00 F on honey bee colonies under semi-field conditions. For this purpose, BAS 758 00 F was applied once at a rate of 1650 mL/ha (equivalent to 407 g total a.s./ha) to full-flowering (BBCH 65) *Phacelia tanacetifolia*. Additionally, an untreated control and two reference items were included in the study. Each of the four treatment groups was replicated four times, with one honey bee colony per tunnel set up 4 days prior exposure in the evening after bee flight when *Phacelia* was close to full-flowering (BBCH 63-65). Mortality of the honey bees was assessed daily from 3 days before to 41 days after treatment (DAT), respectively. Foraging activity was assessed daily during pre-exposure and exposure phase in tunnels. Sub-lethal effects were recorded daily during entire study period. Colony development (colony strength and brood and food status) was assessed 2 days prior exposure phase and on DAT 4, DAT 7, DAT 13, DAT 21, DAT 29 and DAT 41. Furthermore, a detailed

assessment of the brood development of single brood cells was performed on DAT -2, DAT 4, DAT 7, DAT 13 and DAT 21, which is equal to BFD 0, BFD 6, BFD 9, BFD 15 and BFD 23.

Pre-exposure mortality was similarly low in control, test item and reference item treatments, indicating well adapted and comparable colonies. No statistically significant differences between the three treatment groups were observed during the pre-exposure phase (DAT -3 to DAT 0ba).

No increased mortality in the test item treatment and reference item I compared to the control was observed at any time during the test; neither during exposure phase (DAT 0aa to DAT 7), nor during post-exposure between DAT 8 and DAT 41, nor at overall comparisons. Exposure of honey bees to reference item II was a statistically significant increase in the number of dead adults during exposure phase between DAT 0aa and DAT 7 and the overall mean of mortality between DAT 0aa and DAT 41.

During the pre-exposure, exposure and post-exposure phase, no dead pupae were found in the control, test item treatment and reference item II. Dead pupae were only observed from DAT 7 in all replicates of reference item I during exposure phase (DAT 0 to DAT 7). Predominantly pupal mortality occurred during the post-exposure phase in all other replicates of reference item I treatment. The overall mean pupal mortality amounted to 13.2 dead pupae/colony for the entire post-application phase, which was increased in comparison with the control.

Foraging activity was assessed during the tunnel phase. During pre-exposure phase (DAT -3 to DAT 0ba) the overall daily mean foraging activity was on an adequate, similar and high level in all treatment groups and amounted to 7.3, 7.8, 8.0 and 7.3 bees/m², indicating that the bee colonies had well adapted to the new environmental conditions. No statistically significant differences occurred in the overall mean foraging activity during pre-exposure phase by comparing the control, test item and reference item against each other.

On the day of application (DAT 0aa) following application of the test item no reduction of the foraging activity was observed throughout the first day after application. During the following days of exposure, no reduction but rather an increase of actively foraging bees occurred when compared to the pre-application level. The overall mean foraging activity for the exposure phase amounted to 9.4 and 9.8 bees/m²/day in the control and test item treatment, which is not statistically significant different compared to the control. The application of reference item I did not result in any reduction in foraging activity immediately after application as well as during the entire exposure phase. Furthermore, the overall mean foraging activity amounted to 7.2 bees/m² which is similar to the control of 9.4 bees/m² and not statistically significant different.

The application of reference item II revealed a distinct reduction in foraging activity on all days of the exposure phase compared to the control. The overall daily foraging activity was statistically significantly lowered compared to the control and amounted to 0.5 bees/m²/day.

Exposure of honey bees to the test item did not result in abnormal behaviour or intoxication symptoms.

The assessment on colony strength before application on BFD 0 (DAT -2) revealed the bee colonies were on similar levels and confirmed an adequate and good strength for the conduction of the tunnel study. The mean estimated colony strength amounted to 7200, 7622, 6947 and 7341 bees/colony for the control, test item and reference item colonies, respectively.

The mean estimated colony strength of the test item treatment revealed a positive development at each assessment when compared to BFD 0. Both, control and test item treatment groups developed in a similar manner until BFD 43 and the mean estimated colony strength increased by +105% and +96% to 14794 and 14963 bees/colony, respectively.

With respect to reference item I and II treatments, the mean estimated colony strength revealed a lower increase of the mean colony strength until BFD 43 compared to the control and test item treatment. The mean estimated colony strength increased by 48% to 10294 bees/colony.

Before application on BFD 0 (DAT -2), the mean brood status of the single stages as well as the entire brood (area occupied by eggs, larvae and pupae) was on a comparable level in all treatment. The mean brood area amounted to 7400, 7142, 6962 and 7220 cm²/colony for the control group, test item group, reference item I and reference item II, respectively. During the first investigated brood cycle until BFD 23, the mean comb area covered with brood stages in the control and test item treatment groups was on a similar level and developed within the range of natural variability, amounting to 10520 and 10829 cm²/colony for the control and test item groups, respectively.

Compared to the pre-application level, the mean brood area increased by 42% and 52% in the control and test item group, respectively. The mean comb area covered with brood stages showed a lower increase in the reference item I during the first investigated brood cycle compared to the control and amounted to 8328 cm²/colony (+20%). The mean comb area covered with brood stages of reference item II treatment group slightly decreased by -4% to 6910 cm²/colony compared to the pre-application level.

At the last assessment on BFD 43 the mean brood area amounted to 8947, 9257, 7349 and 5569 cm²/colony for the control, test item, reference item I and reference item II, respectively. Compared to the pre-application level, the mean brood area changed by +21%, +30%, +6% and -23% in the control, test item, reference item I and reference item II, respectively.

The assessment of the areas covered with pollen and nectar/honey before application (BFD 0) revealed similar levels and a sufficient supply of all colonies with food. Further assessments displayed a distinct increase of nectar stores occurred from BFD 23 onwards which coincided with the flowering of linden trees in the vicinity of the monitoring site. The total food storages increased by +234%, +139%, +83% and +150% compared to BFD 0 and amounted to 10675, 9437, 7426 and 8715 cm²/colony for the control, test item, reference item I and reference item II, respectively.

The evaluation of the development of initially labelled brood cells (eggs) was expressed by the following brood indices: Brood termination rate [BTR], brood index [BI] and brood compensation index [BCI].

The mean BTR of initially labelled eggs amounted to 11.8 and 9.1% for the control and test item groups, respectively, on BFD 6, which increased up to 12.8 and 9.8%, respectively, until the last assessment on BFD 23. Therefore, the termination of labelled eggs was on a similar level and within a natural range of variability, without any statistically significant differences between control and test item treatment. In contrast, reference item I revealed a high brood termination rate of 71.8% at BFD 23, which was statistically significantly different when compared to the control group.

The BI displays a negative correlation with the BTR: the higher the BTR the lower the BI and vice versa. Therefore, the mean BI of initially labelled eggs was slightly higher in the test item treatment and amounted to 4.5 and 4.4 for the control and test item group, respectively, without any statistically significant differences during the study. In contrast, reference item I revealed a much lower brood index of 1.4, which is statistically significantly different compared to the control.

The BCI was on a similar level for both control and test item treatment and amounted to 4.4 and 4.6 in the control and test item treatment, respectively and therefore without any statistically significant difference, indicating most of terminated brood-cells were refilled with new eggs. In contrast, reference item I revealed a distinct statistically significant lower brood compensation index of 2.2, which means only few emptied cells were refilled with new eggs.

Under semi-field conditions (tunnel test), BAS 758 00 F was applied once at a rate of 1650 mL/ha (equivalent to 407 g total a.s./ha) to full-flowering *Phacelia tanacetifolia*. No unacceptable effects on mortality, foraging activity, colony development, colony strength or bee brood were observed after exposure. Overall, based on the results of this study it can be concluded that BAS 758 00 F does not adversely affect honey bee colonies.

I. MATERIALS AND METHODS

A. MATERIALS

Test item: BAS 758 00 F, batch no. FD-200124-0004, content of a.s.: mefentrifluconazole (BAS 750 F, Reg. No. 5 834 378): 66.7 g/L analyzed (66.6 g/L nominal); metrafenone (BAS 560 F, Reg. No. 4 037 710): 94.9 g/L analyzed (100.0 g/L nominal); , pyraclostrobin (BAS 500 F, Reg. No. 304 428): 81.0 g/L analyzed (80.0 g/L nominal); density: 1.092 g/cm³.

B. STUDY DESIGN

Test species: Honey bees (*Apis mellifera* L. Buckfast); healthy and queen-right bee colonies with 5513 to 9900 bees/colony, colonies consisted of two hive bodies containing 11 combs ("Deutsch-Normal-Maß", German standard size of 37 cm x 22.3 cm), comprising all life stages of adults and juvenile bees (eggs, larvae, pupae, brood nest area per colony: 6085 - 9179 cm² including eggs of 413 - 2063 cm²). Health and good condition of all bee colonies was confirmed. Due to the acceptable food status (food stores per colony: 2682 - 4951 cm² including pollen of 516 - 1753 cm² and nectar of 1547 - 3919 cm²); of the colonies, no additional food was provided and therefore, all assessments represent the natural state of each colony. Source: in-house hives.

Test plots: The test site was located in Posthausen near Leipzig, Germany; separate tunnels (with at least 3 m distance) for the different groups and replicates; tunnel size: 24 m x 6 m x 2.5 m (length x width x height); effective crop area: 2 x 63.25 m² (= 126.5 m²); for post-exposure (DAT 8 to DAT 41), the colonies were moved to a monitoring site without flowering crops or intensive agriculture in Polenz near Leipzig, Germany, where further assessments were performed.

Test design: Honey bee semi-field test in *Phacelia tanacetifolia*; four treatment groups (untreated control, test item group, two reference items) with four replicates (tunnels) for all treatment groups; additionally, a fifth replicate for residue analysis was set up for the control and the test item treatment, respectively; application of test item once at BBCH 65 (full flowering) of *Phacelia* during daily bee flight in separate tunnels; honey bee colonies were introduced to the tunnels at DAT -4 (DAT = days after treatment); pre-exposure phase was 3 days (from DAT -3 to DAT 0ba (ba = before application)); exposure phase was 7 days (from DAT 0aa (aa = after application) to DAT 7); post-exposure phase was 34 days (from DAT 8 to DAT 41); daily assessments of mortality during entire study period until DAT 41 in dead bee traps and on linen sheets until DAT 7, additional assessments of mortality were conducted 2 and 6 hours after application and in the evening after bee flight at nightfall on DAT 0aa; daily assessment of foraging activity from DAT -3 to DAT 7 on three 1 m² plots/tunnel, additional assessments were carried out once, shortly before application, two times within the 1st hour after application and after about 2, 4 and 6 hours after application; daily assessments on behaviour; assessments on colony development: colony strength, general brood and food status on DAT -2, DAT 4, DAT 7, DAT 13, DAT 21, DAT 29 and DAT 41 which is equal to BFD 0, BFD 6, BFD 9, BFD 15, BFD 23, BFD 31 and BFD 43 (BFD = brood area fixing day). For residue analyses, samples of flowers, pollen and nectar were taken. Flowers were sampled on DAT 0aa (within 4 hours after application on DAT 0aa). Pollen and nectar were gained on DAT 0aa.

Endpoints: Mortality: daily, dead bee trap during entire study period until DAT 41, linen sheets until DAT 7;
Foraging activity: daily assessment (on 3 x 1 m² per tunnel during tunnel phase);
Sublethal effects: behavioural changes were monitored daily until test end;
Colony assessments: (general food and brood status, colony strength): on DAT -2, DAT 4, DAT 7, DAT 13, DAT 21, DAT 29 and DAT 41, which is equal to BFD 0, BFD 6, BFD 9, BFD 15, BFD 23, BFD 31 and BFD 43;
Detailed brood assessments: on BFD 0, BFD 6, BFD 9, BFD 15 and BFD 23 for initially labelled eggs.

Reference items: Reference item I: Insegar 25 WG (fenoxycarb, 250 g/L nominal, 24.0% w/w analyzed); reference item II: Dandim Progress (dimethoate (400 g/L nominal, 38.48% w/w analyzed corresponding to 411.20 g/L).

Application rates:

Date (growth stage)	Treatment	Application rate		Applied item
		[mL product/ha]	[g total a.s./ha] *	[mL product/ha]
17.06.2021 (BBCH 65)	Control	--	--	--
	Test item (BAS 758 00 F)	1650	407	1585 - 1679
	Reference item I	1200 g product/ha	300	1183 - 1191
	Reference item II	1200	480	1193 - 1214

* Based on nominal content of the active substance(s).

The control group was treated with tap water. All treatments were applied in 400 L water/ha using a calibrated plot-sprayer.

Test conditions: Natural field conditions. Good weather conditions during application;
 On DAT 0: cloud coverage: 0%; wind: 0.0 - 0.6 m/s; temperature: 25.7 - 27.1°C, relative humidity: 43.8 - 46.0%, no rainfall before or during application;
 No precipitation during the exposure phase except 6 mm precipitation on DAT 4.

Analytics: Analytical analysis of test item concentrations in plant matrices (flowers, pollen and nectar, analytical method L0372/05) as well as analytical verification of the test item concentration in the spray solution (analytical method L0361/03) was conducted using an LC with MS/MS detection.

Statistics: Descriptive statistics; Tukey-test (two-sided) for pre-treatment data evaluation for comparisons between control, test item and reference item treatments.
 Post-treatment data evaluation: pair-wise testing for comparisons between treatments (test item or reference item) separately against control, Student t-test (for variance homogeneous data) or Welch t-test (for variance inhomogeneous data). Mortality and brood termination rate: one-sided greater; brood indices: one-sided smaller. Significance levels of all tests: $\alpha = 0.05$.

C. DESCRIPTION OF THE ANALYTICAL PROCEDURES

Concentrations of BAS 560 F, BAS 750 F and BAS 500 F (contained in BAS 758 00 F) in spray solution and in nectar, pollen and flowers were determined according to the analytical method L0361/03 for spray solution and method L0372/05 for nectar, pollen and flowers. Residues of BAS 560 F, BAS 750 F and BAS 500 F were analysed in spray solution specimens using an aliquot of 5 g (equal to 5 mL) and shaking it with acetonitrile/water/formic acid (400/600/2, v/v/v). An aliquot of the extract is then used for determination by LC-MS/MS with measuring method of L0361/03. Specimens of flowers, pollen, nectar and nectar surrogate were extracted with methanol/water (75/25, v/v) and cleaned-up using QuEChERS dSPE kit prior to LC-MS/MS analysis. Method analysing spray solution has a limit of quantification (LOQ) was 0.1 µg/L and a limit of detection (LOD) was set to 0.02 µg/L. Method analysing bee food items has a limit of quantification (LOQ) was 0.01 mg/kg and a limit of detection (LOD) was set to 0.003 mg/kg. Matrix effects were not assessed during this study. However, matrix-matched standards were used for quantification of BAS 560 F, BAS 750 F and BAS 500 F. The storage stability of the analytes in the solvent systems used throughout this study was assessed during the corresponding validation studies.

Details on measured fortification samples and obtained procedural recoveries of BAS 560 F, BAS 750 F and BAS 500 F are given in the table below.

Table A 79: Procedural recoveries for BAS 560 F, BAS 750 F and BAS 500 F in spray solutions

Matrix	Analyte	Fortification level [mg/kg]	n	Mean recovery [%]	RSD [%]
Spray solution	BAS 560 F	Control	2	< LOD	-
		0.1	7	102	9.0
		495758	5	110	2.3
		Overall	12	105	8.1
	BAS 750 F	Control	2	< LOD	-
		0.1	7	81.6	13
		324362	5	104	3.7
		Overall	12	90.7	16
	BAS 500 F	Control	2	< LOD	-
		0.1	7	96.7	4.4
		386925	5	110	3.5
		Overall	12	103	6.9

Table A 80: Procedural recoveries for BAS 560 F, BAS 750 F and BAS 500 F in bee food items.

Matrix	Analyte	Fortification level (mg/kg)	n	Mean Recoveries (%)	RSD (%)
Flowers	BAS 560 F	Control	2	< LOD	-
		0.010	6	105	2.6
		0.50	5	97.3	6.2
		10	1	82.0	-
		50	1	106	-
		Overall	13	100	7.6
	BAS 750 F	Control	2	< LOD	-
		0.010	6	106	2.7
		0.50	5	96.0	5.5
		10	1	84.2	-
		50	1	109	-
		Overall	13	101	7.9
	BAS 500 F	Control	2	< LOD	-
		0.010	6	105	4.2
		0.50	5	97.2	5.1
		10	1	83.9	-
		50	1	103	-
		Overall	13	100	7.3
Nectar	BAS 560 F	Control	2	< LOD	-
		0.010	5	94.4	8.8
		0.50	5	96.4	4.1
		Overall	10	95.4	6.5
	BAS 750 F	Control	2	< LOD	-
		0.010	5	89.2	12
		0.50	5	99.2	4.2
		Overall	10	94.2	10
	BAS 500 F	Control	2	< LOD	-
		0.010	5	99.2	4.9
		0.50	5	103	3.2
		Overall	10	101	4.4
Pollen	BAS 560 F	Control	2	< LOD	-
		0.010	5	93.0	9.7
		0.50	5	96.5	2.6
		10	1	101	-
		50	1	70.6	-
		Overall	12	93.3	10
	BAS 750 F	Control	2	< LOD	-
		0.010	5	89.5	2.3
		0.50	5	94.1	4.4
		10	1	100	-
		50	1	81.1	-

		Overall	12	91.6	5.9
	BAS 500 F	Control	2	< LOD	-
		0.010	5	94.8	4.2
		0.50	5	95.8	2.6
		10	1	100	-
		50	1	77.1	-
		Overall	12	94.2	6.7

II. RESULTS AND DISCUSSION

Mortality

Pre-exposure mortality was similarly low in control, test item and reference item treatments, indicating well adapted and comparable colonies. No statistically significant differences between the three treatment groups were observed during the pre-exposure phase (DAT -3 to DAT 0ba) (Tukey test, two-sided, $\alpha = 0.05$).

No increased mortality in the test item treatment and reference item I compared to the control was observed at any time during the test; neither during exposure phase (DAT 0aa to DAT 7), nor during post-exposure between DAT 8 and DAT 41, nor at overall comparisons (Student t- or Welch t-test, one-sided greater, $\alpha = 0.05$). Exposure of honey bees to reference item II was a statistically significant increase in the number of dead adults during exposure phase between DAT 0aa and DAT 7 and the overall mean of mortality between DAT 0aa and DAT 41. (Student t- or Welch t-test, one-sided greater, $\alpha > 0.05$).

During the pre-exposure, exposure and post-exposure phase, no dead pupae were found in the control, test item treatment and reference item II. Dead pupae were only observed from DAT 7 in all replicates of reference item I during exposure phase (DAT 0 to DAT 7). Predominantly pupal mortality occurred during the post-exposure phase in all other replicates of reference item I treatment. The overall mean pupal mortality amounted to 13.2 dead pupae/colony for the entire post-application phase, which was increased in comparison with the control.

Foraging activity

Foraging activity was assessed during the tunnel phase. During pre-exposure phase (DAT -3 to DAT 0ba) the overall daily mean foraging activity was on an adequate, similar and high level in all treatment groups and amounted to 7.3, 7.8, 8.0 and 7.3 bees/m², indicating that the bee colonies had well adapted to the new environmental conditions. No statistically significant differences occurred in the overall mean foraging activity during pre-exposure phase (Tukey-test, two-sided, $\alpha = 0.05$) by comparing the control, test item and reference item against each other.

On the day of application (DAT 0aa) following application of the test item no reduction of the foraging activity was observed throughout the first day after application. During the following days of exposure, no reduction but rather an increase of actively foraging bees occurred when compared to the pre-application level. The overall mean foraging activity for the exposure phase amounted to 9.4 and 9.8 bees/m²/day in the control and test item treatment, which is not statistically significant different compared to the control (Student-t test, one sided smaller, $\alpha = 0.05$).

The application of reference item I did not result in any reduction in foraging activity immediately after application as well as during the entire exposure phase. Furthermore, the overall mean foraging activity amounted to 7.2 bees/m² which is similar to the control of 9.4 bees/m² and not statistically significant different (Student-t test, one sided smaller, $\alpha = 0.05$).

The application of reference item II revealed a distinct reduction in foraging activity on all days of the exposure phase compared to the control. The overall daily foraging activity was statistically significantly lowered compared to the control and amounted to 0.5 bees/m²/day (Welch t-test, one sided smaller, $\alpha = 0.05$).

The effects on honey bee mortality and foraging activity are summarized in Table A 81.

Table A 81: Effects of BAS 758 00 F on honey bee mortality and foraging activity under semi-field conditions (tunnel test)

Assessment		Control		BAS 758 00 F [1.65 L/ha]		Reference item I [1.2 kg/ha]		Reference item II [1.2 kg/ha]	
		Mean ¹⁾	± SD	Mean ¹⁾	± SD	Mean ¹⁾	± SD	Mean ¹⁾	± SD
Adult mortality [bees/colony/ day]	Pre-exposure phase ²⁾ DAT -3 to DAT 0ba	6.3a	2.9	6.6a	0.5	7.2a	3.1	4.6a	1.2
	Exposure phase ²⁾ DAT 0aa to DAT 7	9.4	2.1	7.1	2.2	8.9	3.3	67.7*	7.4
	Post-exposure phase ³⁾ DAT 8 to DAT 41	11.3	5.8	9.3	2.0	6.2	1.6	16.2	7.7
	Overall, after application DAT 0aa to DAT 41	10.9	4.9	8.9	1.9	6.7	0.8	26.0*	6.2
Pupal mortality [bees/colony/ day]	Pre-exposure phase ²⁾ DAT -3 to DAT 0ba	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Exposure phase ²⁾ DAT 0aa to DAT 7	0.0	0.0	0.0	0.0	0.8	0.5	0.0	0.0
	Post-exposure phase ³⁾ DAT 8 to DAT 41	0.0	0.0	0.0	0.0	16.1	5.9	0.0	0.0
	Overall, after application DAT 0aa to DAT 41	0.0	0.0	0.0	0.0	13.2	4.8	0.0	0.0
Foraging activity [bees/m ² /col ony]	Pre-exposure phase ²⁾ DAT -3 to DAT 0ba	7.3a	1.0	7.8a	0.8	8.0a	0.8	7.3a	1.2
	Exposure phase ²⁾ DAT 0aa to DAT 7	9.4	0.9	9.8	1.2	7.2**	0.8	0.5**	0.2

Statistical analyses were performed with rounded values.

a: same letters indicate that groups are not statistically significant different (Tukey-test, $\alpha = 0.05$) at pre-application period

*: statistically significant different when comparing treatment against control via Student t-test at exposure phase and entire study period; one-sided greater: mortality, brood termination rate ($\alpha = 0.05$).

***: statistically significant different when comparing treatment against control via Student t-test for reference item I and Welch t-test for reference item II at exposure phase, one-sided smaller: foraging activity, brood index and brood compensation index ($\alpha = 0.05$).

¹⁾ mean of four replicates.

²⁾ sum of dead bees of dead bee trap and on linen sheets in the tunnels

³⁾ dead honey bees found in dead bee trap, only

Bee behavior

Exposure of honey bees to the test item did not result in abnormal behaviour or intoxication symptoms.

Colony strength

The assessment on colony strength before application on BFD 0 (DAT -2) revealed the bee colonies were on similar levels and confirmed an adequate and good strength for the conduction of the tunnel study. The mean estimated colony strength amounted to 7200, 7622, 6947 and 7341 bees/colony for the control, test item and reference item colonies, respectively.

The mean estimated colony strength of the test item treatment revealed a positive development at each assessment when compared to BFD 0. Both, control and test item treatment groups developed in a similar manner until BFD 43 and the mean estimated colony strength increased by +105% and +96% to 14794 and 14963 bees/colony, respectively.

With respect to reference item I and II treatments, the mean estimated colony strength revealed a lower

increase of the mean colony strength until BFD 43 compared to the control and test item treatment. The mean estimated colony strength of reference item I increased by 48% to 10294 bees/colony. The mean estimated colony strength of reference item II increased by 33% to 9788 bees/colony.

The effects on honey bee colony strength are summarized in Table A 82.

Table A 82: Colony strength: estimated average number of bees/colony

Treatment group		BFD 0 (DAT -2)	BFD 6 (DAT 4)	BFD 9 (DAT 7)	BFD 15 (DAT 13)	BFD 23 (DAT 21)	BFD 31 (DAT 29)	BFD 43 (DAT 41)
Control	Mean ¹⁾	7200	7931	7847	11138	13444	13697	14794
	± SD	1236	325	1494	1994	1465	2553	3039
	% ²⁾	-	+10	+9	+55	+87	+90	+105
BAS 758 00 F	Mean ¹⁾	7622	7734	8269	10913	13584	13106	14963
	± SD	1096	1690	1527	1750	1877	1505	1518
	% ²⁾	-	+1	+8	+43	+78	+72	+96
Reference item I	Mean ¹⁾	6947	6834	6553	8466	9534	8494	10294
	± SD	1081	1206	1505	2161	2399	2145	4188
	% ²⁾	-	-2	-6	+22	+37	+22	+48
Reference item II	Mean ¹⁾	7341	6638	6384	9338	9084	9563	9788
	± SD	1849	1578	472	1529	2107	2197	4068
	% ²⁾	-	-10	-13	+27	+24	+30	+66

DAT: day after treatment; BFD: Brood area fixing day;

¹⁾ mean of 4 replicates.

²⁾ relative change [%] in comparison with DAT -2 calculated from the respective mean values.

General brood and food development

Before application on BFD 0 (DAT -2), the mean brood status of the single stages as well as the entire brood (area occupied by eggs, larvae and pupae) was on a comparable level in all treatment. The mean brood area amounted to 7400, 7142, 6962 and 7220 cm²/colony for the control group, test item group, reference item I and reference item II, respectively. During the first investigated brood cycle until BFD 23, the mean comb area covered with brood stages in the control and test item treatment groups was on a similar level and developed within the range of natural variability, amounting to 10520 and 10829 cm²/colony for the control and test item groups, respectively. Compared to the pre-application level, the mean brood area increased by 42% and 52% in the control and test item group, respectively. The mean comb area covered with brood stages showed a lower increase in the reference item I during the first investigated brood cycle compared to the control and amounted to 8328 cm²/colony (+20%). The mean comb area covered with brood stages of reference item II treatment group slightly decreased by -4% to 6910 cm²/colony compared to the pre-application level.

At the last assessment on BFD 43 the mean brood area amounted to 8947, 9257, 7349 and 5569 cm²/colony for the control, test item, reference item I and reference item II, respectively. Compared to the pre-application level, the mean brood area changed by +21%, +30%, +6% and -23% in the control, test item, reference item I and reference item II, respectively.

The assessment of the areas covered with pollen and nectar/honey before application (BFD 0) revealed similar levels and a sufficient supply of all colonies with food. Further assessments displayed a distinct increase of nectar stores occurred from BFD 23 onwards which coincided with the flowering of linden trees in the vicinity of the monitoring site. The total food storages increased by +234%, +139%, +83% and +150% compared to BFD 0 and amounted to 10675, 9437, 7426 and 8715 cm²/colony for the control, test item, reference item I and reference item II, respectively. The effects on brood and food development are summarized in Table A 83.

Table A 83: Brood and food development: Estimated total brood (eggs, larvae + pupae) or food (nectar + pollen) area per colony [cm²/colony] ¹⁾

Treatment group		BFD 0 (DAT -2)	BFD 6 (DAT 4)	BFD 9 (DAT 7)	BFD 15 (DAT 13)	BFD 23 (DAT 21)	BFD 31 (DAT 29)	BFD 43 (DAT 41)
Eggs								
Control	Mean ¹⁾	1624	748	954	1599	2295	1263	2553
	± SD	515	176	155	434	479	893	508
	% ²⁾	-	-54	-41	-2	+41	-22	+57
BAS 758 00 F	Mean ¹⁾	1263	928	1289	2424	2114	1650	2269
	± SD	522	377	598	536	737	367	647
	% ²⁾	-	-27	+2	+92	+67	+31	+80
Reference item I	Mean ¹⁾	1135	825	619	1470	1392	902	1573
	± SD	484	357	266	308	707	494	535
	% ²⁾	-	-27	-45	+30	+23	-20	+39
Reference item II	Mean ¹⁾	722	1109	1057	1392	774	1238	1057
	± SD	332	865	1103	1135	724	819	899
	% ²⁾	-	+54	+46	+93	+7	+71	+46
Larvae								
Control	Mean ¹⁾	2140	2578	1856	2192	2759	2578	2063
	± SD	701	1007	367	818	706	168	326
	% ²⁾	-	+20	-13	+2	+29	+20	-4
BAS 758 00 F	Mean ¹⁾	2682	2553	1985	1908	2991	2243	2269
	± SD	1035	787	319	399	188	580	509
	% ²⁾	-	-5	-26	-29	+12	-16	-15
Reference item I	Mean ¹⁾	2398	1367	1573	1573	2450	2217	2114
	± SD	586	598	389	341	721	676	810
	% ²⁾	-	-43	-34	-34	+2	-8	-12
Reference item II	Mean ¹⁾	2604	1444	1057	1573	2063	1547	1392
	± SD	519	362	781	1103	1400	1191	1186
	% ²⁾	-	-45	-59	-40	-21	-41	-47
Pupae								
Control	Mean ¹⁾	3636	3971	4899	4925	5466	7658	4332
	± SD	330	425	309	1621	1318	1338	734
	% ²⁾	-	+9	+35	+35	+50	+111	+19
BAS 758 00 F	Mean ¹⁾	3197	4564	4693	5080	5724	7220	5080
	± SD	253	814	1093	1242	487	739	839
	% ²⁾	-	+43	+47	+59	+79	+126	+59
Reference item I	Mean ¹⁾	3429	3765	3378	2553	4486	5647	3661
	± SD	676	562	341	769	1253	1330	1857
	% ²⁾	-	+10	-2	-26	+31	+65	+7
Reference item II	Mean ¹⁾	3893	4564	4564	3249	4074	4667	3120
	± SD	676	984	362	1222	3071	3242	2206
	% ²⁾	-	+17	+17	-17	+5	+20	-20

Treatment group		BFD 0 (DAT -2)	BFD 6 (DAT 4)	BFD 9 (DAT 7)	BFD 15 (DAT 13)	BFD 23 (DAT 21)	BFD 31 (DAT 29)	BFD 43 (DAT 41)
Entire brood (eggs, larvae + pupae)								
Control	Mean ¹⁾	7400	7297	7710	8715	10520	11500	8947
	± SD	701	1273	573	1144	1812	1777	881
	% ²⁾		-1	+4	+18	+42	+55	+21
BAS 758 00 F	Mean ¹⁾	7142	8045	7967	9411	10829	11113	9257
	± SD	1015	1176	1189	711	1241	1201	479
	% ²⁾	-	+13	+12	+32	+52	+56	+30
Reference item I	Mean ¹⁾	6962	5956	5569	5595	8328	8767	7349
	± SD	844	573	589	609	2382	2394	2710
	% ²⁾	-	-14	-20	-20	+20	+26	+6
Reference item II	Mean ¹⁾	7220	7116	6678	6214	6910	7452	5569
	± SD	487	1759	1857	3353	5125	5126	4254
	% ²⁾	-	-1	-7	-14	-4	+3	-23
Nectar								
Control	Mean ¹⁾	2192	2991	2450	2888	9179	8999	8483
	± SD	244	838	721	1035	2699	2597	2482
	% ²⁾	-	+36	+12	+32	+319	+311	+287
BAS 758 00 F	Mean ¹⁾	2965	2269	2682	2604	7374	7967	7838
	± SD	716	223	357	155	2496	2131	2448
	% ²⁾	-	-23	-10	-12	+149	+169	+164
Reference item I	Mean ¹⁾	2810	2862	2475	2501	6111	5569	5827
	± SD	987	950	903	676	1959	2125	2801
	% ²⁾	-	+2	-12	-11	+117	+98	+107
Reference item II	Mean ¹⁾	2450	2398	2166	2089	7632	7374	7271
	± SD	465	746	332	744	3067	2022	3456
	% ²⁾	-	-2	-12	-15	+212	+201	+197
Pollen								
Control	Mean ¹⁾	1006	851	593	1831	954	1650	2192
	± SD	479	229	472	924	508	1031	950
	% ²⁾	-	-15	-41	+82	-5	+64	+118
BAS 758 00 F	Mean ¹⁾	980	1212	980	1573	1238	1263	1599
	± SD	434	912	797	852	903	567	425
	% ²⁾	-	+24	±0	+61	+26	+29	+63
Reference item I	Mean ¹⁾	1238	1263	980	1135	1057	980	1599
	± SD	404	361	598	484	573	682	702
	% ²⁾	-	+2	-21	-8	-15	-21	+29
Reference item II	Mean ¹⁾	1031	645	284	1392	902	1212	1444
	± SD	332	60	119	702	417	1073	546
	% ²⁾	-	-38	-73	+35	-13	+18	+40

Treatment group		BFD 0 (DAT -2)	BFD 6 (DAT 4)	BFD 9 (DAT 7)	BFD 15 (DAT 13)	BFD 23 (DAT 21)	BFD 31 (DAT 29)	BFD 43 (DAT 41)
Entire food (nectar + pollen)								
Control	Mean ¹⁾	3197	3842	3043	4719	10133	10649	10675
	± SD	589	864	909	1440	3106	3529	3247
	% ²⁾	-	+20	-5	+48	+217	+233	+234
BAS 758 00 F	Mean ¹⁾	3945	3481	3661	4177	8612	9231	9437
	± SD	792	950	1030	928	2773	2113	2248
	% ²⁾	-	-12	-7	+6	+118	+134	+139
Reference item I	Mean ¹⁾	4048	4126	3455	3636	7168	6549	7426
	± SD	881	863	1043	916	2533	2703	3415
	% ²⁾	-	+2	-15	-10	+77	+62	+83
Reference item II	Mean ¹⁾	3481	3043	2450	3481	8535	8586	8715
	± SD	315	781	309	1393	3290	2417	2952
	% ²⁾	-	-13	-30	±0	+145	+147	+150

DAT: day after treatment; BFD: Brood area fixing day.

¹⁾ Mean of 4 replicates.

²⁾ Relative change [%] in comparison with BFD 0 (DAT -2) calculated from the respective mean values

Detailed brood development of individually labelled brood cells

The evaluation of the development of initially labelled brood cells (eggs) was expressed by the following brood indices: Brood termination rate [BTR], brood index [BI] and brood compensation index [BCI].

Brood termination rate [BTR]

The mean BTR of initially labelled eggs amounted to 11.8 and 9.1% for the control and test item group, respectively, on BFD 6, which increased up to 12.8 and 9.8%, respectively, until the last assessment on BFD 23. Therefore, the termination of labelled eggs was on a similar level and within a natural range of variability, without any statistically significant differences between control and test item treatment (Student-t test, one sided greater, $\alpha = 0.05$).

In contrast, reference item I revealed a high brood termination rate of 71.8% at BFD 23, which was statistically significantly different when compared to the control group (Student-t test, one sided greater, $\alpha = 0.05$).

Brood index [BI]

The BI displays a negative correlation with the BTR: the higher the BTR the lower the BI and vice versa. Therefore, the mean BI of initially labelled eggs was slightly higher in the test item treatment and amounted to 4.5 and 4.4 for the control and test item group, respectively, without any statistically significant differences during the study (Student-t test, one sided smaller, $\alpha = 0.05$).

In contrast, reference item I revealed a much lower brood index of 1.4, which is statistically significantly different compared to the control (Student-t test, one sided smaller, $\alpha = 0.05$).

Brood compensation index [BCI]

The BCI was on a similar level for both control and test item treatment and amounted to 4.4 and 4.6 in the control and test item treatment, respectively and therefore without any statistically significant difference, indicating most of terminated brood-cells were refilled with new eggs (Student-t test, one sided smaller, $\alpha = 0.05$).

In contrast, reference item I revealed a distinct statistically significant lower brood compensation index of 2.2, which means only few emptied cells were refilled with new eggs. (Student-t test, one sided smaller, $\alpha = 0.05$).

The results are summarized in Table A 84 below.

Table A 84: Detailed brood developments (single cell assessments): BTR, BI and BCI on BFD 23¹⁾

Assessment	Control		BAS 758 00 F [1.650 L/ha]		Reference Item I [1200 g/ha]	
	Mean ²⁾	± SD	Mean ²⁾	± SD	Mean ²⁾	± SD
Brood termination rate (BTR) [%]	12.8	10.6	9.8	9.2	71.8*	17.3
Brood-index (BI)	4.4	0.5	4.5	0.5	1.4*	0.9
Brood compensation index (BCI)	4.4	0.5	4.6	0.3	2.2*	1.2

Statistical analyses were performed with rounded values.

*: statistically significant different when comparing treatment against control via Student t-test at post-application period; one-sided greater: brood termination rate; one-sided smaller: brood index and brood compensation index ($\alpha=0.05$).

¹⁾ at the last relevant assessment when development is expected to be completed, *i.e.* BFD 23 for marked eggs.

²⁾ mean of 4 replicates.

Quality criteria:

Quality criteria ¹⁾	Obtained in this study
Reference item treatment: brood termination > 50% and < 90% or distinct increase in pupal and adult mortality compared to the control	Ref. item I (fenoxycarb): 71.8% brood termination at BFD 23 13.2 dead pupae/colony/day (0 dead pupae/colony/day in the control) in the post-treatment phase Ref. item II (dimethoate): 26.0 dead adult bees/colony/day (10.9 dead adult bees/colony/day in the control) in the post-treatment
Flight density of ≥ 5 bees/m ² shortly before the application	6.7 to 10.7 bees/m ² in all treatment groups

¹⁾ There are no validity criteria listed in OECD 75 (2007). Nevertheless, general criteria to assess the quality of honey bee (semi-)field studies can be considered.

III. CONCLUSION

Under semi-field conditions (tunnel test), BAS 758 00 F was applied once at a rate of 1650 mL/ha (equivalent to 407 g total a.s./ha) to full-flowering *Phacelia tanacetifolia*. No unacceptable effects on mortality, foraging activity, colony development, colony strength or bee brood were observed after exposure. Overall, based on the results of this study it can be concluded that BAS 758 00 F does not adversely affect honey bee colonies.

A 2.4.1.6 KCP 10.3.1.6 Field tests with honeybees

BAS 758 00 F poses no unacceptable risk to bees. Further studies are not necessary

A 2.4.2 KCP 10.3.2 Effects on non-target arthropods other than bees

A 2.4.3 KCP 10.3.2.1 Standard laboratory testing for non-target arthropods

A 2.4.3.1 Study 1

Comments of zRMS:	The study follows the guideline specified by Blümel <i>et al.</i> (2000) and according to the principles of GLP. The study is considered to be valid.
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Reference:	CP 10.3.2.1/1
Report	Effects of BAS 758 00 F on the predatory mite <i>Typhlodromus pyri</i> Scheuten in a laboratory test, Roehlig, U., 2020 Report No 876359, 2048NTL0010 BASF DocID 2020/2037663 Authority registration No
Guideline(s):	IOBC (Bluemel et al. 2000)
Deviations:	No
GLP:	yes (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany),
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive Summary

In a worst-case laboratory study, protonymphs of *Typhlodromus pyri* Scheuten (Acaria: Phytoseiidae) were exposed to dried residues of BAS 758 00 F on glass plates. The test item was applied at test rates of 0.28125, 0.5625, 1.125, 2.25 and 4.5 L BAS 758 00 F/ha. Additional test units were treated with deionized water as control and with DANADIM PROGRESS (dimethoate) as reference item. Mortality was assessed 3 and 7 days after treatment by the number of surviving, dead and escaped predatory mites.

After 7 days, in the water-treated control a mortality of 2.0% was observed. In the test item treatments mortality ranged between 1.0% and 94.0%. This resulted in corrected mortality rates between -1.0% and 93.9%. Statistically significant differences in mortality compared to the control were observed at 1.125, 2.25 and 4.5 L BAS 758 00 F/ha.

In a worst-case laboratory study with BAS 758 00 F, the LR_{50} for *Typhlodromus pyri* was determined to be 1.748 L BAS 758 00 F/ha.

I. MATERIALS AND METHODS

A. MATERIALS

Test item: BAS 758 00 F; batch no. FD-200124-0004; content of a.s.: metrafenone (BAS 560 F, Reg. No. 4 037 710): 94.9 g/L analyzed (100.0 g/L nominal), mefentrifluconazole (BAS 750 F, Reg. No. 5 834 378): 66.7 g/L analyzed (66.6 g/L nominal), pyraclostrobin (BAS 500 F, Reg. No. 304 428): 81.0 g/L analyzed (80.0 g/L nominal); density: 1.092 g/cm³.

B. STUDY DESIGN

Test species: Predatory mite (*Typhlodromus pyri* Scheuten), protonymphs, less than 24 hours old, source: source (in the stage of eggs): Katz Biotech AG, 15837 Baruth, Germany.

Test design: Exposure of protonymphs via air-dried residues on treated glass plates; 7 treatment groups (5 test item rates, 1 water treated control and 1 reference item) with 5 replicates (consisting of 20 protonymphs) per treatment; Assessments of mortality on day 3 and 7 after treatment.

Endpoints: Mortality after exposure over 7 days, including determination of the LR₅₀ (Lethal Rate 50%, rate resulting in 50% mortality).

Reference item: DANADIM PROGRESS (a.s.: dimethoate, analyzed content: 411.2 g/L, nominal: 400 g/L).

Test rates: Control: deionized water, test item: 0.28125, 0.5625, 1.125, 2.25 and 4.5 L BAS 758 00 F/ha, reference item: 15 mL/ha.

BAS 758 00 F [L product/ha]	BAS 560 F [g a.s./ha]*	BAS 750 F [g a.s./ha]*	BAS 500 F [g a.s./ha]*
0.28125	281.25	18.75	22.5
0.5625	562.5	37.5	45
1.125	112.5	75	90
2.25	225	150	180
4.5	450	300	360

* Based on nominal content of active substance and a test item density of 1.092 g/cm³.

All substances were applied in 200 L water/ha and sprayed onto glass plates via a laboratory spraying equipment and air dried afterwards.

Test conditions: Temperature: 23 °C – 25 °C, relative humidity: 68% - 72%; photoperiod: 16 h light : 8 h dark; light intensity: 2060 lux; food: pollen: pine (*Pinus nigra*) and birch (*Betula pendula*), 1:1.

Analytics: No analytical verification of the test item is required according to the current test guideline. Hence, no analytical verification was conducted.

Statistics: Descriptive statistics. Multiple Sequentially-rejective Chi² 2x2 Table Test with Bonferroni-Holm ($\alpha = 0.05$) for mortality, Probit Analysis LR₅₀ calculation.

II. RESULTS AND DISCUSSION

After 7 days, in the water-treated control a mortality of 2.0% was observed. In the test item treatments mortality ranged between 1.0% and 94.0%. This resulted in corrected mortality rates between -1.0% and 93.9%. Statistically significant differences in mortality compared to the control were observed at 1.125, 2.25 and 4.5 L BAS 758 00 F/ha (Multiple Sequentially-rejective Chi² 2x2 Table Test with Bonferroni-Holm, $\alpha = 0.05$). The results are summarized below in Table A 85.

Table A 85: Effects on predatory mites (*Typhlodromus pyri*) exposed to BAS 758 00 F in a laboratory trial

Test item	Rate ¹⁾ [L/ha]	Mortality ²⁾ [%]	Corrected mortality ³⁾ [%]
Control	--	2.0	--
BAS 758 00 F	0.28125	1.0	-1.0
	0.5625	3.0	1.0
	1.125	22.0 *	20.4
	2.25	73.0 *	72.4
	4.5	94.0 *	93.9
Endpoint [L BAS 758 00 F/ha]			
LR ₅₀ [95% CL]	1.748 [1.597 - 1.915]		

CL = Confidence Limits

* Mortality statistically significant different compared to the control (Multiple Sequentially-rejective Chi² 2x2 Table Test with Bonferroni-Holm, $\alpha = 0.05$).

1) Application rate in 200 L water/ha.

2) Mortality after 7 days of exposure to BAS 758 00 F on glass surface.

3) Corrected mortality according to Abbott (1925).

The reference item caused a mortality of 80.0% of exposed mites, resulting in a corrected mortality of 79.6%.

Validity criteria:

Validity criteria according to Bluemel et al (2000)	Obtained in this study
Control mortality $\leq 20\%$ on day 7	2.0%
Corrected mortality in the reference group 50-100% on day 7	79.6%

All validity criteria were met.

III. CONCLUSION

In a worst-case laboratory study with BAS 758 00 F, the LR₅₀ for *Typhlodromus pyri* was determined to be 1.748 L BAS 758 00 F/ha.

A 2.4.3.2 Study 2

Comments of zRMS:	The study follows the guideline specified by Mead Briggs <i>et al.</i> and according to the principles of GLP. The study is considered to be valid.
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Reference:	CP 10.3.2.1/2
Report	Effects of BAS 758 00 F on the parasitic wasp <i>Aphidius rhopalosiphi</i> (DESTEFANI-PEREZ) in a laboratory test, Roehlig, U., 2020 Report No 876361, 2048NAL0012 BASF DocID 2020/2037662 Authority registration No
Guideline(s):	IOBC (Mead-Briggs et al. 2000)
Deviations:	No
GLP:	yes (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany),
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive Summary

In a worst-case laboratory study, adults of *Aphidius rhopalosiphi* (Hymenoptera: Braconidae) were exposed to dried residues of 0.28125, 0.5625, 1.125, 2.25 and 4.5 L BAS 758 00 F/ha. Additional test units were treated with deionized water as control and with DANADIM PROGRESS (dimethoate) as reference item. Mortality was assessed 2, 24 and 48 hours after exposure.

After 48 hours in the water-treated control a mortality of 5.0% was observed. In the test item treatments mortality ranged between 5.0% and 100%. This resulted in corrected mortality rates between 0% and 100%. Statistically significant differences in mortality compared to the control were observed all rates above 0.28125 L BAS 758 00 F/ha.

In a worst-case laboratory study with BAS 758 00 F, the LR₅₀ for *Aphidius rhopalosiphi* was determined to be 0.61621 L BAS 758 00 F/ha.

I. MATERIALS AND METHODS

A. MATERIALS

Test item: BAS 758 00 F; batch no. FD-200124-0004; content of a.s.: metrafenone (BAS 560 F, Reg. No. 4 037 710): 94.9 g/L analyzed (100.0 g/L nominal), mefentrifluconazole (BAS 750 F, Reg. No. 5 834 378): 66.7 g/L analyzed (66.6 g/L nominal), pyraclostrobin (BAS 500 F, Reg. No. 304 428): 81.0 g/L analyzed (80.0 g/L nominal); density: 1.092 g/cm³.

B. STUDY DESIGN

Test species: Parasitic wasp (*Aphidius rhopalosiphii* DeStephani-Perez), adults (< 48 hours old); source (in the stage of mummies): Katz Biotech AG, 15837 Baruth, Germany.

Test design: Exposure of parasitoids via air-dried residues on treated glass plates; 7 treatment groups (5 test item rates, water treated control, reference item) with 4 replicates per treatment; each replicate containing 7 females and 3 males. Assessment of mortality 2, 24 and 48 hours after test initiation.

Endpoints: Mortality after exposure over 48 hours including the determination of LR₅₀.

Reference item: DANADIM PROGRESS (a.s.: dimethoate, analyzed content: 411.2 g/L, nominal: 400 g/L).

Test rates: Control: deionized water, test item: 0.28125, 0.5625, 1.125, 2.25 and 4.5 L BAS 758 00 F/ha, reference item: 0.3 mL/ha.

BAS 758 00 F [L product/ha]	BAS 560 F [g a.s./ha]*	BAS 750 F [g a.s./ha]*	BAS 500 F [g a.s./ha]*
0.28125	281.25	18.75	22.5
0.5625	562.5	37.5	45
1.125	112.5	75	90
2.25	225	150	180
4.5	450	300	360

* based on nominal content of active substance and a test item density of 1.092 g/cm³.

All substances were applied in 200 L water/ha. The substances were sprayed onto glass plates via laboratory spraying equipment and left air dried afterwards.

Test conditions: Temperature: 19.0 °C - 22.0 °C; relative humidity: 68% - 72%; photoperiod: 16 h light : 8 h dark; light intensity: 2060 lux; food: 25% w/w aqueous fructose solution.

Analytics: No analytical verification of the test item is required according to the current test guideline. Hence, no analytical verification was conducted.

Statistics: Descriptive statistics. Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm ($\alpha = 0.05$) for mortality, Spearman-Kärber for LR₅₀ calculation.

II. RESULTS AND DISCUSSION

After 48 hours in the water-treated control a mortality of 5.0% was observed. In the test item treatments mortality ranged between 5.0% and 100%. This resulted in corrected mortality rates between 0% and 100%. Statistically significant differences in mortality compared to the control were observed all rates above 0.28125 L BAS 758 00 F/ha (Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm, $\alpha = 0.05$). The results are summarized in Table A 86.

Table A 86: Effects on parasitoids (*Aphidius rhopalosiphi*) exposed to BAS 758 00 F in a laboratory trial

Test item	Rate ¹⁾ [L/ha]	Mortality ²⁾ [%]	Corrected mortality ³⁾ [%]
Control	--	5.0	--
BAS 758 00 F	0.28125	5.0	0
	0.5625	40.0 *	36.8
	1.125	100 *	100
	2.25	100 *	100
	4.5	100 *	100
Endpoint [L BAS 758 00 F/ha]			
LR ₅₀ [95% CL]	0.61621 [0.55439 - 0.68494]		

CL = Confidence Limits

* Mortality statistically significant different compared to the control (Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm, $\alpha = 0.05$).

¹⁾ Application rate in 200 L water/ha.

²⁾ Mortality after 48 hours of exposure to BAS 758 00 F on glass surface.

³⁾ Corrected mortality according to Abbott (1925).

The reference item caused a mortality of 100% of exposed wasps, resulting in a corrected mortality of 100%.

Validity criteria:

Validity criteria according to Mead-Briggs M. et al. (2000)	Obtained in this study
Control mortality < 13% (48h)	5.0%
Corrected mortality in the reference item group 50 - 100% (48h)	100%

All validity criteria were met.

III. CONCLUSION

In a worst-case laboratory study with BAS 758 00 F, the LR₅₀ for *Aphidius rhopalosiphi* was determined to be 0.61621 L BAS 758 00 F/ha.

A 2.4.4 KCP 10.3.2.2 Extended laboratory testing, aged residue studies with non-target arthropods

A 2.4.4.1 Study 1

Comments of zRMS:	The study was conducted to guidance and according to the principles of GLP. All validity criteria were met. The study is considered to be reliable and suitable for the risk assessment.
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Reference:	CP 10.3.2.2/1
Report	Effects of BAS 758 00 F on the parasitic wasp <i>Aphidius rhopalosiphi</i> (DESTEFANI-PEREZ) in an extended laboratory test, Roehlig, U., 2021 Report No 876362, 2148NAE0008 BASF DocID 2021/2015154 Authority registration No
Guideline(s):	IOBC (Mead-Briggs et al. 2009)
Deviations:	No
GLP:	yes (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany),
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive Summary

In an extended laboratory study, adults of the parasitic wasp *Aphidius rhopalosiphi* were exposed to fresh, dried residues of BAS 758 00 F on potted barley plants. The test item was applied at application rates of 0.5, 0.75, 1.0, 1.5, 2.0 and 3.0 L BAS 758 00 F/ha. Additional test units were treated with deionized water as control and with DANADIM PROGRESS (dimethoate) as reference item. Assessment of mortality of wasps was conducted 2, 24 and 48 h after test initiation by the number of surviving, affected, moribund and dead wasps. For the reproduction assessment 15 females from each treatment were transferred individually to pots with untreated, aphid-infested wheat plants for 24 h and then removed. The number of parasitized aphid mummies was recorded 11 days later.

After 48 hours, in the water-treated control a mortality of 6.7% was observed. In the test item treatments mortality ranged between 3.3% and 10.0%. This resulted in corrected mortality rates between -3.6% and 3.6%. No statistically significant effects on mortality were determined in all test item treatments. The mean number of mummies per female in the test item treatments was between 18.9 and 22.3 in comparison to the control with 21.4 mummies per female. No statistically significant effects on reproductive capacity were determined in all test item treatments.

In an extended laboratory study with BAS 758 00 F, the LR₅₀ for *Aphidius rhopalosiphi* was > 3.0 L BAS 758 00 F/ha. The ER₅₀ for reproduction was estimated to be > 3.0 L BAS 758 00 F/ha.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 758 00 F; batch no. FD-200124-0004; content of a.s.: metrafenone (BAS 560 F, Reg. No. 4 037 710): 94.9 g/L analyzed (100.0 g/L nominal), mefentrifluconazole (BAS 750 F, Reg. No. 5 834 378): 66.7 g/L analyzed (66.6 g/L nominal), pyraclostrobin (BAS 500 F, Reg. No. 304 428): 81.0 g/L analyzed (80.0 g/L nominal); density: 1.092 g/cm³.

B. STUDY DESIGN

Test species: Parasitic wasp (*Aphidius rhopalosiphi* DeStephani-Perez), adults (< 48 hours old); source (in the stage of mummies): Katz Biotech AG, 15837 Baruth, Germany.

Test design: Exposure of the adults was achieved via air-dried spray residues on treated, potted barley plants. 8 treatment groups (6 test item rates, water treated control, reference item) with 6 replicates (consisting of 5 females) per treatment. Mortality assessments were carried out 2, 24 and 48 hours after start of exposure of the wasps. At 48 hours, surviving wasps (15 females per treatment) were removed and their reproductive capacity was assessed by confining them individually over untreated wheat plants infested with adult and nymphal aphids (*Rhopalosiphum padi*). Assessment of reproduction capacity, i.e. number of mummies per female, was made for the control and all treated groups (1 assessment, 14 days after application).

Endpoints: Mortality: number of dead wasps, including the determination of the LR₅₀. Reproductive capacity: number of mummies per female, including the determination of the ER₅₀.

Reference item: DANADIM PROGRESS (a.s.: dimethoate, analyzed content: 411.2 g/L, nominal: 400 g/L).

Test rates: Control: deionized water, test item: 0.5, 0.75, 1.0, 1.5, 2.0 and 3.0 L BAS 758 00 F/ha, reference item: 10 mL/ha.

BAS 758 00 F [L product/ha]	BAS 560 F [g a.s./ha]*	BAS 750 F [g a.s./ha]*	BAS 500 F [g a.s./ha]*
0.5	50	33.30	40
0.75	75	49.95	60
1.0	100	66.60	80
1.5	150	99.90	120
2.0	200	133.2	160
3.0	300	200.0	240

* Based on nominal content of active substance and a test item density of 1.092 g/cm³.

All substances were applied in 400 L water/ha. The substances were sprayed on potted barley plants via laboratory spraying equipment and air dried afterwards.

Test conditions: Temperature: 19 °C - 22 °C; relative humidity 66% - 72%; photoperiod 16 h light : 8 h dark; light intensity: 1050 lux (mortality phase), 2550 lux (parasitization phase), 6680 lux (reproduction phase); food: 10% w/w aqueous fructose solution.

Analytics:	No analytical verification of the test item is required according to the current test guideline. Hence, no analytical verification was conducted.
Statistics:	Descriptive statistics. Chi ² 2x2 Table Test with Bonferroni Correction ($\alpha = 0.05$) for mortality, Williams t-test for reproductive capacity ($\alpha = 0.05$).

II. RESULTS AND DISCUSSION

After 48 hours, in the water-treated control a mortality of 6.7% was observed. In the test item treatments mortality ranged between 3.3% and 10.0%. This resulted in corrected mortality rates between -3.6% and 3.6%. No statistically significant effects on mortality were determined in all test item treatments (Chi² 2x2 Table Test with Bonferroni Correction, $\alpha = 0.05$). The mean number of mummies per female in the test item treatments was between 18.9 and 22.3 in comparison to the control with 21.4 mummies per female. No statistically significant effects on reproductive capacity were determined in all test item treatments (Williams t-test, $\alpha = 0.05$).

The results are summarized in Table A 87.

Table A 87: Effects of BAS 758 00 F on parasitoids (*Aphidius rhopalosiphi*) in an extended laboratory trial

Treatment	Rate [L/ha] ¹⁾	Mortality [%] ²⁾	Mortality corr. [%] ³⁾	Reproduction [mummies/female] ⁴⁾	Effect on Reproduction [%] ⁵⁾
Control	--	6.7	--	21.4	--
BAS 758 00 F	0.5	6.7	0	22.1	-3.3
	0.75	3.3	-3.6	20.2	5.6
	1.0	3.3	-3.6	20.6	3.7
	1.5	6.7	0	22.3	-4.2
	2.0	10.0	3.6	20.1	6.1
	3.0	6.7	0	18.9	11.7
Endpoints [L BAS 758 00 F/ha]					
LR ₅₀	> 3.0				
ER ₅₀	> 3.0				

¹⁾ Application rate in 400 L water/ha.

²⁾ Mortality after 48 h of exposure to BAS 758 00 F on treated barley plants.

³⁾ Corrected mortality according to Abbott (1925).

⁴⁾ Reproduction: mean number of parasitized aphids (mummies)/surviving female. The result is compared to a control (Williams t-test, $\alpha = 0.05$).

⁵⁾ Change in mean number of mummies per female, relative to control. A negative value indicates an increase and a positive value indicates a decrease relative to the control.

The reference item caused a mortality of 96.7% of exposed wasps, resulting in a corrected mortality of 96.4%.

Validity criteria:

Validity criteria according to Mead-Briggs M. et al. (2009)	Obtained in this study
Control mortality < 10% (48h)	6.7%
Corrected mortality in the reference item group 50-100% (48h)	96.4%
Number of mummies in the control ≥ 5 mummies/female and no more than two zero values	21.4; no more than 2 zero values

All validity criteria were met.

III. CONCLUSION

In an extended laboratory study with BAS 758 00 F, the LR_{50} for *Aphidius rhopalosiphi* was > 3.0 L BAS 758 00 F/ha. The ER_{50} for reproduction was estimated to be > 3.0 L BAS 758 00 F/ha.

A 2.4.4.2 Study 2

Comments of zRMS:	The study was conducted to guidance and according to the principles of GLP. All validity criteria were met. The study is considered to be reliable and suitable for the risk assessment.
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Reference:	CP 10.3.2.2/2
Report	Effects of BAS 758 00 F on the green lacewing <i>Chrysoperla carnea</i> STEPH. in an extended laboratory test, Roehlig, U., 2021 Report No 876358, 2148NCE0004 BASF DocID 2021/2015155 Authority registration No
Guideline(s):	IOBC (Vogt et al. 2000) modified for the exposure on natural substrate (extended laboratory test)
Deviations:	No
GLP:	yes (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany),
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive Summary

In an extended laboratory study, larvae of *Chrysoperla carnea* (Neuroptera: Chrysopidae) were exposed to fresh, dried residues of BAS 758 00 F on bean leaves. The test item was applied at rates of 0.5, 0.75, 1.0, 1.5, 2.0 and 3.0 L BAS 758 00 F/ha. Additional test units were treated with deionized water as control and with DANADIM PROGRESS (dimethoate) as reference item. Assessment of mortality was conducted regularly until hatching of the adult lacewings by the number of dead larvae and pupae. For the control and all test item groups up to and including 1.5 L BAS 758 00 F/ha the reproductive performance, i.e. egg deposition and hatching rate, was assessed (2 assessments/week, 24 h period each).

A mortality of 6.0% was observed in the control group. In the test item treatment groups, mortalities ranged from 18.0% to 76.0% resulting in corrected mortality rates between 12.8% and 74.5%. Statistically significant effects on mortality were observed in all test item treatment groups above 0.5 L BAS 758 00 F/ha. No effects on reproduction of *Chrysoperla carnea* occurred, when the test item was applied at rates up to and including 1.0 L BAS 758 00 F/ha. In the control and the test item treatments up to and including 1.0 L product/ha the number of eggs per female per day was > 15 and the hatching rate was > 70 %, whereas the reproduction at the rate of 1.5 L product/ha was < 15 eggs per female.

In an extended laboratory study with BAS 758 00 F, the LR₅₀ for *Chrysoperla carnea* was 1.37 L BAS 758 00 F/ha in 200 L water/ha. No unacceptable effects on reproduction were observed up to and including an application rate of 1.0 L BAS 758 00 F/ha in 200 L water/ha.

I. MATERIALS AND METHODS

A. MATERIALS

Test item: BAS 758 00 F; batch no. FD-200124-0004; content of a.s.: metrafenone (BAS 560 F, Reg. No. 4 037 710): 94.9 g/L analyzed (100.0 g/L nominal), mefentrifluconazole (BAS 750 F, Reg. No. 5 834 378): 66.7 g/L analyzed (66.6 g/L nominal), pyraclostrobin (BAS 500 F, Reg. No. 304 428): 81.0 g/L analyzed (80.0 g/L nominal); density: 1.092 g/cm³.

B. STUDY DESIGN

Test species: Green lacewing (*Chrysoperla carnea* Steph), larvae (2 - 3 days old), source: reared in the laboratory of the test facility.

Test design: Exposure of the larvae was reached via air-dried spray residues on treated bean leaves (*Phaseolus vulgaris*). 8 treatment groups (6 test item, water treated control, reference item); 50 replicates (consisting of one larva per replicate) per treatment group. Exposure lasted until pupae were transferred to oviposition units for development of adults. Mortality assessments were carried out regularly until hatching of the adult lacewings. In addition, for the control and all test item groups up to and including 1.5 L BAS 758 00 F/ha the reproductive performance, i.e. egg deposition and hatching rate, was determined (2 assessments/week, 24 h period each).

Endpoints: Pre-imaginal mortality including the estimation of a LR₅₀ (Lethal Rate 50%, rate resulting in 50% mortality), Reproductive performance: number of produced eggs per female per day and hatching rate.

Reference item: DANADIM PROGRESS (a.s.: dimethoate, analyzed content: 411.2 g/L, nominal: 400 g/L).

Test rates: Control: deionized water, test item: 0.5, 0.75, 1.0, 1.5, 2.0 and 3.0 L BAS 758 00 F/ha, reference item: 40 mL/ha.

BAS 758 00 F [L product/ha]	BAS 560 F [g a.s./ha]*	BAS 750 F [g a.s./ha]*	BAS 500 F [g a.s./ha]*
0.5	50	33.30	40
0.75	75	49.95	60
1.0	100	66.60	80
1.5	150	99.90	120
2.0	200	133.2	160
3.0	300	200.0	240

* Based on nominal content of active substance and a test item density of 1.092 g/cm³.

All substances were applied in 200 L water/ha. The substances were sprayed on bean leaves via laboratory spraying equipment and air dried afterwards.

Test conditions: Temperature: 23 °C - 26 °C; relative humidity: 67% - 73%; photoperiod: 16 h light : 8 h dark; light intensity: 1080 lux; food: larvae: *Sitotroga cerealella* eggs (UV-sterilized), adults: artificial diet.

Statistics: Descriptive statistics, Multiple Sequentially-rejective Fisher test after Bonferroni-Holm ($\alpha = 0.05$) for mortality, Probit analysis for LR₅₀ calculation.

II. RESULTS AND DISCUSSION

A mortality of 6.0% was observed in the control group. In the test item treatment groups, mortalities ranged from 18.0% to 76.0% resulting in corrected mortality rates between 12.8% and 74.5%. Statistically significant effects on mortality were observed in all test item treatment groups above 0.5 L BAS 758 00 F/ha (Multiple Sequentially-rejective Fisher test after Bonferroni-Holm, $\alpha = 0.05$). No effects on reproduction of *Chrysoperla carnea* occurred, when the test item was applied at rates up to and including 1.0 L BAS 758 00 F/ha. In the control and the test item treatments up to and including 1.0 L product/ha the number of eggs per female per day was > 15 and the hatching rate was $> 70\%$, whereas the reproduction at the rate of 1.5 L product/ha was < 15 eggs per female. The results are summarized in Table A 88.

Table A 88 Effects on lacewings (*Chrysoperla carnea*) exposed to BAS 758 00 F in an extended laboratory trial

Treatment	Rate [L/ha] ¹⁾	Mortality [%] ²⁾	Mortality corr. [%] ³⁾	Reproduction [eggs/female/day]	Hatching rate [%]
Control	--	6.0	--	20.8	74.7
BAS 758 00 F	0.5	18.0	12.8	21.3	74.6
	0.75	30.0 *	25.5	22.1	75.0
	1.0	44.0 *	40.4	17.5	73.0
	1.5	58.0 *	55.3	12.0	72.7
	2.0	76.0 *	74.5	n.d.	--
	3.0	72.0 *	70.2	n.d.	--
Endpoint [L BAS 758 00 F/ha]					
LR ₅₀	1.37 [1.17-1.61]				
ER ₅₀ ⁴⁾	> 1.0				

* Statistically significant difference from the control (Multiple Sequentially-rejective Fisher test after Bonferroni-Holm, $\alpha = 0.05$).

¹⁾ Application rate in 200 L water/ha.

²⁾ Mortality: percentage of individuals, which did not reach maturity.

³⁾ Corrected mortality according to Abbott (1925).

⁴⁾ ER₅₀ not given in the study report but estimated based on the raw data.

n.d. not determined

The reference item caused a mortality of 62.0% of exposed lacewings, resulting in a corrected mortality of 59.6%.

Validity criteria:

Validity criteria according to Vogt <i>et al.</i> (2000)	Obtained in this study
Pre-imaginal mortality in the control group should not exceed 20%	6.0%
Mean egg production in the control should be ≥ 15 eggs per female per day	20.8
Mean hatching rate of the eggs in the control should be $\geq 70\%$	74.7%
Corrected mortality in the toxic reference treatment should be $\geq 50\%$	59.6%

All validity criteria were met.

III. CONCLUSION

In an extended laboratory study with BAS 758 00 F, the LR_{50} for *Chrysoperla carnea* was 1.37 L BAS 758 00 F/ha in 200 L water/ha. No unacceptable effects on reproduction were observed up to and including an application rate of 1.0 L BAS 758 00 F/ha in 200 L water/ha.

A 2.4.4.3 Study 3

Comments of zRMS:	The study was conducted to guidance and according to the principles of GLP. All validity criteria were met. The study is considered to be reliable and suitable for the risk assessment.
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Reference:	CP 10.3.2.2/3
Report	Effects of BAS 758 00 F on the green lacewing <i>Chrysoperla carnea</i> STEPH. In an extended laboratory test (under semi-field conditions aged residues on potted bean plants), Roehlig, U., 2021 Report No 922408, 2148NCR0001 BASF DocID 2021/2027050 Authority registration No
Guideline(s):	IOBC (Vogt H. et al. 2000) - Modified for extended laboratory conditions
Deviations:	No
GLP:	yes (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany),
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive Summary

In an aged residue extended laboratory study, the effects and the duration of the residual activity of the test item BAS 758 00 F on the green lacewing *Chrysoperla carnea* were investigated. Green lacewing larvae were exposed on detached bean leaves to an untreated control and to freshly dried and dried residues of BAS 758 00 F at application rates of 1.5, 2.0 and 3.0 L product/ha in groups of 50 replicates for the untreated control and the treatments. Additional test units were treated with deionized water as control and with DANADIM PROGRESS (dimethoate) as reference item. Assessment of mortality was conducted regularly until hatching of the adult lacewings by the number of dead larvae and pupae. For the control and test item groups of 2.0 and 3.0 L/ha BAS 758 00 F the reproductive performance, *i.e.* egg deposition and hatching rate, was assessed (2 assessments/week, 24 h period each).

In the 0 DAT bioassay, 14.0% mortality was observed in the control group. In the test item treatment rates of 1.5, 2.0 and 3.0 L product/ha, correct mortalities were 32.0%, 42.0% and 48.0%, respectively, resulting in corrected mortalities of 20.9%, 32.6% and 39.5%, respectively. Statistically significant difference compared to the control group was observed for all test item rates. Reproduction was 23.7, 22.9 and 14.2 eggs/female/day in the test item treatment rates of 1.5, 2.0 and 3.0 L product/ha, respectively, compared to 24.0 eggs/female/day in the control group. Hatching rate of 74.4% was observed in the control group. In the test item treatment rates of 1.5, 2.0 and 3.0 L product/ha, hatching rates were 74.3%, 73.7% and 72.3%, respectively. In the 7 DAT bioassay, 10.0% mortality was observed in the control group. In the test item treatment rates of 2.0 and 3.0 L product/ha, mortalities of 12.0% and 14.0% were observed resulting in corrected mortalities of 2.2% and 2.4%, respectively.

No statistically significant difference compared to the control group was observed for any test item rates. Reproduction was 22.0 and 22.8 eggs/female/day in the test item treatment rates of 2.0 and 3.0 L product/ha, respectively, compared to 23.2 eggs/female/day in the control group. Hatching rate of 73.8% was observed in the control group. In the test item treatment rates of 2.0 and 3.0 L product/ha, hatching rates were 74.1% and 74.0%, respectively.

In an aged residue extended laboratory study no effects of exposure to freshly dried residues (0 DAT) and aged residues (7 DAT) of BAS 758 00 F on survival of *Chrysoperla carnea* were observed. No unacceptable effects on reproduction were detected after exposure to freshly dried residues of BAS 351 32 H at application rates of 1.5, 2.0 and 3.0 L product/ha. No unacceptable effects on reproduction were detected after exposure to aged residues of BAS 351 32 H at application rates of 2.0 and 3.0 L product/ha.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 758 00 F; batch no. FD-200124-0004; content of a.s.: mefentrifluconazole (BAS 750 F, Reg. No. 5 834 378): 66.7 g/L analyzed (66.6 g/L nominal), metrafenone (BAS 560 F, Reg. No. 4 037 710): 94.9 g/L analyzed (100.0 g/L nominal), pyraclostrobin (BAS 500 F, Reg. No. 304 428): 81.0 g/L analyzed (80.0 g/L nominal); density: 1.092 g/cm³.

B. STUDY DESIGN

Test species: Green lacewing (*Chrysoperla carnea*); larvae, 2 - 3 days old; source: in-house culture, originally obtained from “Neudorff GmbH”, Emmerthal, Germany.

Test design: Extended laboratory trial using fresh and aged residues of the test item. Exposure of larvae via freshly dried and aged residues of test item on treated bean leaves. 3 test item treatment groups and 1 deionized water control; with 1 larva per replicate, 50 replicates per treatment group; the treatments were sprayed onto potted bean plants via a calibrated plot sprayer and air dried afterwards under semi-field outdoor conditions (outdoor under UV light-permeable rain protection). The bioassays were initiated within 1 h after treatment (0 days after treatment, DAT 0), as well as 7 and 14 days after treatment (DAT 7 and DAT 14). Exposure of larvae to the spray residues on the bean leaves lasted until pupation, then they were transferred to oviposition units for development of adults. Mortality assessments were carried out regularly until hatching of the adult lacewings. For the assessment of reproduction, *i.e.* egg deposition and hatching rate, was determined (2 assessments/week, 24 h period each).

Endpoints: Pre-imaginal mortality: number of dead larvae and pupae.
Reproduction assessment: number of eggs per female per day and hatching rate.

Reference item: DANADIM PROGRESS (a.s. dimethoate, 401.7 g/L analyzed, 400 g/L nominal).

Test rates: Control (deionised water), test item rates: 1.0, 2.0 and 3.0 L BAS 758 00 F/ha. The reference item was applied at an application rate of 80 mL/ha. All substances were applied in 400 L water/ha. The substances were sprayed onto potted bean plants via a calibrated spray equipment for small plot applications (plot-sprayer) equipment air dried afterwards.

Test conditions:	<u>Controlled-environment test room:</u> Temperature: 23 - 26°C (DAT 0 and DAT 7); relative humidity: 64 - 73% (DAT 0 and DAT 7); photoperiod: 16 hours light, 8 hours dark; light intensity: 1120 lx (DAT 0), 1080 lx (DAT 7). <u>Semi-field (outdoor) conditions (non-GLP): (valid for application on DAT 0 only):</u> Temperature mean (min/max): 14.9 - 15.3°C; relative humidity (min/max): 74.9 - 76.4%; Wind velocity (min/max): 0.3 - 0.4 m/s. <u>Outdoor weather conditions (non-GLP): (valid for the full time of ageing):</u> Temperature (mean/day): 14.8 - 18.9°C, temperature (min/max): 7.8 - 27.1°C, relative humidity (mean/day): 62 - 87 %. Food: larvae: <i>Sitotroga cerealella</i> eggs (UV sterilized), adults: artificial food.
Statistics:	Descriptive statistics. Step-down Cochran-Armitage test for mortality on DAT 0 and Chi ² -2x2 Table Test with Bonferroni Correction on DAT 7 of the test item ($\alpha = 0.05$).

II. RESULTS AND DISCUSSION

In the 0 DAT bioassay, 14.0% mortality was observed in the control group. In the test item treatment rates of 1.5, 2.0 and 3.0 L product/ha, mortalities were 32.0%, 42.0% and 48.0%, respectively, resulting in corrected mortalities of 20.9%, 32.6% and 39.5%, respectively. Statistically significant difference compared to the control group was observed for all test item rates (Step-down Cochran-Armitage test, $\alpha = 0.05$). Reproduction was 23.7, 22.9 and 14.2 eggs/female/day in the test item treatment rates of 1.5, 2.0 and 3.0 L product/ha, respectively, compared to 24.0 eggs/female/day in the control group. Hatching rate of 74.4% was observed in the control group. In the test item treatment rates of 1.5, 2.0 and 3.0 L product/ha, hatching rates were 74.3%, 73.7% and 72.3%, respectively.

In the 7 DAT bioassay, 10.0% mortality was observed in the control group. In the test item treatment rates of 2.0 and 3.0 L product/ha, mortalities of 12.0% and 14.0% were observed resulting in corrected mortalities of 2.2% and 2.4%, respectively. No statistically significant difference compared to the control group was observed for any test item rate. Reproduction was 22.0 and 22.8 eggs/female/day in the test item treatment rates of 2.0 and 3.0 L product/ha, respectively, compared to 23.2 eggs/female/day in the control group. Hatching rate of 73.8% was observed in the control group. In the test item treatment rates of 2.0 and 3.0 L product/ha, hatching rates were 74.1% and 74.0%, respectively.

The results are summarized in Table A 89.

Table A 89: Effects on *Chrysoperla carnea* exposed to freshly dried and aged residues of BAS 758 00 F on bean leaves in extended laboratory trials

Bioassay	Rate ¹⁾ [L BAS 758 00 F/ha]	Mortality ²⁾ [%]	Corrected mortality ³⁾ [%]	Reproduction [eggs/female/day] ⁴⁾	Hatching rate [%]
DAT 0	Control	14.0	-	24.0	74.4
	1.5	32.0*	20.9	23.7	74.3
	2.0	42.0*	32.6	22.9	73.7
	3.0	48.0*	39.5	14.2	72.3
DAT 7	Control	10.0	-	23.2	73.8
	2.0	12.0	2.2	22.0	74.1
	3.0	14.0	4.4	22.8	74.0

* Statistically significant different compared to the control: Step-down Cochran-Armitage test ($\alpha = 0.05$).

¹⁾ Application rate in 400 L water/ha.

²⁾ Percentage of individuals which did not reach maturity.

³⁾ Corrected mortality according to Abbott (1925).

⁴⁾ Mean number of eggs/female/day from the number of eggs on the gauze and on the glass

The reference item caused a corrected mortality of 60.5% of exposed wasps in the 0 DAT bioassay.

Validity criteria:

Validity criteria according to Vogt <i>et al.</i> (2000)	Obtained in this study
Pre-imaginal mortality in the control group should not exceed 20%	DAT 0: 14.0% DAT 7: 10.0%
Mean egg production in the control should be ≥ 15 eggs per female per day	DAT 0: 24.0 DAT 7: 23.2
Mean hatching rate of the eggs in the control should be $\geq 70\%$	DAT 0: 74.4% DAT 7: 73.8%
Mortality in the toxic reference treatment should be $\geq 50\%$	DAT 0: 60.5%

All validity criteria were met.

III. CONCLUSION

In an aged residue extended laboratory study no effects of exposure to freshly dried residues (0 DAT) and aged residues (7 DAT) of BAS 758 00 F on survival of *Chrysoperla carnea* were observed. No unacceptable effects on reproduction were detected after exposure to freshly dried residues of BAS 351 32 H at application rates of 1.5, 2.0 and 3.0 L product/ha. No unacceptable effects on reproduction were detected after exposure to aged residues of BAS 351 32 H at application rates of 2.0 and 3.0 L product/ha.

A 2.4.5 KCP 10.3.2.3 Semi-field studies with non-target arthropods

BAS 758 00 F poses no unacceptable risk to non-target arthropods. Further studies are not necessary

A 2.4.6 KCP 10.3.2.4 Field studies with non-target arthropods

BAS 758 00 F poses no unacceptable risk to non-target arthropods. Further studies are not necessary

A 2.4.7 KCP 10.3.2.5 Other routes of exposure for non-target arthropods

BAS 758 00 F poses no unacceptable risk to non-target arthropods. Further studies are not necessary

A 2.5 KCP 10.4 Effects on non-target soil meso- and macrofauna

A 2.5.1 KCP 10.4.1 Earthworms

A 2.5.1.1 KCP 10.4.1.1 Earthworms - sub-lethal effects

A 2.5.1.1.1 Study 1

The following chronic toxicity study performed with pyraclostrobin is provided in support of the assessment and was recently submitted under the Annex I renewal process for pyraclostrobin (AIR 3). The study summary was provided in the revised Renewal Assessment Report of pyraclostrobin (RAR, Vol. 3, B.9, September 2021).

Comments of zRMS:	Study was evaluated and accepted for the regulatory use in RR for BAS 751 00 F.
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Reference:	CP 10.4.1.1/1
Report	Sublethal toxicity of BAS 500 F (Pyraclostrobin) to the earthworm <i>Eisenia fetida</i> in artificial soil, Friedrich, S., 2014 Report No EU-141048010S,EU-423850,14 10 48 010 S BASF DocID 2014/1000461 Authority registration No
Guideline(s):	OECD 222 (2004)
Deviations:	No
GLP:	yes (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany),
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive Summary

The effects of BAS 500 F on mortality, biomass development and reproduction of the earthworm *Eisenia fetida* (Annelida: Oligochaeta) were investigated in a 56-day reproduction study. Five concentrations of the test item (8.0, 13.6, 23.1, 39.3 and 66.8 mg a.s./kg dry soil) were incorporated into the soil (10% peat) with 4 replicates per treatment and 8 replicates for the solvent control (each containing 10 worms). Assessment of adult worm mortality, biomass development and feeding activity was carried out after 28 days, assessment of reproduction (number of juveniles) was carried out after 56 days.

The mortality of adult worms was about 0 – 5.0% in the five test item treatments and 1.3% in the solvent control group. No statistically significant differences compared to the solvent control were observed at any test item concentration. The weight change of adult worms was about -6.8 – 36.4% in the test item treatments and 35.2% in the solvent control group. Statistically significant differences on worm weight of *Eisenia fetida* were determined at concentrations of 39.3 and 66.8 mg a.s./kg dry soil. The reproduction rate was statistically significantly different compared to the solvent control at concentrations of 39.3 and 66.8 mg a.s./kg dry soil. No behavioural abnormalities were observed in any of the treatment groups. The feeding activity of adult worms was reduced at the concentrations of 39.3 and 66.8 mg a.s./kg dry soil.

In a 56-day earthworm reproduction study with BAS 500 F on earthworms (*Eisenia fetida*), the NOEC for mortality was determined to be 66.8 mg a.s./kg soil dry weight. The NOEC for biomass and reproduction was determined to be 23.1 mg test item/kg dry soil.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 500 F, Reg. No. 304 428, batch No. COD-001236; purity: 99.2% (tolerance \pm 1.0 %).

B. STUDY DESIGN

Test species: Earthworm (*Eisenia fetida*), adult worms (with clitellum); weight: 301 mg – 498 mg), age: approximately 3 months old; source: “W. Neudorff GmbH KG” followed by in-house culture.

Test design: 56-day test in treated artificial soil according to OECD 222 (10 % peat); 5 different concentrations of the test item were incorporated into the soil; 6 treatment groups (5 test item concentrations, solvent control); 4 replicates for the test item treatments, 8 replicates for the solvent control, 10 worms each. Assessment of adult worm mortality, behavioural effects and biomass development was done after 28 days. Reproduction rate was determined after additional 28 days (assessed 56 days after application).

Endpoints: NOEC; effects on mortality, weight change, reproduction rate, feeding activity.

Reference item: Nutdazim 50 FLOW (carbendazim, SC 500). The effects of the reference item were investigated in a separate study.

Test concentrations: Control, 8.0, 13.6, 23.1, 39.3 and 66.8 mg a.s./kg dry soil.

Test conditions: Artificial soil according to OECD 222 (with 10% peat); pH 5.99 - 6.06 at test initiation, pH 5.70 – 5.76 at test termination; water content: 56.4% - 56.5% of maximum water holding capacity (WHC) at test initiation, 55.6% - 56.2% of WHC at test termination; temperature: 18.6 °C – 21.8 °C; photoperiod: 16 h light : 8 h dark, light intensity: 580 lux; food: horse manure.

Analytics: No analytical verification of the test item is required according to the current test guideline.

Statistics: Descriptive statistics, Fisher’s Exact Binomial Test for mortality ($\alpha = 0.05$), Williams-t-test for weight change and reproduction ($\alpha = 0.05$).

II. RESULTS AND DISCUSSION

The mortality of adult worms was about 0 – 5.0% in the five test item treatments and 1.3% in the solvent control group. No statistically significant differences compared to the solvent control were observed at any test item concentration (Fisher's Exact Binomial Test with Bonferroni Correction, $\alpha = 0.05$, one-sided greater). The weight change of adult worms was about -6.8 – 36.4% in the test item treatments and 35.2% in the solvent control group. Statistically significant differences on worm weight of *Eisenia fetida* were determined at concentrations of 39.3 and 66.8 mg a.s./kg dry soil (Williams-t-test, $\alpha = 0.05$, one-sided smaller). The reproduction rate was statistically significantly different compared to the solvent control at concentrations of 39.3 and 66.8 mg a.s./kg dry soil (Williams-test, $\alpha = 0.05$, one-sided smaller). No behavioural abnormalities were observed in any of the treatment groups. The feeding activity of adult worms was reduced at the concentrations of 39.3 and 66.8 mg a.s./kg dry soil. The results are summarized below.

Table A 90: Effects of BAS 500 F on earthworms (*Eisenia fetida*) in a 56-day reproduction study

BAS 500 F [mg/kg dry soil]	Solvent control	8.0	13.6	23.1	39.3	66.8
Mortality (day 28) [%]	1.3	0.0	0.0	2.5	5.0	2.5
Weight change (day 28) [%]	35.2	36.4	32.7	28.0	9.4 *	-6.8 *
Mean no. of juveniles (day 56)	118.8	127.8	123.8	107.5	36.8 *	14.8 *
Reproduction (day 56) [% of control]	--	107.6	104.2	90.5	30.9 *	12.4 *
Endpoints [mg BAS 500 F/kg dry soil]						
EC ₁₀ reproduction (day 56)	22.2					
EC ₂₀ reproduction (day 56)	25.8					
EC ₅₀ reproduction (day 56)	34.2					
NOEC _{mortality} (day 28)	66.8					
NOEC _{weight} (day 28)	23.1					
NOEC _{reproduction} (day 56)	23.1					

* Statistically significantly different from solvent control (Williams-t-test, $\alpha = 0.05$, one-sided smaller).

Validity criteria:

Validity criteria according to OECD 222 (2016)	Obtained in this study
Adult mortality in the control $\leq 10\%$	1.3% (Solvent control)
Number of juveniles per control replicate ≥ 30 (with 10 adults per replicate)	84 to 159
Coefficient of variation of reproduction in the control $\leq 30\%$	19.7%

All validity criteria were met.

III. CONCLUSION

In a 56-day earthworm reproduction study with BAS 500 F on earthworms (*Eisenia fetida*), the NOEC for mortality was determined to be 66.8 mg a.s./kg soil dry weight. The NOEC for biomass and reproduction was determined to be 23.1 mg test item/kg dry soil.

A 2.5.1.1.2 Study 2

The following chronic toxicity study performed with BF 500-6 (metabolite of BAS 500 F, pyraclostrobin) is provided in support of the assessment and was recently submitted under the Annex I renewal process for pyraclostrobin (AIR 3). The study summary was provided in the revised Renewal Assessment Report of pyraclostrobin (RAR, Vol. 3, B.9, September 2021).

Comments of zRMS:	Study was evaluated and accepted for the regulatory use in RR for BAS 751 00 F. In the current evaluation, the study was considered as not essential for the risk assessment.
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Reference:	CP 10.4.1.1/2
Report	Effects of Reg.No. 364380 (metabolite of BAS 500 F, Pyraclostrobin) on reproduction and growth of earthworms <i>Eisenia fetida</i> in artificial soil, Ganssmann, M., 2013 Report No EU-77511022, EU-423855,77511022 BASF DocID 2013/1003174 Authority registration No
Guideline(s):	EC 1107/2009 (14 June 2011), ISO 11268-2 (1998), OECD 222 - Earthworm reproduction Test (2004)
Deviations:	No
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden),
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive Summary

The effects of BF 500-6 (Reg. No. 364 380, synonym: 500M01), a metabolite of pyraclostrobin, on mortality, biomass development and reproduction of the earthworm *Eisenia fetida* (Annelida: Oligochaeta) were investigated in a 56-day reproduction study. Five concentrations (20, 40, 80, 160 and 320 mg BF 500-6/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. Assessment of adult worm mortality, biomass development and feeding activity was carried out after 28 days, assessment of reproduction (number of juveniles) was carried out after 56 days.

After 28 days of exposure a slight mortality of 2.5% was observed at the test item treatment concentration of 80 mg BF 500-6/kg dry soil, which was not statistically significantly different compared to the control. The body weight changes and reproduction rates were also not statistically significantly different compared to the control up to and including the highest concentration tested. Neither behavioural abnormalities nor effects on feeding activity were observed in any of the treatment groups.

In a 56-day earthworm reproduction study with BF 500-6 (Reg. No. 364 380, a metabolite of pyraclostrobin) on earthworms (*Eisenia fetida*), the NOEC for mortality, biomass, feeding activity and reproduction was determined to be ≥ 320 mg BF 500-6/kg dry soil.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Reg. No. 364 380 (metabolite of pyraclostrobin, BF 500-6, synonym: 500M01) batch no. 01311-142; purity: 99.2% (tolerance ± 1.0 %).

B. STUDY DESIGN

Test species: Earthworm (*Eisenia fetida*), adult worms (with clitellum); weight: 300 mg – 592 mg), age: approximately 10 to 11 months old; source: in-house culture.

Test design: 56-day test in treated artificial soil according to OECD 222 (10% peat); different concentrations of the test item were incorporated into the soil; 6 treatment groups (5 test item concentrations, control); 4 replicates for the test item treatments, 8 replicates for the control, 10 worms each. The artificial soil was treated and filled into test vessels, before the earthworms were introduced on the top of the soil. Assessment of worm mortality, behavioural effects and biomass development after 28 days; assessment of reproduction rate (number of offspring) after another 28 days (56 days after application).

Endpoints: NOEC; effects on mortality, weight change, reproduction rate, feeding activity.

Reference item: Luxan Carbendazim 500 FC (carbendazim, 500 g/L nominal). The effects of the reference item were investigated in a separate study.

Test concentrations: Control, 20, 40, 80, 160 and 320 mg Reg. No. 364 380/kg dry soil.

Test conditions: Artificial soil according to OECD 222 (with 10% peat); pH 6.4 - 6.5 at test initiation, pH 6.0 - 6.1 at test termination; water content: 54.3% - 54.9% of maximum water holding capacity (WHC) at test initiation, 52.5% - 58.5% of WHC at test termination; temperature: 18 °C - 22 °C; photoperiod: 16 h light: 8 h dark, light intensity: 400 lux to 800 lux; food: cattle manure.

Analytics: No analytical verification of the test item is required according to the current test guideline.

Statistics: Descriptive statistics, Fisher's Exact Test for mortality ($\alpha = 0.05$), Williams t-test for weight change and reproduction ($\alpha = 0.05$).

II. RESULTS AND DISCUSSION

After 28 days of exposure a slight mortality of 2.5% was observed at the test item treatment concentration of 80 mg BF 500-6 /kg dry soil, which was not statistically significantly different compared to the control (Fisher`s Exact Test, $\alpha = 0.05$). The body weight changes and reproduction rates were also not statistically significantly different compared to the control (Williams t-test, $\alpha = 0.05$) up to and including the highest concentration tested. Neither behavioural abnormalities nor effects on feeding activity were observed in any of the treatment groups. The results are summarized below.

Table A 91: Effects of BF 500-6 (Reg. No. 364 380), a metabolite of pyraclostrobin, on earthworms (*Eisenia fetida*) in a 56-day reproduction study

BF 500-6 [mg/kg dry soil]	Control	20	40	80	160	320
Mortality (day 28) [%]	0.0	0.0	0.0	2.5	0.0	0.0
Weight change (day 28) [%]	51.7	52.7	52.4	55.9	56.7	50.1
Mean no. of juveniles (day 56)	276	324	330	308	293	303
Reproduction (day 56) [% of control]	--	117.4	119.5	111.5	106.2	109.7
Food consumption [g]	25.0	25.0	25.0	25.0	25.0	25.0
Endpoints [mg BF 500-6/kg dry soil]						
NOEC _{mortality, weight} (day 28)	≥ 320					
NOEC _{reproduction} (day 56)	≥ 320					

Commission Regulation 283/2013 requires EC_{10/20} values for chronic toxicity studies. Both values are not provided in the original study report. However, no effects on mortality or reproduction were observed in any test item treatment group and hence, a calculation of EC_{10/20} is not feasible. Therefore, the NOEC is considered the appropriate and relevant endpoint for this study.

Validity criteria:

Validity criteria according to OECD 222 (2016)	Obtained in this study
Adult mortality in the control $\leq 10\%$	0% (Water control)
Number of juveniles per control replicate ≥ 30 (with 10 adults per replicate)	229 to 322
Coefficient of variation of reproduction in the control $\leq 30\%$	10.1%

All validity criteria were met.

III. CONCLUSION

In a 56-day earthworm reproduction study with BF 500-6 (Reg. No. 364 380, a metabolite of pyraclostrobin) on earthworms (*Eisenia fetida*), the NOEC for mortality, biomass, feeding activity and reproduction was determined to be ≥ 320 mg BF 500-6/kg dry soil.

A 2.5.1.1.3 Study 3

The following chronic toxicity study performed with BF 500-7 (metabolite of BAS 500 F, pyraclostrobin) is provided in support of the assessment and was recently submitted under the Annex I renewal process for pyraclostrobin (AIR 3). The study summary was provided in the revised Renewal Assessment Report of pyraclostrobin (RAR, Vol. 3, B.9, September 2021).

Comments of zRMS:	Study was evaluated and accepted for the regulatory use in RR for BAS 751 00 F. In the current evaluation, the study was considered as not essential for the risk assessment.
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Reference:	CP 10.4.1.1/3
Report	Effects of Reg.No. 369315 (metabolite of BAS 500 F, Pyraclostrobin) on reproduction and growth of earthworms <i>Eisenia fetida</i> in artificial soil with 10% peat, Ganssmann, M., 2013 Report No EU-77612022, EU-423856,77612022 BASF DocID 2013/1224029 Authority registration No
Guideline(s):	ISO 11268-2 (2012), OECD 222 - Earthworm reproduction Test (2004)
Deviations:	No
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden),
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive Summary

The effects of BF 500-7 (Reg. No. 369 315, synonym: 500M02), a metabolite of pyraclostrobin, on mortality, biomass development and reproduction of the earthworm *Eisenia fetida* (Annelida: Oligochaeta) were investigated in a 56-day reproduction study. Five concentrations (20, 40, 80, 160 and 320 mg BF 500-7/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. Assessment of adult worm mortality, biomass development and feeding activity was carried out after 28 days, assessment of reproduction (number of juveniles) was carried out after 56 days.

After 28 days of exposure a slight mortality of 2.5% was observed at the test item treatment concentrations of 20, 40 and 320 mg BF 500-7/kg dry soil, respectively, which was not statistically significantly different compared to the control, where 1.3% of the worms died. The body weight changes and reproduction rates were also not statistically significantly different compared to the control up to and including the highest concentration tested. Neither behavioural abnormalities nor effects on feeding activity were observed in any of the treatment groups.

In a 56-day earthworm reproduction study with BF 500-7 (Reg. No. 369 315, a metabolite of pyraclostrobin) on earthworms (*Eisenia fetida*), the NOEC for mortality, biomass, feeding activity and reproduction was determined to be ≥ 320 mg BF 500-7 /kg dry soil.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Reg. No. 369 315 (metabolite of pyraclostrobin, BF 500-7, synonym: 500M02) batch no. L83-168; purity: 96.2%.

B. STUDY DESIGN

Test species: Earthworm (*Eisenia fetida*), adult worms (with clitellum); weight: 301 mg – 598 mg), age: approximately 7 months old; source: in-house culture.

Test design: 56-day test in treated artificial soil according to OECD 222 (10% peat); different concentrations of the test item were incorporated into the soil; 6 treatment groups (5 test item concentrations, control); 4 replicates for the test item treatments, 8 replicates for the control, 10 worms each. The artificial soil was treated and filled into test vessels, before the earthworms were introduced on the top of the soil. Assessment of worm mortality, behavioural effects and biomass development after 28 days; assessment of reproduction rate (number of offspring) after another 28 days (56 days after application).

Endpoints: NOEC; effects on mortality, weight change, reproduction rate, feeding activity.

Reference item: Luxan Carbendazim 500 FC (carbendazim, 500 g/L nominal). The effects of the reference item were investigated in a separate study.

Test concentrations: Control, 20, 40, 80, 160 and 320 mg Reg. No. 369 315/kg dry soil.

Test conditions: Artificial soil according to OECD 222 (with 10% peat); pH 6.0 at test initiation and termination; water content: 51.2% - 53.7% of maximum water holding capacity (WHC) at test initiation, 55.4% - 61.5% of WHC at test termination; temperature: 18 °C - 22 °C; photoperiod: 16 h light: 8 h dark, light intensity: 400 lux to 800 lux; food: cattle manure.

Analytics: No analytical verification of the test item is required according to the current test guideline.

Statistics: Descriptive statistics, Fisher's Exact Test for mortality ($\alpha = 0.05$), Williams t-test for weight change and reproduction ($\alpha = 0.05$).

II. RESULTS AND DISCUSSION

After 28 days of exposure a slight mortality of 2.5% was observed at the test item treatment concentrations of 20, 40 and 320 mg BF 500-7/kg dry soil, respectively, which was not statistically significantly different compared to the control, where 1.3% of the worms died (Fisher's Exact Test, $\alpha = 0.05$). The body weight changes and reproduction rates were also not statistically significantly different compared to the control (Williams t-test, $\alpha = 0.05$) up to and including the highest concentration tested. Neither behavioural abnormalities nor effects on feeding activity were observed in any of the treatment groups. The results are summarized below.

Table A 92: Effects of BF 500-7 (Reg. No. 369 315), a metabolite of pyraclostrobin, on earthworms (*Eisenia fetida*) in a 56-day reproduction study

BF 500-7 [mg/kg dry soil]	Control	20	40	80	160	320
Mortality (day 28) [%]	1.3	2.5	2.5	0.0	0.0	2.5
Weight change (day 28) [%]	21.5	26.6	22.4	20.5	26.7	13.6
Mean no. of juveniles (day 56)	239	233	301	312	293	253
Reproduction (day 56) [% of control]	--	97.6	125.9	130.5	122.8	105.8
Food consumption [g]	25.0	25.0	25.0	25.0	25.0	25.0
Endpoints [mg BF 500-7/kg dry soil]						
NOEC _{mortality, weight} (day 28)	≥ 320					
NOEC _{reproduction} (day 56)	≥ 320					

Commission Regulation 283/2013 requires EC_{10/20} values for chronic toxicity studies. Both values are not provided in the original study report. However, no effects on mortality or reproduction were observed in any test item treatment group and hence, a calculation of EC_{10/20} is not feasible. Therefore, the NOEC is considered the appropriate and relevant endpoint for this study.

Validity criteria:

Validity criteria according to OECD 222 (2016)	Obtained in this study
Adult mortality in the control $\leq 10\%$	1.3% (Water control)
Number of juveniles per control replicate ≥ 30 (with 10 adults per replicate)	197 to 288
Coefficient of variation of reproduction in the control $\leq 30\%$	13.8%

All validity criteria were met.

III. CONCLUSION

In a 56-day earthworm reproduction study with BF 500-7 (Reg. No. 369 315, a metabolite of pyraclostrobin) on earthworms (*Eisenia fetida*), the NOEC for mortality, biomass, feeding activity and reproduction was determined to be ≥ 320 mg BF 500-7 /kg dry soil.

A 2.5.1.1.4 Study 4

The following chronic toxicity study performed with BAS 560 02 F (formulation of BAS 500 F, pyraclostrobin) is provided in support of the assessment and was recently submitted under the Annex I renewal process for metrafenone (AIR 3). The study summary was provided in the revised Renewal Assessment Report of metrafenone (RAR, Vol. 3, B.9, December 2019).

Comments of zRMS:	Study not evaluated by zRMS.
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Reference:	CP 10.4.1.1/4
Report	Effects of BAS 560 02 F on reproduction and growth of earthworms <i>Eisenia fetida</i> in artificial soil with 5% peat, Witte, B., 2011 Report No EU-62321022,EU-2053354,EU-397985 BASF DocID 2011/1000384 Authority registration No
Guideline(s):	ISO 11268-2 (1998), OECD 222 (2004)
Deviations:	No
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden),
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive Summary

The chronic toxicity of BAS 560 02 F to the earthworm *Eisenia fetida* was determined in a 56-day laboratory study. The earthworms were exposed to BAS 560 02 F incorporated into the soil (5% peat) at concentrations of 15, 30, 60, 120 and 240 mg BAS 560 02 F/kg soil dry weight (dw); corresponding to 6.35, 12.70, 25.40, 50.80 and 101.61 mg metrafenone/kg soil dw. Four replicates were tested for each test substance concentration and eight replicates were included for the negative control. The reference item Luxan Carbendazim 500 FC was tested in a separate study. Assessment of adult earthworm mortality, behavioral effects, body weight and feeding activity was carried out after 28 days, and assessment of reproduction (number of juveniles) was carried out after 56 days.

After 28 days of exposure to adult earthworms, no mortality was observed in any test item group. Body weight of earthworms exposed to BAS 560 02 F was not statistically significantly different compared to the control up to the highest test concentration of 240 mg BAS 560 02 F/kg soil dw. No behavioral abnormalities were observed in any of the treatment groups and the feeding activity in all the treated groups was comparable to the control. The reproduction rates, assessed after 56 days, were not significantly different compared to the control up to and including the highest test concentration of 240 mg BAS 560 02 F/kg soil dw.

In a 56-day reproduction study on BAS 560 02 F exposure to earthworms (*Eisenia fetida*), the no-observed-effect concentration (NOEC) for mortality, reproduction and biomass was determined to be 240 mg BAS 560 02 F/kg soil dw, the highest concentration tested, which is equivalent to 101.61 mg a.s./kg soil dw. The lowest-observable-effect concentration (LOEC) for mortality, reproduction and biomass was > 240 mg BAS 560 02 F/kg soil dw.

I. MATERIAL AND METHODS

A. MATERIALS

- 1. Test material:** BAS 560 02 F
Batch: 0002643758
Purity: 490.0 g metrafenone/L (analyzed; nominal: 500.0 g/L)
Description: Beige liquid, SC formulation
- 2. Test concentrations:** 0 (control), 15, 30, 60, 120 and 240 mg BAS 560 02 F/kg soil dry weight; corresponding to 0, 6.35, 12.70, 25.40, 50.80 and 101.61 mg metrafenone/kg soil dry weight
- 3. Vehicle:** Deionized water
- 4. Reference material:** Luxan Carbendazim 500 FC; the effects were evaluated in a separate study
- 5. Artificial soil:** Artificial soil according to OECD 222: 5% sphagnum peat, 20% Kaolin clay, 74.8% fine quartz sand and 0.2% CaCO₃
Test units: Plastic boxes (18.3 x 13.6 cm x 6 cm) containing 615.2 g soil (500 g dry weight plus 10.2 g water plus 5 g food). Transparent perforated lids were used.
- 6. Test organism:**
Species: Earthworm (*Eisenia fetida*)
Age: Adult worms, with clitellum, 11 months old at test initiation
Source: In house culture
Weight: 306 mg to 600 mg (at test initiation)
Acclimation: One day, in artificial soil, under test conditions.
Diet: Cattle manure, weekly

B. STUDY DESIGN

1. Environmental conditions:

- Temperature:** 18 – 22 °C
pH of soil: 6.4 at test initiation, 6.2 - 6.4 at test termination
Water content of soil: At test initiation: 49.0% - 57.3%, at test termination 45.0% - 65.3% (of maximum water holding capacity)
Photoperiod: 16 hours light: 8 hours darkness (400 to 800 lux)

2. Animal assignment and treatment

Different concentrations of the test item were mixed homogeneously into the soil, which was then filled in the test units before the earthworms were introduced on top of the soil. For the five concentrations of the test item four replicates were tested, with ten earthworms each. The negative control group, treated with deionized water only, consisted of eight replicates, with ten earthworms per replicate. After 28 days adult

earthworms were removed and the remaining soil was returned to the respective test units for an additional 28 days.

3. Dose preparation

A stock solution was prepared by adding the test item to deionized water at a concentration of 2.063 mg test item/g. A magnetic stirrer was used to obtain a homogenous dispersion of this stock solution. Predetermined amounts of the stock solution were then added to 2050 g dry soil to prepare the target nominal concentrations of 15, 30, 60, 120 and 240 mg BAS 560 02 F/kg soil dry weight (dw).

4. Measurements and observations

Assessment of worm mortality, behavioral effects and biomass development was performed after 28 days of exposure. After an additional 28 days, at the end of the test on day 56, the number of juveniles was counted to assess the reproduction rate.

Soil water content was checked weekly and pH was determined on the day of application and at study termination.

5. Statistics

Dunnett-test was used for biomass and reproduction data and Fisher exact test for mortality data ($\alpha = 0.05$).

II. RESULTS AND DISCUSSION

A. MORTALITY AND BEHAVIOR

After 28 days of exposure no mortality was observed in any test item group (see table below). No behavioral abnormalities were observed in any of the treatment groups and the feeding activity in all the treated groups was comparable to the control.

B. BODY WEIGHT

Body weight of adult earthworms exposed to BAS 560 02 F was not statistically significantly different compared to the control up to the highest test concentration of 240 mg BAS 560 02 F/kg dry soil (see table below).

C. REPRODUCTION

The reproduction rates were not significantly different compared to the control up to and including the highest test concentration of 240 mg BAS 560 02 F/kg dry soil.

The results are summarized in the table below.

Table A 93: Effects on earthworm (*Eisenia fetida*) exposed to BAS 560 02 F in a 56-day reproduction study

Test parameter	Test concentration (mg BAS 560 02 F/kg soil dw)					
	Control	15	30	60	120	240
Mortality (28 d) (%)	1.3	0	0	0	0	0
Weight change (28 d) (%)	26.8	23.5	21.8	24.5	20.2	20.8
Number of juveniles (56 d)	334	325	304	279	309	318
Reproduction (% of control) (56 d)	--	97.4	91.0	83.6	92.6	95.3
Endpoints (mg BAS 560 02 F/kg soil dw)						
NOEC _{mortality, biomass} (28 d)	240					
NOEC _{reproduction} (56 d)	240					
LOEC (56 d)	> 240					

In the most recent test with the reference item LUXAN Carbendazim 500 FC, there were statistically significant effects on reproduction at a concentration of 1.0 mg carbendazim/kg soil dry weight and higher. The EC₅₀ for reproduction was calculated to be 1.21 mg carbendazim/kg soil dry weight.

D. DEFICIENCIES

None.

III. CONCLUSION

In a 56-day reproduction study with BAS 560 02 F on earthworms (*Eisenia fetida*), the no-observed-effect concentration (NOEC) for mortality, reproduction and biomass was determined to be 240 mg BAS 560 02 F/kg soil dw (corresponding to 101.61 mg a.s./kg soil dw), the highest concentration tested. The lowest-observable-effect concentration (LOEC) for mortality, reproduction and biomass was > 240 mg BAS 560 02 F/kg soil dw.

A 2.5.1.1.5 Study 5

The following chronic toxicity study performed with CL 377160 (metabolite of metrafenone) is provided in support of the assessment and was recently submitted under the Annex I renewal process for metrafenone (AIR 3). The study summary was provided in the revised Renewal Assessment Report of metrafenone (RAR, Vol. 3, B.9, December 2019).

Comments of zRMS:	Study not evaluated by zRMS.
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Reference:	CP 10.4.1.1/5
Report	CL377160 - Determination of chronic toxicity to the earthworm <i>Eisenia andrei</i> in an artificial soil substrate, McCormac, A., 2014 Report No BASF-13-7 BASF DocID 2014/1093923 Authority registration No
Guideline(s):	OECD 222 (2004)
Deviations:	No
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom),
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive Summary

In a chronic toxicity study, adults of *Eisenia andrei*, were exposed to CL377160 (metabolite of metrafenone). The test item was dissolved in acetone and then mixed into artificial soil (5% peat) at nominal concentrations of 204, 305, 458, 688 and 1032 mg test item/kg soil dry weight (dw); corresponding to 198, 296, 444, 667 and 1000 mg a.s./kg soil dw. In parallel an untreated and solvent control were tested. In the test, there were four replicate chambers of soil prepared for each variant concentration of the test item, each holding 10 adult worms. For controls, there were eight replicate chambers of soil, each holding 10 adult worms. Assessment of adult earthworm mortality, behavioral effects, body weight and feeding activity was carried out after 28 days, and assessment of reproduction (number of juveniles) was carried out after 56 days.

After 28 days, there were no significant effects on survival or change in biomass of earthworms exposed to the test item at levels up to and including 1000 mg a.s./kg soil dw. CL 377160 exposure had no statistically significant effects on the reproductive capacity of the confined worms at treatment concentrations up to and including 1000 mg a.s./kg soil dw.

Thus, the no-observed-effect concentration (NOEC) relating to mortality, change in biomass, behavior and reproductive capacity for CL377160 was found to be 1000 mg a.s./kg soil dw, the highest concentration tested. The lowest-observable-effect concentration (LOEC) was > 1000 mg a.s./kg soil dw.

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test material:** CL 377160 (metabolite of metrafenone)
Batch: AC12387-108
Purity: 96.9% w/w
Description: Light-brown crystalline solid
2. **Test concentrations:** 0 (Control and solvent control), 204, 305, 458, 688 and 1032 mg test item/kg soil dry weight (dw); corrected for purity 0, 198, 296, 444, 667 and 1000 mg a.s./kg soil dw
3. **Vehicle:** Acetone
4. **Reference item:** Delsene 50 Flo (500 g carbendazim/L), the effects were evaluated in a separate study
5. **Artificial soil:** Artificial soil according to OECD 222: 5% sphagnum peat, 20% kaolinite clay, 74.81% horticultural silver sand and 0.19% CaCO₃
Test units: Polystyrene boxes (6 cm deep with sides of 17.1 cm x 11.3 cm = 193.2 cm² surface area). Transparent perforated lids were used.
6. **Test organism:**
Species: Earthworm (*Eisenia andrei*) (Oligochaeta: Lumbricidae)
Age: Adult worms, with clitellum (5.5 months old), at test initiation
Source: In house culture
Weight: 300 mg to 600 mg (at test initiation)
Acclimation: Three days, in artificial soil, under test conditions.
Diet: Finely ground oats, weekly

B. STUDY DESIGN

1. **Environmental conditions:**
Temperature: 20.4-28.7 °C (adults); 19.8-20.7 °C (juveniles)
pH of soil: 6.0 ± 0.5
Water content of soil: 50-55.0%
Photoperiod: 16 hours light: 8 hours darkness (490 to 755 lux)

2. Animal assignment and treatment

Different concentrations of the test item were mixed homogeneously into the soil after being dissolved in acetone, which was filled in the test units before the earthworms were introduced on top of the soil. In total, seven treatment groups were set up (five concentrations of the test item, water treated control, and solvent control) with four replicates for the test item treatment group and eight replicates for the controls and ten earthworms per replicate.

After 28 days adult earthworms were removed and the remaining soil was returned to the respective test units for an additional 28 days.

3. Dose preparation

The test item dissolved fully in the acetone and thorough mixing and agitation of each solution was carried out to ensure homogeneity. This test item solution was mixed through an aliquot of the artificial soil and the acetone was allowed to evaporate over a period of up to one hour. Once the treated sand was dry, it was mixed with the rest of the artificial soil to create the equivalent of 500 g dry weight of soil. Then, purified water was added to the individual batches of soil to achieve a final soil moisture content of 50% of its pre-determined maximum water-holding capacity (WHC_{max}).

4. Measurements and observations

Assessment of earthworm mortality, behavioral effects and biomass development was performed after 28 days of exposure. At the end of the test on day 56, the number of juveniles was counted to assess the reproduction rate.

Soil water content and pH was determined on the day of application and at study termination.

5. Statistics

Fisher's Exact Test ($\alpha = 0.05$) was performed on adult mortality data. One-way analysis of variance ($\alpha = 0.05$) was performed on percentage change in adult biomass. One-way analysis of variance ($\alpha = 0.05$) was performed on the number of juveniles.

II. RESULTS AND DISCUSSION

A. MORTALITY AND BEHAVIOUR

After 28 days of exposure, adult mortality in the control and solvent control was 3.8 and 5.0%. The mortality in the individual test-item treatments was compared to that in the untreated control and none of the concentrations differed significantly (see table below). The LC₅₀ was considered to be > 1000 mg/kg soil dw.

There was no loss in condition (open wounds, etc.) or change in behavior observed in worms treated with the test item at concentrations up to and including the maximum of 1000 mg/kg soil dw.

B. BODY WEIGHT

There were no significant changes in adult-worm biomass, relative to the pooled control data, in any of the test-item treatments.

C. REPRODUCTION CAPACITY

The mean number of juveniles produced per arena was 121.9 in the untreated control and 125.1 in the acetone control. There were no significant effects on reproduction, relative to the pooled control data, in any of the test-item treatments. The NOEC was therefore 1000 mg/kg soil dw.

The results are summarized in the table below.

Table A 94: Effects on earthworms (*Eisenia Andrei*) exposed to CL377160 in a 56-day reproduction study

Test parameter	Test concentration (mg CL377160/kg soil dw)						
	Control	Solvent control	198	296	444	667	1000
Mortality (28 d) (%)	3.8	5.0	0.0	0.0	2.5	0.0	5.0
Corrected mortality (%)	-	1.3	0.0	0.0	0.0	0.0	1.3
Mean weight change (and standard deviation) (28 d) (%)	49.7 (14.9)	47.7 (10.6)	47.7 (9.3)	50.7 (14.4)	47.1 (13.5)	47.7 (10.0)	52.0 (13.2)
Number of juveniles / container (56 d)	121.9	125.1	120.5	123.0	122.8	105.5	121.3
Reproduction (% of control) (56 d) ^a	-	-3	1	-1	-1	13	1
Endpoints (mg CL377160/kg soil dw)							
LC ₅₀	> 1000						
NOEC	1000						
LOEC	> 1000						

^a Positive values indicates a decrease, and negative values an increase in reproduction, relative to the control.

The toxic reference bioassay indicated a reproduction EC₅₀ of 3.6 mg a.s. (carbendazim)/kg soil dw (95% CL 3.3 and 3.9 mg a.s./kg), i.e. the performance of the earthworm culture was consistent with the expectations of the OECD guidelines.

D. DEFICIENCIES

Due to a malfunction of the controlled-environment room in which the test was run, the upper temperature threshold was exceeded for a period of 65 hours during the first 28 days of the experiment, briefly reaching a maximum of 28.7 °C. However, as all treatments were subject to the same temperature regime and the validity criteria were met, it was considered that this deviation did not affect the integrity of the study.

III. CONCLUSION

In a 56-day reproduction study with the metabolite CL377160 exposure to earthworms (*Eisenia andrei*), the no-observed-effect concentration (NOEC) relating to mortality, change in biomass, behavior and reproductive capacity was found to be 1000 mg/kg soil dw, the highest test concentration. The lowest-observable-effect concentration (LOEC) was > 1000 mg /kg soil dw.

A 2.5.1.1.6 Study 6

The following chronic toxicity study performed with CL 3000402 (metabolite of BAS 560 F, metrafenone) is provided in support of the assessment and was recently submitted under the Annex I renewal process for metrafenone (AIR 3). The study summary was provided in the revised Renewal Assessment Report of metrafenone (RAR, Vol. 3, B.9, December 2019).

Comments of zRMS:	Study not evaluated by zRMS.
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Reference:	CP 10.4.1.1/6
Report	CL 3000402 - Determination of chronic toxicity to the earthworm <i>Eisenia andrei</i> in an artificial soil substrate, McCormac, A., 2015 Report No EU-BASF-15-14,BASF-15-14 BASF DocID 2015/1041971 Authority registration No
Guideline(s):	OECD 222 - Earthworm reproduction Test (2004)
Deviations:	No
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom),
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive Summary

In a chronic toxicity study, adults of *Eisenia andrei* were exposed to CL 3000402 (metabolite of metrafenone). The test item was mixed into artificial soil (10% peat) at nominal concentrations of 4.76, 8.57, 15.43, 27.78 and 50.0 mg a.s./kg soil dry weight (dw). In the test, there were four replicate chambers of soil prepared for each variant concentration of the test item, each holding 10 adult earthworms. In parallel an untreated control was tested, with eight replicate chambers of soil, each holding 10 adult earthworms. Assessment of adult earthworm mortality, behavioral effects, body weight and feeding activity was carried out after 28 days, and assessment of reproduction (number of juveniles) was carried out after 56 days.

After 28 days, the test item treatments up to and including 50.0 mg/kg soil dw did not have any significant effects on survival and change in biomass of the adult earthworms. CL 3000402 exposure had no statistically significant effects on the reproductive capacity of the earthworms, except for the earthworms exposed to the highest treatment concentration (50.0 mg/kg soil dw).

Thus, the no-observed-effect concentration (NOEC) relating to reproductive capacity for CL 3000402 was found to be 27.78 mg/kg soil dw. The lowest-observable-effect concentration (LOEC) based on reproduction was 50.00 mg/kg soil dw.

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test material:** CL 3000402 (metabolite of metrafenone)
Batch: L87-50
Purity: 97.1% w/w (analyzed)
Description: White powder
2. **Test concentrations:** 0 (Control), 4.76, 8.57, 15.43, 27.78 and 50.0 mg CL 3000402/kg soil dry weight (dw)
3. **Vehicle:** Purified water
4. **Reference item:** Effects of technical grade carbendazim were evaluated in a separate study
5. **Artificial soil:** Artificial soil according to OECD 222: 10% sphagnum peat, 20% kaolinite clay, 69.65% horticultural silver sand and 0.35% CaCO₃
Test units: Polystyrene boxes (6 cm deep with sides of 17.1 cm x 11.3 cm = 193.2 cm² surface area) with ventilated lids were used containing 500 g of dry soil.
6. **Test organism:**
Species: Earthworm (*Eisenia andrei*) (Oligochaeta: Lumbricidae)
Age: Adult worms, with clitellum (approximately 5 months old at test initiation)
Source: In-house culture
Weight: 341 mg to 474 mg (at test initiation)
Acclimation: One day, in artificial soil, under test conditions.
Diet: Finely ground oats, for the first 4 weeks of the test

B. STUDY DESIGN

1. **Environmental conditions:**
Temperature: 20.4-21.7 °C (adults); 19.8-20.7 °C (juveniles)
pH of soil: 5.82-6.32
Water content of soil: 56.5-58.0%
Photoperiod: 16 hours light: 8 hours darkness (550 to 770 lux)

2. Animal assignment and treatment

One day prior to treatment application, earthworms were moved from the culture medium to the artificial soil medium, to acclimate to the different substrate. Immediately following preparation of the test soil, earthworms were randomly selected from this acclimatized population and distributed between treatments. The study design included four replicates of 10 earthworms per treatment concentration and 8 replicates of 10 worms for control treatment. After one day, food was provided and further food was supplied on a weekly basis.

At 28 days after treatment (DAT), the numbers of surviving earthworms and their fresh weights were recorded. Any apparent change in the behaviour or physical condition of the confined earthworms was noted. Adult earthworms were removed and the test soil with any cocoons or juvenile worms was returned to the test chambers. Food was provided on the soil surface. After a further 28 days (i.e. 56 DAT) the number of juvenile earthworms in each replicate arena was recorded.

3. Dose preparation

The test item was diluted in purified water shortly before application, at a concentration of 0.515g product/L purified water. This dilution was placed into a sonicating water-bath until the product was observed to have formed a fine homogeneous dispersion and was maintained on a magnetic stirrer whilst aliquots were removed for further dilutions, to obtain the four other required test dilutions. All dilutions were thoroughly agitated to ensure their homogeneity before taking aliquots to treat the test soil, to obtain the soil concentrations equivalent to 4.76, 8.57, 15.43, 27.78 and 50.00 mg CL 3000402/kg soil dw (based on the measured purity of the test item). The replicate arenas were treated individually. To make up each replicate batch of treated soil, the equivalent of 500 g dry soil was partially moistened with purified water, followed by the appropriate test item dilution. This resulted in bringing the total soil moisture content to 50% of the maximum water-holding capacity (WHC).

The treated soil was mixed well using an electrical mixer. No direct measurement was made of test item homogeneity in the soil, but care was taken to mix both the test item dilutions and the treated soil thoroughly. No measurement was made of the stability of the test item in the soil, but the earthworms were placed onto the soil immediately after the treatment of each arena, and all arenas had been treated within 1.5 hours of first making the test item dilutions.

4. Measurements and observations

Assessment of earthworm mortality, behavioral effects and biomass development was performed after 28 days of exposure. After an additional 28 days, at the end of the test on day 56, the number of juveniles was counted to assess the reproduction rate.

5. Statistics

Fisher's Exact Test ($\alpha = 0.05$) was performed on the adult mortality data and T-test for independent samples ($\alpha = 0.05$) or Mann-Whitney *U*-test ($\alpha = 0.05$) on percentage change were used to assess the adult biomass data. One-way analysis of variance (one-sided, $\alpha = 0.05$) and Probit analysis were used to evaluate the number of juveniles. All statistical analyses were carried out using SPSS (2013).

II. RESULTS AND DISCUSSION

A. MORTALITY AND BEHAVIOUR

After 28 days of exposure, adult mortality in the control was 0.0%. The mortality in the test item treatment groups did not differ statistically significantly from the control group (see table below). Hence, the LC_{50} was considered to be > 50.0 mg/kg soil dw.

There was no loss in condition (open wounds, etc.) or change in behavior observed in earthworms treated with the test item at concentrations up to and including the maximum of 50.0 mg/kg soil dw.

B. BODY WEIGHT

There were no statistically significant changes in the biomass of the adult earthworms in any of the treatment groups, relative to the control group (see table below).

C. REPRODUCTION CAPACITY

The mean number of juveniles produced per arena was 129.0 in the control and the coefficient of variation for the reproduction in the control group was 25.5%. In terms of numbers of offspring found at 56 days, statistically significant effects compared to the control were only observed at the treatment concentration of 50.00 mg/kg soil dw.

Thus, the LOEC for reproductive effects was 50.00 mg/kg soil dw, and the NOEC was 27.78 mg /kg soil dw. The EC₅₀ was > 50.00 mg CL 3000402/kg soil dw and the EC₂₀ and EC₁₀ for effects on reproduction were calculated to be 39.2 and 33.4 mg/kg soil dw, respectively. The results are summarized in the table below.

Table A 95: Effects on earthworms (*Eisenia Andrei*) exposed to CL 3000402 in a 56-day reproduction study

Test parameter	Test concentration ^a (mg CL 3000402/kg soil dw)					
	Control	4.76	8.57	15.43	27.78	50.0
Mortality (28 d) (%)	0.0	0.0	0.0	0.0	0.0	0.0
Mean fresh weight change (and standard deviation) (28 d) (%) ^b	23.8 (13.4)	27.7 (26.3)	29.1 (7.5)	35.4 (14.7)	38.6 (3.0)	18.6 (8.0)
Number of juveniles / container (56 d) ^c	129.0	132.8	141.8	149.0	162.5	74.0*
Reproduction (% of control) (56 d) ^d	-	-2.9	-9.9	-15.5	-26.0	42.6
Endpoints (mg CL 3000402/kg soil dw)						
LC ₅₀	> 50.00					
EC ₅₀ ^e	> 50.00					
EC ₂₀ (95% CL) ^e	39.2 (30.2 and 45.4)					
EC ₁₀ (95% CL) ^e	33.4 (21.4 and 39.1)					
NOEC	27.78					
LOEC	50.00					

^a Application concentration in terms of mg CL 3000402/kg soil dw, based on measured purity.

^b The mean change in adult worm weight in each arena between 0 and 28 DAT. A positive value indicates an increase in fresh weight. Standard Deviation given in parentheses. Individual treatments compared to the control by t-test for independent samples ($\alpha = 0.05$) or Mann-Whitney U-test ($\alpha = 0.05$). There were no significant differences.

^c Mean number of juvenile worms per replicate arena. The results for the individual treatments were compared to the control by one-way ANOVA and Dunnett's t-test (one-sided, $\alpha = 0.05$). An asterisk indicates a significant difference.

^d Positive values indicates a decrease, and negative values an increase in reproduction, relative to the control.

^e Values for the reproduction EC₅₀, EC₂₀ and EC₁₀ effect concentrations and their 95% confidence limits (where applicable). Only data from the active part of the response curve (shaded values) were included in the Probit regression analysis used to calculate EC₂₀ and EC₁₀ values.

The toxic reference bioassay indicated a reproduction EC₅₀ of 2.4 mg a.s. (carbendazim)/kg soil dw (95% CL 1.9 and 3.0 mg a.s./kg), i.e. the performance of the earthworm culture was consistent with the expectations of the OECD guidelines.

D. DEFICIENCIES

None.

III. CONCLUSION

In a 56-day reproduction study with the metabolite CL 3000402 exposure to earthworms (*Eisenia andrei*), statistically significant effects were noted for reproductive capacity at the highest test concentration, 50 mg/kg soil dw. Thus the no-observed-effect concentration (NOEC) relating to reproductive capacity was found to be 27.78 mg a.s./kg soil dw. The lowest-observable-effect concentration (LOEC) was 50.00 mg a.s./kg soil dw.

A 2.5.1.1.7 Study 7

Comments of zRMS:	The study was conducted to OECD guidance 222 and according to the principles of GLP. All validity criteria were met. The study is considered to be reliable and suitable for the risk assessment.
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Reference:	CP 10.4.1.1/7
Report	Effects of BAS 758 00 F on the reproduction of the earthworm <i>Eisenia andrei</i> in artificial soil, Friedrich, S., 2021 Report No 876346, 2148TEC0001 BASF DocID 2020/2037658 Authority registration No
Guideline(s):	OECD 222 (2016)
Deviations:	No
GLP:	yes (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany),
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive Summary

In a chronic toxicity study, adults of *Eisenia andrei* (Annelida: Oligochaeta) were exposed to BAS 758 00 F. The test item was mixed into artificial soil (10% peat) at eight test concentrations of 25.0, 37.5, 56.2, 84.4, 126.5, 189.8, 284.7 and 427.1 mg BAS 758 00 F/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. Assessment of worm mortality, body weight and feeding activity was carried out after 28 days, assessment of reproduction (number of juveniles) was carried out after 56 days.

After 28 days of exposure, no mortality was observed in the test item treatment groups and in the control. The weight change of adult worms was between -8.0 and 28.5% in the test item treated groups and 27.1% in the control group. The test item caused a statistically significant change in biomass compared to the control at the three highest concentrations. The feeding activity of adult worms was reduced at 284.7 and 427.1 mg BAS 758 00 F/kg dry soil. The reproduction rate was significantly different compared to the control at all concentrations of above 84.4 mg BAS 758 00 F/kg dry soil. No pathological symptoms and no further effects on behavior of the worms were observed.

In a 56-day earthworm reproduction study with BAS 758 00 F, the NOEC for reproduction was determined to be 84.4 mg BAS 758 00 F/kg dry soil. The EC₁₀ for reproduction was calculated to be 86.6 mg BAS 758 00 F/kg dry soil.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 758 00 F; batch no. FD-200124-0004; content of a.s.: metrafenone (BAS 560 F, Reg. No. 4 037 710): 94.9 g/L analyzed (100.0 g/L nominal), mefentrifluconazole (BAS 750 F, Reg. No. 5 834 378): 66.7 g/L analyzed (66.6 g/L nominal), pyraclostrobin (BAS 500 F, Reg. No. 304 428): 81.0 g/L analyzed (80.0 g/L nominal); density: 1.092 g/cm³.

B. STUDY DESIGN

Test species: Earthworm (*Eisenia andrei*), adult worms (with clitellum), weight: 300 – 499 mg/worm, age: approx. 4 months old; source: W. Neudorff GmbH KG, followed by in-house culture.

Test design: 56-day test in treated artificial soil according to OECD 222 (10% peat); different concentrations of the test item are mixed homogeneously into the soil; 9 treatment groups were set up (8 test item concentrations, untreated control) with 4 replicates for the test item treatments and 8 replicates for the control, 10 worms per replicate. Assessment of worm mortality, behavioral effects, and biomass development after 28 days of exposure; after an additional 28 days (56 days after application) reproduction (number of juveniles) was assessed.

Endpoints: Mortality (LC₅₀, NOEC), weight change (EC₅₀, NOEC), feeding activity, reproduction rate (EC_{10, 20 and 50}, NOEC).

Reference item: Maypon Flow (Carbendazim, SC 500). The effects of the reference item were investigated in a separate study.

Test concentrations: Control: untreated soil; test item: 25.0, 37.5, 56.2, 84.4, 126.5, 189.8, 284.7 and 427.1 mg BAS 758 00 F/kg dry soil, (spacing factor: 1.5).

BAS 758 00 F [mg/kg dry soil]	Total active substances [mg/kg dry soil]*	BAS 560 F [mg/kg dry soil]*	BAS 750 F [mg/kg dry soil]*	BAS 500 F[mg/kg dry soil]*
25.0	5.64	2.29	1.52	1.83
37.5	8.47	3.43	2.29	2.75
56.2	12.7	5.15	3.43	4.12
84.4	19.1	7.73	5.15	6.18
126.5	28.6	11.6	7.72	9.27
189.8	42.9	17.4	11.6	13.9
284.7	64.3	26.1	17.4	20.9
427.1	96.4	39.1	26.0	31.3

* Based on nominal content of active substances and a test item density of 1.092 g/cm³, calculations were done with unrounded values.

Test conditions: Artificial soil according to OECD 222 (10% peat); pH 5.97 – 6.05 at test initiation, 5.52 – 5.77 at test end; water content 55.6 – 55.7% of maximum water holding capacity (WHC) at test start and 54.5 – 55.3% of WHC at test end; temperature: 18.2 °C – 20.5 °C; photoperiod: 16 hours light: 8 hours dark, light intensity: 630 lux, food: horse manure.

Analytics:	No analytical verification of the test item is required according to the current test guideline. Hence, no analytical verification was conducted.
Statistics:	Descriptive statistics; Williams t-test for weight change and reproduction ($\alpha = 0.05$, one-sided smaller); Probit analysis for calculation of EC_x .

II. RESULTS AND DISCUSSION

After 28 days of exposure, no mortality was observed in the test item treatment groups and in the control. The weight change of adult worms was between -8.0 and 28.5% in the test item treated groups and 27.1% in the control group. The test item caused a statistically significant change (Williams-t-test, $\alpha = 0.05$, one-sided smaller) in biomass compared to the control at the three highest concentrations. The feeding activity of adult worms was reduced at 284.7 and 427.1 mg BAS 758 00 F/kg dry soil. The reproduction rate was significantly different compared to the control at all concentrations of above 84.4 mg BAS 758 00 F/kg dry soil (Williams-t-test, $\alpha = 0.05$, one-sided smaller). No pathological symptoms and no further effects on behavior of the worms were observed. The results are summarized in Table A 96.

Table A 96: Effects of BAS 758 00 F on *Eisenia andrei* in a 56-day reproduction study

BAS 758 00 F [mg/kg dry soil]	Control	25.0	37.5	56.2	84.4	126.5	189.8	284.7	427.1
Mortality (28 d) [%]	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Weight change (28 d) [%]	27.1	26.1	28.5	25.7	27.2	26.8	21.9 *	9.8 *	-8.0 *
Number of juveniles (56 d)	273.6	282.8	265.8	273.5	274.0	164.5 *	109.3 *	33.8 *	0.0 *
Coefficient of variation [%]	10.6	16.0	15.5	12.3	8.1	15.1	11.2	17.0	-
Reproduction [% of control] (56 d)	100	103.3	97.1	100.0	100.1	60.1	39.9	12.3	0.0
Endpoints [mg BAS 758 00 F/kg dry soil]									
NOEC (28 d) ^{mortality}	≥ 427.1								
NOEC (28 d) ^{biomass}	126.5								
NOEC (56 d)	84.4								
LC ₅₀ (28 d) ¹	> 427.1								
EC ₁₀ (56 d) ²	86.6 (95 % confidence limits 68.5 – 109.3)								
EC ₂₀ (56 d) ²	106.9 (95 % confidence limits 89.7 – 127.3)								
EC ₅₀ (56 d) ²	159.9 (95 % confidence limits 142.8 – 179.0)								

* Statistically significantly different from control (Williams-t-test for weight change and reproduction, $\alpha = 0.05$; one-sided smaller).

¹ Based on estimation of the data.

² Based on Probit analysis.

In a separate study, the reference item Maypon Flow (Carbendazim, SC 500) had a significant effect on biomass increase and reproduction of earthworms. The reproduction rate was clearly inhibited by 56.5% and 99.6% compared to the control at the tested concentrations of 5 and 10 mg product/kg dry soil.

Validity criteria:

Validity criteria according to OECD 222 (2016)	Obtained in this study
Adult mortality in the control $\leq 10\%$	0%
Number of juveniles per control replicate ≥ 30 (with 10 adults per replicate)	219 to 315
Coefficient of variation of reproduction in the control $\leq 30\%$	10.6%

All validity criteria were met.

III. CONCLUSION

In a 56-day earthworm reproduction study with BAS 758 00 F, the NOEC for reproduction was determined to be 84.4 mg BAS 758 00 F/kg dry soil. The EC₁₀ for reproduction was calculated to be 86.6 mg BAS 758 00 F/kg dry soil.

A 2.5.1.2 KCP 10.4.1.2 Earthworms - field studies

BAS 758 00 F poses no unacceptable risk to earthworms. Further studies are not necessary.

A 2.5.2 KCP 10.4.2 Effects on non-target soil meso- and macrofauna (other than earthworms)

A 2.5.2.1 KCP 10.4.2.1 Species level testing

A 2.5.2.1.1 Study 1

The following chronic toxicity study performed with BF 500-6 (metabolite of BAS 500 F, pyra-clostrobin) is provided in support of the assessment and was recently submitted under the Annex I renewal process for pyraclostrobin (AIR 3). The study summary was provided in the revised Renewal Assessment Report of pyraclostrobin (RAR, Vol. 3, B.9, September 2021).

Comments of zRMS:	Study was evaluated and accepted for the regulatory use in RR for BAS 751 00 F. In the current evaluation, the study was considered as not essential for the risk assessment.
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Reference:	CP 10.4.2.1/1
Report	Effects of Reg. No. 364380 (Metabolite of BAS 500 F, Pyraclostrobin) on Reproduction of the Collembola Folsomia candida in Artificial Soil with 5% Peat, Ganssmann, M., 2013 Report No 77512016 BASF DocID 2013/1068054 Authority registration No
Guideline(s):	ISO 11267 (1999), OECD 232 (2009)
Deviations:	No
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden),
Acceptability:	Yes
Duplication (if vertebrate study)	No

Reference:	CP 10.4.2.1/2
Report	Addendum to study BASF DocID: 2013/1068054 - Effects of Reg. No. 364380 (Metabolite of BAS 500 F, Pyraclostrobin) on Reproduction of the <i>Collembola Folsomia candida</i> in Artificial Soil with 5% Peat, Braaker, S., 2019 Report No BASF DocID 2019/2034464 Authority registration No
Guideline(s):	None, not relevant for addendum
Deviations:	None, not relevant for addendum
GLP:	No, not relevant for addendum
Acceptability:	Yes
Duplication (if vertebrate study)	Not relevant

Executive Summary

The effects of BF 500-6 (Reg. No. 364 380, synonym: 500M01), a metabolite of pyraclostrobin, on mortality and reproduction of *Collembola (Folsomia candida)* were investigated in a laboratory study over 28 days. Five concentrations (62.5, 125, 250, 500 and 1000 mg BF 500-6 /kg dry soil) were incorporated into the soil with 4 replicates per test item treatment. An untreated control with 8 replicates was included. All replicates contained 10 collembolans. Assessment of mortality, reproduction rate (number of juveniles) and behavior was carried out after 28 days.

After 28 days of exposure a slight mortality of up to 15% was observed in the test item treated groups, which was not statistically significantly different compared to the control, where 8% of the *Collembola* died. Reproduction of the collembolans exposed to BF 500-6 was not statistically significantly different compared to the control up to and including the highest test concentration of 1000 mg/kg dry soil. No behavioural abnormalities were observed in any of the treatment groups.

In a 28-day *Collembola* reproduction study with BF 500-6 (Reg. No. 364 380, a metabolite of pyraclostrobin) the NOEC based on mortality and reproduction was determined to be ≥ 1000 mg BF 500-6/kg dry soil, the highest concentration tested.

I. MATERIAL AND METHODS

A. MATERIALS

Test item:	Reg. No. 364 380 (metabolite of pyraclostrobin, BF 500-6, synonym: 500M01) batch no. 01311-142; purity: 99.2% (tolerance ± 1.0 %).
Test species:	<i>Collembola (Folsomia candida)</i> , juveniles (10 - 12 days old); source: in-house culture.

B. STUDY DESIGN

Test design:	28-day chronic laboratory test in treated artificial soil (5% peat) according to OECD 232 and ISO 11267; different concentrations of the test item were mixed homogenously into artificial soil and filled into glass vessels after which collembolans were introduced on top of the soil; 6 treatment groups (5 test item concentrations, control); 4 replicates for each test item treatment and 8 replicates for the control, each containing 10 collembolans. Assessment of adult mortality, reproduction (number of juveniles) and behavioural effects after 28 days.
Endpoints:	Mortality, reproduction rate after 28 days.
Reference item:	Boric acid (100 % analyzed). The effects of the reference item were investigated in a separate study.
Test rates:	Control, 62.5, 125, 250, 500 and 1000 mg Reg. No. 364 380/kg dry soil.
Test conditions:	Artificial soil according to OECD 232 (peat: 5 %); pH 6.1 at test initiation, pH 5.9 - 6.0 at test termination; water content at study initiation 52.2 % - 54.6 % of maximum water holding capacity and 46.6 % - 52.7 % of maximum WHC at test termination; temperature: 18 C – 22 C; photoperiod: 16 h light : 8 h dark, light intensity: 400 lux - 800 lux; food: 2 mg granulated dry yeast at the start of the test and after 14 days.
Analytics:	No analytical verification of the test item is required according to the current test guideline.
Statistics:	Descriptive statistics; Fisher's Exact Test for mortality ($\alpha = 0.05$), Williams t-test for reproduction data ($\alpha = 0.05$).

II. RESULTS AND DISCUSSION

After 28 days of exposure a slight mortality of up to 15% was observed in the test item treated groups, which was not statistically significantly different compared to the control, where 8% of the Collembola died (Fisher's Exact test, $\alpha = 0.05$, one-sided greater).

Reproduction of the collembolans exposed to BF 500-6 was not statistically significantly different compared to the control up to and including the highest test concentration of 1000 mg/kg dry soil (Williams t-test, $\alpha = 0.05$, one-sided smaller).

No behavioural abnormalities were observed in any of the treatment groups. The results are summarized below.

Table A 97: Effect of BF 500-6 (Reg. No. 364 380) on Collembola (*Folsomia candida*) in a 28-day reproduction study

BF 500-6 [mg/kg dry soil]	Control	62.5	125	250	500	1000
Mortality (day 28) [%]	8	8	5	10	15	10
No. of juveniles (day 28)	437	471	467	527	451	419
Reproduction (day 28) [% of control]	--	108	107	121	103	96
Endpoints [mg BF 500-6/kg dry soil]						
NOEC _{mortality, weight} (day 28)	≥ 1000					
NOEC _{reproduction} (day 28)	≥ 1000					

Commission Regulation 283/2013 requires EC_{10/20} values for chronic toxicity studies. Both values are not provided in the original study report. However, no effects on reproduction were observed in any test item treatment group and hence, a calculation of EC_{10/20} is not feasible. Regarding mortality, 5 – 15% mortality was observed in the test item concentrations. However, since 8% mortality was observed in the control a calculation of the LC_{10/20} is not feasible as well. This is confirmed by a statistical re-evaluation (for details please refer to BASF DocID 2019/2034464). Therefore, the NOEC is considered the appropriate and relevant endpoint for this study.

Validity criteria:

Validity criteria according to OECD 232 (2016)	Obtained in this study
Mean adult mortality in the control ≤ 20%	8.0%
Mean number of juveniles per control replicate ≥ 100	398 to 500
Coefficient of variation of reproduction in the control ≤ 30%	8.7%

All validity criteria were met.

III. CONCLUSION

In a 28-day Collembola reproduction study with BF 500-6 (Reg. No. 364 380, a metabolite of pyraclostrobin) the NOEC based on mortality and reproduction was determined to be ≥ 1000 mg BF 500-6/kg dry soil, the highest concentration tested.

A 2.5.2.1.2 Study 2

The following chronic toxicity study performed with BF 500-7 (metabolite of BAS 500 F, pyra-clostrobin) is provided in support of the assessment and was recently submitted under the Annex I renewal process for pyraclostrobin (AIR 3). The study summary was provided in the revised Renewal Assessment Report of pyraclostrobin (RAR, Vol. 3, B.9, September 2021).

Comments of zRMS:	Study was evaluated and accepted for the regulatory use in RR for BAS 751 00 F. In the current evaluation, the study was considered as not essential for the risk assessment.
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Reference:	CP 10.4.2.1/3
Report	Effects of Reg.No. 369315 (metabolite of BAS 500 F, Pyraclostrobin) on reproduction of the collembola Folsomia candida in artificial soil with 5% peat, Ganssmann, M., 2013 Report No EU-77611016, EU-426695,77611016 BASF DocID 2013/1224030 Authority registration No
Guideline(s):	OECD 232 (2009)
Deviations:	No
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden),
Acceptability:	Yes
Duplication (if vertebrate study)	No

Reference:	CP 10.4.2.1/4
Report	Addendum to study BASF DocID: 2013/1224030 - Effects of Reg. No 369315 (Metabolite of BAS 500 F, pyraclostrobin) on Reproduction of the Collembola <i>Folsomia candida</i> in Artificial Soil with 5% Peat, Braaker, S., 2019 Report No BASF DocID 2019/2034467 Authority registration No
Guideline(s):	None, not relevant for addendum
Deviations:	None, not relevant for addendum
GLP:	No, not relevant for addendum
Acceptability:	Yes
Duplication (if vertebrate study)	Not relevant

Executive Summary

The effects of BF 500-7 (Reg. No. 369 315, synonym: 500M02, a metabolite of pyraclostrobin, on mortality and reproduction of Collembola (*Folsomia candida*) were investigated in a laboratory study over 28 days. Five concentrations (50, 100, 200, 400 and 800 mg BF 500-7/kg dry soil) were incorporated into the soil with 4 replicates per test item treatment. An untreated control with 8 replicates was included. All replicates contained 10 collembolans. Assessment of mortality, reproduction rate (number of juveniles) and behavior was carried out after 28 days.

After 28 days of exposure a slight mortality of up to 15% was observed in the test item treated groups, which was not statistically significantly different compared to the control, where 6% of the Collembola died. Reproduction of the collembolans exposed to BF 500-7 was not statistically significantly different compared to the control up to and including the highest test concentration of 800 mg/kg dry soil. No behavioural abnormalities were observed in any of the treatment groups.

In a 28-day Collembola reproduction study with BF 500-7 (Reg. No. 369 315, a metabolite of pyraclostrobin) the NOEC based on mortality and reproduction was determined to be ≥ 800 mg BF 500-7/kg dry soil, the highest concentration tested.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Reg. No. 369 315 (metabolite of pyraclostrobin, BF 500-7, synonym: 500M02) batch no. L83-168; purity: 96.2%.

Test species: Collembola (*Folsomia candida*), juveniles (10 - 12 days old); source: in-house culture.

B. STUDY DESIGN

Test design: 28-day chronic laboratory test in treated artificial soil (5% peat) according to OECD 232 and ISO 11267; different concentrations of the test item were mixed homogenously into artificial soil and filled into glass vessels after which collembolans were introduced on top of the soil; 6 treatment groups (5 test item concentrations, control); 4 replicates for each test item treatment and 8 replicates for the control, each containing 10 collembolans. Assessment of adult mortality, reproduction (number of juveniles) and behavioural effects after 28 days.

Endpoints: Mortality, reproduction rate after 28 days.

Reference item: Boric acid (100.3 % analyzed). The effects of the reference item were investigated in a separate study.

Test rates: Control, 50, 100, 200, 400 and 800 mg Reg. No. 369 315/kg dry soil.

Test conditions: Artificial soil according to OECD 232 (peat: 5 %); pH 6.1 - 6.2 at test initiation, pH 6.0 - 6.1 at test termination; water content at study initiation 43.6 % - 45.7 % of maximum water holding capacity and 41.4 % - 44.3 % of maximum WHC at test termination; temperature: 18 C – 22 C; photoperiod: 16 h light : 8 h dark, light intensity: 400 lux - 800 lux; food: 2 mg granulated dry yeast at the start of the test and after 14 days.

Analytics: No analytical verification of the test item is required according to the current test guideline.

Statistics: Descriptive statistics; Fisher's Exact Test for mortality ($\alpha = 0.05$), Williams t-test for reproduction data ($\alpha = 0.05$).

II. RESULTS AND DISCUSSION

After 28 days of exposure a slight mortality of up to 15% was observed in the test item treated groups, which was not statistically significantly different compared to the control, where 6% of the Collembola died (Fisher's Exact test, $\alpha = 0.05$, one-sided greater).

Reproduction of the collembolans exposed to BF 500-7 was not statistically significantly different compared to the control up to and including the highest test concentration of 800 mg/kg dry soil (Williams t-test, $\alpha = 0.05$, one-sided smaller).

No behavioural abnormalities were observed in any of the treatment groups. The results are summarized below:

Table A 98: Effect of BF 500-7 (Reg. No. 369 315) on Collembola (*Folsomia candida*) in a 28-day reproduction study

BF 500-7 [mg/kg dry soil]	Control	50	100	200	400	800
Mortality (day 28) [%]	6	8	10	10	13	15
No. of juveniles (day 28)	886	861	1041	921	873	860
Reproduction (day 28) [% of control]	--	97	117	104	98	97
Endpoints [mg BF 500-7/kg dry soil]						
NOEC _{mortality, weight} (day 28)	≥ 800					
NOEC _{reproduction} (day 28)	≥ 800					

Commission Regulation 283/2013 requires EC_{10/20} values for chronic toxicity studies. Both values are not provided in the original study report. However, no effects on reproduction were observed in any test item treatment group and hence, a calculation of EC_{10/20} is not feasible. Regarding mortality, 8 – 15% mortality was observed in the test item concentrations. However, since 6% mortality was observed in the control a calculation of the LC_{10/20} is not feasible as well. This is confirmed by a statistical re-evaluation (for details please refer to BASF DocID 2019/2034467). Therefore, the NOEC is considered the appropriate and relevant endpoint for this study.

Validity criteria:

Validity criteria according to OECD 232 (2016)	Obtained in this study
Mean adult mortality in the control $\leq 20\%$	6.0%
Mean number of juveniles per control replicate ≥ 100	768 to 1084
Coefficient of variation of reproduction in the control $\leq 30\%$	11.4%

All validity criteria were met.

III. CONCLUSION

In a 28-day Collembola reproduction study with BF 500-7 (Reg. No. 369 315, a metabolite of pyraclostrobin) the NOEC based on mortality and reproduction was determined to be ≥ 800 mg BF 500-7/kg dry soil, the highest concentration tested.

A 2.5.2.1.3 Study 3

The following chronic toxicity study performed with BAS 560 02 F is provided in support of the assessment and was recently submitted under the Annex I renewal process for metrafenone (AIR 3). The study summary was provided in the revised Renewal Assessment Report of metrafenone (RAR, Vol. 3, B.9, December 2019).

Comments of zRMS:	Study not evaluated by zRMS.
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Reference:	CP 10.4.2.1/5
Report	Effects of BAS 560 02 F on Reproduction of the Collembola <i>Folsomia candida</i> in Artificial Soil with 5% peat, Ganssmann, M., 2013 Report No EU-62322016,EU-408125,62322016 BASF DocID 2013/1003203 Authority registration No
Guideline(s):	ISO 11267 (1999), OECD 232 (2009)
Deviations:	No
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden),
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive Summary

The toxicity of BAS 560 02 F to the collembola *Folsomia candida* was determined in a 28-day laboratory study. The collembolans were exposed to BAS 560 02 F incorporated into the soil (5% peat) at concentrations of 62.5, 125, 250, 500 and 1000 mg BAS 560 02 F/kg soil dw. Four replicates were tested for each test substance concentration and eight replicates were included for the negative control. The reference item boric acid was tested in a separate study.

The adult collembolans exposed to BAS 560 02 F did not show any statistically significant increase in mortality rate compared to the control. In addition, their behavior was comparable to the control group. The number of juveniles was also not statistically significantly different between collembolans in the control group and the collembolans exposed to BAS 560 02 F.

In the 28-day study, the no-observed-effect concentration (NOEC) for mortality and reproduction of *Folsomia candida* exposed to BAS 560 02 F was 1000 mg BAS 560 02 F/kg soil dw (equivalent to 422.65 mg a.s./kg soil dw), the highest concentration tested. The lowest-observable-effect concentration for mortality and reproduction was determined to be > 1000 mg BAS 560 02 F/kg soil dw.

I. MATERIAL AND METHODS

A. MATERIALS

- 1. Test material:** BAS 560 02 F
Batch number: 0008024566
Purity: 504.5 g metrafenone/L (analyzed)
Description: Beige liquid
- 2. Test concentrations:** 0 (control), 62.5, 125, 250, 500 and 1000 mg BAS 560 02 F/kg soil dry weight (equivalent to 0, 26.42, 52.83, 105.66, 211.33 and 422.65 mg a.s./kg soil dw)
- 3. Vehicle:** Deionized water
- 4. Reference item:** Boric acid, tested in a separate study
- 5. Artificial soil:** According to OECD 232; containing 5% sphagnum-peat, 20% kaolin clay, 74.8% fine quartz-sand and 0.2% calcium carbonate, and with a maximum water holding capacity (WHC) of 36% of the dry weight
Test units: Glass containers (100 mL volume, 5 cm diameter), closed tightly to avoid water evaporation and filled with 30 g of test soil wet weight
- 6. Test organism:**
Species: *Collembola Folsomia candida*
Age/life stage: 10 to 12 days old
Source: In-house culture
Diet: Granulated dried yeast, spread over the soil surface at test initiation and after fourteen days

B. STUDY DESIGN

1. Environmental conditions:

- Temperature:** 18 – 22 °C
pH of soil: 5.8 – 5.9 at test initiation; 5.5 at test termination
Water content of soil: 18.8 – 20.2% at test initiation; 17.4 – 19.5% at test termination
Photoperiod: 16 h light: 8 h darkness (light intensity between 400 and 800 lux)

2. Assignment and treatment:

At test initiation, collembolans were randomly assigned to batches of ten and then each batch of ten collembolans was placed on the surface of a test unit. Four replicate test units were included for the test substance and eight replicates were tested in the control group. The collembolans were exposed for 28 days.

3. Dose preparation:

A stock solution was prepared by adding BAS 560 02 F to deionized water, at a concentration of 7.9365 mg BAS 560 02 F/g. This stock solution was further diluted to obtain the five different nominal test concentrations of 62.5, 125, 250, 500 and 1000 mg BAS 560 02 F/kg soil dry weight (dw). The negative control group was exposed to deionized water. The test solutions and (additional) deionized water were mixed through the soil using a laboratory mixer.

4. Measurements and observations:

Mortality and behavioral abnormalities were recorded for the adult collembolans, at test termination. Missing adult collembolans were assumed to be dead. In addition, the number of juvenile collembolans was recorded at day 28.

Soil water content was determined on day fourteen and it was not necessary to replenish to compensate for loss of water. The pH was measured at test initiation and test termination.

5. Statistics:

Mortality data were statistically analyzed using Fisher's Exact test. Reproduction data were tested for normal distribution and homogeneity of variance using Shapiro-Wilk's test and Levene's test, respectively. Williams t-test was used to compare treatment groups with the control group. These calculations were performed using ToxRat Professional 2.10.05.

II. RESULTS AND DISCUSSION

A. MORTALITY AND BEHAVIOUR

The mean mortality in the control group was 8%. The mortality in the groups exposed to BAS 560 02 F was not statistically significantly different compared to the control group (see table below). No abnormal behavior was observed with the surviving collembolans in the control group and all the BAS 560 02 F groups.

B. REPRODUCTION

In the control group, the number of juveniles per replicate ranged between 243 and 361, and the coefficient of variance of reproduction was 13.1%. The reproduction rate in the groups exposed to BAS 560 02 F was not statistically significantly different compared to the control group.

The results for mortality and reproduction for the control group and the groups exposed to BAS 560 02 F are shown in the table below.

Table A 99: Mortality and reproduction of *Folsomia candida* exposed to BAS 560 02 F

Test parameter	Test concentration (mg BAS 560 02 F/kg soil dw)					
	Control	62.5	125	250	500	1000
Mortality (mean % \pm SD)	8 \pm 7	8 \pm 10	10 \pm 8	3 \pm 5	8 \pm 10	13 \pm 5
Juveniles/replicate (mean number \pm SD)	283 \pm 37	301 \pm 28	303 \pm 35	272 \pm 16	289 \pm 15	269 \pm 16
Reproduction as % of control	-	106	107	96	102	95
Endpoints (mg BAS 560 02 F/kg dry soil)						
NOEC	1000					
LOEC	1000					

The reference item boric acid demonstrated an EC₅₀ for reproduction of 59.9 mg a.s./ kg soil dw. This is close to the value discussed in the OECD 232 guideline (100 mg/kg soil dw).

C. DEFICIENCIES

None.

III. CONCLUSION

The no-observed-effect concentration (NOEC) for mortality and reproduction of *Folsomia candida* exposed to BAS 560 02 F for 28 days was 1000 mg BAS 560 02 F/kg soil dw (equivalent to 422.65 mg a.s./kg soil dw), the highest concentration tested. The lowest-observable-effect concentration (LOEC) for mortality and reproduction was determined to be > 1000 mg BAS 560 02 F/kg soil dw. The EC_{10/20} calculation not applicable, as NOEC_{mortality} and NOEC_{reproduction} at highest dose tested.

A 2.5.2.1.4 Study 4

Comments of zRMS:	The study was conducted to OECD guidance 232 and according to the principles of GLP. All validity criteria were met. The study is considered to be reliable and suitable for the risk assessment.
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Reference:	CP 10.4.2.1/6
Report	Effects of BAS 758 00 F on the reproduction of the collembolan <i>Folsomia candida</i> , Friedrich, S., 2020 Report No 876347, 2048TCC0047 BASF DocID 2020/2037659 Authority registration No
Guideline(s):	OECD 232 (2016)
Deviations:	No
GLP:	yes (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany),
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive Summary

The effects of BAS 758 00 F on survival and reproduction of the collembolan *Folsomia candida* were investigated in a laboratory study over 28 days. The test item was mixed into artificial soil (5% peat) at concentrations of 20.0, 30.0, 45.0, 67.5, 101.2, 151.9, 227.8 and 341.7 mg BAS 758 00 F/kg dry soil with 4 replicates per treatment. An untreated control with 8 replicates were included. Each replicate contained 10 juvenile collembolans. Assessment of adult mortality, reproduction (number of juveniles) and behavior was carried out after 28 days.

After 28 days of exposure, mortalities ranging from 0.0% to 10.0% were observed in the test item treatments compared to 1.3% in the control. No statistically significant effect on mortality were observed. In the control, a mean of 1415 juveniles were counted. In the treatment groups, a mean number of juveniles between 930 and 1438 was counted. This is corresponding to a reproduction relative to the control between 65.7% and 101.7%. Statistically significant reductions in the number of juveniles were observed in the test concentrations of 227.8 and 341.7 mg BAS 758 00 F/kg dry soil. No behavioral abnormalities were observed at any tested concentration.

In a 28-day collembolan reproduction study with BAS 758 00 F the NOEC for reproduction was 151.9 mg/kg dry soil. For reproduction, the EC₁₀ was determined to be 191.7 mg/kg dry soil.

I. MATERIALS AND METHODS

A. MATERIALS

Test item: BAS 758 00 F; batch no. FD-200124-0004; content of a.s.: metrafenone (BAS 560 F, Reg. No. 4 037 710): 94.9 g/L analyzed (100.0 g/L nominal), mefentrifluconazole (BAS 750 F, Reg. No. 5 834 378): 66.7 g/L analyzed (66.6 g/L nominal), pyraclostrobin (BAS 500 F, Reg. No. 304 428): 81.0 g/L analyzed (80.0 g/L nominal); density: 1.092 g/cm³.

B. STUDY DESIGN

Test species: Collembola (*Folsomia candida*), juveniles, 9 - 12 days old; source: in-house culture.

Test design: 28-day exposure in treated artificial soil (5% peat); different concentrations of the test item were mixed homogeneously into the soil, then filled in glass vessels before springtails were introduced on top of the soil surface; 9 treatment groups (8 concentrations of the test item, untreated control) with 4 replicates for the test item treatment groups and 8 replicates for the control, with 10 collembolans per replicate. Assessment of adult mortality, reproduction (number of juveniles) and behavioral effects after 28 days.

Endpoints: Mortality (NOEC, LOEC, LC₅₀) and reproduction rate (NOEC, LOEC, EC₁₀, 20 and 50) after 28 days.

Reference item: Boric acid - the effects of the reference item were investigated in a separate study.

Test concentrations: Control: untreated soil, test item: 20.0, 30.0, 45.0, 67.5, 101.2, 151.9, 227.8 and 341.7 mg BAS 758 00 F/kg dry soil, (spacing factor: 1.5).

BAS 758 00 F [mg/kg dry soil]	Total active substances [mg/kg dry soil]*	BAS 560 F [mg/kg dry soil]*	BAS 750 F [mg/kg dry soil]*	BAS 500 F [mg/kg dry soil]*
20.0	4.52	1.83	1.22	1.47
30.0	6.77	2.75	1.83	2.20
45.0	10.2	4.12	2.74	3.30
67.5	15.2	6.18	4.12	4.94
101.2	22.9	9.27	6.17	7.42
151.9	34.3	13.9	9.26	11.13
227.8	51.4	20.9	13.9	16.7
341.7	77.2	31.3	20.8	25.0

* Based on nominal content of active substances and a test item density of 1.092 g/cm³, calculations were done with unrounded values.

Test conditions: Artificial soil according to OECD 232 (5% peat); pH 5.93 - 6.05 at test initiation and pH 5.70 - 5.83 at test termination; water content at test start 57.2 - 57.5% of maximum water holding capacity (WHC) and at test end 55.6 - 56.8% of maximum WHC; temperature: 18.1 - 21.3 °C; photoperiod: 16 h light : 8 h dark, light intensity: 640 lux; food: approx. 2 mg granulated dry yeast at the start of the test and after 14 days.

Analytics: No analytical verification of the test item is required according to the current test

guideline. Hence, no analytical verification was conducted.

Statistics: Descriptive statistics; Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm (one-sided greater, $\alpha = 0.05$) for mortality, Williams-t-test for reproduction (one-sided smaller, $\alpha = 0.05$), Logit analysis for reproduction.

II. RESULTS AND DISCUSSION

After 28 days of exposure, mortalities ranging from 0.0% to 10.0% were observed in the test item treatments compared to 1.3% in the control. No statistically significant effect on mortality were observed (Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm, $\alpha = 0.05$, one-sided greater).

In the control, a mean of 1415 juveniles were counted. In the treatment groups, a mean number of juveniles between 930 and 1438 was counted. This is corresponding to a reproduction relative to the control between 65.7% and 101.7%. Statistically significant reductions in the number of juveniles were observed in the test concentrations of 227.8 and 341.7 mg BAS 758 00 F/kg dry soil (Williams-t-test, one-sided smaller, $\alpha = 0.05$). No behavioral abnormalities were observed at any tested concentration. The results are summarized in Table A 100.

Table A 100: Effects of BAS 758 00 F on *Folsomia candida* in a 28-day reproduction study

BAS 758 00 F [mg/kg dry soil]	Control	20.0	30.0	45.0	67.5	101.2	151.9	227.8	341.7
Mortality (day 28) [%]	1.3	0.0	2.5	2.5	2.5	5.0	0.0	5.0	10.0
No. of juveniles (day 28)	1415	1438	1399	1427	1412	1372	1438	1144*	930*
Coefficient of variation [%]	11.0	6.8	8.2	5.2	8.9	1.6	8.7	6.6	14.0
Reproduction [% of control] (day 28)	100	101.7	98.9	100.9	99.8	96.9	101.6	80.9	65.7
Endpoints [mg BAS 758 00 F/kg dry soil]									
NOEC _{mortality}	≥ 341.7								
LOEC _{mortality}	> 341.7								
NOEC _{reproduction}	151.9								
LOEC _{reproduction}	227.8								
LC ₅₀ ¹ (95% confidence limits)	> 341.7								
EC ₁₀ ² (95% confidence limits)	191.7 (156.2 – 235.2)								
EC ₂₀ ² (95% confidence limits)	257.2 (229.7 – 288.0)								
EC ₅₀ ² (95% confidence limits)	> 341.7								

* Statistically significant differences compared to the control (Williams-t-test for reproduction; $\alpha = 0.05$, one-sided smaller).

¹ Due to negligible effects and lacking dose response the value was estimated to be above the highest test concentration.

² Based on Logit analysis.

In a separate, the EC₅₀ (reproduction) of the reference item boric acid was calculated to be 107 mg/kg dry soil.

Validity criteria:

Validity criteria according to OECD 232 (2016)	Obtained in this study
Mean adult mortality in the control $\leq 20\%$	1.3%
Mean number of juveniles per control replicate ≥ 100	1415
Coefficient of variation of reproduction in the control $\leq 30\%$	11.0%

All validity criteria were met.

III. CONCLUSION

In a 28-day collembolan reproduction study with BAS 758 00 F the NOEC for reproduction was 151.9 mg/kg dry soil. For reproduction, the EC₁₀ was determined to be 191.7 mg/kg dry soil.

A 2.5.2.1.5 Study 7

Comments of zRMS:	The study was conducted to OECD guidance 226 and according to the principles of GLP. All validity criteria were met. The study is considered to be reliable and suitable for the risk assessment.
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Reference:	CP 10.4.2.1/7
Report	Effects of BAS 758 00 F on the reproduction of the predatory mite <i>Hypoaspis aculeifer</i> , Schulz, L., 2020 Report No 876348, 2048THC0040 BASF DocID 2020/2037660 Authority registration No
Guideline(s):	OECD 226 (2016)
Deviations:	No
GLP:	yes (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany),
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive Summary

The effects of BAS 758 00 F on mortality and reproduction of the soil mite (*Hypoaspis aculeifer*) were investigated in a laboratory study over 14 days. The test item was mixed into artificial soil (5% peat) at rates of 40.0, 64.0, 102.4, 163.8, 262.1, 419.4, 671.1 and 1073.7 mg BAS 758 00 F/kg dry soil, with 4 replicates per treatment. An untreated control with 8 replicates was included. Each replicate contained 10 adult mites. Assessment of adult mortality and reproduction (number of juveniles) was carried out after 14 days.

After 14 days of exposure, mortality rates ranging from 0% to 7.5% were observed in the test item treatments compared to 2.5% in the control. No statistically significant differences on mortality were observed at concentrations of up to and including 1073.7 mg BAS 758 00 F/kg dry soil. Differences in the behavior of the mites in the control and the test item treatments groups were not observed. In the control, a mean of 238.0 juveniles was counted. In the treatment groups, mean numbers of juveniles between 188.8 and 238.5 were counted. This is corresponding to a reproduction relative to the control ranging from 79% and 100%. Statistically significant reductions in the number of juveniles were observed in the test concentrations of 419.4, 671.1 and 1073.7 mg BAS 758 00 F/kg dry soil.

In a 14-day *Hypoaspis aculeifer* reproduction study with BAS 758 00 F, the LC₅₀ value for mortality was calculated to be > 1073.7 mg/kg dry soil. The EC₁₀ value for reproduction was calculated to be 397.5 mg/kg dry soil. The NOEC for reproduction was determined to be 262.1 mg/kg dry soil.

I. MATERIALS AND METHODS

A. MATERIALS

Test item: BAS 758 00 F; batch no. FD-200124-0004; content of a.s.: metrafenone (BAS 560 F, Reg. No. 4 037 710): 94.9 g/L analyzed (100.0 g/L nominal), mefentrifluconazole (BAS 750 F, Reg. No. 5 834 378): 66.7 g/L analyzed (66.6 g/L nominal), pyraclostrobin (BAS 500 F, Reg. No. 304 428): 81.0 g/L analyzed (80.0 g/L nominal); density: 1.092 g/cm³.

B. STUDY DESIGN

Test species: Soil mite (*Hypoaspis aculeifer* CANESTRINI), adult mites with an age difference of 2 days; source: Katz Biotech AG, Baruth, Germany.

Test design: 14-day chronic laboratory test (according to OECD 226) on effects of BAS 758 00 F 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; 9 treatment groups (8 test item concentrations, 1 control); 8 replicates/control group and 4 replicates/test item treatments, each with 10 soil mites; Assessment of adult mortality and reproduction effects (number of juveniles) after 14 days

Endpoints: Mortality (NOEC, LOEC, LC₅₀) and reproduction rate (NOEC, LOEC, EC₁₀, 20 and 50) after 28 days.

Reference item: Dimethoate (98.8% ± 0.5%, analyzed). The effects of the reference item were investigated in a separate study.

Test rates: Control, 40.0, 64.0, 102.4, 163.8, 262.1, 419.4, 671.1 and 1073.7 mg BAS 758 00 F/kg dry soil (spacing factor: 1.6).

BAS 758 00 F [mg/kg dry soil]	Total active substances [mg/kg dry soil]*	BAS 560 F [mg/kg dry soil]*	BAS 750 F [mg/kg dry soil]*	BAS 500 F [mg/kg dry soil]*
40.0	9.0	3.7	2.4	2.9
64.0	14.5	5.9	3.9	4.7
102.4	23.1	9.4	6.2	7.5
163.8	37.0	15.0	10.0	12.0
262.1	59.2	24.0	16.0	19.2
419.4	94.7	38.4	25.6	30.7
671.1	151.5	61.5	40.9	49.2
1073.7	242.5	98.3	65.5	78.7

* Based on nominal content of active substances and a test item density of 1.092 g/cm³, calculations were done with unrounded values.

Test conditions: Artificial soil according to OECD 226 (5% peat); pH 6.4 – 6.5 at test initiation, pH 6.2 – 6.5 at test termination; water content 46.16% - 47.98% of max. water holding capacity (WHC) at test initiation and at test termination 46.94% – 47.93% of max. WHC; temperature: 19.4 – 21.4 °C; photoperiod: 16 h light : 8 h dark, light intensity: 523 lux; food: cheese mite (*Tyrophagus putrescentiae*) supplied twice to three times a week.

Analytics:	No analytical verification of the test item is required according to the current test guideline. Hence, no analytical verification was conducted.
Statistics:	Descriptive statistics, Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm ($\alpha = 0.05$, one-sided greater) for mortality, Williams Multiple Sequential t-test Procedure ($\alpha = 0.05$, one-sided smaller) for reproduction, Logit analysis using linear maximum likelihood regression for EC _x determination.

II. RESULTS AND DISCUSSION

After 14 days of exposure, mortality rates ranging from 0% to 7.5% were observed in the test item treatments compared to 2.5% in the control. No statistically significant differences on mortality were observed at concentrations of up to and including 1073.7 mg BAS 758 00 F/kg dry soil (Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm, $\alpha = 0.05$, one-sided greater). Differences in the behavior of the mites in the control and the test item treatments groups were not observed.

In the control, a mean of 238.0 juveniles was counted. In the treatment groups, mean numbers of juveniles between 188.8 and 238.5 were counted. This is corresponding to a reproduction relative to the control ranging from 79% and 100%. Statistically significant reductions in the number of juveniles were observed in the test concentrations of 419.4, 671.1 and 1073.7 mg BAS 758 00 F/kg dry soil (Williams Multiple Sequential t-test Procedure, $\alpha = 0.05$, one-sided smaller). The results are summarized in Table A 101.

Table A 101: Effects of BAS 758 00 F on soil mite (*Hypoaspis aculeifer*) in a 14-day reproduction study

BAS 758 00 F [mg/kg dry soil]	Control	40.0	64.0	102.4	163.8	262.1	419.4	671.1	1073.7
Mortality (day 14) [%]	2.5	7.5	0.0	0.0	2.5	2.5	0.0	2.5	0.0
No. of juveniles (day 14)	238.0	230.5	228.8	230.8	225.3	238.5	214.3*	198.8*	188.8*
Coefficient of variation [%]	9.5	4.1	8.7	3.5	4.8	9.4	6.0	11.8	4.7
Reproduction [% of control] (day 14)	100	97	96	97	95	100	90	84	79
Endpoints [mg BAS 758 00 F/kg dry soil]									
NOEC _{mortality}	≥ 1073.7								
LOEC _{mortality}	> 1073.7								
NOEC _{reproduction}	262.1								
LOEC _{reproduction}	419.4								
LC ₅₀ ¹	> 1073.7								
EC ₁₀ ² (95% confidence limits)	397.5 (282.7 – 559.0)								
EC ₂₀ ² (95% confidence limits)	1013.8 (727.6 - 1412.6)								
EC ₅₀ ²	> 1073.7								

* Statistically significant compared to control (Williams Multiple Sequential t-test Procedure, $\alpha = 0.05$, one-sided smaller).

¹ Due to negligible effects and lacking dose response this value was estimated to be above the highest test concentration.

² Based on Logit analysis using linear maximum likelihood regression.

³ Due to negligible effects this value was estimated to be above the highest test concentration.

In a separate study, the EC₅₀ (reproduction) of the reference item dimethoate (98.8% ± 0.5%, analyzed) was calculated to be 6.3 mg a.s./kg dry soil. The results of the reference test demonstrate the sensitivity of the test system.

Validity criteria:

Validity criteria according to OECD 226 (2016)	Obtained in this study
Mean adult mortality in the control ≤ 20%	2.5%
Mean number of juveniles per control replicate ≥ 50	238.0
Coefficient of variation of reproduction in the control ≤ 30%	9.5%

All validity criteria were met.

III. CONCLUSION

In a 14-day *Hypoaspis aculeifer* reproduction study with BAS 758 00 F, the LC₅₀ value for mortality was calculated to be > 1073.7 mg/kg dry soil. The EC₁₀ value for reproduction was calculated to be 397.5 mg/kg dry soil. The NOEC for reproduction was determined to be 262.1 mg/kg dry soil, respectively.

A 2.5.2.2 KCP 10.4.2.2 Higher tier testing

BAS 758 00 F poses no unacceptable risk to non-target soil meso- and macro-organisms other than earthworms. Further studies are not necessary.

A 2.6 KCP 10.5 Effects on soil nitrogen transformation

A 2.6.1 Study 1

The following nitrogen transformation study performed with CL3000402 is provided in support of the assessment and was recently submitted under the Annex I renewal process for metrafenone (AIR 3). The study summary was provided in the revised Renewal Assessment Report of metrafenone (RAR, Vol. 3, B.9, December 2019).

Comments of zRMS:	Study not evaluated.
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Reference:	CP 10.5/1
Report	CL3000402: Effects on the activity of the soil microflora under laboratory conditions (nitrogen transformation), Schoebinger U., 2015 Report No EU-S15-01808,S15-01808 BASF DocID 2015/1132053 Authority registration No
Guideline(s):	OECD 216 (2000)
Deviations:	No
GLP:	yes (certified by Landesamt fuer Umwelt, Messungen und Naturschutz Baden Wuerttemberg, Karlsruhe, Germany),
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive Summary

The effect of CL3000402 on nitrogen transformation by soil microflora was determined in a medium silty sand soil (DIN 4220) in the laboratory over the course of 28 days. CL3000402 was applied to samples of the soil at nominal test concentrations of 0.200 and 1.00 mg/kg soil dry weight (dw). A control and a solvent control (0.3 mL acetone/kg soil wet weight) were tested in parallel. Nitrogen transformation (measured as NO₃-N production) was determined 0, 7, 14 and 28 days after application.

There were no effects above 25 % deviation to the solvent control on the nitrogen transformation for the application of 0.200 and 1.00 mg test item/kg soil dw at the end of the 28-day incubation period.

CL 3000402 applied to a field soil up to a test concentration of 1.00 mg/kg soil dw caused no adverse effects (deviation from the solvent control < 25 %) on soil nitrogen transformation (measured as NO₃-N production) at the end of the 28-day incubation period.

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test material:** CL 3000402
Description: White solid
Lot/Batch: L87-50
Purity: 97.1% w/w
2. **Test concentrations:** Water control (untreated quartz sand), solvent control (solvent treated quartz sand), 0.200 and 1.00 mg CL3000402 /kg soil dw
3. **Vehicle:** Acetone
4. **Reference item:** Sodium Chloride. The reference item was tested in a separate study at a rate of 20 g a.s./kg soil dw
5. **Test soil:**
Source: Offenbach, Rhineland-Palatinate, Germany, latitude E 439713, longitude N 5449850 (no plant protection products had been applied for at least 4 years)
Type: Medium silty sand soil (DIN 4220)
pH: 7.0
Total organic carbon: 0.58%
Nitrate content: 13.9 mg/kg soil dw
Total nitrogen: 0.08%
Cation exchange capacity: 6.8 meq/100 g
Maximum water holding capacity: 36.61%
Microbial biomass: 42.3 mg C/100 g soil dw
Test vessels: Glass vessels loosely closed with screw caps

B. STUDY DESIGN

1. **Environmental conditions:**
Temperature: 19.8 - 21.3 °C
pH of soil: 6.8-7.1
Water content: Approximately 42% of maximum water holding capacity. Measured water content: 13.4 – 15.6 g/100 g soil
Photoperiod: Continuous darkness

2. **Preparation of soil:**

The test soil was sieved to a particle size of 2 mm. Prior to the initiation of the study, the moisture content of the soil and the amount of water needed to adjust the soil moisture content to 42 % WHC_{max} (maximum water holding capacity) were calculated. The test item was applied at a concentration of 0.20 and 1.00 mg/kg soil dw, respectively. Untreated control and solvent control (0.3 mL acetone/kg soil wet weight) groups were tested in parallel.

For the nitrogen turnover the soil and finely ground lucerne meal were thoroughly mixed before application by hand. The final concentration of the dried lucerne meal was 0.5 % of the soil dw.

After treating the soil and mixing thoroughly, three equally sized soil sub-samples from each treatment group (approx. 1100 g) were placed in appropriate glass bottles. The bottles were closed loosely with screw caps and weighed for the determination of the starting weight.

3. Dose preparation:

The test item solution T2 was prepared by diluting the necessary amount of the test item with acetone. An aliquot of T2 was further diluted with acetone to obtain test item solution T1. The test item solutions were homogenized by shaking. For application, 1 mL of the test item solutions were added to quartz sand and mixed thoroughly with the test soil. To achieve 42 % of WHC_{max}, 77.2 g deionized water were added to the respective treatment groups. Each treatment group (soil with the test item added at two different concentrations and untreated controls) contained 3300 g pre-moistened soil for the nitrogen turnover.

4. Measurements and observations:

Samples of the treated soil were taken from each replicate on days 0, 7, 14 and 28 after treatment. Approximately 50 g was used for determination of nitrogen content. Soil nitrification was determined by measuring the NO₃⁻ contents of aqueous soil extracts by means of Segmented Flow Analysis (SFA) using photometric measurement of nitrate. The concentrations of NO₃-N in the soil were then calculated from the measured values.

At each sampling data, pH and water content were determined. The amount of moisture loss was determined and water was added when required to adjust vessels to the starting weight.

5. Statistics:

Descriptive statistics.

I. RESULTS AND DISCUSSION

A. NITRIFICATION

No adverse effects of CL3000402 on nitrogen transformation in soil could be observed in both test concentrations (0.200 and 1.00 mg/kg soil dw) after 28 days of exposure. The results are summarized in the table below.

Table A 102: Summary of CL 3000402 effects on nitrogen transformation

Interval ^a Sampling days	Solvent Control	Water Control		0.200 mg CL3000402 / kg soil dw		1.00 mg CL3000402 / kg soil dw	
	Mean formation rate ^b						
	mg NO ₃ -N/ kg soil dw /day	mg NO ₃ -N/ kg soil dw /day	Dev. % ^c	mg NO ₃ -N/ kg soil dw /day	Dev. % ^c	mg NO ₃ -N/ kg soil dw /day	Dev. % ^c
0-7	-1.05	-1.07	-1.60	-1.29	-22.0	-1.48	-40.4
0-14	1.02	0.994	-2.52	0.764	-25.1	0.671	-34.2
0-28	1.19	1.17	-2.01	1.01	-15.1	0.987	-17.2

^a time interval from test start (day 0) until measurement

^b calculated from the mean values of NO₃-N content between day 0 and the sampling date

^c deviation from solvent control

- = inhibition; + = stimulation

The toxic reference item (sodium chloride), tested in a separate study, had significant effects on the soil nitrogen turnover (decrease of the nitrate transformation rate for the interval of 0 to 28 days of 96.7 %, decrease of the nitrate transformation rate for the interval of 14 to 28 days of 85.1 %) and the short-term respiration (89.5 % inhibition) in a field soil tested at a concentration of 20.0 g/kg soil dry weight.

B. DEFICIENCIES

CaO and MgO had been applied to the field soil within 6 months prior to sampling to increase the soil pH in the field. It is considered that this has not impacted on the validity of the study.

III. CONCLUSION

CL 3000402 applied to a field soil up to a test concentration of 1.00 mg/kg soil dry weight caused no adverse effects (deviation from the solvent control < 25 %) on the soil nitrogen transformation (measured as NO₃-N production) at the end of the 28-day incubation period.

A 2.6.2 Study 2

Comments of zRMS:	The study was conducted to OECD guidance 216 and according to the principles of GLP. All validity criteria were met. The study is considered to be reliable and suitable for the risk assessment.
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Reference:	CP 10.5/2
Report	Effects of BAS 758 00 F on the activity of soil microflora (Nitrogen Transformation test), Schulz, L., 2020 Report No 876345, 2048SMN0047 BASF DocID 2020/2037661 Authority registration No
Guideline(s):	OECD 216 (2000)
Deviations:	No
GLP:	yes (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany),
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive Summary

In a soil microbial activity study, the effect of BAS 758 00 F on nitrogen transformation was tested in loamy sand soil. BAS 758 00 F was applied to samples of the soil at nominal concentrations of 8 mg and 40 mg/kg dry soil. Triplicate samples of each treatment were removed for analysis of NH₄-nitrogen and NO₃-nitrogen 0, 7, 14 and 28 days after application.

No unacceptable effects of BAS 758 00 F on nitrogen transformation in soil were observed at both test concentrations (8 mg and 40 mg/kg dry soil) after 28 days. Only negligible deviations from the control of +6.1% and +4.4% (at test concentration 8 mg and 40 mg/kg dry soil, respectively) were observed in both treatment groups after 28 days.

Based on the results of this study, BAS 758 00 F caused no unacceptable effects (< 25% deviation from control according to OECD 216) on the soil nitrogen transformation (measured as NO₃-N production) in a loamy sand soil tested up to a concentration of 40 mg/kg dry soil at the end of the 28-day incubation period.

I. MATERIALS AND METHODS

A. MATERIALS

Test item: BAS 758 00 F; batch no. FD-200124-0004; content of a.s.: metrafenone (BAS 560 F, Reg. No. 4 037 710): 94.9 g/L analyzed (100.0 g/L nominal), mefentrifluconazole (BAS 750 F, Reg. No. 5 834 378): 66.7 g/L analyzed (66.6 g/L nominal), pyraclostrobin (BAS 500 F, Reg. No. 304 428): 81.0 g/L analyzed (80.0 g/L nominal); density: 1.092 g/cm³.

B. STUDY DESIGN

Test soil: Biologically active agricultural soil: loamy sand (DIN 4220) / loam (USDA), soil pH 6.3, C_{org} 1.42%, WHC: 38.20%.

Test design: Determination of the N-transformation (NO₃-nitrogen production) in soil enriched with lucerne meal (concentration in soil 0.5%). Three treatment groups were set up (untreated control, 2 test item concentrations) with 3 replicates per treatment. Comparison of test item treated soil with a non-treated soil. NH₄-nitrogen formed from organically bound nitrogen and NO₃-nitrogen from the nitrification process was determined by using an Autoanalyzer (SEAL Analytical). Sampling scheme: 0, 7, 14 and 28 days after treatment; sub-samples were withdrawn from the bulk batches and subjected to the measurement.

Endpoints: Effects on the NO₃-nitrogen production 0, 7, 14 and 28 days after exposure.

Test rates: Control, 8 mg and 40 mg BAS 758 00 F/kg dry soil.

Reference item: Dinoterb (purity: 99.28% (g/g) analyzed). The reference item was tested in a separate study at rates of 6.80, 13.60 and 27.20 mg/kg dry soil.

Test conditions: Water content: approx. 45% of its maximum water holding capacity; measured water content: 17.08 – 17.57 g/100 g dry soil; pH 6.1 - 6.2. Soil samples were incubated at 19.4 °C – 20.7 °C while stored in glass flasks in the dark.

Analytics: No analytical verification of the test item is required according to the current test guideline. Hence, no analytical verification was conducted.

Statistics: Descriptive statistics.

II. RESULTS AND DISCUSSION

No unacceptable effects of BAS 758 00 F on nitrogen transformation in soil were observed at both test concentrations (8 mg and 40 mg/kg dry soil) after 28 days. Only negligible deviations from the control of +6.1% and +4.4% (at test concentration 8 mg and 40 mg/kg dry soil, respectively) were observed in both treatment groups after 28 days. The results are summarized below in Table A 103.

Table A 103: Effects of BAS 758 00 F on soil micro-organisms (nitrogen transformation rate) for the intervals 0 - 7, 0 - 14 and 0 - 28

Soil (interval)	Control	8 mg BAS 758 00 F/kg dry soil		40 mg BAS 758 00 F/kg dry soil	
	NO ₃ -N/day [mg/kg dry soil/d] ¹	NO ₃ -N/day [mg/kg dry soil/d] ¹	% Deviation from control	NO ₃ -N/day [mg/kg dry soil/d] ¹	% Deviation from control
Loamy sand (0 - 7 d)	3.30	3.21	-2.9	3.50	+6.1
Loamy sand (0 - 14 d)	2.64	2.56	-3.1	3.11	+17.7
Loamy sand (0 - 28 d)	2.00	2.12	+6.1	2.08	+4.4

¹ (measured values sampling day “x” - measured values sampling day 0) / days, mean of 3 replicates

- = inhibition, + = stimulation.

NO₃-N-contents - measured values

Samp-ling day	Treatment group	Repl.	Measured values	Mean value	SD	CV	[mg NO ₃ -N/ kg soil d.w.]
			[mg NO ₃ -N/100 g soil d.w.]				[%]
0	Control	1	4.84	4.90	0.11	2.3	49.0
		2	4.83				
		3	5.03				
	BAS 758 00 F 8 mg/kg	1	4.99	4.95	0.04	0.7	49.5
		2	4.92				
		3	4.94				
	BAS 758 00 F 40 mg/kg	1	4.95	4.89	0.09	1.7	48.9
		2	4.92				
		3	4.79				
7	Control	1	7.37	7.21	0.20	2.8	72.1
		2	7.28				
		3	6.99				
	BAS 758 00 F 8 mg/kg	1	7.32	7.20	0.21	3.0	72.0
		2	7.32				
		3	6.95				
	BAS 758 00 F 40 mg/kg	1	7.27	7.34	0.07	1.0	73.4
		2	7.41				
		3	7.34				
14	Control	1	8.96	8.60	0.34	3.9	86.0
		2	8.53				
		3	8.30				
	BAS 758 00 F 8 mg/kg	1	8.78	8.53	0.32	3.8	85.3
		2	8.65				
		3	8.17				
	BAS 758 00 F 40 mg/kg	1	9.17	9.24	0.06	0.6	92.4
		2	9.26				
		3	9.28				
28	Control	1	10.79	10.49	0.26	2.5	104.9
		2	10.37				
		3	10.31				
	BAS 758 00 F 8 mg/kg	1	10.72	10.88	0.30	2.8	108.8
		2	11.23				
		3	10.70				
	BAS 758 00 F 40 mg/kg	1	10.54	10.72	0.17	1.6	107.2
		2	10.88				
		3	10.74				

Limit of quantification (= LOQ): 0.84 mg/100 g soil d.w.

SD = Standard Deviation

CV [%] = Coefficient of Variation

Repl. = Replicate

soil d.w. = soil dry weight

In a separate study the reference item Dinoterb produced a stimulation of nitrogen transformation of +28.3% and +48.6% at 6.80 mg and 13.60 mg/kg dry soil determined 28 days after application.

Validity criteria:

Validity criteria according to OECD 216 (2000)	Obtained in this study
Coefficient of variation in the control for NO ₃ -N ≤ 15%	max. 3.9% (loamy sand)

All validity criteria were met.

III. CONCLUSION

Based on the results of this study, BAS 758 00 F caused no unacceptable effects (< 25% deviation from control according to OECD 216) on the soil nitrogen transformation (measured as NO₃-N production) in a loamy sand soil tested up to a concentration of 40 mg/kg dry soil at the end of the 28-day incubation period.

A 2.7 KCP 10.6 Effects on terrestrial non-target higher plants

A 2.7.1 KCP 10.6.1 Summary of screening data

Tests on non-target plants have been conducted. The data point is covered by Appendix 2.6.2 (KCP 10.6.2).

A 2.7.2 KCP 10.6.2 Testing on non-target plants

A 2.7.2.1 Study 1

Comments of zRMS:	The study was conducted to OECD guidance 227 and according to the principles of GLP. All validity criteria were met. The study is considered to be reliable and suitable for the risk assessment.
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Reference:	CP 10.6.2/1
Report	Effects of BAS 758 00 F on vegetative vigour of ten species of terrestrial plants under greenhouse conditions, Teresiak-Baumgart, P., 2020 Report No 876350, AC/BASF/20/19 BASF DocID 2020/2037665 Authority registration No
Guideline(s):	OECD 227 July 2006, EPA 850.4150 (2012)
Deviations:	No
GLP:	yes (certified by Land Brandenburg Ministerium der Justiz und fuer Europa und fuer Verbraucherschutz, Potsdam, Germany),
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive Summary

In a vegetative vigor test, six species of dicotyledonous plants (carrot, lettuce, oilseed rape, cabbage, soybean and tomato) and four species of monocotyledonous plants (onion, ryegrass, wheat and corn) were exposed to BAS 758 00 F to evaluate the phytotoxic potential. BAS 758 00 F was applied post-emergence at BBCH 12 to 14 at a limit rate of 1.5 L BAS 758 00 F/ha. After application, the plants were cultivated for 21 days under greenhouse conditions. Assessment of plant damage (phytotoxicity) and plant survival were done 7, 14 and 21 days after treatment (DAT). Assessments of plant length and plant dry weight were done at study termination 21 DAT.

After exposure to BAS 758 00 F, no treatment-related symptoms of plant survival were observed for all tested plant species following the application of 1.5 L BAS 758 00 F/ha. No reduction on plant length were not observed for any of the tested plant species except tomato. Tomato showed statistically significant plant length reduction with 4% at the tested rate. No influence of BAS 758 00 F on plant biomass was observed for all tested plant species except carrot following the application of the tested rate. Carrot showed statistically significant plant biomass reduction with 8% at the tested rate.

Post-emergence application of BAS 758 00 F conducted under worst-case greenhouse conditions did not result in relevant effects on phytotoxicity, plant survival, plant dry weight and length. The ER₅₀ based on phytotoxicity, plant survival, plant dry weight and length was > 1.5 L BAS 758 00 F/ha for all tested plant species (the highest rate tested).

I. MATERIALS AND METHODS

A. MATERIALS

Test item: BAS 758 00 F; batch no. FD-200124-0004; content of a.s.: metrafenone (BAS 560 F, Reg. No. 4 037 710): 94.9 g/L analyzed (100.0 g/L nominal), mefentrifluconazole (BAS 750 F, Reg. No. 5 834 378): 66.7 g/L analyzed (66.6 g/L nominal), pyraclostrobin (BAS 500 F, Reg. No. 304 428): 81.0 g/L analyzed (80.0 g/L nominal); density: 1.092 g/cm³.

B. STUDY DESIGN

Test species: Dicotyledonous: Carrot (*Daucus carota* L.), lettuce (*Lactuca sativa* L.), oilseed rape (*Brassica napus* L. spp. *napus*), cabbage, white (*Brassica oleracea* L. var. *capitata* L. f. *alba*), soybean (*Glycine max* L.), tomato (*Solanum lycopersicum* L.).
Monocotyledonous: onion (*Allium cepa* L.), ryegrass (*Lolium multiflorum* L.), wheat (*Triticum aestivum* L.), corn (*Zea mays* L.).

Test design: Greenhouse trial, limit test; 1 test item rate and 1 tap water control; 5 replicates per treatment; 1, 2 or 3 pots per replicate and 2, 3, or 6 plants per pot depending on the plant species; test item applied post emergence at BBCH 12 - 14 using a laboratory spray chamber at a water volume of 248 L/ha. Assessments for plant damage (phytotoxicity) and plant survival were done 7, 14 and 21 DAT. Plant length and shoot dry weight was determined 21 DAT.

Endpoints: Phytotoxicity (NOER), Plant weight and length (NOER, ER₅₀).

Test rates: Control: tap water, 1.5 L BAS 758 00 F/ha.

Test conditions: Greenhouse conditions, daily average temperature: 22.6 °C – 27.1 °C; daily mean relative humidity: 55.3% - 75.7%; photoperiod: day length ≥ 16 h; additional light supply automatically for 16 hours in maximum when indoor illumination was less than 300 µmol.

Analytics: Analytical verification of the a.s. BAS 750 F present in application solutions prepared from the test item BAS 758 00 F was conducted according to BASF method L0361/01 using LC-MS/MS.

Statistics: Descriptive statistics; Student t-test (one-sided smaller, $\alpha = 0.05$) for metric data; NOER for phytotoxicity was estimated; Phytotoxicity values < 10% were considered as insignificant.

C. DESCRIPTION OF THE ANALYTICAL PROCEDURES

Concentrations of BAS 750 F (contained in BAS 758 00 F) in application solution were determined according to the analytical method L0361/01. The method is validated within the study. 1 mg/mL stock solution was prepared by dissolving approx. 7 and 140 mg BAS 758 00 F in a volumetric flask, dissolved in some 30 μ L silicon antifoam emulsion in 1.0 L of tap water, and diluted with solvent (acetonitrile/water/formic acid of 20/80/0.1, v/v). A high total dilution factor was used to bring the concentration of the present application solutions in the linear range of the respective LC-MS/MS calibration curve(s). Aliquot of the final solution (total dilution factor: 200,000) was directly injected into the LC-MS/MS system for determination of BAS 750 F. The limit of quantification (LOQ) was 0.045 g/L, and the limit of detection (LOD) was calculated to be 0.010 g/L. The analysis of the application solutions was performed within 90 ± 1 days after preparation of the samples. Thus, no additional storage stability testing is necessary. Due to the high total dilution factor, no relevant matrix effects were expected for the LC-MS/MS determination of BAS 750 F within this study. Thus, calibration solutions in solvent were applied for analysis. Details on measured fortification samples and obtained procedural recoveries for BAS 750 F are given in the table below.

Table A 104 Procedural recoveries for BAS 750 F (based on BAS 758 00 F)

Matrix	Mean fortification level [g BAS 750 F/L] (nominal)	n	Recoveries [%]	Mean recovery [%]	RSD [%]
Application solution	0.045	5	93, 90, 93, 90, 90	91	1.75
	0.862	5	90, 91, 91, 90, 91	91	1.01

Abbreviations: RSD = relative standard deviation

II. RESULTS AND DISCUSSION

After exposure to BAS 758 00 F, no effects to plant survival were observed for all tested plant species following the application of 1.5 L BAS 758 00 F/ha. No reduction on plant length were not observed for any of the tested plant species except tomato. Tomato showed statistically significant plant length reduction with 4% at the tested rate of 1.5 L BAS 758 00 F/ha (Student t-test, one-sided smaller, $\alpha = 0.05$). No influence of BAS 758 00 F on plant biomass was observed for all tested plant species except carrot following the application of 1.5 L BAS 758 00 F/ha. Carrot showed statistically significant plant biomass reduction with 8% at the tested rate (Student t-test, one-sided smaller, $\alpha = 0.05$). These results are summarized in

Table A 105 and Table A 106.

Table A 105: Effects of BAS 758 00 F on phytotoxicity, plant survival, plant length and biomass 21 DAT

BAS 758 00 F [L/ha]	Carrot	Lettuce	Oilseed rape	Cabbage	Soy-bean	Tomato	Onion	Rye-grass	Wheat	Corn
Phytotoxic damages [%]										
Control	0	0	0	0	0	0	0	0	0	0
1.5	0	0	0	0	0	0	0	0	0	0
Plant survival [% to control]										
Control	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
1.5	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Plant length [% to control]										
Control	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
1.5	101.9	103.0	101.7	102.1	104.2	96.0*	101.6	95.5	100.3	98.8
Plant dry weight [% to control]										
Control	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
1.5	92.5*	97.1	98.1	100.1	100.4	96.1	103.5	92.7	101.2	95.7

* Statistically significant difference compared to the control (Student t-test, one-sided smaller, $\alpha = 0.05$)

Table A 106: NOER and ER₅₀ of BAS 758 00 F for non-target plants 21 DAT

	Carrot	Lettuce	Oilseed rape	Cabbage	Soy-bean	Tomato	Onion	Rye-grass	Wheat	Corn
Phytotoxicity * [L/ha]										
NOER	≥ 1.5	≥ 1.5	≥ 1.5	≥ 1.5	≥ 1.5	≥ 1.5	≥ 1.5	≥ 1.5	≥ 1.5	≥ 1.5
ER ₅₀	> 1.5	> 1.5	> 1.5	> 1.5	> 1.5	> 1.5	> 1.5	> 1.5	> 1.5	> 1.5
Plant survival [L/ha]										
NOER	≥ 1.5	≥ 1.5	≥ 1.5	≥ 1.5	≥ 1.5	≥ 1.5	≥ 1.5	≥ 1.5	≥ 1.5	≥ 1.5
ER ₅₀	> 1.5	> 1.5	> 1.5	> 1.5	> 1.5	> 1.5	> 1.5	> 1.5	> 1.5	> 1.5
Plant length [L/ha]										
NOER	≥ 1.5	≥ 1.5	≥ 1.5	≥ 1.5	≥ 1.5	< 1.5	≥ 1.5	≥ 1.5	≥ 1.5	≥ 1.5
ER ₅₀	> 1.5	> 1.5	> 1.5	> 1.5	> 1.5	> 1.5	> 1.5	> 1.5	> 1.5	> 1.5
Plant dry weight [L/ha]										
NOER	< 1.5	≥ 1.5	≥ 1.5	≥ 1.5	≥ 1.5	≥ 1.5	≥ 1.5	≥ 1.5	≥ 1.5	≥ 1.5
ER ₅₀	> 1.5	> 1.5	> 1.5	> 1.5	> 1.5	> 1.5	> 1.5	> 1.5	> 1.5	> 1.5

* Estimated from assessment data.

Validity criteria:

Validity criteria according to OECD 227	Obtained in this study
Seedling emergence is at least 70%	yes (88% to 100%)
In the controls:	
The plants do not exhibit visible phytotoxic effects (e.g. chlorosis, necrosis, wilting, leaf and stem deformations). Plants exhibit only normal variation in growth and morphology for that particular species	yes (0%)
Mean plant survival at least 90% for the duration of the study	yes (100%)
Environmental conditions for a particular species are identical and growing media contain the same amount of soil matrix, support media, or substrate from the same source	yes

All validity criteria were met.

III. CONCLUSION

Post-emergence application of BAS 758 00 F conducted under worst-case greenhouse conditions did not result in relevant effects on phytotoxicity, plant survival, plant dry weight and length. The ER₅₀ based on phytotoxicity, plant survival, plant dry weight and length was > 1.5 L BAS 758 00 F/ha for all tested plant species (the highest rate tested).

A 2.7.2.2 Study 2

Comments of zRMS:	The study was conducted to OECD guidance 208 and according to the principles of GLP. All validity criteria were met. The study is considered to be reliable and suitable for the risk assessment.
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Reference:	CP 10.6.2/2
Report	Effect of BAS 758 00 F on seedling emergence and seedling growth of ten species of terrestrial plants under greenhouse conditions, Teresiak-Baumgart, P., 2020 Report No 876349, AC/BASF/20/18 BASF DocID 2020/2037664 Authority registration No
Guideline(s):	OECD 208 (2006), EPA 850.4100 (2012)
Deviations:	No
GLP:	yes (certified by Land Brandenburg Ministerium der Justiz und fuer Europa und fuer Verbraucherschutz, Potsdam, Germany),
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive Summary

In a seedling emergence test, six species of dicotyledonous plants (carrot, lettuce, oilseed rape, cabbage, soybean and tomato) and four species of monocotyledonous plants (onion, ryegrass, wheat and corn) were exposed to BAS 758 00 F to evaluate the phytotoxic potential. BAS 758 00 F was applied pre-emergence at a limit rate of 1.5 L BAS 758 00 F/ha. After 50% emergence of the untreated control, the plants were cultivated for 21 days under greenhouse conditions. Assessments for seedling emergence, phytotoxicity and plant survival were done 7, 14 and 21 days after emergence (DAE). Plant dry weight and plant length was determined at study termination 21 DAE.

After the application of BAS 758 00 F at pre-emergence, none of the tested plant species was affected concerning seedling emergence, plant survival and plant length. No influence of BAS 758 00 F on plant dry weight was observed for all tested species, except ryegrass, following the pre emergence application of 1.5 L BAS 758 00 F/ha. Ryegrass showed statistically significant biomass reduction with 9% at the tested rate of 1.5 L BAS 758 00 F/ha. The NOER for plant emergence, plant survival, plant length and plant biomass for all tested plant species is equal or higher than the tested rate of 1.5 L BAS 758 00 F/ha, except for plant biomass of ryegrass. The NOER for plant biomass reduction for ryegrass is < 1.5 L BAS 758 00 F/ha.

Pre-emergence application of BAS 758 00 F conducted under worst-case greenhouse conditions did not result in relevant effects on seedling emergence, survival, phytotoxicity, plant length and dry weight of all tested plant species. The ER₅₀ based on seedling emergence, phytotoxicity, plant survival, plant dry weight and length was > 1.5 L BAS 758 00 F/ha for all tested plant species (the highest rate tested).

I. MATERIALS AND METHODS

A. MATERIALS

Test item: BAS 758 00 F; batch no. FD-200124-0004; content of a.s.: metrafenone (BAS 560 F, Reg. No. 4 037 710): 94.9 g/L analyzed (100.0 g/L nominal), mefentrifluconazole (BAS 750 F, Reg. No. 5 834 378): 66.7 g/L analyzed (66.6 g/L nominal), pyraclostrobin (BAS 500 F, Reg. No. 304 428): 81.0 g/L analyzed (80.0 g/L nominal); density: 1.092 g/cm³.

B. STUDY DESIGN

Test species: Dicotyledonous: Carrot (*Daucus carota* L.), lettuce (*Lactuca sativa* L.), oilseed rape (*Brassica napus* L. spp. *napus*), cabbage, white (*Brassica oleracea* L. var. *capitata* L. f. *alba*), soybean (*Glycine max* L.), tomato (*Solanum lycopersicum* L.).
Monocotyledonous: onion (*Allium cepa* L.), ryegrass (*Lolium multiflorum* L.), wheat (*Triticum aestivum* L.), corn (*Zea mays* L.).

Test design: Greenhouse study; limit test; 1 test item rate and 1 tap water control; 4 replicates per treatment; 1 or 2 pot/replicate, each pot with 5 or 10 seeds per pot (depending on species); test item applied pre-emergence shortly after sowing using a laboratory spray cabin at a mean water rate of 248 L/ha. Assessments for seedling emergence, plant survival, plant development (BBCH) and visual plant damage (phytotoxicity) were done 7, 14 and 21 days after 50% emergence of the untreated control (DAE). Shoot dry weight and single plant length were determined 21 DAE.

Endpoints: Phytotoxicity (NOER), plant emergence, plant weight and length (NOER, ER₅₀).

Test rates: Control: tap water; test rate 1.5 L BAS 758 00 F/ha.

Test conditions: Greenhouse conditions, daily average temperature: 22.6 °C – 27.1 °C; daily mean relative humidity: 50.9% - 75.7%; photoperiod: day length ≥ 16 h; additional light supply automatically for 16 hours in maximum when indoor illumination was less than 300 µmol.

Analytics: Analytical verification of the a.s. BAS 750 F present in application solutions prepared from the test item BAS 758 00 F was conducted according to BASF method L0361/01 using LC-MS/MS.

Statistics: Descriptive statistic; Student t-test (one-sided smaller, $\alpha = 0.05$) for metric data; Two-sample Fisher's Exact test (one-sided greater, $\alpha = 0.05$) for quantal data; NOER for visual plant phytotoxicity was estimated; Phytotoxicity values < 10% were considered as insignificant.

C. DESCRIPTION OF THE ANALYTICAL PROCEDURES

Concentrations of BAS 750 F (contained in BAS 758 00 F) in application solution was determined according to the analytical method L0361/01. The method is validated in a separate study (BASF-DocID: 2017/1065621). 1 mg/mL stock solution was prepared by dissolving approx. 7 and 140 mg BAS 758 00 F in a volumetric flask, dissolved in some 30 µL silicon antifoam emulsion in 1.0 L of tap water, and diluted with solvent (acetonitrile/water/formic acid of 20/80/0.1, v/v). A high total dilution factor was used to bring the concentration of the present application solutions in the linear range of the respective LC-MS/MS calibration curve(s). Aliquot of the final solution (total dilution factor: 200,000) was directly injected into the LC-MS/MS system for determination of BAS 750 F. The limit of quantification (LOQ) was 0.045 g/L. Whereas the limit of detection (LOD) was calculated to be 0.01 g/L. The analysis of the application solutions was performed within 90 ± 1 days after preparation of the samples. Thus, no additional storage stability testing is considered to be necessary. Due to the high total dilution factor (200,000), no relevant matrix effects are expected for the LC-MS/MS determination of BAS 750 F. Therefore, results were evaluated using calibration solutions in solvent. Details on measured fortification samples and obtained procedural recoveries for BAS 750 F are given in the table below.

Table A 107: Procedural recoveries for BAS 750 F (based on BAS 758 00 F)

Matrix	Fortification level (g BAS 750 F/L)	n	Recoveries (%)	Mean recoveries (%)	RSD (%)
Application solution	0.045	5	90, 92, 90, 92, 90	91	1.13
	0.865	5	92, 92, 92, 94, 91	92	1.19

Abbreviations: RSD = relative standard deviation

II. RESULTS AND DISCUSSION

After the application of BAS 758 00 F at pre-emergence, none of the tested plant species was affected concerning seedling emergence, plant survival and plant length. No influence of BAS 758 00 F on plant dry weight was observed for all tested species, except ryegrass, following the pre emergence application of 1.5 L BAS 758 00 F/ha. Ryegrass showed statistically significant biomass reduction with 9% at the tested rate of 1.5 L BAS 758 00 F/ha (Student t-test, one-sided smaller, $\alpha = 0.05$). The NOER for plant emergence, plant survival, plant length and plant biomass for all tested plant species is equal or higher than the tested rate of 1.5 L BAS 758 00 F/ha, except for plant biomass of ryegrass. The NOER for plant biomass reduction for ryegrass is < 1.5 L BAS 758 00 F/ha. The results are summarized in Table A 108 and

Table A 109.

Table A 108: Effect of BAS 758 00 F on seedling emergence, phytotoxicity, plant survival, plant length and plant dry weight 21 DAE

BAS 758 00 F [L/ha]	Carrot	Lettuce	Oilseed rape	Cabbage	Soy-bean	Tomato	Onion	Rye-grass	Wheat	Corn
Seedling emergence rate [%]										
Control	100	100	100	100	100	100	100	100	100	100
1.5	100	98	97	97	97	100	90	95	98	100
Visual Phytotoxicity [%]										
Control	0	0	0	0	0	0	0	0	0	0
1.5	0	0	0	0	0	0	0	0	0	0
Plant survival [%]										
Control	100	100	100	100	100	100	100	100	100	100
1.5	100	100	100	100	100	100	100	100	100	100
Plant length [% to control]										
Control	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
1.5	104.3	106.6	100.1	102.6	98.2	102.3	102.8	105.3	97.5	99.7
Plant dry weight [% to control]										
Control	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
1.5	105.7	104.1	98.6	105.4	101.0	99.9	91.7	91.2*	87.6	99.1

* Statistically significantly different to the untreated control (Student t-test, one-sided smaller, $\alpha = 0.05$).

Table A 109: NOER, ER₂₅, and ER₅₀ of BAS 758 00 F for non-target plants 21 DAE

	Carrot	Lettuce	Oilseed rape	Cabbage	Soy-bean	Tomato	Onion	Rye-grass	Wheat	Corn
Seedling emergence [L/ha]										
NOER	≥ 1.5	≥ 1.5	≥ 1.5	≥ 1.5	≥ 1.5	≥ 1.5	≥ 1.5	≥ 1.5	≥ 1.5	≥ 1.5
ER ₅₀	> 1.5	> 1.5	> 1.5	> 1.5	> 1.5	> 1.5	> 1.5	> 1.5	> 1.5	> 1.5
Phytotoxicity* [L/ha]										
NOER	≥ 1.5	≥ 1.5	≥ 1.5	≥ 1.5	≥ 1.5	≥ 1.5	≥ 1.5	≥ 1.5	≥ 1.5	≥ 1.5
Plant survival [L/ha]										
NOER	≥ 1.5	≥ 1.5	≥ 1.5	≥ 1.5	≥ 1.5	≥ 1.5	≥ 1.5	≥ 1.5	≥ 1.5	≥ 1.5
ER ₅₀	> 1.5	> 1.5	> 1.5	> 1.5	> 1.5	> 1.5	> 1.5	> 1.5	> 1.5	> 1.5
Plant length [L/ha]										
NOER	≥ 1.5	≥ 1.5	≥ 1.5	≥ 1.5	≥ 1.5	≥ 1.5	≥ 1.5	≥ 1.5	≥ 1.5	≥ 1.5
ER ₅₀	> 1.5	> 1.5	> 1.5	> 1.5	> 1.5	> 1.5	> 1.5	> 1.5	> 1.5	> 1.5
Plant dry weight [L/ha]										
NOER	≥ 1.5	≥ 1.5	≥ 1.5	≥ 1.5	≥ 1.5	≥ 1.5	≥ 1.5	< 1.5	≥ 1.5	≥ 1.5
ER ₅₀	> 1.5	> 1.5	> 1.5	> 1.5	> 1.5	> 1.5	> 1.5	> 1.5	> 1.5	> 1.5

* Estimated from assessment data.

Validity criteria:

Validity criteria according to OECD 208	Obtained in this study
Seedling emergence is at least 70% in the control	yes (93% to 100%)
Seedlings do not exhibit visible phytotoxic effects (e.g. chlorosis, necrosis, wilting, leaf and stem deformations) in the control and control plants exhibit only normal variation in growth and morphology for that particular species	yes (0%)
Mean survival of emerged control seedlings at least 90% for the duration of the study	yes (100%)
Environmental conditions for a particular species are identical and growing media contain the same amount of soil matrix, support media, or substrate from the same source	yes

All validity criteria were met.

III. CONCLUSION

Pre-emergence application of BAS 758 00 F conducted under worst-case greenhouse conditions did not result in relevant effects on seedling emergence, survival, phytotoxicity, plant length and dry weight of all tested plant species. The ER₅₀ based on seedling emergence, phytotoxicity, plant survival, plant dry weight and length was > 1.5 L BAS 758 00 F/ha for all tested plant species (the highest rate tested).

A 2.7.3 KCP 10.6.3 Extended laboratory studies on non-target plants

Further studies are not necessary.

A 2.7.4 KCP 10.6.4 Semi-field and field tests on non-target plants

Further studies are not necessary.

A 2.8 KCP 10.7 Effects on other terrestrial organisms (flora and fauna)

No studies conducted.

A 2.9 KCP 10.8 Monitoring data

No studies conducted.