FINAL REGISTRATION REPORT

Part B

Section 9

Ecotoxicology

Detailed summary of the risk assessment

Product code: BAS 768 00 F

Product name(s): Revytur

Chemical active substance(s):

Mefentrifluconazole, 25 g/L

Sulfur, 600 g/L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(authorization)

Applicant: BASF

Submission date: August 2023

MS Finalisation date: 11/12/2023

Version history

When	What
03/2023	Initial dRR – BASF DocID 2023/2009575
04/2023	Dossier sent for evaluation
08/2023	Update dRR – BASF DocID 2023/2036796
08/2023	zRMS evaluation of dRR
12/2023	Final version prepared by zRMS after Commenting period

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Evaluator comments:
The text highlighted in grey was provided by the evaluator.

9 Ecotoxicology (KCP 10)

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 safener/ 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 arthropods	Soil organisms	Non-target plants
Zonal uses (field or outdoor uses, certain types of protected crops)																				
1	DE, AT, IE, NL	wheat TRZAW, TRZAS TRZDU, TRZSP	F	*Zymoseptoria tritici* - SEPTTR *Blumeria graminis* - ERYSGR *Puccinia triticina* - PUCCRT *Puccinia striiformis* - PUCCST	Spraying (SP)	30 - 59	a) 2 b) 2	14	a) 4 b) 8	a) 0.100 [1)]/ 2.400 [2)] b) 0.200 [1)]/ 4.800 [2)]	100 - 300	F*								
2	IE	wheat TRZAW, TRZAS TRZDU, TRZSP	F	*P. tritici-repentis* - PYRNTR	Spraying (SP)	30 - 59	a) 2 b) 2	14	a) 4 b) 8	a) 0.100 [1)]/ 2.400 [2)] b) 0.200 [1)]/ 4.800 [2)]	100 - 300	F*								
3	PL	wheat TRZAW, TRZAS TRZDU, TRZSP	F	*Zymoseptoria tritici* - SEPTTR *Blumeria graminis* - ERYSGR *Puccinia triticina* - PUCCRT	Spraying (SP)	30 - 59	a) 2 b) 2	14	a) 4 b) 8	a) 0.100 [1)]/ 2.400 [2)] b) 0.200 [1)]/ 4.800 [2)]	100 - 300	F*								

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
4	DE, AT, IE, NL	barley HORVW, HORVS	F	*Ramularia collo-cygni* - RAMUCC *Pyrenophora teres* - PYRNTE *Puccinia hordei* – PUCCHD *Rhynchosporium secalis* - RHYNSE	Spraying (SP)	30 - 59	a) 2 b) 2	14	a) 4 b) 8	a) 0.100 [1)]/ 2.400 [2)] b) 0.200 [1)]/ 4.800 [2)]	100 - 300	F*								
5	DE, AT, IE, NL	triticale TTLWI	F	*Septoria species* - SEPTSP *Blumeria graminis* - ERYSGR *Puccinia triticina* - PUCCRT *Puccinia striiformis* - PUCCST	Spraying (SP)	30 - 59	a) 2 b) 2	14	a) 4 b) 8	a) 0.100 [1)]/ 2.400 [2)] b) 0.200 [1)]/ 4.800 [2)]	100 - 300	F*								
6	PL	triticale TTLWI	F	*Septoria species* - SEPTSP *Blumeria graminis* - ERYSGR *Puccinia triticina* - PUCCRT	Spraying (SP)	30 - 59	a) 2 b) 2	14	a) 4 b) 8	a) 0.100 [1)]/ 2.400 [2)] b) 0.200 [1)]/ 4.800 [2)]	100 - 300	F*								
7	CZ	wheat TRZAW, TRZAS TRZDU, TRZSP	F	*Zymoseptoria tritici* - SEPTTR *Blumeria graminis* - ERYSGR *Puccinia triticina* - PUCCRT *Puccinia striiformis* - PUCCST	Spraying (SP)	30 - 59	a) 1 b) 1		a) 3 - 4 b) 3 - 4	a) 0.100 [1)]/ 2.400 [2)] b) 0.100 [1)]/ 2.400 [2)]	100 - 300	F*								
8	CZ	barley HORVW, HORVS	F	*Ramularia collo-cygni* - RAMUCC *Pyrenophora teres* - PYRNTE *Puccinia hordei* – PUCCHD *Rhynchosporium secalis* - RHYNSE	Spraying (SP)	30 - 59	a) 1 b) 1		a) 3 - 4 b) 3 - 4	a) 0.100 [1)]/ 2.400 [2)] b) 0.100 [1)]/ 2.400 [2)]	100 - 300	F*								

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	F	*Septoria species* - SEPTSP *Blumeria graminis* - ERYSGR *Puccinia triticina* - PUCCRT *Puccinia striiformis* - PUCCST	Spraying (SP)	30 - 59	a) 1 b) 1		a) 3 - 4 b) 3 - 4	a) 0.100 [1)]/ 2.400 [2)] b) 0.100 [1)]/ 2.400 [2)]	100 - 300	F*								

* 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)] Sulfur

F* = Defined by latest application timinig. Fixed by professional use.

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

Comments of zRMS:	Taking into consideration that in August 2023 the new LoEP for sulphur is available, the new endpoints were used in risk assessment, if relevant. If the endpoint is the same as in EFSA, 2008, no changes are provided by the evaluator. If the new one was agreed , the relevant notes are added.

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

Based on the substance-specific characteristics of sulfur no long-term studies are required and the reproductive risk from sulfur to birds is regarded as very low.

Exposure to combined active substances

In the tier 1 acute risk assessment for combined toxicity of the active substances (virtual compound approach) all TER_A values are above the trigger of 10 for acceptability of effects.

Based on the substance-specific characteristics of sulfur no long-term studies are required and the reproductive risk from sulfur to birds is regarded as very low. The reproductive risk from the formulation BAS 768 00 F can therefore be predicted based on the reproductive risk assessment for mefentrifluconazole.

Therefore, the acute and reproductive dietary risks to birds from BAS 768 00 F according to the proposed use pattern are acceptable.

Drinking water risk assessment

Following EFSA/2009/1438, the puddle scenario is considered relevant for applications of BAS 768 00 F according to the proposed use pattern. Since the ratios of the effective application rate to the relevant toxicity endpoints are below the value of 3000 for both mefentrifluconazole and sulfur, a quantitative risk assessment for the proposed use pattern of BAS 768 00 F is not necessary.

Secondary poisoning and biomagnification

The log P_{ow} of the active substance mefentrifluconazole is 3.4, which triggers an assessment of the potential risk from secondary poisoning. According to the tier 1 risk assessment for earthworm- and fish-eating birds, the TER values for mefentrifluconazole are above the trigger value of 5, indicating an acceptable risk for

the intended use of BAS 768 00 F. Based on the substance-specific characteristics of sulfur, a risk assessment for effects due to secondary poisoning is not required.

Low potential for accumulation of both mefentrifluconazole and sulfur 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 768 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 both mefentrifluconazole and sulfur and all TER_{LT} values for mefentrifluconazole exceed the triggers set by Commission regulation (EU) 546/2011 for acceptability of effects.

Based on the substance-specific characteristics of sulfur no long-term studies are required and the reproductive risk from sulfur to mammals is regarded as very low.

Exposure to combined active substances

In the acute screening step risk assessment for combined toxicity of the active substances (virtual compound approach) the TER_A value is above the trigger of 10 for acceptability of effects.

Based on the substance-specific characteristics of sulfur no long-term studies are required and the reproductive risk from sulfur to mammals is regarded as very low. The reproductive risk from the formulation BAS 768 00 F can therefore be predicted based on the reproductive risk assessment for mefentrifluconazole.

Therefore, the acute and reproductive dietary risks to mammals from BAS 768 00 F according to the proposed use pattern are 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 toxicity endpoints are below the value of 3000 for both mefentrifluconazole and sulfur, a quantitative risk assessment for the proposed use pattern of BAS 768 00 F is not necessary.

Secondary poisoning and biomagnification

The log P_{ow} of the active substance mefentrifluconazole is 3.4, which triggers an assessment of the potential risk from secondary poisoning. According to the tier 1 risk assessment for earthworm- and fish-eating mammals, the TER values for mefentrifluconazole are above the trigger value of 5, indicating an acceptable risk for the intended use of BAS 768 00 F. Based on the substance-specific characteristics of sulfur, a risk assessment for effects due to secondary poisoning is not required.

Low potential for accumulation of both mefentrifluconazole and sulfur 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 768 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.

In the absence of toxicity data on mefentrifluconazole and sulfur, the active substances in the formulation BAS 768 00 F, and considering the lack of guidance for risk assessment, it is assumed that the risk assessments for birds and mammals are protective for terrestrial life-stages of amphibians and reptiles, an approach that is also used by US-EPA (2004).

Reference

US-EPA 2004. Overview of the ecological risk assessment process in the Office of Pesticide Programs, U.S. Environmental Protection Agency. Endangered and Threatened Species Effects Determinations. Office of Prevention, Pesticides and Toxic Substances; Office of Pesticide Programs, Washington, D.C. 92 pp.

9.1.1.2 Effects on aquatic organisms (KCP 10.2)

The standard risk assessment for the active substances mefentrifluconazole and sulfur indicates an acceptable risk for all groups of aquatic organisms following the intended uses of BAS 768 00 F 'in spring and winter cereals' with no need for additional mitigation measures.

The PEC/RAC ratios for the relevant metabolites of mefentrifluconazole 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.

The PEC/RAC ratios for the relevant metabolite of sulfur are significantly below the trigger of 1 based on a conservative 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 768 00 F does not indicate a significantly higher (or unexpected) toxicity than predicted based on the results of the active substances. The formulation risk assessment revealed an acceptable risk to aquatic organisms following the intended uses of BAS 768 00 F in 'spring and winter cereals' with no need for additional mitigation measures.

The standard risk assessment for the fungicidal product BAS 768 00 F, the active substances mefentrifluconazole and sulfur and the relevant metabolites demonstrates that the application of BAS 768 00 F in 'spring and winter cereals' according to good agricultural practice is of low risk to aquatic ecosystems with no need for any mitigation measures.

9.1.1.3 Effects on bees (KCP 10.3.1)

The risk to honey bees from the use of mefentrifluconazole, sulfur and BAS 768 00 F was assessed using the maximum single application rate and the LD_{50} 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 768 00 F and the active substances mefentrifluconazole and sulfur for acute oral and acute contact exposure of honey bees are considerably below the Commission Regulation (EU) 546/2011 trigger value of 50. Additionally, all calculated TERs exceed the suggested trigger, except for the honey bee larvae. The remaining risk for honey bee larvae can be attributed to sulfur and was addressed with a semi-field study with sulfur showing no negative effects on bee larvae and brood / overall colony development. The results of the higher tier study indicate that low risk to honey bee colonies is expected after application of BAS 768 00 F.

The hazard quotients for BAS 768 00 F and the active substances mefentrifluconazole and sulfur 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 768 00 F according to the proposed uses. This is confirmed by a risk assessment following EPPO (2010) and supported by the results of a semi-field study with sulfur showing no negative effects on bee larvae and brood / overall colony development.

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 768 00 F is based on Tier I tests with the standard test species *Typhlodromus pyri and Aphidius rhopalosiphi* and Tier II test on *A. rhopalosiphi*. 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 768 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 768 00 F, mefentrifluconazole, sulfur and the relevant metabolite 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 768 00 F, mefentrifluconazole, sulfur and the relevant metabolite 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 768 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 768 00 F, mefentrifluconazole, sulfur and the relevant metabolite to soil micro-organisms was assessed by comparing the maximum PEC_{soil} values with the maximum concentration with effects ≤ 25%.

For the formulation BAS 768 00 F, the active substances mefentrifluconazole and sulfur as well as their relevant metabolite, 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 768 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 768 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 as 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, 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 768 00 F is used to calculate the maximum off-field predicted environmental rate ($PER_{off\text{-}field}$). The potential risk of BAS 768 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 768 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 768 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).

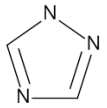
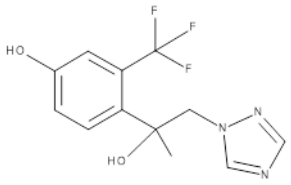
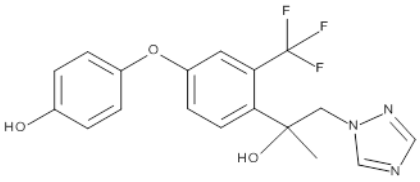
Table 9.1-2: Critical use pattern of BAS 768 00 F grouped according to applications

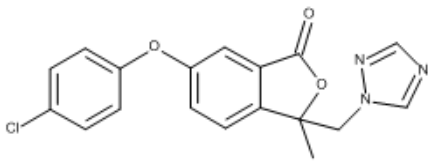
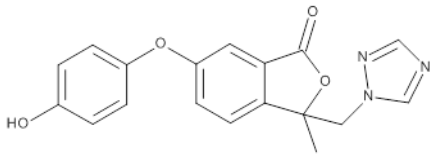
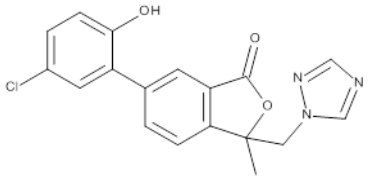
Grouping according to worst-case application				
Area	**Group**	**Intended uses**	**Relevant use parameters for grouping**	**Relevant parameter or value for sorting**
Birds and mammals	Application rate and number of applications	All intended uses	EFSA crop group: Cereals	Maximum worst-case application rate = 2 x 4.0 L/ha (corresponding to 2 x 0.1 kg mefentrifluconazole/ha and 2 x 2.4 kg sulfur/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 2 x 4.0 L/ha (corresponding to 2 x 0.100 kg mefentrifluconazole and 2 x 2.4 kg sulfur/ha), which covers all other intended uses.	Maximum application rate = 4.0 L/ha
Non-target arthropods	Application rate	All intended uses	Risk assessments are based on the maximum application rate of 2 x 4.0 L/ha (corresponding to 2 x 0.100 kg mefentrifluconazole and 2 x 2.4 kg sulfur/ha), which covers all other intended uses	Maximum application rate = 2 x 4.0 L/ha
Soil macro- and micro-organisms	Worst case PEC_{soil} value	All intended uses	Risk assessments are based on the worst case PEC_{soil} value which covers all other intended uses	Worst case PEC_{soil} derived from Section 8, chapter 8.7

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 768 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

9.2 Effects on birds (KCP 10.1.1)

zRMS Comments:	The risk assessment was performed in accordance with the B & M Guidance, EFSA (2009). All relevant used endpoints were agreed at the EU level. **Mefentrifluconazole**. The used endpoints were agreed at the EU level (EFSA conclusion, 2018). The acute and long-term risk were submitted at screening step. The submitted acute and long-term risk to birds was accepted. The TER_A and TER_{LT} values are ~~below~~ above the trigger values of 10 and 5, respectively, indicating an acceptable risk for birds. **Sulphur**. The new endpoint for acute risk assessment was used (EFSA conclusion, 2023). In relevant tables, in references the corrected source of data should be pointed EFSA Journal 2023;21(3):7385. The TER_A and TER_{LT} values are ~~below~~ above the trigger values of 10 and 5, respectively, indicating an acceptable risk for birds. For both active substances and their relevant metabolites, a risk assessment for fish-eating and earthworm-eating birds was performed and the TER_{LT} values are above the trigger value of 5. The risks due to bioaccumulation of both active substances via the food chain for birds is acceptable. **Mixture toxicity**. The combined acute toxicity was submitted. The surrogate LD_{50} and for the mixture of active substances was assessed and accepted. For combined reproductive toxicity the justification was submitted and accepted. The risk to birds following application of formulation BAS 768 00 F in accordance with proposed uses is acceptable.

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 and sulfur. Full details of the studies with mefentrifluconazole are provided in the respective EU Draft Assessment Report (DAR) and related documents.

Data on acute oral and short-term dietary toxicity of sulfur to birds are based on the first EU review of sulfur. The resulting endpoints were cited in the EFSA Scientific Report (2008) 221, 1-70 and in the List of Endpoints (LoE) in the Addendum to the DAR for Confirmatory Data (April 2012). The acute oral and short-term dietary toxicity studies with birds were evaluated in the recent renewal process of sulfur and are still considered relevant. Following the previous and current conclusions from the EU evaluation of sulfur reproductive toxicity studies with birds are not required (see EFSA Scientific Report 2008 and revised RAR by RMS France from June 2022).

Active substances

An overview of the EU agreed endpoints is given in Table 9.2-1 (mefentrifluconazole) and Table 9.2-2 (sulfur).

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

Species	Substance	Exposure System	Results	Reference [BASF DocID]
Colinus virginianus	Mefentrifluconazole	Oral, 1 d Acute	LD_{50} = 816 mg/kg bw	EFSA Journal 2018;16(7):5379 [2014/1095701]
Anas platyrhynchos	Mefentrifluconazole	Oral, 1 d Acute	LD_{50} > 2000 mg/kg bw	EFSA Journal 2018;16(7):5379 [2014/1095700]
Serinus canaria	Mefentrifluconazole	Oral, 1 d Acute	LD_{50} > 2860 mg/kg bw	EFSA Journal 2018;16(7):5379 [2015/1085493]
Colinus virginianus	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]
Anas platyrhynchos	Mefentrifluconazole	Dietary, 8d Short-term	LC_{50} = 8347 mg/kg diet LDD_{50} = 1213 mg/kg bw/d	DAR (2017) [2014/1117035]
Colinus virginianus	Mefentrifluconazole	Dietary Reproductive toxicity	NOEL = 25.3 mg/kg bw/d	EFSA Journal 2018;16(7):5379 [2013/1281276]
Anas platyrhynchos	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**	**LD_{50} = 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: Sulfur (BAS 175 F): Endpoints relevant for the risk assessment for birds

Species	Substance	Exposure System	Results	Reference [Study no.]
Coturnix coturnix japonica	Sulphur Dust [1)]	Oral 1 d Acute	LD_{50} > 2000 mg /kg bw, eq. LD_{50} > 1970 mg a.s./kg bw	EFSA Scientific Report (2008) 221, 1-70 [Study no. 4263/05]
Coturnix coturnix japonica	Sulphur Dust [1)]	Oral 1 d Acute	LD_{50} > 3500 mg/kg bw, eq. LD_{50} >3451 mg a.s./kg bw	Addendum to DAR for Confirmatory Data (2012) [Study no. 7715]
			LD_{50} (extrapolated) = 5570 mg a.s./kg bw	
Coturnix coturnix japonica	Sulphur Dust [1)]	Dietary 8 d Short-term	LDD_{50} > 3633.5 mg/kg bw/d, eq. > 3579 mg a.s./kg bw/d	Addendum to DAR for Confirmatory Data (2012) [Study no. 7714]
Colinus virginianus	Sulfur	Dietary 8 d Short-term	LDD_{50} >5339 mg a.s./kg feed, eq. > 1334.75 mg a.s./kg bw/d	EFSA Scientific Report (2008) 221, 1-70 Supplemental endpoint
Endpoint used for acute assessment	**Sulfur Dust**	**Oral, 1 d Acute**	**Tier 1: LD_{50} > 3451 mg a.s./kg bw LD_{50} (extrapolated) = 5570 mg a.s./kg bw**	**Addendum to DAR for Confirmatory Data and Sulphur_RAR_45_LoEP_2022-06 [Study no. 7715]**

[1)] This study has been conducted with 'Sulphur Dust' as a surrogate for technical sulfur since the minimum content of the a.s. is specified as 985 g/kg and the only co-formulant is an inert carrier.

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 sulfur

During the first peer-review of sulfur, the expert meeting concluded that the most relevant residue on treated crops was elemental sulphur. As sulfur is a mineral essential in the metabolism of birds and mammals the consideration of sulfur as metabolites is not applicable (***EFSA Scientific Report* (2008) 221, 1-70**). This conclusion was confirmed in the RAR by RMS France (Status July 2022). Transformation products of sulfur in soil and water are mainly sulfates, which are part of the sulfur cycle with no potential for bioaccumulation in birds or mammals and thus no risk of secondary poisoning via consumption of earthworms or fish.

Therefore, the only relevant substance birds and mammals may be exposed on potential food items is the parent compound, which will be covered in the risk assessment.

Formulation toxicity

The acute oral toxicity of the formulation BAS 768 00 F to mammals can be predicted based on toxicity

data from the active ingredients and co-formulants available for mammals. The availability of acute oral toxicity data of BAS 768 00 F individual components is provided in chapter 6, Appendix 2.

None of the active substances or co-formulants are classified for acute oral toxicity in mammals. Hence, on the basis of the formulation composition, it can be concluded that the acute oral toxicity of BAS 768 00 F is low and the predicted rat acute oral LD_{50} is > 2000 mg/kg bw/d (see chapter 6, Appendix 2, A 2.2). As no increased toxicity is expected for the formulation for mammals no acute bird formulation study for the formulation BAS 768 00 F is required. The acute risk from the formulation is covered by the acute risk assessment for the active substances mefentrifluconazole and sulfur.

9.2.1.1 Justification for new endpoints

Mefentrifluconazole

Acute – Not applicable. Endpoint is EU agreed.

Reproductive – Not applicable. Endpoint is EU agreed.

Sulfur

Acute - Not applicable. Endpoints are EU agreed.

Reproductive – In the last EU review no long-term toxicity of sulfur for terrestrial vertebrates was expected mainly based on the lack of acute, short-term and subchronic toxicity in birds and mammals, its natural occurrence in all three environmental compartments (air, soil, water), the relevance as essential element in the metabolism of vertebrates and the long-term use of sulfur related products in agriculture. Experts agreed in principle that long-term studies can be waived (EFSA Scientific Report (2008) 221, 1-70), which is a conclusion supported by further arguments provided during the confirmatory data process. This argumentation along with the current evaluation by RMS France in the revised RAR (June 2022) from the renewal process further supports the conclusion that no long-term studies are required and any long-term risk for birds is considered low and acceptable.

9.2.2 Risk assessment for spray applications

Proposed use pattern for the risk assessments

The proposed use pattern for the use of BAS 768 00 F is summarized in Table 9.2-3. The detailed use pattern table is presented at the beginning of the ecotoxicology chapter (section 9.1).

Table 9.2-3: Proposed use pattern

Crop	Crop group according to EFSA/2009/1438	Application time (BBCH growth stage)	Max-number of applications	Min. interval between applications [d]	Max. application rate per application		
					Mefentrifluconazole [kg/ha]	Sulfur [kg/ha]	BAS 768 00 F [L/ha]
Wheat, barley, triticale	Cereals	30-59	2	14	0.1	2.4	4.0

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 TER_A values are presented in Table 9.2-4 for mefentrifluconazole and in Table 9.2-5 for sulfur. All the TER_A values at the screening step (mefentrifluconazole) or tier 1 (sulfur) are above the relevant trigger of 10 for acceptability of acute effects.

Table 9.2-4: Mefentrifluconazole: Screening step calculation of the acute risk for birds due to the use of BAS 768 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 9.2-5: Sulfur: Screening step and tier 1 calculations of the acute risk for birds due to the use of BAS 768 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	2.4	2	14	10.0	>3451.0	
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
	Small omnivorous bird	158.8	381.12	1.2	457.34	**> 7.5**	
First Tier Risk Assessment:							
Calculate TER for each generic focal species	**Crop**	**Generic focal species**			**Short cut 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			12.0	> 99.9	
	Cereals BBCH ≥ 40	Small omnivorous bird "lark" Combination (invertebrates with interception) 25% crop leaves 25% weed seeds 50% ground arthropods			7.2	> 166.4	

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

Reproductive risk assessment

The TER_{LT} value for the screening step as presented in Table 9.2-6 for mefentrifluconazole is above the relevant trigger of 5 for acceptability of reproductive effects.

Please note, as outlined above, that no long-term toxicity of sulfur in birds is expected because sulfur is naturally present in all compartments, an essential element in the animal metabolism and has lately been assessed in the EU review as safe for terrestrial vertebrates. Therefore, long-term exposure of birds to sulfur is not expected to present any unacceptable risk (see chapter 9.2.1).

Table 9.2-6: Mefentrifluconazole: Screening step calculation of the long-term/reproductive risk for birds due to the use of BAS 768 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 bw/d)**	**Time weighted average (TWA)**
	Cereals	0.1	2	14	10.0	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	

The conclusions for the first-tier dietary risk assessments for birds for each of the active substances are as follows: acceptable acute risks for birds were shown at the screening level and/or tier 1 for both mefentrifluconazole and sulfur, and an acceptable reproductive risk for birds was shown at the screening level for mefentrifluconazole. Based on the substance-specific characteristics of sulfur no long-term studies are required and therefore no long-term risk needs to be assessed for wild birds. 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 768 00 F because this formulation contains more than one active substance.

Following Appendix B (step 1) in EFSA/2009/1438 a surrogate LD_{50} = 3056.2 mg/kg bw is calculated based on the assumption of dose additivity (Table 9.2-7). 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 768 00 F this does not apply because the deviation for both active substances is more than 10% (Table 9.2-7).

Table 9.2-7: Calculation of surrogate LD_{50} 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	25	0.04	816	0.00005	**3056.2**	20400.0	567
Sulfur	600	0.96	> 3451	0.00028		3594.8	18

Since there is no experimental data on acute toxicity of the formulation BAS 768 00 H to birds (see justification in chapter 9.2.1), the surrogate LD_{50} = 3056.2 mg/kg bw will be used for the acute risk assessment below.

Exposure and acute risk assessment for the combined active substances (<u>virtual compound approach</u>)

The potential exposure to the combined substances follows step 4 of Appendix B of EFSA/2009/1438. The maximum application rate of formulation BAS 768 00 F is 4.0 L product/ha (corresponding to 0.1 kg/ha mefentrifluconazole and 2.4 kg/ha sulfur for the use in cereals); applying the concept for dose additivity to the exposure calculations results in a combined application rate of 2.5 kg virtual compound/ha.

The dietary TER acute values for the tier 1 risk assessment presented in Table 9.2-8 are above the trigger of 10. Therefore, the acute risk to birds from combined effects of the two active substances in BAS 768 00 F is acceptable.

Table 9.2-8: Screening step and tier 1 calculations of the acute risk for birds due to the use of BAS 768 00 F in the crop group "cereals" – 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	2.5	2	14	10.0	3056.2	
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	397.00	1.2	476.40	**6.4**	
First Tier Risk Assessment:							
Calculate TER for each generic focal species	**Crop**	**Generic focal species**		**Short cut 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		12.0	84.9		
	Cereals BBCH ≥ 40	Small omnivorous bird "lark" Combination (invertebrates with interception) 25% crop leaves 25% weed seeds 50% ground arthropods		7.2	141.5		

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

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 $TER_{A\ combi}$ = trigger/((trigger/$TER_{A\ substance\ 1}$)+(trigger/$TER_{A\ substance\ 2}$)).

Combined reproductive toxicity

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 requested.

Based on the substance-specific characteristics of sulfur no long-term toxicity for birds is expected. The reproductive risk for birds exposed to sulfur is considered low and a chronic combination toxicity assessment of sulfur with mefentrifluconzaole is therefore not required. The overall reproductive risk of BAS 768 00 F can reliably be based on the reproductive risk assessment for mefentrifluconazole. The TER_{LT} value for the screening step scenario of the reproductive risk assessment (see **Table 9.2-6**) is above the trigger, showing a high margin of safety. Thus, it can be concluded that the reproductive risk for birds from the combined exposure to the two active substances after application of BAS 768 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 tier 1 risk assessments for all scenarios.

9.2.2.3 Drinking water exposure

Leaf scenario

Since BAS 768 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} ≥ 500 L/kg).

The ratio calculations for effective application rate to relevant endpoint are detailed in Table 9.2-9 (mefentrifluconazole) and Table 9.2-10 (sulfur). The ratios for acute and reproductive endpoints for mefentrifluconazole (0.24 and 7.7, respectively) and for the acute endpoint for sulfur (< 1.4) do not exceed the threshold value of 3000, thus no specific calculations of exposure for birds through drinking water for the puddle scenario are necessary. Therefore, a quantitative drinking water risk assessment for the puddle scenario is not triggered.

Table 9.2-9: Assessment of the risk for birds due to exposure to mefentrifluconazole via contaminated drinking water in puddles

Parameter	Mefentrifluconazole	Reference
K_{oc} (geometric mean) [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	--
LD_{50} [mg/kg bw]	816	Chapter 9.2.1
Ratio (acute) [3)]	0.24	--
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} = Application rate (g/ha) x MAF_m
3) Ratio of AR_{eff} and relevant toxicity endpoint

Table 9.2-10: Assessment of the risk for birds due to exposure to sulfur via contaminated drinking water in puddles

Parameter	Sulfur	Reference
K_{oc} [L/kg]	3615.3	Document Sulphur_RAR_45_LoEP_2022-06
DT_{50} (soil) [days] [4)]	n.a.	Chapter 8.9; Document Sulphur_RAR_27_Volume_3CP_SULPHUR DUST_B-9_2022-06
Number of applications	n.a.	--
Interval [days]		--
MAF_m [1)]		--
Max use rate [g/ha]	2400	--
AR_{eff} [g/ha] [2)]	4800	--
LD_{50} [mg/kg bw]	>3451	Chapter 9.2.1
Ratio (acute) [3)]	<1.4	--
Trigger	3000	--
Drinking water assessment required [Yes/No]	No	--

n.a. = not applicable
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) For sulfur a soil DT_{50} is not available (see p. 43 in the document 'Sulphur_RAR_27_Volume_3CP_SULPHUR DUST_B-9_2022-06'), therefore as a worst-case approach the maximum yearly application rate for Sulphur Dust (2x 2.4 kg/ha/year) is assumed to be the maximum effective application rate. Please note that this approach is in line with that evaluated by the RMS in the document 'Sulphur_RAR_27_Volume_3CP_SULPHUR DUST_B-9_2022-06'.
3) Ratio of AR_{eff} and relevant toxicity endpoint
4) According to EFSA/2009/1438 the DT_{50} soil as used in PEC_{sw} calculations has to be selected. However, due to the low water solubility of sulfur no PEC_{sw} was calculated. For details see chapter 8.9.

In conclusion, the risk to birds via drinking water from the intended use of BAS 768 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 exceeds the trigger value of 3. A risk assessment for effects due to secondary poisoning is required.

In the LoEP of the RAR from June 2022 (Sulphur_RAR_45_LoEP_2022-06), RMS France concluded that "*the potential of sulfur bioaccumulation is considered to be negligible as sulfur is a naturally occurring*

mineral and an essential element in the metabolism of all living organisms. Furthermore, sulfur is of low toxicity to birds and mammals." Therefore, a risk assessment for effects due to secondary poisoning is not 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 9.2-11, the TER_{LT} for mefentrifluconazole exceeds the relevant trigger of 5 for acceptability of effects, indicating an acceptable risk to earthworm-eating birds via secondary poisoning.

Table 9.2-11: Assessment of the risk for earthworm-eating birds due to exposure to mefentrifluconazole via bioaccumulation in earthworms (secondary poisoning) for the intended use

Parameter	Mefentrifluconazole	Reference
PEC_{soil} (accu) [mg/kg soil] [1)]	0.092	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)]	0.039	--
Daily dose [mg/kg bw/d] [4)]	0.041	--
NO(A)EL [mg/kg bw/d]	25.3	Chapter 9.2.1
TER_{LT} [5)]	623.3	--

1) PEC_{soil} (accu) value calculated for an application scenario of 2 x 100 g a.s./ha in cereals at a plateau soil depth of 20 cm. For details see chapter 8.7.
2) Bioconcentration factor (BCF) = $(0.84 + 0.012 \times K_{ow}) / (f_{oc} \times K_{oc})$
3) $PEC_{worm} = PEC_{soil}$ (accu) 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 9.2-12 the TER_{LT} for mefentrifluconazole exceeds the relevant trigger of 5 for acceptability of effects, indicating an acceptable risk to fish-eating birds via secondary poisoning.

Table 9.2-12: 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 d) [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) Worst-case PEC_{sw} (twa, 21 d) value calculated for a multiple application scenario of 2 x 100 g a.s./ha to cereals from FOCUS Step 2 (Northern Europe scenario). For details see chapter 8.9.
2) PEC_{fish} = PEC_{sw} (twa, 21d) 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 (EFSA Journal 2018; 16(7): 5379).

The potential for bioaccumulation of sulfur is negligible as sulfur is a naturally occurring element in all three environmental compartments (air, soil, water) and an essential mineral in the metabolism of terrestrial vertebrates. Furthermore, sulfur is of low toxicity to birds (RMS in LoEP of the RAR from June 2022).

Since the bioaccumulation potential of both mefentrifluconazole and sulfur is low no further assessment on biomagnification in the food chain 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 768 00 F according to good agricultural practice is acceptable.

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

zRMS Comments:	The risk assessment was performed in accordance with the B & M Guidance, EFSA (2009). All relevant used endpoints were agreed at the EU level. **Mefentrifluconazole**. The used endpoints were agreed at the EU level (EFSA conclusion, 2018). The acute and long-term risk for mammals were submitted and accepted at screening step and first tier, respectively. The TER_A and TER_{LT} values are below the trigger values of 10 and 5, respectively, indicating an acceptable risk for mammals. **Sulphur**. The new endpoint for acute risk assessment was used (EFSA conclusion, 2023). In relevant tables, in references the corrected source of data should be pointed EFSA Journal 2023;21(3):7385. The TER_A and TER_{LT} values are below the trigger values of 10 and 5, respectively, indicating an acceptable risk for mammals. For both active substances, a risk assessment for fish-eating and earthworm-eating mammals was performed and the TER_{LT} values are above the trigger value of 5. The risks due to bioaccumulation of both active substances via the food chain for birds is acceptable. **Mixture toxicity**. The combined acute toxicity was submitted. The surrogate LD_{50} and for the mixture of active substances was assessed and accepted. For combined reproductive toxicity the justification was submitted and accepted. The risk to mammals following application of formulation BAS 768 00 F in accordance with proposed uses is acceptable.

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 and sulfur. Full details of the studies with mefentrifluconazole are provided in the respective EU Draft Assessment Report (DAR) and related documents.

The endpoints of the studies with sulfur for the risk assessment are based on the previous EU review process (EFSA Scientific Report (2008)). The key study to address the acute toxicity was included in the Addendum to the DAR for Confirmatory Data (April 2012). In case additional data or evaluations were taken up by the RMS in the recent EU review process of sulfur, it is considered below.

Active substances

An overview of the EU agreed endpoints is given in Table 9.2-13 (mefentrifluconazole) and Table 9.2-14 (sulfur).

Table 9.2-13: 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 9.2-14: Sulfur (BAS 175 F): Endpoints relevant for the risk assessment for mammals

Species	Substance	Exposure System	Results	Reference BASF DocID
Rat	Sulfur	Oral 1 d Acute	LD_{50} > 1760 mg a.s./kg bw	EFSA Scientific Report (2008) 221, 1-70
Rat	Sulfur	Oral 1 d Acute	LD_{50} > 5000 mg a.s./kg bw	EFSA Scientific Report (2008) 221, 1-70
Rat	Sulphur Dust [1)]	Oral 1 d Acute	LD_{50} > 2000 mg/kg bw, eq. LD_{50} > 1970 mg a.s./kg bw [3)]	EFSA Scientific Report (2008) 221, 1-70 [Study no. 4257/05]
Rat	Sulphur Dust [1)]	Oral 1 d Acute	LD_{50} > 35000 mg/kg bw, eq. LD_{50} > 34475 mg a.s./kg bw [2)]	Addendum to DAR for Confirmatory Data (2012) [Study no. 8390]
Rat	Sulfur	Short-term	NOEL (90 d) ≥ 1000 mg a.s./kg bw/d	EFSA Scientific Report (2008) 221, 1-70 [Study no. 4191/05]
Rat	Sulphur Dust [1)]	Short-term	NOEL (28 d) ≥ 1000 mg/kg bw/d, eq. 985 mg a.s./kg bw/d	EFSA Scientific Report (2008) 221, 1-70 [Study no. 4264/05]
Endpoint used for acute assessment	**Sulphur Dust**	**Oral 1 d Acute**	**LD_{50} > 34475 mg a.s./kg bw [2)]**	Addendum to DAR for Confirmatory Data (2012) [Study no. 8390]

1) This study has been conducted with 'Sulphur Dust' as a surrogate for technical sulfur since the minimum content of the a.s. is specified as 985 g/kg and the only co-formulant is an inert carrier.
2) Endpoints relevant for the risk assessment according to the document 'Sulphur_RAR_45_LoEP_2022-06', chapter "Effects on birds and other terrestrial vertebrates".
3) Please note, in EFSA Conclusion only the endpoint based on the Sulfur Dust formulation is stated. In addition, the endpoint was re-calculated for the active substance sulfur.

Metabolites

Metabolites of mefentrifluconazole

See section 9.2.1 in the bird chapter.

Metabolites of sulfur

See section 9.2.1 in the bird chapter.

Formulation toxicity

The acute oral toxicity of the formulation BAS 768 00 F to mammals can be predicted based on toxicity data from the active ingredients and co-formulants available for mammals. The availability of acute oral toxicity data of BAS 768 00 F individual components is provided in chapter 6, Appendix 2.

None of the active substances or co-formulants are classified for acute oral toxicity in mammals. Hence, on the basis of the formulation composition, it can be concluded that the acute oral toxicity of BAS 768 00 F is low and the predicted rat acute oral LD_{50} is > 2000 mg/kg bw/d (see chapter 6, Appendix 2, A 2.2). As no increased toxicity is expected for the formulation the acute risk assessment for mammals can reliably be predicted on the basis of the acute endpoints for the active ingredients.

9.3.1.1 Justification for new endpoints

Mefentrifluconazole

Acute – Not applicable. Endpoint is EU agreed.

Reproductive – Not applicable. Endpoint is EU agreed.

Sulfur

Acute - Not applicable. Endpoints are EU agreed.

Reproductive – In the last EU review no long-term toxicity of sulfur for terrestrial vertebrates was expected mainly based on the lack of acute, short-term and subchronic toxicity in birds and mammals, its natural occurrence in all three environmental compartments (air, soil, water), the relevance as essential element in the metabolism of vertebrates and the long-term use of sulfur related products in agriculture. Experts agreed in principle that long-term studies can be waived (EFSA Scientific Report (2008) 221, 1-70), which is a conclusion supported by further arguments provided during the confirmatory data process. This argumentation along with the current evaluation by RMS France in the revised RAR (June 2022) from the renewal process further supports the conclusion that no long-term studies are required and any long-term exposure for wild mammals is considered acceptable.

9.3.2 Risk assessment for spray applications

Proposed use pattern for the risk assessments

The proposed use pattern for the use of BAS 768 00 F is summarized in Table 9.2-15. The detailed use pattern table is presented at the beginning of the ecotoxicology chapter (section 9.1).

Table 9.2-15: Proposed use pattern

Crop	Crop group according to EFSA/2009/1438	Application time (BBCH growth stage)	Max. number of applications	Min. interval between applications [d]	Max. application rate per application		
					Mefentrifluconazole [kg/ha]	Sulfur [kg/ha]	BAS 768 00 F [L/ha]
Wheat, barley, triticale, rye	Cereals	30-59	2	14	0.1	2.4	4.0

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 TER_A values are presented in Table 9.2-16 for mefentrifluconazole and in

Table **9.2-17** for sulfur. All the TER_A values at the screening step are above the relevant trigger of 10 for acceptability of acute effects.

Table 9.2-16: Mefentrifluconazole: Screening step calculation of the acute risk for mammals due to the use of BAS 768 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	14.21	>140.8	

Table 9.2-17: Sulfur: Screening step calculation of the acute risk for mammals due to the use of BAS 768 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	2.4	2	14	10.0	>34475.0	
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	284.16	1.2	340.99	>101.1	

Reproductive risk assessment

The TER_{LT} values for the tier 1 risk assessment as presented in Table 9.2-18 for mefentrifluconazole are above the relevant trigger of 5 for acceptability of reproductive effects.

Please note, as outlined above, that no long-term toxicity of sulfur in mammals is expected because sulfur is naturally present in all compartments, an essential element in the animal metabolism and has lately been assessed in the EU review as safe for terrestrial vertebrates. Therefore, long-term exposure for mammals to sulfur is not expected to present any unacceptable risk (see chapter 9.3.1).

Table 9.2-18: Mefentrifluconazole: Screening step and tier 1 calculations of the long-term/reproductive risk for mammals due to the use of BAS 768 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 bw/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
	Small herbivorous mammal	48.3	4.83	1.4	3.58	**4.2**	

First Tier Risk Assessment:

	Crop	**Generic focal species**	**Short cut value**	**TER**	
Calculate TER for each generic focal species	Cereals BBCH ≥ 20	Small insectivorous mammal "shrew" ground dwelling invertebrates with interception 100% ground arthropods	1.9	106.4	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	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 in **bold** are below the trigger.

The conclusions for the first-tier dietary risk assessments for mammals for each of the active substances are as follows: acceptable acute risks were shown at the screening step for both mefentrifluconazole and sulfur, and an acceptable reproductive risk was shown at tier 1 for mefentrifluconazole. Based on the substance-specific characteristics of sulfur no long-term studies are required and therefore no long-term risk needs to be assessed for wild mammals. 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 768 00 F because this formulation contains more than one active substance.

Following Appendix B (step 1) in EFSA/2009/1438 a surrogate LD_{50} = 20900.3 mg/kg bw is calculated based on the assumption of dose additivity (Table 9.2-19). 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 768 00 F this does not apply because the deviation for both active substances is more than 10% (Table 9.2-19).

Table 9.2-19: Calculation of surrogate LD_{50} 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	25	0.04	> 2000	0.00002	**20900.3**	50000	139
Sulfur	600	0.96	> 34475	0.00003		35911.5	72

Since there is no experimental data on acute toxicity of the formulation BAS 768 00 H to rats (see justification in chapter 9.3.1), the surrogate LD_{50} = 20900.3 mg/kg bw will be used for the acute risk assessment below.

Exposure and acute risk assessment for combined active substances (<u>virtual compound approach</u>)

The potential exposure to the combined substances follows step 4 of Appendix B of EFSA/2009/1438. The maximum application rate of formulation BAS 768 00 F is 4.0 L product/ha (corresponding to 0.1 kg/ha mefentrifluconazole and 2.4 kg/ha sulfur for the use in cereals); applying the concept for dose additivity to the exposure calculations results in a combined application rate of 2.5 kg virtual compound/ha.

The dietary TER acute value for the screening step presented in Table 9.2-20 is above the trigger of 10. Therefore, the acute risk to mammals from combined effects of the two active substances in BAS 768 00 F is acceptable.

Table 9.2-20: Screening step calculation of the acute risk for mammals due to the use of BAS 768 00 F in "cereals" – 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	2.5	2	14	10.0	20900.3	
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 herbivorous mammal	118.4	296.00	1.2	355.20	58.8	

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 $TER_{A\ combi}$ = trigger/((trigger/$TER_{A\ substance\ 1}$)+(trigger/$TER_{A\ substance\ 2}$)).

Combined reproductive toxicity

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 requested.

Based on the substance-specific characteristics of sulfur no long-term toxicity for mammals is expected. The reproductive risk for mammals exposed to sulfur is considered low and a chronic combination toxicity

assessment of sulfur with mefentrifluconzaole is therefore not required. The overall reproductive risk of BAS 768 00 F can reliably be based on the reproductive risk assessment for mefentrifluconazole. The TER_{LT} values for the tier 1 scenarios of the reproductive risk assessment as presented in Table 9.2-18 are well above the trigger (by a factor > 1.8). Thus, it can be concluded that the reproductive risk for mammals from the combined exposure to the two active substances after application of BAS 768 00 F according to good agricultural practice is low and acceptable.

9.3.2.2 Higher-tier risk assessment

Not necessary as acceptable acute and reproductive risks were shown with the screening and/or tier 1 risk assessments for all scenarios.

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} ≥ 500 L/kg).

The ratio calculations for effective application rate to relevant endpoint are detailed in Table 9.2-21 (mefentrifluconazole) and Table 9.2-22 (sulfur). The ratios for acute and reproductive endpoints for mefentrifluconazole (< 0.1 and 13.0, respectively) and for the acute endpoint for sulfur (< 0.1) do not exceed the threshold values of 3000, 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 9.2-21: Assessment of the risk for mammals due to exposure to mefentrifluconazole via contaminated drinking water in puddles

Parameter	Mefentrifluconazole	Reference
K_{oc} (geometric mean) [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	--
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} = Application rate (g/ha) x MAF_m
3) Ratio of AR_{eff} and relevant toxicity endpoint

Table 9.2-22: Assessment of the risk for mammals due to exposure to sulfur via contaminated drinking water in puddles

Parameter	Sulfur	Reference
K_{oc} [L/kg]	3615.3	Document Sulphur_RAR_45_LoEP_2022-06
DT_{50} (soil) [days] [4]	n.a.	Chapter 8.9; Document Sulphur_RAR_27_Volume_3CP_SULPHUR DUST_B-9_2022-06
Number of applications	n.a.	--
Interval [days]		--
MAF_m [1]		--
Max use rate [g/ha]	2400	
AR_{eff} [g/ha] [2]	4800	--
LD_{50} [mg/kg bw]	>34475	Chapter 9.3.1
Ratio (acute) [3]	<0.1	--
Trigger	3000	--
Drinking water assessment required [Yes/No]	No	--

n.a. = not applicable

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) For sulfur a soil DT_{50} is not available (see p. 43 in the document 'Sulphur_RAR_27_Volume_3CP_SULPHUR DUST_B-9_2022-06'), therefore as a worst-case approach the maximum yearly application rate for Sulphur Dust (2x 2.4 kg/ha/year) is assumed to be the maximum effective application rate. Please note that this approach is in line with that evaluated by the RMS in the document 'Sulphur_RAR_27_Volume_3CP_SULPHUR DUST_B-9_2022-06'.

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

4) According to EFSA/2009/1438 the DT_{50} soil as used in PEC_{sw} calculations has to be selected. However, due to the low water solubility of sulfur no PEC_{sw} was calculated. For details see chapter 8.9.

In conclusion, the risk to mammals via drinking water from the intended use of BAS 768 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 exceeds the trigger value of 3. A risk assessment for effects due to secondary poisoning is required.

In the LoEP of the RAR from June 2022 (Sulphur_RAR_45_LoEP_2022-06), RMS France concluded that "*the potential of sulfur bioaccumulation is considered to be negligible as sulfur is a naturally occurring mineral and an essential element in the metabolism of all living organisms. Furthermore, sulfur is of low toxicity to birds and mammals.*" Therefore, a risk assessment for effects due to secondary poisoning is not 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.

As shown in the following Table 9.2-23, the TER_{LT} for mefentrifluconazole exceeds the relevant trigger of 5 for acceptability of effects, indicating an acceptable risk to earthworm-eating mammals via secondary poisoning.

Table 9.2-23: Assessment of the risk for earthworm-eating mammals due to exposure to mefentrifluconazole via bioaccumulation in earthworms (secondary poisoning) for the intended use

Parameter	Mefentrifluconazole	Reference
PEC_{soil} (accu) [mg/kg soil] [1)]	0.092	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)]	0.039	--
Daily dose [mg/kg bw/d] [4)]	0.049	--
NO(A)EL [mg/kg bw/d]	15.0	Chapter 9.3.1
TER_{LT} [5)]	303.1	--

1) PEC_{soil} (accu) value calculated for an application scenario of 2 x 100 g a.s./ha in cereals at a plateau soil depth of 20 cm. For details see chapter 8.7.
2) Bioconcentration factor (BCF) = $(0.84 + 0.012 \times K_{ow}) / (f_{oc} \times K_{oc})$
3) $PEC_{worm} = PEC_{soil}$ (accu) 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 9.2-24, the TER_{LT} for mefentrifluconazole exceeds the relevant trigger of 5 for acceptability of effects, indicating an acceptable risk to fish-eating mammals via secondary poisoning.

Table 9.2-24: 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} (twa, 21 d) [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)]	0.110	--
NO(A)EL [mg/kg bw/d]	15	Chapter 9.3.1
TER_{LT} [4)]	135.8	--

1) Worst-case PEC_{sw} (twa, 21 d) value calculated for a multiple application scenario of 2 x 100 g a.s./ha to cereals from FOCUS Step 2 (Northern Europe scenario). For details see chapter 8.9.
2) $PEC_{fish} = PEC_{sw}$ (twa, 21 d) 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).

The potential for bioaccumulation of sulfur is negligible as sulfur is a naturally occurring element in all three environmental compartments (air, soil, water) and an essential mineral in the metabolism of terrestrial vertebrates. Furthermore, sulfur is of low toxicity to mammals (RMS in LoEP of the RAR from June 2022).

Since the bioaccumulation potential of both mefentrifluconazole and sulfur is low no further assessment on biomagnification in the foodchain 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 768 00 F according to good agricultural practice is acceptable.

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

zRMS Comments:	The submitted iformation was accepted. There is no agreed guidance for risk assessment to terrestrial vertebrate wildlife.

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 terrestrial life-stages of amphibians and reptiles shall be addressed, yet toxicity testing is not required.

In general, information on the toxicity of chemicals to terrestrial life-stages of amphibians is scarce. However, in the cases where terrestrial life-stages of amphibians were tested in the same type of study as birds and mammals, the general pattern is that amphibians are less sensitive than the latter two taxa (see Table 12 and 13 in Fryday and Thompson, 2012). A review compiling data on 26 chemicals for birds, mammals and amphibians confirmed this pattern (Crane et al., 2016).

For reptiles, there is even less information available than for amphibians (see the review by Fryday and Thompson, 2009).

For the time being, it is assumed that the risk assessments for birds and mammals are protective for terrestrial life-stages of amphibians and reptiles; an approach that is also used by US-EPA (US-EPA 2004).

References

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US-EPA 2004. Overview of the ecological risk assessment process in the Office of Pesticide Programs, U.S. Environmental Protection Agency: Endangered and Threatened Species Effects Determinations. Office of Prevention, Pesticides and Toxic Substances; Office of Pesticide Programs, Washington, D.C. 92 pp.

9.5 Effects on aquatic organisms (KCP 10.2)

zRMS Comments:	The submitted information and justification were accepted. The following application pattern was taken into consideration: • Winter cereals at early (BBCH 30) and late (BBCH 59) single and multiple (2 x) applications at rates of 100 g mefentrifluconazole /ha and 1 x 2400 g sulphur/ha, • Spring cereals at early (BBCH 30) and late (BBCH 59) single and multiple (2 x) applications at rates of 100 g mefentrifluconazole /ha and 1 x 2400 g sulphur/ha. All relevant metabolites were taken into consideration. All changes were provided in dRR in grey. **Mefentrifluconazole**. The endpoints used for risk assessment were agreed at the EU level. The relevant metabolites were taken into consideration. The PECsw and PECsed assessment was provided in accordance with FOCUS Surface Water guidance in Step1 & 2 and Step 3. No mitigation measures were proposed in risk assesment. **Sulphur**. The endpoints used for risk assessment were agreed at the EU level. In relevant tables, in references the corrected source of data should be pointed: EFSA Journal 2023;21(3):7385. **Metabolite of sulphur**. The new endpoint for *Chironomus riparius* was added for sulfates (EFSA, 2023). The risk assessment for sediment dwelling organisms was submitted. **Mixture toxicity/Formulation toxicity**. The submitted risk assessment was accepted. No mitigation measures are required. An acceptable risk to aquatic organisms is expected if the application of the BAS 768 00 F is in accordance with proposed pattern use.

9.5.1 Toxicity data

Studies on the toxicity to aquatic organisms have been carried out with the formulation BAS 768 00 F, the active substances mefentrifluconazole (BAS 750 F) and sulfur (BAS 175 F) and the 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) and sulfur (DAR, Vol.3, B.9 (March 2008); Final Addendum to the DAR (December 2008); EFSA Scientific Report (2008) 221, 1-70; Addendum to the DAR for Confirmatory Data (April 2012); Addendum to the DAR for Post Annex I Data (April 2012); the revised EC Review report (after the assessment of the confirmatory data), SANCO/2676/08 – final, July 2012) as well as in Appendix 2 of this document (new studies).

The selection of studies and endpoints and the risk assessments for the active substances mefentrifluconazole and sulfur and the relevant metabolites are in general 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. Justification for new endpoints is provided in section 9.5.1.1.

Effects on aquatic organisms of product BAS 768 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 768 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 9.2.4-1.

Table 9.2.4-1: Endpoints and effect values relevant for the risk assessment for aquatic organisms – mefentrifluconazole and relevant metabolites

Species	Substance	Exposure System	Results	Reference / BASF DocID
Oncorhynchus mykiss	mefentrifluconazole	96 h, f	**LC_{50} = 0.532 mg a.s./L $_{mm}$**	EFSA Journal 2018;16(7):5379 / 2014/1036951
Cyprinus carpio	mefentrifluconazole	96 h, f	LC_{50} = 1.126 mg a.s./L $_{mm}$	EFSA Journal 2018;16(7):5379 / 2015/1249071
Cyprinodon variegatus [1)]	mefentrifluconazole	96 h, ss	LC_{50} = 0.761 mg a.s./L $_{mm}$	EFSA Journal 2018;16(7):5379 / 2014/7002810
Danio rerio	mefentrifluconazole	96 h, s	LC_{50} = 0.906 mg a.s./L $_{mm}$	EFSA Journal 2018;16(7):5379 / 2015/1001581
Pimephales promelas	mefentrifluconazole	96 h, s	LC_{50} = 0.65 mg a.s./L $_{mm}$	New study – not EU evaluated / xxxxxxxxxx
D. rerio (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
C. variegatus [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
D. rerio (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
D. rerio (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
O. mykiss (BCF study)	mefentrifluconazole	14 d uptake, 7 d depuration	$BCF_{KLg\ (whole\ fish)}$ = 385	EFSA Journal 2018;16(7):5379 / 2015/1122811
Daphnia magna	mefentrifluconazole	48 h, s	**EC_{50} = 0.944 mg a.s./L $_{mm}$**	EFSA Journal 2018;16(7):5379 / 2013/1250866
Americamysis bahia [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**
Crassostrea virginica [1)]	mefentrifluconazole	96 h, f	EC_{50} = 0.947 mg a.s./L $_{mm}$	EFSA Journal 2018;16(7):5379 / 2015/7000021
D. magna	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 $_{mm}$ **EC_{10} = 0.0161 mg a.s./L $_{mm}$**	EFSA Journal 2018;16(7):5379 / 2014/1098028
A. bahia [1)]	mefentrifluconazole	28 d, f	NOEC ≥ 0.0132 mg a.s./L $_{mm}$	EFSA Journal 2018;16(7):5379 / 2016/7001293
D. pulex	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
D. longispina	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
Chironomus dilutus (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
Hyalella azteca (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
Leptocheirus plumulosus [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
C. riparius (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
C. dilutus (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
Pseudokirchneriella subcapitata [2)]	mefentrifluconazole	72 h, s	E_rC_{50} = 1.352 mg a.s./L $_{mm}$ E_yC_{50} = 0.777 mg a.s./L $_{mm}$	EFSA Journal 2018;16(7):5379 / 2013/1250865

Species	**Substance**	**Exposure System**	**Results**	**Reference / BASF DocID**
Skeletonema costatum [1), 2)]	mefentrifluconazole	72 h, s	**E_rC_{50} = 0.679 mg a.s./L $_{mm}$** E_yC_{50} = 0.479 mg a.s./L $_{mm}$	EFSA Journal 2018;16(7):5379 / 2015/7000620 + 2016/1292092 (re-calculation)
Navicula pelliculosa [2)]	mefentrifluconazole	72 h, s	E_rC_{50} = 1.347 mg a.s./L $_{mm}$ E_yC_{50} = 0.671 mg a.s./L $_{mm}$	EFSA Journal 2018;16(7):5379 / 2015/7000618 + 2016/1292093 (re-calculation)
Anabaena flos-aquae [2)]	mefentrifluconazole	72 h, s	E_rC_{50} & E_yC_{50} > 3.08 mg a.s./L $_{mm}$	EFSA Journal 2018;16(7):5379 / 2015/7000617
Lemna gibba [2)]	mefentrifluconazole	7 d, s	**E_rC_{50}** & E_yC_{50} **> 2.017 mg a.s./L $_{im}$**	EFSA Journal 2018;16(7):5379 / 2014/1001322 + 2018/1220943
O. mykiss	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
O. mykiss	M750F005 (Reg. No. 6003433)	96 h, s	LC_{50} > 5 mg/L $_{nom}$	New study – not EU evaluated / xxxxxxxxxxxxxxxx
O. mykiss	M750F006 (Reg. No. 5863469)	96 h, s	LC_{50} = 6.2 mg/L $_{mm}$	EFSA Journal 2018;16(7):5379 / 2016/1128152
O. mykiss	M750F007 (Reg. No. 6003432)	96 h, s	LC_{50} > 7.2 mg/L $_{mm}$	EFSA Journal 2018;16(7):5379 / 2015/1001489
O. mykiss	1,2,4-triazole	28 d, ss	NOEC = 3.2 mg/L $_{nom}$	EFSA Journal 2018;16(7):5379 / 2002/1007850
D. magna	1,2,4-triazole	48 h, s	EC_{50} > 100 mg/L $_{nom}$	EFSA Journal 2018;16(7):5379 / 1995/1001851
D. magna	M750F003	48 h, s	EC_{50} > 100 mg/L $_{nom}$	EFSA Journal 2018;16(7):5379 / 2016/1289876
D. magna	M750F005	48 h, s	EC_{50} > 8.58 mg/L $_{mm}$	EFSA Journal 2018;16(7):5379 / 2015/1001490
D. magna	M750F006	48 h, s	EC_{50} = 4.42 mg/L $_{mm}$	EFSA Journal 2018;16(7):5379 / 2015/1001492
D. magna	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
D. magna	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**
C. riparius	M750F003	28 d, s	NOEC ≥ 1.944 mg/kg dry sediment $_{im}$	EFSA Journal 2018;16(7):5379 / 2015/1003916 +2017/1044237
P. subcapitata [2)]	1,2,4-triazole	72 h, s	E_rC_{50} = 22.5 mg/L$^{-3)}$ $_{mm}$	EFSA Journal 2018;16(7):5379 / 2001/1022266
P. subcapitata [2)]	M750F003	72 h, s	E_rC_{50} > 100 mg/L $_{nom}$	EFSA Journal 2018;16(7):5379 / 2016/1289875
P. subcapitata [2)]	M750F005	72 h, s	E_rC_{50} > 8.57 mg/L $_{mm}$	EFSA Journal 2018;16(7):5379 / 2015/1184816
P. subcapitata [2)]	M750F006	72 h, s	E_rC_{50} = 1.42 mg/L $_{mm}$	EFSA Journal 2018;16(7):5379 / 2015/1184815
P. subcapitata [2)]	M750F007	72 h, s	E_rC_{50} > 10 mg/L $_{nom}$	EFSA Journal 2018;16(7):5379 / 2015/1003914
P. subcapitata [2)]	M750F008	72 h, s	E_rC_{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 ± 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_rC_{50} > 31 mg/L) and the endpoint reported in the Annex I approval of mefentrifluconazole (*i.e.,* E_rC_{50} = 22.5 mg/L). For the risk assessment the EU agreed endpoint (E_rC_{50} > 22.5 mg/L, based on mean measured concentrations) is used.

Sulfur (BAS 175 F)

The results from toxicity tests with representative aquatic species conducted with the active substance sulfur found in aquatic systems are summarized in Table 9.2.4-2.
The endpoints for aquatic organisms for the active substance sulfur were derived from studies with the formulated products 'Sulphur Dust' and/or 'Sulfur 80% WG'. Since both chemical preparations consist predominantly of the active substance sulfur, i.e., 98.5% (w/w) and 80% (w/w), respectively, the results of ecotoxicity studies with the formulated products can be extrapolated to effects of sulfur without restrictions. This approach was already accepted in the first EU review of sulfur and is also used for the renewal approval application.

Generally, the risk to aquatic organisms is considered low because the solubility of sulfur in water is very low and no effects were observed at concentrations, which exceeded the water solubility by several orders of magnitude. During the first EU evaluation it was agreed to set the effect values for acute and chronic exposure of all groups of aquatic organisms at the solubility limit of elemental sulfur in water (*i.e.*, 0.063 mg a.s./L), reflecting, on the one hand, the lack of relevant effects in most of the studies, and, on the other hands, the obvious experimental problems in producing stable solutions with reliable concentrations. A newly generated study was submitted in the Annex I renewal (AIR4) process which results in water solubility limit of 0.016 mg a.s./L (see revised RAR, June 2022, Volume 3. CA, B.2.5, Rigamonti, E. (2018); KCA 2.5/03). In contrast to the old study, the new value is analytically verified by using a method completely validated according to SANCO/3029/99. Thus, the new value is considered more reliable. Accordingly, the lower (more conservative) endpoint for effects on aquatic organisms in the water column is adjusted to 0.016 mg a.s./L and used in the risk assessment below.
Since sulfur might absorb to the sediment after entering the surface water, an assessment of the exposure in the sediment compartment as well as the risk for sediment-dwelling organisms was considered necessary (see EFSA Conclusion for sulfur, 2008). To confirm the risk assessment a study on effects on sediment-dwelling organisms exposed to sulfur and sulphate applied as 'Sulphur Dust' and sodium sulphate, respectively, has been submitted with the confirmatory data.

Table 9.2.4-2: Endpoints and effect values relevant for the risk assessment for aquatic organisms – sulfur

Species	Substance	Exposure System	Results	Reference / BASF DocID
Oncorhynchus mykiss	Sulfur (tested as 80% WG)	96 h, s	LC_{50} > 0.016 mg a.s./L [1)]	EFSA Scientific Report (2008) 221, 57-70 (revised endpoint based on new water solubility study)
Cyprinus carpio	Sulfur (tested as 80% WG)	96 h, s	LC_{50} > 0.016 mg a.s./L [1)]	EFSA Scientific Report (2008) 221, 57-70 (revised endpoint based on new water solubility study)
C. carpio	Sulfur (tested as 80% WG)	96 h, s	LC_{50} > 0.016 mg a.s./L [1)]	EFSA Scientific Report (2008) 221, 57-70 (revised endpoint based on new water solubility study)

Species	**Substance**	**Exposure System**	**Results**	**Reference / BASF DocID**
O. mykiss	Sulfur (tested as Sulphur Dust)	96 h, ss	LC_{50} > 0.016 mg a.s./L [1)]	EFSA Scientific Report (2008) 221, 57-70 (revised endpoint based on new water solubility study)
O. mykiss	Sulfur (tested as 80% WG)	28 d, f	NOEC > 0.016 mg a.s./L [1)]	EFSA Scientific Report (2008) 221, 57-70 (revised endpoint based on new water solubility study)
Daphnia magna	Sulfur (tested as 80% WG)	48 h,s	EC_{50} > 0.016 mg a.s./L [1)]	EFSA Scientific Report (2008) 221, 57-70 (revised endpoint based on new water solubility study)
Daphnia magna	Sulfur (tested as Sulphur Dust)	48 h,s	EC_{50} > 0.016 mg a.s./L [1)]	EFSA Scientific Report (2008) 221, 57-70 (revised endpoint based on new water solubility study)
Daphnia magna	Sulfur (tested as 80% WG)	48 h,s	EC_{50} > 0.016 mg a.s./L [1)]	EFSA Scientific Report (2008) 221, 57-70 (revised endpoint based on new water solubility study)
Daphnia magna	Sulfur (tested as Sulphur Dust)	21 d, ss	NOEC > 0.016 mg a.s./L [1)]	Addendum to the DAR for Post Annex I Data (2012) (revised endpoint based on new water solubility study)
Daphnia magna	Sulfur (tested as 80% WG)	21 d, ss	NOEC > 0.016 mg a.s./L [1)]	New study – not EU evaluated / 2011/1023625 (revised endpoint based on new water solubility study)
Chironomus riparius	Sulfur (tested as Sulphur Dust)	28 d, spiked-sediment, s	NOEC = 949 mg a.s./kg dry sediment $_{nom}$ **Recalculated: NOEC = 592.9 mg a.s./kg dry sediment $_{mm}$** [2)]	Addendum to DAR for Confirmatory Data (2012) + new recalculation [2)]

Species	**Substance**	**Exposure System**	**Results**	**Reference / BASF DocID**
Scenedesmus subspicatus	Sulfur (tested as 80% WG)	72 h, s	E_rC_{50} & E_bC_{50} > 0.016 mg a.s./L [1)]	EFSA Scientific Report (2008) 221, 57-70 (revised endpoint based on new water solubility study)
Desmodesmus subspicatus	Sulfur (tested as Sulphur Dust)	72 h, s	E_rC_{50} & E_bC_{50} > 0.016 mg a.s./L [1)]	EFSA Scientific Report (2008) 221, 57-70 (revised endpoint based on new water solubility study)
Chironomus riparius	Sodium sulphate	28 d, spiked-water, s	NOEC = 100 mg a.s./kg dry sediment $_{nom}$ **Recalculated: NOEC = 81.9 mg a.s./kg dry sediment $_{mm}$** [2)]	Addendum to DAR for Confirmatory Data (2012) + new recalculation [2)]

Abbreviations: s: static; ss: semi-static; f: flow-through

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

1) The water solubility limit of sulfur is the relevant endpoint for acute and chronic exposure of all groups of aquatic organisms in the water column (*i.e.*, water solubility limit of 0.016 mg a.s./L derived from new study submitted with the dossier for renewal of the approval (AIR4) of sulfur (see revised RAR, June 2022, Volume 3. CA, B.2.5, Rigamonti, E. (2018); KCA 2.5/03).

2) As measured concentrations varied more than ± 20% during the test, the endpoint has been recalculated to mean measured concentrations by the zRMS in AIR4.

Formulated product (BAS 768 00 F)

The results from the toxicity tests with representative aquatic species conducted with the formulation BAS 768 00 F found in aquatic systems are summarized in Table 9.2.4-3.

Table 9.2.4-3: Endpoints and effect values relevant for the risk assessment for aquatic organisms – BAS 768 00 F

Species	Substance	Exposure System	Results	Reference / BASF DocID
Oncorhynchus mykiss	BAS 768 00 F	96 h, s	LC_{50} = 15.3 mg/L $_{nom}$ (0.299 mg BAS 750 F/L) [1)]	New study – not EU evaluated xxxxxxxxxxxx
Daphnia magna	BAS 768 00 F	48 h, s	EC_{50} > 80 mg/L $_{nom}$ (> 1.56 mg BAS 750 F/L) [1)]	New study – not EU evaluated 2021/2016980
Pseudokirchneriella subcapitata	BAS 768 00 F	72 h, s	E_rC_{50} & E_yC_{50} > 80 mg/L $_{nom}$ (> 1.56 mg BAS 750 F/L) [1)]	New study – not EU evaluated 2021/2016979

Abbreviations: s: static; nom: based on nominal loading rate

1) Formulation endpoints have been recalculated to the active substance mefentrifluconazole (BAS 750 F) based on its analysed content within the formulation (*i.e.,* 26.3 g mefentrifluconazole/L) and the product density of 1.346 g/cm^3 (sulfur was disregarded; for justification, please refer to the risk assessment for the formulated product below).

9.5.1.1 Justification for new endpoints

Mefentrifluconazole and metabolite
In general, for mefentrifluconazole and its metabolites the EU agreed endpoints are used for the risk assessment. A new acute fish study (*P. promelas*) was performed with the active substance for refinement (BASF DocID xxxxxxxxxxxxxxx). Additionally, an acute fish study with the metabolite M750F005 (BASF DocID xxxxxxxxxx) is available. This study was conducted post Annex I inclusion for a different region to address due to an authority request. The study is provided to support the risk assessment of the metabolite. 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.

Sulfur
In addition to the data package for sulfur already evaluated in the first EU review, a new *Daphnia* reproductive toxicity test has been performed (with the formulation 'Sulphur 80% WG') which was not evaluated as part of the first EU review of the active substance. The study was conducted to address a data gap outlined in the EFSA conclusion (2008) and is currently in the evaluation phase of the Annex I renewal process of sulfur (AIR4). Under the conditions of this study, no effects on mortality, offspring or abnormal behavior were observed at concentrations up to 40 mg a.s./L, thus confirming the low aquatic toxicity of sulfur and sulfur containing plant protection products.
Moreover, during the first EU evaluation it was agreed to set the effect values for acute and chronic exposure of all groups of aquatic organisms at the solubility limit of elemental sulfur in water (*i.e.*, 0.063 mg a.s./L). However, a water solubility of 0.016 mg a.s./L based on a new study submitted in the Annex I renewal (AIR4) process (see revised RAR, June 2022, Volume 3. CA, B.2.5, Rigamonti, E. (2018); KCA 2.5/03) is now considered more reliable. Thus, the endpoint for effects on aquatic organisms in the water column is adjusted to the more conservative value of 0.016 mg a.s./L and it used in the risk assessment below.

Summaries of the new studies are provided in Appendix 2.

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 the EU agreed endpoints are considered for the tier 1 risk assessment.

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 (at 1x and 2x 100 g a.s./ha) in 'spring and winter cereals' are given per intended use and each organism group and are presented in Table 9.4-4 to Table 9.4-11. For details on the PEC calculations please refer to Part B, Section 8.9.

Table 9.4-4: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for mefentrifluconazole for each organism group based on standard FOCUS Step 1 - 3 calculations for single application of BAS 768 00 F at 1x 100 g a.s./ha in 'spring cereals' (BBCH 30)

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Higher-Plant**	**Group**	**Sed. dwell. prolonged**
Test species		***O. mykiss***	***D. rerio* (FLC study)**	***D. magna***	***D. magna***	***S. costatum***	***L. gibba***	**Test species**	***C. riparius***
Endpoint		LC_{50}	NOEC	EC_{50}	EC_{10}	E_rC_{50}	E_rC_{50}	**Endpoint**	NOEC
(µg/L)		532	22	944	16.1	679	> 2017	**(µg/kg)**	≥ 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 $_{gl\text{-}sw\ max}$ (µg/L)**	**PEC/RAC (= ETR)**						**PEC $_{gl\text{-}sed\ max}$ (µg/kg)**	**PEC/RAC (= ETR)**
Step 1									
	6.864	**1.3**	**3.1**	0.7	**4.3**	0.1	< 0.03	210.190	**≤ 1.8**
Step 2									
N-Europe	1.164	0.2	0.5	--	0.7	--	--	37.825	≤ 0.3
S-Europe	2.102	0.4	0.96	--	**1.3**	--	--	70.102	≤ 0.6
Step 3									
D3 ditch	0.632	--	--	--	0.4	--	--	0.377	--
D4 pond	0.035	--	--	--	0.02	--	--	0.325	--
D4 stream	0.517	--	--	--	0.3	--	--	0.106	--
D5 pond	0.023	--	--	--	0.01	--	--	0.195	--
D5 stream	0.531	--	--	--	0.3	--	--	0.019	--
R1 pond *	0.069	--	--	--	0.04	--	--	1.207	--
R1 stream *	0.417	--	--	--	0.3	--	--	1.186	--
R3 stream *	0.511	--	--	--	0.3	--	--	0.399	--

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Higher-Plant	Group	Sed. dwell. prolonged
R4 stream	0.602	--	--	--	0.4	--	--	2.152	--

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**

* $PEC_{sw/sed}$ values for the R1 and R3 scenarios are only relevant for Austria and have been calculated using 'spring oilseed rape' and 'legumes', respectively, as surrogate crops since these scenarios are not defined for spring cereals (for details please refer to Part B, Section 8.9).

For the intended single application of BAS 768 00 F at 1x 100 g a.s./ha in 'spring cereals' (at BBCH 30), 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 9.4-5: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for mefentrifluconazole for each organism group based on standard FOCUS Step 1 - 3 calculations for <u>twofold application of BAS 768 00 F at 2x 100 g a.s./ha in 'spring cereals' (BBCH 30)</u>

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Higher-Plant**	**Group**	**Sed. dwell. prolonged**
Test species		***O. mykiss***	***D. rerio* (FLC study)**	***D. magna***	***D. magna***	***S. costatum***	***L. gibba***	**Test species**	***C. riparius***
Endpoint		LC_{50}	NOEC	EC_{50}	EC_{10}	E_rC_{50}	E_rC_{50}	**Endpoint**	NOEC
(µg/L)		532	22	944	16.1	679	> 2017	**(µg/kg)**	≥ 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 $_{gl\text{-}sw\ max}$ (µg/L)**	**PEC/RAC (= ETR)**						**PEC $_{gl\text{-}sed\ max}$ (µg/kg)**	**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	0.553	--	0.3	--	0.3	--	--	0.514	≤ 0.004
D4 pond	0.066	--	0.03	--	0.04	--	--	0.590	≤ 0.005
D4 stream	0.461	--	0.2	--	0.3	--	--	0.225	≤ 0.002
D5 pond	0.032	--	0.01	--	0.02	--	--	0.335	≤ 0.003
D5 stream	0.477	--	0.2	--	0.3	--	--	0.036	≤ 0.0003
R1 pond *	0.131	--	0.06	--	0.08	--	--	2.199	≤ 0.02
R1 stream *	0.536	--	0.2	--	0.3	--	--	2.195	≤ 0.02
R3 stream *	0.481	--	0.2	--	0.3	--	--	0.891	≤ 0.008

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Higher-Plant	Group	Sed. dwell. prolonged
R4 stream	1.106	--	0.5	--	0.7	--	--	3.775	≤ 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**

* $PEC_{sw/sed}$ values for the R1 and R3 scenarios are only relevant for Austria and have been calculated using 'spring oilseed rape' and 'legumes', respectively, as surrogate crops since these scenarios are not defined for spring cereals (for details please refer to Part B, Section 8.9).

For the intended twofold application of BAS 768 00 F at 2x 100 g a.s./ha in 'spring cereals' (at BBCH 30), 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 9.4-6: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for mefentrifluconazole for each organism group based on standard FOCUS Step 1 - 3 calculations for single application of BAS 768 00 F at 1x 100 g a.s./ha in 'spring cereals' (BBCH 59)

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Higher-Plant**	**Group**	**Sed. dwell. prolonged**
Test species		***O. mykiss***	***D. rerio* (FLC study)**	***D. magna***	***D. magna***	***S. costatum***	***L. gibba***	**Test species**	***C. riparius***
Endpoint		LC_{50}	NOEC	EC_{50}	EC_{10}	E_rC_{50}	E_rC_{50}	**Endpoint**	NOEC
(µg/L)		532	22	944	16.1	679	> 2017	**(µg/kg)**	≥ 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 $_{gl\text{-}sw\ max}$ (µg/L)**	**PEC/RAC (= ETR)**						**PEC $_{gl\text{-}sed\ max}$ (µg/kg)**	**PEC/RAC (= ETR)**
Step 1									
	6.864	**1.3**	**3.1**	0.7	**4.3**	0.1	< 0.03	210.191	**≤ 1.8**
Step 2									
N-Europe	1.164	0.2	0.5	--	0.7	--	--	37.825	≤ 0.3
S-Europe	2.102	0.4	0.96	--	**1.3**	--	--	70.102	≤ 0.6
Step 3									
D3 ditch	0.635	--	--	--	0.4	--	--	0.461	--
D4 pond	0.025	--	--	--	0.02	--	--	0.259	--
D4 stream	0.547	--	--	--	0.3	--	--	0.104	--
D5 pond	0.023	--	--	--	0.01	--	--	0.177	--
D5 stream	0.553	--	--	--	0.3	--	--	0.036	--
R1 pond *	0.062	--	--	--	0.04	--	--	1.013	--
R1 stream *	0.419	--	--	--	0.3	--	--	1.245	--
R3 stream *	0.511	--	--	--	0.3	--	--	0.436	--

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Higher-Plant	Group	Sed. dwell. prolonged
R4 stream	0.419	--	--	--	0.3	--	--	1.673	--

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**

* $PEC_{sw/sed}$ values for the R1 and R3 scenarios are only relevant for Austria and have been calculated using 'spring oilseed rape' and 'legumes', respectively, as surrogate crops since these scenarios are not defined for spring cereals (for details please refer to Part B, Section 8.9).

For the intended single application of BAS 768 00 F at 1x 100 g a.s./ha in 'spring cereals' (at BBCH 59), 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 9.4-7: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for mefentrifluconazole for each organism group based on standard FOCUS Step 1 - 3 calculations for <u>twofold application of BAS 768 00 F at 2x 100 g a.s./ha in 'spring cereals' (BBCH 59)</u>

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Higher-Plant**	**Group**	**Sed. dwell. prolonged**
Test species		***O. mykiss***	***D. rerio* (FLC study)**	***D. magna***	***D. magna***	***S. costatum***	***L. gibba***	**Test species**	***C. riparius***
Endpoint		LC_{50}	NOEC	EC_{50}	EC_{10}	E_rC_{50}	E_rC_{50}	**Endpoint**	NOEC
(µg/L)		532	22	944	16.1	679	> 2017	**(µg/kg)**	≥ 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 $_{gl\text{-}sw\ max}$ (µg/L)**	**PEC/RAC (= ETR)**						**PEC $_{gl\text{-}sed\ max}$ (µg/kg)**	**PEC/RAC (= ETR)**
Step 1									
	13.728	**2.6**	**6.2**	**1.5**	**8.5**	0.2	< 0.07	417.549	**≤ 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	0.556	--	0.3	--	0.3	--	--	0.571	≤ 0.005
D4 pond	0.052	--	0.02	--	0.03	--	--	0.494	≤ 0.004
D4 stream	0.474	--	0.2	--	0.3	--	--	0.172	≤ 0.001
D5 pond	0.033	--	0.02	--	0.02	--	--	0.306	≤ 0.003
D5 stream	0.511	--	0.2	--	0.3	--	--	0.141	≤ 0.001
R1 pond *	0.121	--	0.06	--	0.08	--	--	1.917	≤ 0.02
R1 stream *	0.388	--	0.2	--	0.2	--	--	2.428	≤ 0.02
R3 stream *	0.485	--	0.2	--	0.3	--	--	0.895	≤ 0.008

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Higher-Plant	Group	Sed. dwell. prolonged
R4 stream	0.420	--	0.2	--	0.3	--	--	3.159	≤ 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**

* $PEC_{sw/sed}$ values for the R1 and R3 scenarios are only relevant for Austria and have been calculated using 'spring oilseed rape' and 'legumes', respectively, as surrogate crops since these scenarios are not defined for spring cereals (for details please refer to Part B, Section 8.9).

For the intended twofold application of BAS 768 00 F at 2x 100 g a.s./ha in 'spring cereals' (at BBCH 59), 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 9.4-8: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for mefentrifluconazole for each organism group based on standard FOCUS Step 1 - 3 calculations for single application of BAS 768 00 F at 1x 100 g a.s./ha in 'winter cereals' (BBCH 30)

Group		**Fish acute**	**Fish prolonged**	**Inverteb. Acute**	**Inverteb. prolonged**	**Algae**	**Higher-Plant**	**Group**	**Sed. dwell. prolonged**
Test species		***O. mykiss***	***D. rerio* (FLC study)**	***D. magna***	***D. magna***	***S. costatum***	***L. gibba***	**Test species**	***C. riparius***
Endpoint		LC_{50}	NOEC	EC_{50}	EC_{10}	E_rC_{50}	E_rC_{50}	**Endpoint**	NOEC
(µg/L)		532	22	944	16.1	679	> 2017	**(µg/kg)**	≥ 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 $_{gl\text{-}sw\ max}$ (µg/L)**	**PEC/RAC (= ETR)**						**PEC $_{gl\text{-}sed\ max}$ (µg/kg)**	**PEC/RAC (= ETR)**
Step 1									
	6.864	**1.3**	**3.1**	0.7	**4.3**	0.1	< 0.03	210.190	**≤ 1.8**
Step 2									
N-Europe	1.164	0.2	0.5	--	0.7	--	--	37.825	≤ 0.3
S-Europe	2.102	0.4	0.96	--	**1.3**	--	--	70.102	≤ 0.6
Step 3									
D3 ditch	0.632	--	--	--	0.4	--	--	0.390	--
D4 pond	0.034	--	--	--	0.02	--	--	0.302	--
D4 stream	0.467	--	--	--	0.3	--	--	0.116	--
D5 pond	0.023	--	--	--	0.01	--	--	0.193	--
D5 stream	0.504	--	--	--	0.3	--	--	0.019	--
R1 pond	0.043	--	--	--	0.03	--	--	0.591	--
R1 stream	0.416	--	--	--	0.3	--	--	0.746	--
R3 stream	0.585	--	--	--	0.4	--	--	0.910	--

Group		Fish acute	Fish prolonged	Inverteb. Acute	Inverteb. prolonged	Algae	Higher-Plant	Group	Sed. dwell. prolonged
R4 stream	0.418	--	--	--	0.3	--	--	0.978	--

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**

For the intended single application of BAS 768 00 F at 1x 100 g a.s./ha in ‘winter cereals’ (at BBCH 30), 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 9.4-9: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for mefentrifluconazole for each organism group based on standard FOCUS Step 1 - 3 calculations for <u>twofold application of BAS 768 00 F at 2x 100 g a.s./ha in 'winter cereals' (BBCH 30)</u>

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Higher-Plant**	**Group**	**Sed. dwell. prolonged**
Test species		***O. mykiss***	***D. rerio* (FLC study)**	***D. magna***	***D. magna***	***S. costatum***	***L. gibba***	**Test species**	***C. riparius***
Endpoint		LC_{50}	NOEC	EC_{50}	EC_{10}	E_rC_{50}	E_rC_{50}	**Endpoint**	NOEC
(µg/L)		532	22	944	16.1	679	> 2017	**(µg/kg)**	≥ 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 $_{gl\text{-}sw\ max}$ (µg/L)**	**PEC/RAC (= ETR)**						**PEC $_{gl\text{-}sed\ max}$ (µg/kg)**	**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	0.553	--	0.3	--	0.3	--	--	0.486	≤ 0.005
D4 pond	0.069	--	0.03	--	0.04	--	--	0.587	≤ 0.0006
D4 stream	0.417	--	0.2	--	0.3	--	--	0.237	≤ 0.004
D5 pond	0.035	--	0.02	--	0.02	--	--	0.348	≤ 0.0003
D5 stream	0.482	--	0.2	--	0.3	--	--	0.042	≤ 0.004
R1 stream	0.100	--	0.05	--	0.06	--	--	1.372	≤ 0.0009
R1 pond	0.529	--	0.2	--	0.3	--	--	2.094	≤ 0.005
R3 stream	0.509	--	0.2	--	0.3	--	--	1.892	≤ 0.004

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Higher-Plant	Group	Sed. dwell. prolonged
R4 stream	0.761	--	0.3	--	0.5	--	--	2.172	≤ 0.007

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**

For the intended twofold application of BAS 768 00 F at 2x 100 g a.s./ha in ‘winter cereals’ (at BBCH 30), 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 9.4-10: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for mefentrifluconazole for each organism group based on standard FOCUS Step 1 - 3 calculations for single application of BAS 768 00 F at 1x 100 g a.s./ha in 'winter cereals' (BBCH 59)

Group		**Fish acute**	**Fish prolonged**	**Inverteb. Acute**	**Inverteb. prolonged**	**Algae**	**Higher-Plant**	**Group**	**Sed. dwell. prolonged**
Test species		***O. mykiss***	***D. rerio* (FLC study)**	***D. magna***	***D. magna***	***S. costatum***	***L. gibba***	**Test species**	***C. riparius***
Endpoint		LC_{50}	NOEC	EC_{50}	EC_{10}	E_rC_{50}	E_rC_{50}	**Endpoint**	NOEC
(µg/L)		532	22	944	16.1	679	> 2017	**(µg/kg)**	≥ 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 $_{gl\text{-}sw\ max}$ (µg/L)**	**PEC/RAC (= ETR)**						**PEC $_{gl\text{-}sed\ max}$ (µg/kg)**	**PEC/RAC (= ETR)**
Step 1									
	6.864	**1.3**	**3.1**	0.7	**4.3**	0.1	< 0.03	210.191	**≤ 1.8**
Step 2									
N-Europe	1.164	0.2	0.5	--	0.7	--	--	37.825	≤ 0.3
S-Europe	2.102	0.4	0.96	--	**1.3**	--	--	70.102	≤ 0.6
Step 3									
D3 ditch	0.634	--	--	--	0.4	--	--	0.572	--
D4 pond	0.022	--	--	--	0.01	--	--	0.245	--
D4 stream	0.546	--	--	--	0.3	--	--	0.114	--
D5 pond	0.023	--	--	--	0.01	--	--	0.178	--
D5 stream	0.590	--	--	--	0.4	--	--	0.159	--
R1 pond	0.069	--	--	--	0.04	--	--	0.651	--
R1 stream	0.418	--	--	--	0.3	--	--	1.953	--
R3 stream	0.585	--	--	--	0.4	--	--	0.530	--

Group		Fish acute	Fish prolonged	Inverteb. Acute	Inverteb. prolonged	Algae	Higher-Plant	Group	Sed. dwell. prolonged
R4 stream	0.418	--	--	--	0.3	--	--	1.369	--

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**

For the intended single application of BAS 768 00 F at 1x 100 g a.s./ha in ‘winter cereals’ (at BBCH 59), 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 9.4-11: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for mefentrifluconazole for each organism group based on standard FOCUS Step 1 - 3 calculations for <u>twofold application of BAS 768 00 F at 2x 100 g a.s./ha in 'winter cereals' (BBCH 59)</u>

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Higher-Plant**	**Group**	**Sed. dwell. prolonged**
Test species		***O. mykiss***	***D. rerio* (FLC study)**	***D. magna***	***D. magna***	***S. costatum***	***L. gibba***	**Test species**	***C. riparius***
Endpoint		LC_{50}	NOEC	EC_{50}	EC_{10}	E_rC_{50}	E_rC_{50}	**Endpoint**	NOEC
(µg/L)		532	22	944	16.1	679	> 2017	**(µg/kg)**	≥ 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 $_{gl\text{-}sw\ max}$ (µg/L)**	**PEC/RAC (= ETR)**						**PEC $_{gl\text{-}sed\ max}$ (µg/kg)**	**PEC/RAC (= ETR)**
Step 1									
	13.728	**2.6**	**6.2**	**1.5**	**8.5**	0.2	< 0.07	417.549	**≤ 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	0.555	--	0.3	--	0.3	--	--	0.717	≤ 0.006
D4 pond	0.047	--	0.02	--	0.03	--	--	0.467	≤ 0.004
D4 stream	0.473	--	0.2	--	0.3	--	--	0.151	≤ 0.001
D5 pond	0.033	--	0.02	--	0.02	--	--	0.298	≤ 0.003
D5 stream	0.510	--	0.2	--	0.3	--	--	0.177	≤ 0.002
R1 stream	0.143	--	0.07	--	0.09	--	--	1.368	≤ 0.01
R1 pond	0.471	--	0.2	--	0.3	--	--	4.771	≤ 0.04
R3 stream	0.510	--	0.2	--	0.3	--	--	0.980	≤ 0.008

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Higher-Plant	Group	Sed. dwell. prolonged
R4 stream	0.686	--	0.3	--	0.4	--	--	2.784	≤ 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**

For the intended twofold application of BAS 768 00 F at 2x 100 g a.s./ha in ‘winter cereals’ (at BBCH 59), 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 *Daphnia* and algae 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 a 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 9.4-12, the exposure-toxicity ratios (ETRs) for aquatic organisms are given for the use of BAS 768 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. For details on the $PEC_{sw/sed}$ calculations please refer to Part B, Section 8.9.

Table 9.4-12: 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 application (1x and 2x 100 g a.s./ha) of BAS 768 00 F in 'spring and winter cereals'[1)]

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Algae**	**Group**	**Sed. dwell. prolonged**
Test species		***O. mykiss***	***O. mykiss***	***D. magna***	***P. subcapitata***	**Test species**	***C. riparius***
AF		100	10	100	10	**AF**	10
1,2,4-triazole (M750F001)							
Endpoint (µg/L)		LC_{50} 498000	NOEC 3200	EC_{50} > 100000	E_rC_{50} > 22500	**Endpoint (µg/kg)**	NOEC ≥ 1158 [2)]
RAC (µg/L)		4980	320	> 1000	> 2250	**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							
	2.155	0.0004	0.007	< 0.002	< 0.001	1.781	≤ 0.02
M750F003							
Endpoint (µg/L)		LC_{50} 532 [2)]	NOEC n.a.	EC_{50} > 100000	E_rC_{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	16.852	≤ 0.09
M750F005							
Endpoint (µg/L)		LC_{50} > 5000	NOEC n.a.	EC_{50} > 8580	E_rC_{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	143.916	**≤ 1.2**
Step 2							
N-Europe	0.338	--	--	--	--	24.965	≤ 0.2
S-Europe	0.613	--	--	--	--	46.542	≤ 0.4
M750F006							
Endpoint (µg/L)		LC_{50} 6200	NOEC n.a.	EC_{50} 4420	E_rC_{50} 1420	**Endpoint (µg/kg)**	NOEC ≥ 1158 [2)]
RAC (µg/L)		62	--	44.2	142	**RAC (µg/kg)**	≥ 115.8

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Algae**	**Group**	**Sed. dwell. prolonged**
FOCUS Scenario	**PEC $_{gl\text{-}max,\ sw}$ (µg/L)**	**PEC/RAC ratio (= ETR)**				**PEC $_{gl\text{-}sed\ max}$ (µg/kg)**	**PEC/RAC (= ETR)**
Step 1							
	2.927	0.05	--	0.07	0.02	122.338	**≤ 1.06**
Step 2							
N-Europe	0.457	--	--	--	--	21.222	≤ 0.2
S-Europe	0.830	--	--	--	--	39.563	≤ 0.3
M750F007							
Endpoint (µg/L)		LC_{50} > 7200	NOEC n.a.	EC_{50} > 9900	E_rC_{50} > 10000	**Endpoint (µg/kg)**	NOEC ≥ 1158 [2)]
RAC (µg/L)		> 72	--	> 99	> 1000	**RAC (µg/kg)**	≥ 115.8
FOCUS Scenario	**PEC $_{gl\text{-}max,\ sw}$ (µg/L)**	**PEC/RAC ratio (= ETR)**				**PEC $_{gl\text{-}sed\ max}$ (µg/kg)**	**PEC/RAC (= ETR)**
Step 1							
	4.655	< 0.06	--	< 0.05	< 0.005	160.547	**≤ 1.4**
Step 2							
N-Europe	0.747	--	--	--	--	27.850	≤ 0.2
S-Europe	1.358	--	--	--	--	51.920	≤ 0.4
M750F008							
Endpoint (µg/L)		LC_{50} 53.2 [3)]	NOEC n.a.	EC_{50} > 8070	E_rC_{50} 4080	**Endpoint (µg/kg)**	NOEC ≥ 1158 [2)]
RAC (µg/L)		0.532	--	> 80.7	408	**RAC (µg/kg)**	≥ 115.8
FOCUS Scenario	**PEC $_{gl\text{-}max,\ sw}$ (µg/L)**	**PEC/RAC ratio (= ETR)**				**PEC $_{gl\text{-}sed\ max}$ (µg/kg)**	**PEC/RAC (= ETR)**
Step 1							
	0.306	0.6	--	< 0.004	0.0008	32.131	≤ 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 768 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 sulfur (BAS 175 F)

In agreement with the EFSA Scientific Report (2008) 221, 1-70, the risk to aquatic organisms in the water column can be considered in general as low because the water solubility limit of the active substance sulfur is very low, and no effects were observed at concentrations which clearly exceeded the water solubility by several orders of magnitude. Further, there is no risk of bioconcentration of sulfur.
In the previous evaluation of sulfur (EFSA, 2008), the PEC_{sw} values were set to the maximum water solubility, an approach which is used here to determine dissolved sulfur concentrations in surface water (see Part B, Section 8.9). Additionally, total sulfur concentrations (dissolved + non-dissolved) were calculated using FOCUS step 1 and step 2 according to the recent EFSA conclusion (2023). ~~Moreover, it was agreed by the member states and EFSA, that the use of FOCUS modelling is not appropriate for the risk assessment for sulfur while it was experienced that the use of FOCUS for PEC_{sw} calculations lead to concentrations above the water solubility limit (see Final addendum to the Draft Assessment Report (2008) and EFSA Scientific Report (2008) 221, 1-70). This supported the RMS's approach to address the risk assessment to aquatic organisms taking into account an absence of effects to organisms at the highest water solubility limit of sulfur. Therefore, it is not necessary to assess the risk for aquatic organisms by calculating PEC_{sw}/RAC ratios and sulfur can be considered of no concern for aquatic organisms in the water column. Therefore, no PEC_{sw} was calculated but it was set to the water solubility limit (i.e., 0.016 mg a.s./L).~~
~~However, it was further concluded, that a conservative PEC_{sed} estimation should be performed, based on the partition properties of sulfur, the maximum total annual dose, and taking into account the percentage values for entry via drift and run-off/drainage. Therefore, an assessment of exposure in the sediment compartment as well as an assessment of the risk for sediment-dwelling organisms is provided. A simplified static water body scenario on basis of the sum of percentage values for drift and run-off/drainage entry routes on the basis of Steps 1-2 in FOCUS was applied (for details on PEC_{sed} calculations please refer to Part B, Section 8.9). The results of this assessment are presented in the following tables including the ratio between predicted environmental concentrations in sediment (PEC_{sed}) and regulatory acceptable concentrations (RAC) for aquatic organisms.~~

Acceptability of risk

The relevant worst-case predicted environmental concentrations in surface water bodies and sediment (PEC_{sw} and PEC_{sed}), regulatory acceptable concentrations (RAC) for ~~sediment-dwelling~~ aquatic organisms, and the resulting PEC/RAC ratios (ETR) for the intended uses are presented in Table 9.4-13**Table 9.4-16**. The worst-case PEC_{sw} and PEC_{sed} values from calculations for the single/twofold application (at 1x/2x 2400 g a.s./ha) for total sulfur (dissolved and undissolved) in 'winter cereals' are ~~is~~ used in the risk assessment, covering the intended uses in 'spring and winter cereals'. For details on the $PEC_{sw/sed}$ calculations please refer to Part B, Section 8.9.

~~**Table 9.4-13: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for sulfur for sediment-dwelling organisms based on worst-case Step 1 calculation following twofold application (2x 2400 g a.s./ha) of BAS 768 00 F in 'spring and winter cereals'**~~

~~**Group**~~		~~**Sed. dwell. prolonged**~~
~~**Test species**~~		~~***C. riparius***~~
~~**AF**~~		~~10~~
~~**Endpoint (µg/kg)**~~		~~NOEC~~ ~~592900~~
~~**RAC (µg/kg)**~~		~~59290~~
~~**$PEC_{gl\text{-}sed\ max}$ (µg/kg)** [1]~~		~~**PEC/RAC (= ETR)**~~
~~**Step 1**~~	~~12330~~	~~0.2~~

Table 9.4-13: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for total sulfur for aquatic organisms based on standard FOCUS Step 1 - 2 calculations for single/twofold application of BAS 768 00 F at 1-2x 2400 g a.s./ha in 'winter cereals'

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Group**	**Sed. dwell. prolonged**
Test species		***O. mykiss***	***O. mykiss* (28 d)**	***D. magna***	***D. magna***	***D. subspicatus***	**Test species**	***C. riparius***
Endpoint		LC_{50}	NOEC	EC_{50}	EC_{10}	E_rC_{50}	**Endpoint**	NOEC
(µg/L)		> 16	> 16	> 16	> 16	> 16	**(µg/kg)**	592900
AF		100	10	100	10	10	**AF**	10
RAC (µg/L)		> 0.16	> 1.6	> 0.16	> 1.6	> 1.6	**RAC (µg/kg)**	59290
FOCUS Scenario	**PEC $_{gl\text{-}sw\ max}$ [1] (µg/L)**	**PEC/RAC (= ETR)**					**PEC $_{gl\text{-}sed\ max}$ [1] (µg/kg)**	**PEC/RAC (= ETR)**
Step 1								
	159.52	**< 997**	**< 100**	**< 997**	**< 100**	**< 100**	10200	0.172
Step 2								
N-Europe	22.07	**< 138**	**< 14**	**< 138**	**< 14**	**< 14**	1820	
S-Europe	22.07	**< 138**	**< 14**	**< 138**	**< 14**	**< 14**	3390	

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 PEC_{sed} is derived from twofold application, also covering single application.~~ $PEC_{sw/sed}$ values for total sulfur were calculated for single application and twofold application, respectively. For details, please refer to Part B, Section 8.9.

For the intended single and twofold application of BAS 768 00 F at 1 - 2x 2400 g a.s./ha in 'spring and winter cereals', the calculated PEC/RAC ratio for sulfur indicates no acceptable risk for all aquatic organisms except for sediment-dwelling organisms based on tier 1 toxicity data and standard FOCUS Step 1-2 $PEC_{sw/sed}$ values. However, the $PEC_{sw/sed}$ values are based on total sulfur (dissolved and undissolved) and therefore the ETR is overestimated. Furthermore, the endpoint for assessing the risk to all aquatic organisms in the water column is based on dissolved sulfur and is an unbound value that represents the maximum solubility limit. Using the first approach presented in Section 8.9, where the PEC_{sw} values are set to the maximum water solubility, no unacceptable risk can be concluded since no effects were observed at the water solubility limit in toxicity studies. For these reasons, the risk to all aquatic organisms can be considered as low. Therefore, sulfur can be considered of no concern for aquatic organisms. an ~~acceptable risk for sediment-dwelling organisms based on conservative Step 1 assumptions~~. Therefore, no further assessment is necessary.

Risk assessment for the metabolites of sulfur (BAS 175 F)

There are no metabolites of potential concern for the risk assessments of aquatic organisms based on ~~(please refer to the~~ EFSA Scientific Report (2008) 221, 1-70~~)~~. Nevertheless, a pH-dependency of soil sorption cannot be excluded for sulfate, hence a risk to aquatic organisms cannot be excluded for sulfate. Therefore, PEC_{sed} for sulfate were calculated using a minimum default $K_{f,oc}$ value of 0 mL g^{-1} and a maximum default $K_{f,oc}$ value of 10000 mL g^{-1} due to potential pH dependency. A risk assessment for sulfate is shown below for the available sediment-dweller study in sulfate spiked water.

Acceptability of risk

In Table 9.4-14, the exposure-toxicity ratio (ETRs) for sediment dwellers is given for the use of BAS 768 00 F in 'winter cereals' for the metabolite, sulfate. Worst-case PEC_{sw} value from twofold application (2x 2400 g a.s./ha) in 'winter cereals' is used for the risk assessment, and this covers all intended uses. For details on the $PEC_{sw/sed}$ calculations please refer to Part B, Section 8.9.

Table 9.4-14: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for sulfate for sediment-dwellers based on worst-case FOCUS Step 1 - 2 calculations for twofold applications (2x 2400 g a.s./ha) of BAS 768 00 F in 'winter cereals'

Group			**Sed. dwell. prolonged**
Test species			***C. riparius***
Endpoint			NOEC
(µg/L)			81900
AF			10
RAC (µg/L)			8190
K_{oc}	**FOCUS Scenario [1)]**	**PEC gl-sw max [2)] (µg/L)**	**PEC/RAC (= ETR)**
0 and 10000	**Step 1**		
		9720	**1.2**
	Step 2		
	N-Europe	1640	0.2
	S-Europe	3160	0.4

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) Risk envelope: spring cereals are covered by winter cereals at Step 1.

2) Worst-case PEC_{sed} is derived from twofold application, also covering single application. For details, please refer to Part B, Section 8.9.

For the intended twofold application of BAS 768 00 F at 2x 2400 g a.s./ha in 'spring and winter cereals', the calculated PEC/RAC ratio for sulphate indicates an acceptable risk for sediment-dwelling organisms based on conservative Step 2 assumptions. Therefore, no further assessment is necessary.

Risk assessment for the formulated product (BAS 768 00 F)

According to the EFSA AGD (2013) a mixture toxicity RA for the formulated product is required to determine synergistic, additive or antagonistic effects of the formulation. However, the proposed standard approach (*i.e.*, calculation of the model deviation ratios (MDR) using the concentration addition (CA) model) for comparison of the measured and calculated mixture toxicity is not meaningful for BAS 768 00 F because no definitive endpoints are available for the active substance sulfur due to its low water solubility and low toxicity to aquatic organisms. In the risk assessment for sulfur, the limit of its water solubility and toxicity value is considered to be > 0.016 mg a.s./L (see above). However, this is not an adequate value for MDR calculations because in the toxicity studies no effects were observed at concentrations which clearly exceeded the water solubility by several orders of magnitude. In addition, no definitive endpoints were derived from the formulation studies with daphnids and algae.
As an alternative approach, the measured toxicity of the product is compared to the calculated mixture toxicity of the active substance mefentrifluconazole, whereas sulfur is disregarded (see Table 9.2.4-15).

Table 9.2.4-15: Comparison of the measured toxicity of the formulated product BAS 768 00 F and the calculated mixture toxicity of the active substance mefentrifluconazole (sulfur disregarded)

Test species	Test system	Endpoint	Measured toxicity of the a.s. ($EC_{x\ a.s.}$) [µg a.s./L]		Measured toxicity of BAS 768 00 F ($EC_{x\ PPP}$) [µg product/L]	Calculated mixture toxicity ($EC_{x\ mix\text{-}CA}$) [µg mixture/L] 1)	MDR ($EC_{x\ mix\text{-}CA}$ / $EC_{x\ PPP}$)
O. mykiss	acute	LC_{50} (96 h)	mefentri-fluconazole	532	15300	28643	1.87
D. magna	acute	EC_{50} (48 h)	mefentri-fluconazole	944	> 80000	50825	0.64
P. subcapitata	--	E_rC_{50} (72 h)	mefentri-fluconazole	1352 2)	> 80000	72792	0.91

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 substance mefentrifluconazole, its nominal content within the formulation (*i.e.*, 25 g mefentrifluconazole/L) and the product density of 1.346 g/cm^3 (second active substance sulfur is disregarded).

2) For mefentrifluconazole, the slightly higher algae endpoint of the study with the green algae *P. subcapitata* is considered, since the formulation study was conducted with green alga as well.

The calculated MDR values (0.64 - 1.87) indicate that the measured toxicity of the product is comparable to the toxicity expected from the data of mefentrifluconazole. Thus, it can be concluded that sulfur and/or other co-formulants as contained in BAS 768 00 F do not significantly contribute to the toxicity of the formulation. Overall, the results of the formulation studies indicate a low toxicity of BAS 768 00 F to aquatic organisms and no increased toxicity is to be expected by interaction of the active substances. It is therefore appropriate to assess the risk of BAS 768 00 F by considering each of the active substances separately. For the sake of completeness, since the formulation is not stable in the environment and exposure of aquatic organisms to the formulated product can only occur via spray drift, a risk assessment was also conducted on the formulated product comparing the measured formulation endpoints (ECx_{ppp}) and the maximum instantaneous PEC_{sw} values resulting from drift entry following single application. The results are summarized in Table 9.4-16.

Furthermore, 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.

Table 9.4-16: Aquatic organisms: acceptability of risk ($PEC_{ini}/RAC_{PPP} < 1$) for BAS 768 00 F for each organism group based on the maximum instantaneous PEC_{sw} value following single application at 1x 4.0 L product/ha in 'spring and winter cereals'

Group		**Fish acute**	**Inverteb. acute**	**Algae**
Test species		***O. mykiss***	***D. magna***	***P. subcapitata***
Endpoint		$LC_{50\ PPP}$	$EC_{50\ PPP}$	$E_rC_{50\ PPP}$
(µg/L)		15300	> 80000	> 80000
AF		100	100	10
RAC_{PPP} (µg/L)		153	> 800	> 8000
Non-spayed buffer zone	**$PEC_{sw\ ini}$ (µg/L)**	**PEC/RAC ratio (= ETR)**		
1 m (standard distance)	~~23.408~~ 34.950	~~0.2~~ 0.23	~~< 0.04~~ 0.04	~~< 0.004~~ 0.004

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.**

For the intended uses in 'spring and winter cereals', the calculated PEC/RAC ratios for BAS 768 00 F indicate an acceptable risk for all groups of aquatic organisms based on standard assumptions. 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.

Sulfur
No bioconcentration study is available for sulfur. In general, the potential of bioconcentration for sulfur is considered to be negligible, as sulfur is a naturally occurring mineral and an essential element in the metabolism of all living organisms (see DAR (March 2008) and in the Final Addendum to the DAR (2008) for sulfur). Elemental sulfur is known to enter the sulfur cycle immediately after application, *i.e.,* elemental sulfur is transformed by water bacteria into various stages of oxidation which are soluble and thus made available for further uptake by various organisms such as plants and animals. No reliable experimental log K_{ow} value exists (related to its extremely low water solubility). The active substance is not expected to accumulate in fish. In conclusion, the risk arising from bioaccumulation of the active substance is considered to be low.

Thus, residues of the active substances of BAS 768 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 risk assessment for the fungicidal product BAS 768 00 F, the active substances mefentrifluconazole and sulfur and the relevant metabolites demonstrates that the application of BAS 768 00 F in 'spring and winter cereals' according to good agricultural practice is of low risk to aquatic ecosystems with no need for any mitigation measures.

References

EFSA (2013) EFSA Scientific Opinion. Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters. EFSA Journal 2013; 11(7): 3290.

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9.6 Effects on bees (KCP 10.3.1)

zRMS Comments:	The submitted risk assessment based on SANCO, 2002 guidance was accepted. New studies for acute and chronic toxicity for formulation were submitted and accepted. The EU agreed endpoints and accepted endpoints from submitted studies were used in risk assessment performed in accordance with the SANCO guidance, 2002, **Mefentrifluconazole**. In case of chronic toxicity for *Apis mellifera* (larvae) the used endpoints based on EFSA Journal, 2018, represent a worse case compared with new proposed endpoints presented in Draft Assessment Report (DAR) of mefentrifluconazole (January 2018), Vol. 3CA, B.9: NOEC (22 d) = 162.4 µg a.s./kg food EC_{50} (22 d) > 324.8 mg a.s./kg food The lower values were accepted as a worse case and were used in risk assessment. **Sulfur**. All submitted studies, presented in Table 9.6-2 (References) were evaluated during active substance renewal. The correct Reference should be EFSA Journal 2023;21(3):7385. The hazard quotients are below the trigger value of 50 considering SANCO guidance indicating that the active substances and formulation pose an acceptable acute risk to bees. The chronic risk assessment was also performed and accepted. Based on calculation for chronic risk – the risk was not acceptable. Additionally, the semi field tunnel study (2018) was performed. The study was accepted. Based on presented study conclusion the risk for bees is acceptable. Therefore, an acceptable risk to bees is expected from the application of BAS 768 00 F.

9.6.1 Toxicity data

Acute contact and oral toxicity studies on honey bees have been carried out with BAS 768 00 F, the active substances mefentrifluconazole (BAS 750 F) and sulfur (BAS 175 F, tested as Sulphur Dust and Sulphur 80% WG).

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.

For sulfur, a new dossier for EU re-registration within the AIR 4 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 have been carried out with sulfur (tested as Sulphur 80% WG). Moreover, new data from a chronic toxicity study on adult honey bees and two repeated exposure toxicity study (8 d and 22 d) on honey bee larvae are available. Furthermore, a semi-field tunnel test with sulfur (tested as Sulphur 80% WG) has been performed.

Additionally, a chronic toxicity study on adult honey bees and toxicity studies on honey bee larvae as well

as acute studies on bumblebees have been carried out with BAS 768 00 F.

All studies are listed in Table 9.6-1, Table 9.6-2 and Table 9.6-3. 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 9.6-1: Endpoints and effect values for mefentrifluconazole relevant for the risk assessment for bees

Species	Substance	Exposure System	Results	Reference
Apis mellifera (adults)	mefentrifluconazole	acute oral	LD_{50} (48 h) > 100 µg a.s./bee	EFSA Journal 2018;16(7):5379 2015/1128674
Apis mellifera (adults)	mefentrifluconazole	acute contact	LD_{50} (48 h) > 100 µg a.s./bee	EFSA Journal 2018;16(7):5379 2015/1128674
Apis mellifera (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
Apis mellifera (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
Apis mellifera (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 #
Apis mellifera (larvae)	mefentrifluconazole	repeated exposure	NOED (22 d) = 25 µg a.s./larva ED_{50} (22 d) > 50 µg a.s./larva	EFSA Journal 2018;16(7):5379 2017/1045562
Bombus terrestris (adults)	mefentrifluconazole	acute oral	LD_{50} (96 h) > 195.4 µg a.s./bumble bee	EFSA Journal 2018;16(7):5379 2014/1275250
Bombus terrestris (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 9.6-2: Endpoints and effect values for sulfur relevant for the risk assessment for bees

Species	Substance	Exposure System	Results	Reference
Apis mellifera (adults)	sulfur (tested as Sulphur Dust)	acute oral	LD_{50} > 105.2 µg a.s./bee	EFSA Scientific Report (2008) 221, 58-70
Apis mellifera (adults)	sulfur (tested as Sulphur 80% WG)	acute oral	LD_{50} > 100 µg a.s./bee	EFSA Scientific Report (2008) 221, 58-70
Apis mellifera (adults)	sulfur (tested as Sulphur 80% WG)	acute oral	LD_{50} > 700.1 µg a.s./bee	not EU evaluated 2016/1345280
Apis mellifera (adults)	sulfur (tested as Sulphur Dust)	acute contact	LD_{50} > 98.5 µg a.s./bee	EFSA Scientific Report (2008) 221, 58-70
Apis mellifera (adults)	sulfur (tested as Sulphur 80% WG)	acute contact	LD_{50} > 100 µg a.s./bee	EFSA Scientific Report (2008) 221, 58-70
Apis mellifera (adults)	sulfur (tested as Sulphur 80% WG)	acute contact	LD_{50} > 700.1 µg a.s./bee	not EU evaluated 2016/1345280
Apis mellifera (adults)	sulfur (tested as Sulphur 80% WG)	chronic oral	LDD_{50} (10 d) > 149.3 µg a.s./bee/day NOEDD (10 d) ≥ 149.3 µg a.s./bee/day	not EU evaluated 2017/1219185
Apis mellifera (larvae)	sulfur (tested as Sulphur 80% WG)	repeated exposure [1)]	LD_{50} (8 d) = 149.7 µg a.s./larva NOED (8 d) = 60.1 µg a.s./larvae	not EU evaluated 2017/1218043
Apis mellifera (larvae)	sulfur (tested as Sulphur 80% WG)	repeated exposure	ED_{50} (22 d) = 10.4 µg a.s./larva ED_{10} (22 d) = 1.25 µg a.s./larva NOED (22 d) = 0.90 µg a.s./larvae	not EU evaluated 2023/2005062
Higher-tier studies (tunnel test, field studies)				
Apis mellifera (colonies)	sulfur (tested as Sulphur 80% WG)	semi-field	Semi-field tunnel test (*Phacelia tanacetifolia*): no unacceptable lethal or sublethal effects on honey bee colonies exposed to 12.5 kg product/ha (equivalent to 10 kg a.s./ha)	not EU evaluated 2018/1232880

[1)] Study comprised repeated exposure of honey bee larvae over a period of 8 days, therefore no assessment of adult emergence was conducted.

Table 9.6-3: Endpoints and effect values of BAS 768 00 F relevant for the risk assessment for bees

Species	Product	Exposure System	Results	Reference
Apis mellifera (adults)	BAS 768 00 F	acute oral	LD_{50} (48 h) > 845 µg/bee (corresponding to 392 µg total a.s./bee)	~~not~~ EU evaluated 2021/2014196
Apis mellifera (adults)	BAS 768 00 F	acute contact	LD_{50} (48 h) > 1000 µg/bee (corresponding to 464 µg total a.s./bee)	~~not~~ EU evaluated 2021/2014196
Apis mellifera (adults)	BAS 768 00 F	chronic oral	LDD_{50} (10 d) = 367 µg/bee/day (corresponding to 170.4 µg total a.s./bee/day) NOEDD (10 d) = 45.5 µg/bee/day (corresponding to 21.1 µg total a.s./bee/day)	~~not~~ EU evaluated 2021/2014218
Apis mellifera (larvae)	BAS 768 00 F	repeated exposure	ED_{50} (22 d) = 7.7 µg/larva (corresponding to 3.6 µg total a.s./larva) ED_{10} (22 d) = 4.0 µg/larva (corresponding to 1.9 µg total a.s./larva) NOED (22 d) = 2.7 µg/larva (corresponding to 1.3 µg total a.s./larva)	~~not~~ EU evaluated 2021/2014219
Bombus terrestris (adults)	BAS 768 00 F	acute oral	LD_{50} (48 h) > 976.9 µg/bee (corresponding to 453.6 µg total a.s./bee)	~~not~~ EU evaluated 2022/2010697
Bombus terrestris (adults)	BAS 768 00 F	acute contact	LD_{50} (48 h) > 1000.0 µg/bee (corresponding to 464.~~4~~ µg total a.s./bee)	~~not~~ EU evaluated 2022/2010697

9.6.1.1 Justification for new endpoints

Effects of the formulation BAS 768 00 F on honey bees were not evaluated as part of the EU assessment of the active substances mefentrifluconazole or sulfur. 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 sulfur, 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 sulfur (tested as Sulphur 80% WG), are used in the risk assessment in place of the EU agreed endpoints. In the original submission AIR 4, the effects of the repeated exposure honey bee larvae study was only assessed up to day 8. Therefore, the study was repeated to include the assessment of adult emergence (up to day 22).

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 9.6.1.

9.6.2 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 and is under revision by EFSA at the time of application.

The application of BAS 768 00 F is envisioned in cereals. The following risk assessment is based on the worst-case maximum single application rate of 4.0 L BAS 768 00 F/ha (equivalent to 0.100 kg mefentrifluconazole /ha and 2.400 kg sulfur/ha; see Section 9 Chapter 9.1 for details).

9.6.2.1 Hazard quotients for bees

The risk to honey bees from the use of mefentrifluconazole, sulfur and BAS 768 00 F was assessed using the maximum single application rate and the LD_{50} 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.

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

A hazard quotient of less than 50 indicates a low risk to honey bees colonies in the field (see Table 9.6-4 to Table 9.6-6).

Table 9.6-4: First-tier assessment of the risk for bees due to the use of mefentrifluconazole as contained in BAS 768 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: $Q_H \leq 50$**
Oral toxicity	> 100	100	< 1.0
Contact toxicity	> 100		< 1.0

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

Table 9.6-5: First-tier assessment of the risk for bees due to the use of sulfur as contained in BAS 768 00 F according to the proposed use pattern

Intended use	cereals		
Product	sulfur		
Application rate (g a.s./ha)	2 x 2400		
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	> 700.1	2400	< 3.4
Contact toxicity	> 700.1		< 3.4

Table 9.6-6: First-tier assessment of the risk for bees due to the use of BAS 768 00 F according to the proposed use pattern

Intended use	cereals		
Product	BAS 768 00 F		
Application rate (L/ha)	2 x 4.0		
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	> 845	5440 [1)]	< 6.4
Contact toxicity	> 1000		< 5.4

Q_{HO}, Q_{HC}: Hazard quotients for oral and contact exposure.
1) Taking into account a single application of 4.0 L product/ha and the density of BAS 768 00 F of 1.36 g/cm^3.

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) and sulfur (BAS 175 F) as well as BAS 768 00 F. For mefentrifluconazole, chronic toxicity study on honey bees resulted in a NOEDD ≥ 110.5 µg a.s./bee/day. The NOED derived from the repeated exposure study on honey bee larvae was 25 µg a.s./larva. For sulfur, chronic toxicity study on honey bees resulted in a NOEDD ≥ 149.3 µg a.s./bee/day. The NOED derived from the repeated exposure study on honey bee larvae was 0.90 µg a.s./larva. For BAS 768 00 F, the chronic toxicity study on honey bees resulted in a NOEDD of 21.1 µg total a.s./bee/day. The NOED derived from the repeated exposure study on honey bee larvae was 1.3 µg 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 768 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 768 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 µg a.s./bee/day) and the exposure (also in µg a.s./bee/day) is then calculated as follows:

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

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 µg a.s./larva) and the exposure (residues ingested over the five-day feeding period in µg a.s./larva) is calculated by the following equation:

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

Following EPPO (2010) the expected worst-case residue consumption of larvae and adult bees was calculated. For mefentrifluconazole and sulfur, 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 768 00 F (100 g mefentrifluconazole/ha and 2400 g sulfur/ha; see Table 9.6-7).

Table 9.6-7: 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 [1)]	6.37 mg a.s./kg
sulfur (Application rate 2400 g a.s./ha)	63.70 mg a.s./kg [1)]	152.88 mg a.s./kg
BAS 768 00 F (Application rate 100 g mefentrifluconazole/ha and 2400 g sulfur/ha)	63.70 mg total a.s./kg[1)]	159.25 mg total a.s./kg
Nectar		
mefentrifluconazole (Application rate 100 g a.s./ha)	3.99 mg a.s./kg [1)]	0.40 mg a.s./kg
sulfur (Application rate 2400 g a.s./ha)	3.99 mg a.s./kg [1)]	9.58 mg a.s./kg
BAS 768 00 F (Application rate 100 g mefentrifluconazole/ha and 2400 g sulfur/ha)	3.99 mg total a.s./kg [1)]	9.98 mg total a.s./kg

[1)] 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), sulfur (BAS 175 F) and BAS 768 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 768 00 F (see Table 9.6-8 to Table 9.6-10). The calculated chronic TER values are given in Table 9.6-11 to Table 9.6-13. 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, except for the TER calculated for the risk to larvae by sulfur and BAS 768 00 F.**

Table 9.6-8: 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	0.170 µg a.s./bee/day	0.0790 µg a.s./larva
Total residue intake	**0.170 µg a.s./bee/day**	**0.0917 µg a.s./larva**

Table 9.6-9: Total residue intake for adult honey bees and larvae following exposure to BAS 175 F according to the proposed uses

Honey bee stage	Adult	Larva (over 5 days)
Residue in pollen	152.88 mg a.s./kg (= 0.15288 µg a.s./mg)	152.88 mg a.s./kg (= 0.15288 µg a.s./mg)
Pollen consumption	0	2.0 mg/larva
Residue intake through pollen	0 µg a.s./bee/day	0.306 µg a.s./larva
Residue in nectar	9.58 mg a.s./kg (= 0.00958 µg a.s./mg)	9.58 mg a.s./kg (= 0.00958 µg a.s./mg)
Nectar consumption	426.7 mg/bee/day	198.0 mg/larva

Residue intake through nectar	4.09 µg a.s./bee/day	1.90 µg a.s./larva
Total residue intake	**4.09 µg a.s./bee/day**	**2.20 µg a.s./larva**

Table 9.6-10: Total residue intake for adult honey bees and larvae following exposure to BAS 768 00 F according to the proposed uses

Honey bee stage	Adult	Larva (over 5 days)
Residue in pollen	159.25 mg total a.s./kg (= 0.15925 µg total a.s./mg)	159.25 mg total a.s./kg (= 0.15925 µg total a.s./mg)
Pollen consumption	0	2.0 mg/larva
Residue intake through pollen	0 µg total a.s./bee/day	0.319 µg a.s./larva
Residue in nectar	9.98 mg total a.s./kg (= 0.00998 µg total a.s./mg)	9.98 mg total a.s./kg (= 0.00998 µg total a.s./mg)
Nectar consumption	426.7 mg/bee/day	198.0 mg/larva
Residue intake through nectar	4.26 µg a.s./bee/day	1.98 µg a.s./larva
Total residue intake	**4.26 µg a.s./bee/day**	**2.29 µg a.s./larva**

Table 9.6-11: 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	0.170 µg a.s./bee/day	≥ 650	1
Larvae	Oral	25 µg a.s./larva	0.0917 µg a.s./larva	273	1

Table 9.6-12: Chronic risk to adult bees and larvae following the use of BAS 175 F in cereals using TER approach

Honey bee stage	Exposure route	NOED	Worst case residue intake	TER_{ch}	Trigger value
Adult	Oral	≥ 149.3 µg a.s./bee/day	4.09 µg a.s./bee/day	≥ 37	1
Larvae	Oral	0.90 µg a.s./larva	2.20 µg a.s./larva	**0.41**	1

TER values shown in **bold** are below the proposed trigger.

Table 9.6-13: Chronic risk to adult bees and larvae following the use of BAS 768 00 F in cereals using TER approach

Honey bee stage	Exposure route	NOED	Worst case residue intake	TER_{ch}	Trigger value
Adult	Oral	21.1 µg total a.s./bee/day	4.26 µg a.s./bee/day	5	1

Larvae	Oral	1.3 µg total a.s./larva	2.29 µg a.s./larva	**0.57**	1

TER values shown in **bold** are below the proposed trigger.

All calculated TERs for chronic adult and also for bee larvae and BAS 750 F exceed the suggested trigger by at least a factor of 5.

For larvae there is potential risk indicated for BAS 175 F and the product BAS 768 00 F based on the laboratory data and default exposure values. The toxicity data suggest that larval risk is driven by BAS 175 F, also in the product BAS 768 00 F. In fact, considering the content and toxicity of BAS 175 F within BAS 768 00 F, the toxicity of the product can be explained based on this active. Having identified BAS 175 F as the driver for toxicity, the refined assessment can focus on BAS 175 F while also addressing the product BAS 768 00 F. For BAS 175 F a semi-field study with Sulphur 80% WG is available und described below (chapter 9.6.2.2).

Higher-tier risk assessment for bees (tunnel test, field studies)
In addition to the laboratory studies, a higher-tier semi-field tunnel test with sulfur (BAS 175 F, tested as Sulphur 80% WG) (DocID 2018/1232880) has been performed according to OECD guidance document No. 75 (2007) and EPPO Standard PP 1/170(4) (2010). The study was carried out to provide further evidence that the potential toxicity of sulfur covers the effects on honey bee larvae and bee brood development under more realistic conditions for sulfur as well as BAS 768 00 F. Sulfur was applied at a rate of 12.5 kg product/ha (equivalent to 10 kg 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 test item caused no significant differences on adult and pupal bee mortality, foraging activity, behaviour of bees, colony strength and brood development were observed between the test item treatment and the control when applied at a rate of 10 kg a.s./ha under semi-field conditions (tunnel) to *P. tanacetifolia* during active foraging conditions.

The results of the higher tier studies, conducted under more realistic and reliable risk scenarios, conclude that the proposed use of sulfur and by extension BAS 768 00 F, according to good agricultural practice, presents low risk to honey bees and will not adversely affect honey bee colonies.

9.6.3 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 BAS 768 00 F. The oral and contact LD_{50} were determined to be > 976.9 µg/bumble bee and > 1000.0 µg/bumble bee, respectively. Both endpoints exceed or are similar to the acute endpoints for honeybees, suggesting that the active substances mefentrifulconazole and sulfur as contained in BAS 768 00 F poses no unacceptable risk to bumblebees at the proposed use rate.

9.6.4 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.

9.6.5 Overall conclusions

The hazard quotients for BAS 768 00 F and the active substances mefentrifluconazole and sulfur 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 768 00 F according to the proposed uses. This is confirmed by a risk assessment following EPPO (2010) for chronic exposure to adult honey bees. The remaining risk for honey bee larvae can be attributed to sulfur and was addressed with a semi-field study with Sulphur 80% WG showing no negative effects on bee larvae and brood / overall colony development. The results of this semi-field study confirms that low risk is expected to honey bee colonies exposed to BAS 768 00 F according to the proposed uses.

References

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9.7 Effects on arthropods other than bees (KCP 10.3.2)

zRMS Comments:	The submitted risk assessment based on the "Guidance Document on Terrestrial Ecotoxicology", 2002, was accepted. New studies for formulation at Tier 1 and Tier 2 were submitted and accepted. The risk assessment is based on accepted studies endpoints for formulation BAS 768 00 F. The following endpoints at Tier 1 were used in risk assessment: • *Typhlodromus pyri:* LR_{50} > 8.0 L/ha • *Aphidius rhopalosiphi: :* LR_{50} > 8.0 L/ha As the hazard quotients are higher than trigger value (HQ ≤ 2) at tier 1, the refinement at higher tier was provided. indicating that the formulation poses an acceptable risk to arthropods other than bees. Therefore, an acceptable risk to arthropods other than bees is expected if the application of the BAS 768 00 F is in accordance with intended uses.

9.7.1 Toxicity data

The toxicity of BAS 768 00 F to non-target arthropods has been investigated by carrying out Tier I tests on *Aphidius rhopalosiphi* and *Typhlodromus pyri* and a Tier II test on *A. rhopalosiphi*. All studies are listed in Table 9.7-1.

New data submitted with this application are listed in Appendix 1 and summarized in Appendix 2.

Table 9.7-1: Endpoints and effect values for BAS 768 00 F relevant for the risk assessment for non-target arthropods

Species	Product	Exposure System	Results	Reference
Typhlodromus pyri (protonymphs)	BAS 768 00 F	laboratory test glass plates 2D exposure	LR_{50} > 8.0 L/ha Corrected mortality: 0.0% at 0.5 L/ha 1.0% at 1.0 L/ha 1.0% at 2.0 L/ha 2.0% at 4.0 L/ha 11.1% at 8.0 L/ha	not EU evaluated 2021/2014189
Aphidius rhopalosiphi (adults)	BAS 768 00 F	laboratory test glass plates 2D exposure	LR_{50} > 8.0 L/ha Corrected mortality [1)]: 0.0% at 0.5 L/ha 0.0% at 1.0 L/ha -2.6% at 2.0 L/ha 0.0% at 4.0 L/ha 2.6% at 8.0 L/ha	not EU evaluated 2021/2014190
Aphidius rhopalosiphi	BAS 768 00 F	extended laboratory	LR_{50} > 8.0 L/ha	not EU evaluated

Species	Product	Exposure System	Results	Reference
(protonymphs)		test natural substrate (bean plant) 3D exposure	ER_{50} > 8.0 L/ha Corrected mortality: -3.6% at 1.5 L/ha 0.0% at 3 L/ha -3.6% at 4 L/ha 0.0% at 6 L/ha -3.6% at 8 L/ha Effects on reproduction [1]: -4.5% at 1.5 L/ha 0.0% at 3 L/ha -6.1% at 4 L/ha 4.5% at 6 L/ha -1.7% at 8 L/ha	2022/2010703

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

9.7.1.1 Justification for new endpoints

Effects of BAS 768 00 F on non-target arthropods other than bees were not evaluated as part of the EU assessment of the active substances mefentrifluconazole and sulfur. Hence, all relevant data and assessments considering this formulation are provided here and are considered adequate.

9.7.2 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).

9.7.2.1 Risk assessment for in-field exposure

The application of BAS 768 00 F is envisioned in cereals. The following risk assessment is based on the worst-case field application rate of 2 × 4.0 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:

$$\mathrm{PER}_{\mathrm{in-field}} = \text{Application rate [L/ha]} * \mathrm{MAF}$$

Default foliar and soil MAF values following multiple applications are given in the ESCORT 2 Guidance Document and are the following for BAS 768 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 768 00 F (see Section 9 Chapter 9.1 for details), the MAF (soil) will not be considered in the following risk assessment. Thus, the $PER_{in\text{-}field}$ is 6.8 L/ha.

The potential risk for non-target arthropods exposed in-field to BAS 768 00 F was assessed by calculating the hazard quotient (HQ = exposure/toxicity, see Table 9.7-2) for tier I standard laboratory studies according to the formula:

$$HQ_{in\text{-}field} = \frac{PER_{in\text{-}field}\ [L/ha]}{LR_{50}\ [L/ha]}$$

For higher tier laboratory studies risk is acceptable if the $PER_{in\text{-}field}$ is below the relevant endpoint (see Table 9.7-2).

Table 9.7-2: First- and higher-tier assessment of the in-field risk for non-target arthropods due to the use of BAS 768 00 F according to the proposed use pattern

Intended use	cereals		
Product	BAS 768 00 F		
Application rate (L/ha)	2 x 4.0		
MAF	1.7 (vegetation)		
Test species	**Tier I**		
	LR_{50} (lab.) [L/ha]	**$PER_{in\text{-}field}$ [L/ha]**	**$HQ_{in\text{-}field}$ criterion: HQ ≤ 2**
Typhlodromus pyri	> 8.0	6.8	0.9
Aphidius rhopalosiphi	> 8.0		0.9
Test species	**Higher-tier**		
	Endpoint [L/ha]	**$PER_{in\text{-}field}$ [L/ha]**	**$PER_{in\text{-}field}$ below rate with ≤ 50% effect?**
Aphidius rhopalosiphi	LR_{50} > 8.0 ER_{50} > 8.0	6.8	yes yes

MAF: Multiple application factor; PER: Predicted environmental rate; HQ: Hazard quotient..

9.7.2.2 Risk assessment for off-field exposure

Exposure of non-target arthropods living in off-field areas to BAS 768 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:

$$PER_{off\text{-}field} = \frac{\text{maximum } PER_{in\text{-}field} * (\%\ \text{drift}/100)}{\text{vegetation distribution factor}}$$

A vegetation distribution or dilution factor is included in the equation when calculating PER values from toxicity endpoints derived from two-dimensional studies (Table 9.7-3). A dilution factor of 10 is recommended by ESCORT 2.

For 2 applications of BAS 768 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 9.7-3: $PER_{off\text{-}field}$ values following application of BAS 768 00 F

Study type [Exposure scenario]	Maximum $PER_{in\text{-}field}$ [L/ha]	Drift factor [% drift/100]	Vegetation distribution factor	$PER_{off\text{-}field}$ [L/ha]
2D	6.8	0.0238	10	0.01618
3D			--	0.1618

To assess the potential risk of BAS 768 00 F to off-field non-target arthropods (see Table 9.7-4), the $PER_{off\text{-}field}$ (Table 9.7-3) is compared to the toxicity endpoints of tier I standard laboratory studies according to the following equation:

$$HQ_{off\text{-}field} = \frac{PER_{off\text{-}field}\ [L/ha]}{LR_{50}\ [L/ha]} * \text{correction factor}$$

For higher tier laboratory studies risk is acceptable if the $PER_{off\text{-}field}$ 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 9.7-4: First- and higher-tier assessment of the off-field risk for non-target arthropods due to the use of BAS 768 00 F according to the proposed use pattern

Intended use	cereals				
Product	BAS 768 00 F				
Application rate (L/ha)	2 x 4.0				
MAF	1.7 (vegetation)				
vdf	10 (2D exposure) / - (3D exposure)				
Test species	**Tier I**				
	LR_{50} (lab.) [L/ha]	**Drift rate (%)**	**$PER_{off\text{-}field}$ [L/ha]**	**CF**	**$HQ_{off\text{-}field}$ criterion: HQ ≤ 2**
Typhlodromus pyri	> 8.0	2.38	0.01618	10	0.02
Aphidius rhopalosiphi	> 8.0				0.02
Test species	**Higher-tier**				
	Endpoint [L/ha]	**Drift rate (%)**	**$PER_{off\text{-}field}$ [L/ha]**	**CF**	**corr. $PER_{off\text{-}field}$ below rate with ≤ 50 % effect?**
Aphidius rhopalosiphi	LR_{50} > 8.0 ER_{50} > 8.0	2.38	0.1618	5	yes yes

MAF: Multiple application factor; vdf: Vegetation distribution factor; (corr.) PER: (corrected) Predicted environmental rate; CF: Correction factor; HQ: Hazard quotient.

9.7.2.3 Additional higher-tier risk assessment

Not relevant.

9.7.2.4 Risk mitigation measures

No risk mitigation needed.

9.7.3 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 768 00 F according to the proposed use pattern. No unacceptable effects on non-target arthropods are expected in in-field and off-field habitats.

9.8 Effects on non-target soil meso- and macrofauna (KCP 10.4)

zRMS Comments:	The submitted risk assessment was corrected in accordance with PECs assessment (Section B8, p. 8.7). New studies were submitted and accepted. **Sulfur**. All submitted studies, presented in Table 9.8-2 (References) were evaluated during active substance renewal. The correct Reference should be EFSA Journal 2023;21(3):7385. The risk assessment for formulation is based on accepted endpoints. The max PECs values for active substances, their metabolites and formulation (see Section 8. Fate and behavior) were used for acute and long-term risk assessment. Since risk assessment for non-target soil meso- and macrofauna (earthworm and other organisms) is acceptable at Tier 1, then no further assessment was required. An acceptable risk to non-target soil organisms meso- and macrofauna is expected if the formulation is used in accordance with proposed uses.

9.8.1 Toxicity data

Studies on the toxicity to earthworms and other non-target soil organisms (meso- and macrofauna) have been carried out with mefentrifluconazole, sulfur 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 sulfur and as BAS 768 00 F. All studies are listed in Table 9.8-1, Table 9.8-2 and Table 9.8-3.

New data submitted with this application are listed in Appendix 1 and summarized in Appendix 2.

Table 9.8-1: 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 [#]				
Eisenia fetida	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 ~~Draft Assessment Report (DAR) of mefentrifluconazole, Vol. 3, B.9 2015/1003342~~
Chronic				

Eisenia fetida	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
Eisenia fetida	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
Folsomia candida	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
Folsomia candida	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
Hypoaspis aculeifer	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
Hypoaspis aculeifer	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 in accordance with the EPPO earthworm scheme 2002 due to a log P_{OW} >2 of mefentrifluconazole.

Table 9.8-2: Endpoints and effect values of sulfur for the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna)

Species	Substance/metabolite	Exposure System	Results	Reference
Chronic				
Eisenia fetida	sulfur (tested as Sulphur Dust) [1)]	Mixed into substrate 56 d 10% peat content	NOEC ≥ 1000 mg/kg dry soil (equivalent to 985 mg a.s./kg dry soil) **EC_{10} = 740 mg/kg dry soil (equivalent to 728.9 mg a.s./kg)**	~~not~~ EU evaluated 2018/1241025 (please refer to Draft Assessment Report of Sulphur Dust (August 2022), Vol. 3CP, B.9)
Folsomia candida	sulfur (tested as Sulphur Dust) [1)]	Mixed into substrate 28 d 5% peat content	NOEC = 309 mg/kg dry soil (equivalent to 304.4 mg a.s./kg dry soil) **EC_{10} = 144.8 mg/kg dry soil (equivalent to 142.6 mg a.s./dry soil)**	~~not~~ EU evaluated 2018/1241024 (please refer to Draft Assessment Report of Sulphur Dust (August 2022), Vol. 3CP, B.9)
Hypoaspis aculeifer	sulfur (tested as Sulphur Dust) [1)]	Mixed into substrate 14 d 5% peat content	**NOEC ≥ 1000 mg/kg dry soil (equivalent to 985 mg a.s./kg dry soil)** EC > 1000 mg/kg dry soil (equivalent to 985 mg a.s./kg dry soil)	~~not~~ EU evaluated 2017/1229983 (please refer to Draft Assessment Report of Sulphur Dust (August 2022), Vol. 3CP, B.9)

Values shown in **bold** are relevant for the conclusion of the risk assessment.

1) Sulphur Dust is the representative solo-formulation of sulfur containing 98.5% w/w sulfur (purity).

Table 9.8-3: Endpoints and effect values of BAS 768 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				
Eisenia andrei	BAS 768 00 F	Mixed into substrate, 56 d 10% peat content	NOEC = 320 mg/kg dry soil (equivalent to 148.6 mg total a.s./kg dry soil) EC_{10} = 404 mg/kg dry soil (equivalent to 187.6 mg total a.s./kg dry soil) **$NOEC_{CORR}$ = 74.3 mg total a.s./kg dry soil [1)] [*]** $EC_{10\,CORR}$ = 93.8 mg total a.s./kg dry soil [1)] [*]	not EU evaluated 2021/2014184
Folsomia candida	BAS 768 00 F	Mixed into substrate, 28 d 5% peat content	NOEC = 135 mg/kg dry soil (equivalent to 62.7 mg total a.s./kg dry soil) EC_{10} = 154.2 mg/kg dry soil (equivalent to 71.6 mg total a.s./kg dry soil) **$NOEC_{CORR}$ = 31.4 mg total a.s./kg dry soil [1)] [*]** $EC_{10\,CORR}$ = 35.8 mg total a.s./kg dry soil [1)] [*]	not EU evaluated 2021/2014185
Hypoaspis aculeifer	BAS 768 00 F	Mixed into substrate, 14 d 5% peat content	NOEC = 1417 mg/kg dry soil (equivalent to 658.1 mg total a.s./kg dry soil) EC_{10} = 3643.9 mg/kg dry soil (equivalent to 1692.0 mg total a.s./kg dry soil) **$NOEC_{CORR}$ = 329.1 mg total a.s./kg dry soil [1)] [*]** $EC_{10\,CORR}$ = 846.0 mg total a.s./kg dry soil [1)] [*]	not EU evaluated 2021/2014186

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 P_{OW} >2.

1) Endpoint based on sum of active substances (nominal) and taking into account a density of BAS 768 00 F of 1.346 g/cm^3.

9.8.1.1 Justification for new endpoints

Effects of the formulation BAS 768 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 or sulfur. 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 sulfur (tested as Sulphur Dust), a new chronic study on earthworms which was not evaluated as part of the EU assessment of the active substance sulfur on earthworms has been carried out. In addition, new chronic *Folsomia candida* and *Hypoaspis aculeifer* studies on sulfur (tested as Sulphur Dust) are 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.

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 EC_{10} is listed in Chapter 9.8.1.

9.8.2 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’s metabolite 1,2,4-triazole and sulfur is < 2. Therefore, the endpoint was not corrected. The active substance mefentrifluconazole has a log P_{ow} > 2, thus the endpoints were corrected.

9.8.2.1 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 sulfur. In contrast, multi-annual accumulation needs to be considered for mefentrifluconazole and its metabolite 1,2,4-triazole.

The potential risk of BAS 768 00 F, mefentrifluconazole, sulfur and the relevant metabolite 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 9.8-4 to Table 9.8-6).
The TER was calculated as follows:

$$\mathbf{TER} = \frac{\mathbf{Endpoint\ [mg/kg\ dry\ soil]}}{\mathbf{PEC_{soil}\ [mg/kg\ dry\ soil]}}$$

Table 9.8-4: 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 768 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	EC_{10} = 5.3 $\underline{EC_{10\ CORR} = 2.65}$	0.092 *	58 29
Metabolite, Reg. No. 87 084 1,2,4-triazole	NOEC ≥ 1.0	< 0.001 *	≥ 1000
Chronic effects on other soil meso- and macrofauna			
Collembola (*Folsomia candida*)			
Active substance/metabolite	**Endpoint (mg/kg dry soil)**	**PEC_{soil} (mg/kg dry soil)**	**TER_{lt} (criterion TER ≥ 5)**
mefentrifluconazole	NOEC ≥ 400 NOEC $_{CORR}$ ≥ 200	0.092 *	≥ 4348 ≥ 2174
Metabolite, Reg. No. 87 084 1,2,4-triazole	NOEC = 1.8	< 0.001 *	> 1800
Springtail (*Hypoaspis aculeifer*)			
mefentrifluconazole	NOEC ≥ 1000 NOEC $_{CORR}$ ≥ 500	0.092 *	≥ 10870 ≥ 5435
Metabolite, Reg. No. 87 084 1,2,4-triazole	NOEC = 171	< 0.001 *	> 171000

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 9.8-5: First-tier assessment of the chronic risk for earthworms and other non-target soil organisms (meso- and macrofauna) due to the use of sulfur as contained in BAS 768 00 F according to the proposed use pattern

Intended use	2 x 2400 g sulfur/ha in cereals		
Chronic effects on earthworms			
Product/active substance	**Endpoint (mg/kg dry soil)**	**PEC_{soil} (mg/kg dry soil)**	**TER_{lt} (criterion TER ≥ 5)**
sulfur (tested as Sulphur Dust) [1)]	EC_{10} = 728.9	~~0.640 *~~ 1.274	~~1139~~ 564
Chronic effects on other soil macro- and mesofauna			
Product/active substance	**Endpoint (mg/kg dry soil)**	**PEC_{soil} (mg/kg dry soil)**	**TER_{lt} (criterion TER ≥ 5)**
Collembola (*Folsomia candida*)			
sulfur (tested as Sulphur Dust) [1)]	EC_{10} = 142.6	~~0.640~~ * 1.274	~~223~~ 112
Soil mites (*Hypoaspis aculeifer*)			
sulfur (tested as Sulphur Dust) [1)]	NOEC ≥ 985	~~0.640~~ * 1.274	≥ 1539 ≥ 773

Underlined values are relevant for the conclusion of the risk assessment.
* $PEC_{soil, ini.}$ For details please refer to section 8, chapter 8.7.
1) Sulphur Dust is the representative solo-formulation of sulfur containing 98.5% w/w sulfur (purity).

Table 9.8-6: First-tier assessment of the chronic risk for earthworms and other non-target soil organisms (meso- and macrofauna) due to the use of BAS 768 00 F according to the proposed use pattern

Intended use	2 x 4.0 L BAS 768 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 768 00 F	NOEC = 148.6 $NOEC_{CORR}$ = 74.3	~~0.732 [2)]~~ 1.451	~~203~~ ~~102~~ 51
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 (*Folsomia candida*)			
(total) a.s. in BAS 768 00 F	NOEC = 62.7 $NOEC_{CORR}$ = 31.4	~~0.732 [2)]~~ 1.451	~~86~~ ~~43~~ 22
Soil mites (*Hypoaspis aculeifer*)			
(total) a.s. in BAS 768 00 F	NOEC = 658.1 $NOEC_{CORR}$ = 329.1	~~0.732 [2)]~~ 1.451)	~~899~~ ~~450~~ 227

Underlined values are relevant for the conclusion of the risk assessment.
1) Endpoint based on sum of active substances (nominal) and taking into account a density of BAS 768 00 F of 1.346 g/cm^3.
2) Based on the sum of the worst-case active substance PEC_{soil} values.

9.8.2.2 Higher-tier risk assessment

Not relevant.

9.8.3 Overall conclusions

All TER values for BAS 768 00 F, the active substances mefentrifluconazole, sulfur and the relevant metabolite 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 768 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.

9.9 Effects on soil microbial activity (KCP 10.5)

zRMS Comments:	The submitted risk assessment was corrected in accordance with PECs assessment (Section B8, p. 8.7). **Sulfur**. The submitted studies, presented in Table 9.9-2 (References) were evaluated during active substance renewal. The correct Reference should be EFSA Journal 2023;21(3):7385. New study conducted on formulation was submitted and accepted. The formulation BAS 768 00 F poses no adverse effect on nitrate formation in soil. An acceptable risk to soil microorganisms is expected if the BAS 768 00 F application is in accordance with proposed pattern use.

9.9.1 Toxicity data

Studies on the effects on soil microorganisms have been carried out with BAS 768 00 F, the active substances mefentrifluconazole, sulfur and its relevant metabolite. 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 9.9-1, Table 9.9-2 and Table 9.9-3.

Table 9.9-1: 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 9.9-2: Endpoints and effect values of sulfur relevant for the risk assessment for soil microorganisms

Endpoint	Substance/metabolite	Exposure System	Results	Reference
N-mineralization	sulfur (tested as Sulphur 80% WG)	77 d, aerobic loamy sand	Nitrate formation rate at 106.4 mg/kg dry soil -14%	EFSA Scientific Report (2008) 221, 63-70 Dohmen, G.P. (1990) 1990/0146
	sulfur (tested as Sulphur Dust)[1)]	28 d, aerobic loamy sand	Nitrate formation rate at 394 mg/kg dry soil -15.9%	EFSA Scientific Report (2008) 221, 63-70 Reis, K.-H (2005)

+ = stimulation, - = inhibition

1) This study has been conducted with Sulphur Dust as a surrogate for technical sulfur since the minimum content of the a.s. is specified as 985 g/kg and the only co-formulant is inert.

Table 9.9-3: Endpoints and effect values of BAS 768 00 F relevant for the risk assessment for soil microorganisms

Endpoint	Product	Exposure System	Results	Reference
N-mineralization	BAS 768 00 F [1)]	28 d, aerobic loamy sand	Nitrate formation rate 134.60 mg/kg dry soil (equivalent to 62.50 mg total a.s./kg dry soil) [1)] +15.8%	not EU evaluated 2021/2014181

- = inhibition

1) Calculated, based on the nominal content of the total a.s. and considering a density of BAS 768 00 F of 1.346 g/cm^3.

9.9.1.1 Justification for new endpoints

Effects on soil microbial activity of BAS 768 00 F were not evaluated as part of the EU review of mefentrifluconazole or sulfur. Therefore, all relevant data and assessments are provided here and are considered adequate.

9.9.2 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 768 00 F, mefentrifluconazole, sulfur and the relevant metabolite to soil micro-organisms was assessed by comparing the maximum PEC_{soil} values with the maximum concentration with effects ≤ 25 % (see Table 9.9-4 and Table 9.9-6).

Table 9.9-4: Assessment of the risk for effects on soil micro-organisms due to the use of mefentrifluconazole as contained BAS 768 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)	0.092 *	yes
Metabolite, Reg. No. 87 084 1,2,4-triazole	0.333 (at 28 d)	< 0.001 *	yes

* $PEC_{soil, accu.}$ For details please refer to section 8, chapter 8.7.

Table 9.9-5: Assessment of the risk for effects on soil micro-organisms due to the use of sulfur as contained BAS 768 00 F according to the proposed use pattern

Intended use	cereals		
Active substance	sulfur		
Application rate (g a.s./ha)	2 x 2400		
N-mineralization			
Active substance/metabolite	**Max. conc. with effects ≤ 25 % (mg/kg dry soil)**	**PEC_{soil} (mg/kg dry soil)**	**Risk acceptable?**
sulfur (tested as Sulphur 80% WG)	106.4 (at 77 d)	~~0.640 *~~ 1.274	yes
sulfur (tested as Sulphur Dust)	394 (at 28 d)	~~0.640 *~~ 1.274	yes

* $PEC_{soil, ini.}$ For details please refer to section 8, chapter 8.7.

Table 9.9-6: Assessment of the risk for effects on soil micro-organisms due to the use of BAS 768 00 F according to the proposed use pattern

Intended use	cereals		
Product	BAS 768 00 F		
Application rate (L/ha)	2 x 4.0		
N-mineralization			
Product	**Max. conc. with effects ≤ 25 % (mg a.s./kg dry soil)**	**PEC_{soil} (mg a.s./kg dry soil)**	**Risk acceptable?**
mefentrifluconazole and sulfur in BAS 768 00 F	62.50 (at 28 d) [1)]	~~0.732 *~~ [)] 1.451	yes

* Based on the sum of the worst-case active substance PEC_{soil} values

1) Calculated, based on the nominal content of the total a.s. and considering a density of BAS 768 00 F of 1.346 g/cm^3.

9.9.3 Overall conclusions

For the formulation BAS 768 00 F, the active substances mefentrifluconazole and sulfur as well as for the relevant metabolite, 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 768 00 F, will not pose an unacceptable risk to non-target soil micro-organisms, if applied according to good agricultural practice.

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

zRMS Comments:	The new studies were submitted and accepted. Toxicity effects of BAS 768 00 F on the vegetative vigor and seedling emergence were tested. The NOER for plant is higher or equal than the highest tested application rate of 4.0 L BAS 768 00 F /ha for all tested plant species. The NOER for all species tested are ≥ 4.0 Lformulation/ha The ER_{50} for all species tested are > 4.0 Lformulation/ha. No mitigation measure is required.

9.10.1 Toxicity data

Vegetative vigour and seedling emergence studies have been conducted with BAS 768 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 768 00 F is provided in Table 9.10-1 below.

Table 9.10-1: Endpoints and effect values of BAS 768 00 F relevant for the risk assessment for non-target terrestrial plants

Species	Product	Exposure System	Results	Reference
Daucus carota $_{d}$ (carrot) *Lactuca sativa* $_{d}$ (lettuce) *Brassica napus* $_{d}$ (oilseed rape) *Brassica oleracea* $_{d}$ (cabbage) *Glycine max* $_{d}$ (soybean) *Solanum lycopersicum* $_{d}$ (tomato) *Allium cepa* $_{m}$ (onion) *Lolium multiflorum* $_{m}$ (ryegrass) *Triticum aestivum* $_{m}$ (wheat) *Zea mays* $_{m}$ (corn)	BAS 768 00 F	21 d Seedling emergence	ER_{50} emergence > 4.0 L/ha ER_{50} plant weight > 4.0 L/ha ER_{50} plant height > 4.0 L/ha	not EU evaluated 2021/2014236
Daucus carota $_{d}$ (carrot) *Lactuca sativa* $_{d}$ (lettuce) *Brassica napus* $_{d}$ (oilseed rape) *Brassica oleracea* $_{d}$ (cabbage) *Glycine max* $_{d}$ (soybean)	BAS 768 00 F	21 d Vegetative vigour	ER_{50} plant weight > 4.0 L/ha ER_{50} plant height > 4.0 L/ha	not EU evaluated 2021/2014237

Species	**Product**	**Exposure System**	**Results**	**Reference**
Solanum lycopersicum d (tomato) *Allium cepa* m (onion) *Lolium multiflorum* m (ryegrass) *Triticum aestivum* m (wheat) *Zea mays* m (corn)				

m: monocotyledonous; d: dicotyledonous

9.10.1.1 Justification for new endpoints

Effects on non-target plants of BAS 768 00 F were not evaluated as part of the initial Annex I inclusion or the Annex I renewal process of mefentrifluconazole or sulfur. Hence, all relevant data and assessments considering this formulation are provided here and are considered adequate.

9.10.2 Risk assessment

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 situations, as non-target plants are non-crop plants located outside the treated area.

9.10.2.1 Tier-1 risk assessment (based on screening data)

Following SANCO, the first-tier assessment is evaluating effects at highest single application rate for at least 6 species. As a general rule, the risk should be considered acceptable if there are no data indicating more than 50% effect at the maximum application rate. Only if the results show more than 50% effect a quantitative risk assessment would be triggered. BAS 768 00 F shows no effects at 4 L/ha.

9.10.2.2 Tier-2 risk assessment (quantitative risk assessment)

Screening studies indicate no unacceptable risk. Nevertheless, a quantitative risk assessment was conducted.

The application of BAS 768 00 F is envisioned in cereals. The following risk assessment is based on the worst-case single application rate of 4.0 L BAS 768 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 ($PER_{off\text{-}field}$) is calculated as:

$$PER_{off\text{-}field} = \text{maximum single application rate (L/ha)} * (\%\ \text{drift}/100)$$

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 768 00 F is 4.0 L product/ha. The maximum off-field predicted environmental rate ($PER_{off\text{-}field}$) is thus calculated to be 0.1108 L product/ha.

The potential risk of BAS 768 00 F to non-target plants was assessed by comparing the calculated PER value to the ER_{50} values in order to generate the toxicity exposure ratio (TER) as follows.

$$TER = \frac{\text{Endpoint [L/ha]}}{PER_{off-field}\ \text{[L/ha]}}$$

The results of the risk assessment are presented in Table 9.10-2.

Table 9.10-2: Assessment of the risk for non-target plants due to the use of BAS 768 00 F according to the proposed use pattern

Intended use	cereals			
Product	BAS 768 00 F			
Application rate (L/ha)	2 x 4.0			
MAF	n/a			
Test species	**ER_{50} (L/ha) [1)]**	**Drift rate (%)**	**$PER_{off\text{-}field}$ (L/ha)**	**TER criterion: TER ≥ 5**
Daucus carota d (carrot) *Lactuca sativa* d (lettuce) *Brassica napus* d (oilseed rape) *Brassica oleracea* d (cabbage) *Glycine max* d (soybean) *Solanum lycopersicum* d (tomato) *Allium cepa* m (onion) *Lolium multiflorum* m (ryegrass) *Triticum aestivum* m (wheat) *Zea mays* m (corn)	> 4.0	2.77	0.1108	> 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.

9.10.2.3 Higher-tier risk assessment

Not relevant.

9.10.2.4 Risk mitigation measures

No risk mitigation needed.

9.10.3 Overall conclusions

Based on the risk assessment it can be concluded that BAS 768 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 768 00 F applications are not required for the protection of terrestrial non-target plants.

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

Not relevant.

9.12 Monitoring data (KCP 10.8)

According to the knowledge of the applicant, there are currently no monitoring studies available which assess ecotoxicological effects of BAS 768 00 F or of the active substances.

9.13 Classification and Labelling

zRMS Comments:	The proposed classification was accepted: BAS 768 00 F is classified as: Aquatic chronic hazard category 3 (H412)

Plant protection products have to be classified for their acute and chronic environmental hazard according to (EC) No 1272/2008 (CLP). Classification is based primarily on data of the product itself if adequate acute and chronic data is available. When aquatic toxicity data for the formulated product is not available for all three trophic species levels, the summation method is additionally performed, meaning that the content of substances classified with a specific category are added to derive a classification for the product.

For the formulated product BAS 768 00 F acute data (LC/EC_{50}) is available for all trophic levels. Regarding chronic toxicity, adequate data are only available for algae, 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), the active substance mefentrifluconazole (BAS 750 F) is listed with acute hazard category 1 (H400), M = 1 and chronic hazard category 1 (H410), M = 1. According to Annex IV of Commission Regulation (EC) 1272/2008 (CLP), sulfur (BAS 175 F) is not legally classified within the EU. According to EFSA Scientific Report (2008; 221, 57-70), the risk of sulfur to aquatic organisms in the water column was considered low, since the solubility of sulfur in water is very low and no effects were observed at concentrations, which exceeded the water solubility (0.016 mg/L) by several orders of magnitude. Therefore, sulfur is considered to has no aquatic acute and chronic classification.
The formulation contains also the co-formulant #12 (0.07% w/w, for details please refer to chapter 1.2.2, Part C of this dossier). The co-formulant contains 1.48% (w/w) of a reaction mass that is legally classified with both aquatic acute and chronic hazard cat. 1, M = 100. Despite higher toxicity, since the portion of the reaction mass is very low in the formulation (*i.e.* 0.001036% w/w), it has no determining effect on the classification of the product. Hence, the classification is driven generally by the toxicity of the active substance mefentrifluconazole, and therefore, the co-formulant is not listed here.
Table 9.13-1 shows the relevant data for classification purposes.

Table 9.13-1: Ecotoxicology/Environment data relevant for Classification of BAS 768 00 F

Substance tested	Study Type (duration)	Findings	Triggered classification and labelling	Reference
Acute (short-term) & chronic (long-term) aquatic hazard				
BAS 768 00 F	*Oncorhynchus mykiss* (96 h)	LC_{50} = 15.3 mg/L	No aquatic acute hazard cat.	BASF DocID xxxxxxxxxx
BAS 768 00 F	*Daphnia magna* (48 h)	EC_{50} > 80 mg/L	No aquatic acute hazard cat.	BASF DocID 2021/2016980
BAS 768 00 F	*Pseudokirchneriella subcapitata* (72 h)	E_rC_{50} > 80 mg/L	No aquatic acute hazard cat.	BASF DocID 2021/2016979
		E_rC_{10} = 32 mg/L	No aquatic chronic hazard cat.	
Chronic (long-term) aquatic hazard				
Mefentrifluconazole (BAS 750 F) [1)]	--	--	Aquatic chronic hazard cat. 1 (H410); M=1	Legal classification according to Annex VI of (EC) No 1272/2008 (CLP)
	Biodegradation	Not readily biodegradable	--	BASF DocID 2014/71239574
Sulfur [2)] (BAS 175 F)	--	--	No aquatic acute and chronic hazard cat.	EFSA Scientific Report (2008) 221, 57-70
	Biodegradation	Not readily biodegradable	--	

1) Nominal contents within the formulated product (density: 1.36 g/cm^3): 25 g/L (1.84% w/w).

2) Nominal contents within the formulated product (density: 1.36 g/cm^3): 600 g/L (44.12% w/w).

Based on the lowest acute aquatic toxicity endpoint obtained using BAS 768 00 F no aquatic acute hazard category is given according to (EC) No 1272/2008 (CLP).
Regarding chronic classification, mefentrifluconazole (a.s. content of 1.84% w/w within the product), classified as chronic hazard cat. 1 (M=1) and sulfur (a.s. content of 44.12% w/w within the product), has no chronic hazard cat., are considered for the summation method in the 1st, 2nd and 3rd equation according to CLP. The 3rd equation yields a value which is above the trigger of 25%. Hence, BAS 768 00 F is classified as aquatic chronic hazard category 3 (H412). Chronic classification of BAS 768 00 F using the summation method is summarized in Table 9.13-2.

Table 9.13-2: Chronic classification of BAS 768 00 F using the summation method according to (EC) No 1272/2008

Chronic classification of BAS 768 00 F						
Formulation component				Result (% Content x M-Factor)		
Name	Chronic Category	M-Factor	Content in BAS 768 00 F [%]			
BAS 750 F	1	1	1.84	1.84		
BAS 175 F	none	none	44.12	none		
1st equation	SUM *(M x Chronic 1)*			1.84	< 25 %	No aquatic chronic hazard cat.
2nd equation	SUM *(M x 10 x Chronic 1) + chronic 2*			18.4	< 25 %	No aquatic chronic hazard cat.
3rd equation	SUM *(M x 100 x Chronic 1) + (10 x chronic 2) + chronic 3*			184	**≥ 25 %**	**BAS 768 00 F: Aquatic Chronic Hazard Category 3**

Conclusion

Based on the data obtained using the product and the lowest chronic aquatic toxicity endpoints of the active substances within the formulated product, the following classification and labelling, according to GHS following Regulation (EC) No 1272/2008, is proposed for BAS 768 00 F:
No aquatic acute hazard category
Aquatic chronic hazard category 3 (H412)

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 10.2.1/1	xxxxxxxxxxxxx	2016	BAS 750 F - Acute toxicity study in the fathead minnow (Pimephales promelas) xxxxxxxxxxxxxxxxxxxxxxxxx yes Unpublished	Yes	BASF
KCP 10.2.1/2	xxxxxxxxxxxxxx	2019	Reg.No. 6003433 (metabolite of BAS 750 F) - Acute toxicity study in the rainbow trout (Oncorhynchus mykiss) xxxxxxxxxxxxxxxxxxxxxxxxx yes Unpublished	Yes	BASF
KCP 10.2.1/3	xxxxxxxxxxxxx	2022	BAS 768 00 F: Acute Toxicity to Rainbow Trout (Oncorhynchus mykiss) in a 96-hour Static Test xxxxxxxxxxxxxxxxxxxxxxxxxx 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 10.2.1/4	Bebon, R.	2022	BAS 768 00 F: Acute Toxicity to Daphnia magna in a Static 48-hour Immobilisation Test 2021/2016980 ibacon GmbH, Rossdorf, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 10.2.1/5	Bebon, R.	2022	BAS 768 00 F: Toxicity to Pseudokirchneriella subcapitata in an Algal Growth Inhibition Test 2021/2016979 ibacon GmbH, Rossdorf, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 10.2.2/1	Salinas, E.	2011	BAS 175 01 F - Daphnia magna reproduction test 2011/1023625 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 10.3.1.1.1/1	Franke, M.	2016	Acute toxicity of Netzschwefel Stulln (Sulphur 80% WG) to the honeybee Apis mellifera L. under laboratory conditions 2016/1345280 BioChem agrar GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished	No	Sulphur TF

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 10.3.1.1.1/2	Kling, A.	2021	BAS 768 00 F - Acute Oral and Contact Toxicity to the Honey Bee Apis mellifera L. (Hymenoptera, Apidae) under Laboratory Conditions 2021/2014196 Eurofins Agroscience Services Ecotox GmbH, Niefern-Oeschelbronn, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 10.3.1.1.1/3	Amsel, K.	2022	Acute toxicity of BAS 768 00 F to the bumblebee Bombus terrestris L. under laboratory conditions 2022/2010697 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 10.3.1.1.2/1	Franke, M.	2016	Acute toxicity of Netzschwefel Stulln (Sulphur 80% WG) to the honeybee Apis mellifera L. under laboratory conditions 2016/1345280 BioChem agrar GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished	No	Sulphur TF
KCP 10.3.1.1.2/2	Kling, A.	2021	BAS 768 00 F - Acute Oral and Contact Toxicity to the Honey Bee Apis mellifera L. (Hymenoptera, Apidae) under Laboratory Conditions 2021/2014196 Eurofins Agroscience Services Ecotox GmbH, Niefern-Oeschelbronn, 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	Ruhland, S.	2017	Chronic toxicity of Netzschwefel Stulln (Sulphur 80% WG) to the honey bee Apis mellifera L. under laboratory conditions 2017/1219185 BioChem agrar GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished	No	Sulphur TF
KCP 10.3.1.2/2	Dressler, K.	2022	Chronic toxicity of BAS 768 00 F to the honey bee Apis mellifera L. under laboratory conditions 2021/2014218 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Machern OT Gerichshain, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 10.3.1.3/1	Scheller, K.	2017	Microthiol special disperss (Sulphur 80% WG) - Repeated exposure of honey bee (Apis mellifera L.) larvae under laboratory conditions (in vitro) 2017/1218043 BioChem agrar GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished	No	Sulphur TF

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 10.3.1.3/2	Haensel, M.	2021	Microthiol Special Disperss – Repeated exposure of honey bee larvae (Apis mellifera L.) under laboratory conditions 2023/2005062 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Machern OT Gerichshain, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 10.3.1.3/3	Schmidt, K.	2023	Repeated exposure of honey bee (Apis mellifera L.) larvae to BAS 768 00 F under laboratory conditions 2021/2014219 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 10.3.1.5/1	Schnurr, A.	2018	Effects of Microthiol Special Disperss (Sulphur 80% WG) on the honeybee Apis mellifera L. under semi-field conditions (tunnel test) with additional assessments on colony and brood development 2018/1232880 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Machern OT Gerichshain, Germany Fed.Rep. yes Unpublished	No	Sulphur TF

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 10.3.2.1/1	Roehlig, U.	2021	Effects of BAS 768 00 F on the predatory mite Typhlodromus pyri SCHEUTEN in a laboratory test 2021/2014189 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Machern OT Gerichshain, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 10.3.2.1/2	Amsel, K.	2022	Acute toxicity of BAS 768 00 F to the bumblebee Bombus terrestris L. under laboratory conditions 2022/2010697 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 10.3.2.1/3	Roehlig, U.	2021	Effects of BAS 768 00 F on the parasitic wasp Aphidius rhopalosiphi (DESTEPHANI-PEREZ) in a laboratory test 2021/2014190 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 10.3.2.2/1	Röhlig,U	2022	Effects of BAS 768 00 F on the parasitic wasp Aphidius rhopalosiphi (DESTEPHANI-PEREZ) in an extended laboratory test 2022/2010703 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 10.4.1.1/1	Dini, R., Tediosi, E.	2018	Effects of Sulphur Dust on reproduction of earthworm Eisenia fetida in an artificial soil study 2018/1241025 ChemService S.r.l. Controlli e Ricerche, Novate Milanese - MI - Italy, Italy yes Unpublished	No	Sulphur TF
KCP 10.4.1.1/2	Friedrich, S.	2021	Effects of BAS 768 00 F on the reproduction of the earthworm Eisenia andrei in artificial soil 2021/2014184 BioChem agrar GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 10.4.2.1/1	Ponti, B.	2018	Sulphur Dust: Effects on Collembolan reproduction in an artificial soil study 2018/1241024 ChemService S.r.l. Controlli e Ricerche, Novate Milanese - MI - Italy, Italy yes Unpublished	No	Sulphur TF
KCP 10.4.2.1/2	Rossini, L.	2017	Effects of Sulphur Dust on reproduction of the predatory mite Hypoaspis aculeifer in soil 2017/1229983 BioTecnologie B.T. SrI, Todi, Italy yes Unpublished	No	Sulphur TF

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 10.4.2.1/3	Friedrich, S.	2020	Effects of BAS 768 00 F on the reproduction of the collembolan Folsomia candida 2021/2014185 BioChem agrar GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 10.4.2.1/4	Mann, D.	2021	Effects of BAS 768 00 F on the reproduction of the predatory mite Hypoaspis aculeifer 2021/2014186 BioChem agrar GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 10.5/1	Schulz, L.	2021	Effects of BAS 768 00 F on the activity of soil microflora - (Nitrogen transformation test) 2021/2014181 BioChem agrar GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 10.6.2/1	Maleck, A.	2021	Effect of BAS 768 00 F on vegetative vigour of several species of terrestrial plants under greenhouse conditions 2021/2014237 Agro-Check Dr. Teresiak & Erdmann GbR, Lentzke, 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 10.6.2/2	Maleck, A.	2021	Effect of BAS 768 00 F on seedling emergence and seedling growth of several species of terrestrial plants under greenhouse conditions 2021/2014236 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 the reference list.

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 XX	Author	YYYY	Title Company Report N Source GLP/non GLP/GEP/non GEP Published/Unpublished	Y/N	Owner

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

No studies conducted.

A 2.1.1.2 KCP 10.1.1.2 Higher tier data on birds

No studies conducted.

A 2.1.2 KCP 10.1.2 Effects on terrestrial vertebrates other than birds

A 2.1.2.1 KCP 10.1.2.1 Acute oral toxicity to mammals

No studies conducted.

A 2.1.2.2 KCP 10.1.2.2 Higher tier data on mammals

No studies conducted.

A 2.1.3 KCP 10.1.3 Effects on other terrestrial vertebrate wildlife (reptiles and amphibians)

No studies conducted.

A 2.2 KCP 10.2 Effects on aquatic organisms

A 2.2.1 KCP 10.2.1 Acute toxicity to fish, aquatic invertebrates, or effects on aquatic algae and macrophytes

A 2.2.1.1 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:	The study was evaluated and accepted (formulation BAS 751 00 F).

Reference:	CP 10.2.1/1
Report	BAS 750 F - Acute toxicity study in the fathead minnow (Pimephales promelas) xxxxxxxxxx, 2016 Report No xxxxxxxxxxxxx BASF DocID xxxxxxxxxxxxx Authority registration No
Guideline(s):	EC 440/2008 C.1, EPA 72-1, EPA 850.1075, OECD 203
Deviations:	No
GLP:	Yes (xxxxxxxxxxxxxxxxxxxxx)
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 mefentrifluconazole (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 LC_{50} (96 h) of mefentrifluconazole 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).

Materials and methods

Materials

Test item: Mefentrifluconazole (BAS 750 F, Reg. no.: 583 437 8); Batch no. COD-001740; purity: 98.8% (± 1.0%).

Study design and methods

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 bevor 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: LC_{50}, NOEC, mortality and sub-lethal effects.

Test concentrations: Control (dilution water), 4.6, 10, 22, 46 and 100% of a saturated solution of mefentrifluconazole (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 $CaCO_3$/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 LC_{50}.

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 mefentrifluconazole 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 1: 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

Results

Analytical results

Analytical verification of mefentrifluconazole 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 mefentrifluconazole 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 2.

Table A 2: Effects of mefentrifluconazole on fathead minnow (*Pimephales promelas*) after 96 hours of exposure

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 a.s./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

Table A 3: Validity criteria according to OECD 203 (2019)

Criteria	Trigger value	Study value and page no.	Criteria met? Y/N	Guideline
Mortality in the control at end of exposure	≤ 10%	0% (p. 20, 22)	Y	OECD 203
Dissolved oxygen in all vessels	≥ 60%. of the air saturation value	> 60% (6.9 - 8.4 mg/L) (p. 20, 25)	Y	OECD 203
Analytical measurements of test concentrations	see § 24 of OECD 203	Analyses of control and all test item concentrations at the start, after 48 h and at test end; the analytical results are based on mean measured concentrations (p. 12)	Y	OECD 203

All validity criteria were met (see Table A 3 above).

Conclusion

In a static acute toxicity study with fathead minnow the LC_{50} (96 h) of mefentrifluconazole 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 2.2.1.2 Study 2

The following fish acute toxicity study performed with M750F005 (metabolite of mefentrifluconazole) is provided in support of the assessment and has not been previously evaluated on EU level.

Comments of zRMS:	The study was evaluated and accepted (formulation BAS 758 00 F).

Reference:	CP 10.2.1/2
Report	Reg.No. 6003433 (metabolite of BAS 750 F) - Acute toxicity study in the rainbow trout (Oncorhynchus mykiss) xxxxxxxxxxxxx 2019 Report No xxxxxxxxxxxx BASF DocID xxxxxxxxxxxxxxxx Authority registration No
Guideline(s):	EC 440/2008 C.1 Acute Toxicity for Fish, OECD 203
Deviations:	No
GLP:	Yes (xxxxxxxxxxxxxxxxxxxxxxxxxx)
Acceptability:	Yes
Duplication (if vertebrate study)	No

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 LC_{50} (96 h) for M750F005 was determined to be > 5 mg/L based on nominal concentration. The NOEC was determined to be ≥ 5 mg/L based on nominal concentration.

Materials and methods

Materials

Test item: M750F005 (Reg. No. 6003433, metabolite of mefentrifluconazole), Batch no. L87-34, purity: 96.9%.

Study design and methods

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.

Description of the analytical procedures

Concentrations of M750F005 (metabolite of metabolite of mefentrifluconazole) 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 4: 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

Results

Analytical results

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 **5**.

Table A 5: Effects of M750F005 on rainbow trout (*Oncorhynchus mykiss*) after 96 hours of exposure

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

Table A 6: Validity criteria according to OECD 203 (2019)

Criteria	Trigger value	Study value and page no.	Criteria met? Y/N	Guideline
Mortality in the control at end of exposure	≤ 10%	0% (p. 22, 24)	Y	OECD 203
Dissolved oxygen in all vessels	≥ 60%. of the air saturation value	> 60% (7.9 – 10.4 mg/L) (p. 22, 25)	Y	OECD 203
Analytical measurements of test concentrations	see § 24 of OECD 203	Analyses of control and all test item concentrations at the start and at test end; the analytical results are based on nominal loading rates (p. 22, 23)	Y	OECD 203

All validity criteria were met (see Table A 6 above).

III. CONCLUSION

In a 96-h static acute toxicity study with rainbow trout the LC_{50} (96 h) for M750F005 was determined to be > 5 mg/L based on nominal concentration. The NOEC was determined to be ≥ 5 mg/L based on nominal concentration.

A 2.2.1.3 Study 3

The following fish acute toxicity study performed with BAS 768 00 F is provided in support of the assessment and has not been previously evaluated on EU level.

Comments of zRMS:	The submitted study was accepted. The validity criteria were met. No deviation was noted. The following endpoint was derived: LC_{50} / LL_{50} (96 h) for BAS 768 00 F was determined to be 15.3 mg test item/L based on nominal loading rates (equivalent to 0.299 mg BAS 750 F/L).

Reference:	CP 10.2.1/3
Report	BAS 768 00 F: Acute Toxicity to Rainbow Trout (Oncorhynchus mykiss) in a 96-hour Static Test, xxxxxxxxxxxxxxxxx 2022 Report No xxxxxxxxxxxxxxxx BASF DocID xxxxxxxxxxxx Authority registration No
Guideline(s):	EPA 712-C-16-007, EPA 850.1075, OECD 203 (2019)
Deviations:	No
GLP:	Yes (xxxxxxxxxxxxxxxxxxx)
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive summary

In a static acute toxicity laboratory study, the effects of the formulated product BAS 768 00 F on juvenile rainbow trout (*Oncorhynchus mykiss*) were investigated. Fish were exposed for 96 hours to a dilution water control and to BAS 768 00 F at nominal loading rates of 2.1, 4.7, 10.3, 22.7, and 50 mg test item/L in groups of 7 animals with 1 replicate per treatment. Fish were observed for mortality and symptoms of toxicity at test start and after approx. 2, 4.5, 24, 27.5, 48, 51.5, 72, 75.5 and 96 hours.

The biological results are based on the nominal loading rates of the test item. After 96 hours of exposure, in the control and up to and including the nominal loading rate of 4.7 mg test item/L all fish survived and no signs of intoxication occurred. At the nominal loading rate of 10.3 mg test item/L, all fish survived but abnormal swimming behavior, abnormal ventilatory function, abnormal skin pigmentation and mucus secretion was observed. At the nominal loading rate of 22.7 mg test item/L and above, all fish were dead after 96 hours.

In a 96-h static acute toxicity study with rainbow trout (*Oncorhynchus mykiss*), the LC_{50} / LL_{50} (96 h) for BAS 768 00 F was determined to be 15.3 mg test item/L based on nominal loading rates(equivalent to 0.299 mg BAS 750 F/L).

Materials and methods

Materials

Test item: BAS 768 00 F, Batch no. FD-210120-1029; content of a.s.: mefentrifluconazole (BAS 750 F; Reg. no. 5834378): 26.3 g/L (nominal: 25.0 g/L), sulfur (BAS 175 F, Reg. no.: 240586): 614.9 g/L (nominal: 600.0 g/L); density: 1.346 g/cm^3.

Study design and methods

Test species: Rainbow trout (*Oncorhynchus mykiss*); juveniles; mean body length: 3.97 cm ± 0.5 cm (Mean ± SD),); mean body weight: 0.58 g ± 0.2 g (Mean ± SD); source: supplied by 'Forellenzuchtbetrieb Störk', Bad Saulgau, Germany.

Test design: Static system; exposure period: 96 hours; five test item loading rates plus a dilution water control; 7 fish per aquarium (loading 0.403 g fish/L), one replicate (aquarium) per treatment; assessment of mortality and symptoms of toxicity at test start and after approx. 2, 4.5, 24, 27.5, 48, 51.5, 72, 75.5 and 96 hours.

Endpoints: LC_{50} / LL_{50} related to mortality and assessment of sub-lethal effects.

Test concentrations: Dilution water control; test item concentrations: 2.1, 4.7, 10.3, 22.7, and 50 mg test item/L (nominal loading rates) The term "loading rate" (mass to volume ratio of the test chemical to test medium) is used as recommended in OECD GD 23 to express exposures to poorly water-soluble test chemicals dosed above water solubility that may not form a stable dispersion or emulsion over the tested range (OECD GD 23, 2019).

A stock solution of 50 mg test item/L was prepared by stirring for 24 hours. To remove any suspended undissolved particles and obtain a water-soluble fraction for testing as recommended in OECD GD 23 (2019), the stock solution was filtered. The filtered stock solution was diluted by factor 2.2, 4.84, 10.65 and 23.43 to obtain the nominal loading rates.

Dosage of test item: The test medium was prepared at a loading rate of 50 mg test item/L to obtain maximum dissolved concentrations of the poorly soluble active ingredients. The test item was mixed into test water by intense stirring for 24 hours. The stock solutions were then filtered using a cellulose acetate filter (capsule, combination of 0.65 and 0.45 µm) to remove undissolved material (OECD GD 23) to avoid any potential physical effects of suspended particles on the test organisms. The remaining filtrate contains all toxicologically relevant soluble components of the formulation that would be expected to cause toxicity to aquatic organisms in a realistic exposure scenario. One filtered stock solution was used directly as the highest treatment and lower treatments were prepared as dilutions of the other filtered stock solution.

Test conditions:	12 L glass aquaria; test volume: 10 L; test medium: reconstituted water (ISO medium); temperature: 12.4 - 12.8 °C; pH: 7.6 - 7.8; oxygen content: 92 - 100% of the air saturation value; hardness: 160.2 mg $CaCO_3$/L; alkalinity: 43.6 mg HCO_3^-/L; conductivity: 495 µS/m; photoperiod: 12 h light : 12 h dark; light intensity: 750 - 840 lux; slight aeration during the test; no feeding.
Analytics:	Analytical verification of test item concentrations was conducted using an HPLC-method with MS/MS-detection (method no. L0361/01) and an HPLC-method with UV-detection (method no. L0477/01).
Statistics:	Descriptive statistics; calculation of LC_{50} / LL_{50} as geometric mean of the highest concentration causing no mortality and the lowest concentration resulting in 100% mortality.

Description of the analytical procedures

The concentrations of mefentrifluconazole (BAS 750 F) and sulfur (BAS 175 F; contained in BAS 768 00 F) in dilution water were determined according to the analytical method L0361/01 and L0477/01, respectively. The analytical methods are fully validated in separate studies (BASF DocID: 2017/1065621 and 2021/2016981, respectively). The validation of the analytical methods is described in the study report.
Method L0361/01 was used for the analysis of mefentrifluconazole in test samples and was adapted to the requirements of the study by using a HPLC column with a similar stationary phase but with a changed inner diameter and from a different supplier. The aqueous test samples were initially diluted with acetonitrile/HPLC water/formic acid (= D1; 400/600/2; v/v/v) by factor 2 and further diluted with D1/test water (= D2; 1/1; v/v). Final determination was accomplished by LC-MS/MS. The limit of quantification (LOQ) was 0.10 µg mefentrifluconazole/L (equiv. to 0.005 mg test item/L) and the limit of detection (LOD) was set to 0.020 µg mefentrifluconazole/L (equiv. to 1.03 µg test item/L).
Method L0477/01 was used for the analysis of sulfur in test samples. The aqueous test samples were initially diluted with acetonitrile by factor 2 and further diluted with acetonitrile/test water (1/1; v/v) as appropriate. Final determination was accomplished by HPLC-UV. The limit of quantification (LOQ) was 0.050 mg sulfur/L (equiv. to 0.11 mg test item/L) and the limit of detection (LOD) was set to 10.0 µg sulfur/L (equiv. to 21.89 µg test item/L).
No peak interferences occurred at the retention time and mass transitions of mefentrifluconazole. No peak interference occurred at the retention time of sulfur. Potential matrix effects were not investigated since matrix-matched standards were used and matrix effect for mefentrifluconazole has already been investigated for comparable matrices within method L0361/01 (see 2017/1065621) and for sulfur in comparable matrices within method L0477/01 (see 2021/2016981). Samples for mefentrifluconazole analysis were stored for a maximum duration of 21 days within this study. The storage stability of sulfur in test water/acetonitrile (1/1; v/v) was shown for up to 37 days when stored frozen (≤ -20 °C). Mean recoveries of the procedural recovery samples were between 79.5% and 102% for mefentrifluconazole and between 106% and 112% for sulfur. Details on measured fortification samples and obtained procedural recoveries for the formulated product BAS 768 00 F (based on measurements of mefentrifluconazole and sulfur) are provided in Table A 7 and Table A 8.

Table A 7: Procedural recoveries for BAS 768 00 F (based on measurement of mefentrifluconazole)

Matrix	Fortification level [mg test item/L]	n	Mean recovery [%]	RSD [%]
Dilution water	0.005	5	79.5	18.0
Dilution water	60.0	5	102	9.15

Abbreviations: n: number of replicates; RSD: relative standard deviation

Table A 8: Procedural recoveries for BAS 768 00 F (based on measurement of sulfur)

Matrix	Fortification level [mg test item/L]	n	Mean recovery [%]	RSD [%]
Dilution water	0.110	8	112	2.65
Dilution water	60.0	8	106	6.15

Abbreviations: n: number of replicates; RSD: relative standard deviation

Results

Analytical results

Analytical verification of test item concentrations (based on the active substances mefentrifluconazole and sulfur) was conducted in the control and in the test media samples from each test item concentration (filtrate of test item stock solution and the subsequent dilutions of the filtrate) at test initiation and at the end of the test. At test end, samples were taken from unstirred and stirred test media since both dissolved and undissolved test material can be bioavailable to the test organism. Measured concentrations for mefentrifluconazole ranged from 28.0 to 667 µg a.s./L at test initiation and were between 83.8% and 98.9% of the initial measured concentrations at test termination, demonstrating that concentrations were maintained within ±20% over the duration of the study. Measured concentrations for sulfur ranged from 74.9 to 738 µg a.s./L at test initiation and were between 50.3% and 96.6% of the initial measured concentrations at test termination. In the nominal loading rate of 2.1 mg test item/L, measured concentrations for sulfur were < LOD at test termination. At the higher two test concentrations, concentrations were maintained within ±20% over the duration of the study, and at 10.3 mg test item/L, the concentration at test end was only slightly below 80% of the initial measured sulfur concentration (76.7%). At all nominal loading rates, the geometric mean measured concentration of sulfur was well above the solubility limit. The biological results are based on nominal loading rates of the test item (since a filtrate of a stock solution and subsequent dilutions of the filtrate were tested), according to OECD GD 23 (2019), for application solutions that may contain ingredients above the solubility limit which are partly removed by filtration.

Biological results

After 96 hours of exposure, in the control and up to and including the nominal loading rate of 4.7 mg test item/L, all fish survived and no signs of intoxication occurred. At the nominal loading rate of 10.3 mg test item/L, all fish survived but abnormal swimming behavior, abnormal ventilatory function, abnormal skin pigmentation and mucus secretion was observed. At the nominal loading rate of 22.7 mg test item/L and above, all fish were dead after 96 hours. The results are summarized inTable A 9.

Table A 9: Effects of BAS 768 00 F on rainbow trout (*Oncorhynchus mykiss*) after 96 hours of exposure

Loading rate [mg test item/L] (nominal)	**Dilution water control**	**2.1**	**4.7**	**10.3**	**22.7**	**50**
Total initial number of fish	7	7	7	7	7	7
Cumulative number of dead fish after 96 h [individuals]	0	0	0	0	7	7
Cumulative mortality after 96 h [%]	0	0	0	0	100	100
Symptoms after 96 h [1)]	none	none	none	SB, VF, SP, MS	n.d.	n.d.
	Endpoints [mg BAS 768 00 F/L] (nominal)					
LC_{50} / LL_{50} (96 h)	15.3 (95% confidence limits: 10.3 - 22.7) (equivalent to 0.299 mg BAS 750 F/L)					

Abbreviations: n.d.: not determined

1) Symptoms after 96 h: SB: abnormal swimming behavior, VF: abnormal ventilatory function, SP: abnormal skin pigmentation, MS: mucus secretion, n.d.: not determined since all fish had died.

Table A 10: Validity criteria according to OECD 203 (2019)

Criteria	**Trigger value**	**Study value and page no.**	**Criteria met? Y/N**	**Guideline**
Mortality in the control at end of exposure	≤ 10%	0% (p. 23)	Y	OECD 203
Dissolved oxygen in all vessels	≥ 60%. of the air saturation value	≥ 92% of max. saturation (p. 23)	Y	OECD 203
Analytical measurements of test concentrations	see § 24 of OECD 203	Analyses of control and all test item concentrations at the start and at test end; the analytical results are based on nominal loading rates (p. 23)	Y	OECD 203

All validity criteria were met (see Table A 10 above).

Conclusion

In a 96-h static acute toxicity study with rainbow trout (*Oncorhynchus mykiss*), the LC_{50} / LL_{50} (96 h) for BAS 768 00 F was determined to be 15.3 mg test item/L based on nominal loading rates (equivalent to 0.299 mg BAS 750 F/L).

A 2.2.1.4 Study 4

The following acute toxicity study on *D. magna* performed with BAS 768 00 F is provided in support of the assessment and has not been previously evaluated on EU level.

Comments of zRMS:	The submitted study was accepted. The validity criteria were met. No deviations were noted. The following endpoints were derived: 48h EC_{50} > 80 mg test item /L based on nominal loading rates (equivalent to > 1.56 mg BAS 750 F/L). 48h EC_{10} = 71 mg test item /L based on nominal loading rates (equivalent to 1.39 mg BAS 750 F/L). 48h NOEC = 36 mg test item /L based on nominal loading rates (equivalent to 0.703 mg BAS 750 F/L).

Reference:	CP 10.2.1/4
Report	BAS 768 00 F: Acute Toxicity to Daphnia magna in a Static 48-hour Immobilisation Test, Bebon, R., 2022 Report No 894517, 162361220 BASF DocID 2021/2016980 Authority registration No
Guideline(s):	EPA 850.1000, EPA 850.1010, OECD Guidelines for testing of chemicals No. 202. Daphnia sp., OECD Series on Testing and Assessment No. 23 (2000) - Aquatic Toxicity Testing of Difficult Substances and Mixtures, OECD-ENV/JM/MONO/(2007)17, SANTE/2020/12830 rev. 1
Deviations:	No
GLP:	Yes (certified by Hessisches Ministerium fuer Umwelt, Klimaschutz, Landwirtschaft und Verbraucherschutz, Wiesbaden)
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive summary

In a static acute toxicity laboratory study, the effects of the formulated product BAS 768 00 F on water flea neonates (*Daphnia magna*) were investigated. Daphnids were exposed for 48 hours to a dilution water control and to BAS 768 00 F at nominal loading rates of 3.4, 7.5, 16.5, 36 and 80 mg test item/L in groups of 5 animals with 4 replicates per treatment. Daphnids were observed for immobility and other symptoms of toxicity 24 hours and 48 hours after start of exposure.

The biological results are based on the nominal loading rates of the test item. After 48 hours of exposure, no immobilization of the test animals was observed in the control and up to and including the nominal

loading rate of 36 mg test item/L. At the nominal loading rate of 80 mg test item/L, 5 animals were immobile. At 80 mg test item/L, sublethal effects like slow movement were also observed.

In a 48-h static acute toxicity study with water flea (*Daphnia magna*), the EC_{50} / EL_{50} (48 h) for BAS 768 00 F was determined to be > 80 mg test item/L based on nominal loading rates (equivalent to > 1.56 mg BAS 750 F/L).

Materials and methods

Materials

Test item: BAS 768 00 F, Batch no. FD-210120-1029; content of a.s.: mefentrifluconazole (BAS 750 F; Reg. no. 5834378): 26.3 g/L (nominal: 25.0 g/L), sulfur (BAS 175 F, Reg. no.: 240586): 614.9 g/L (nominal: 600.0 g/L); density: 1.346 g/cm^3.

Study design and methods

Test species: Water flea (*Daphnia magna*); neonates, 0.50 to 15.75 hours old at test initiation and not first brood progeny; source: in-house culture.

Test design: Static system; exposure period: 48 hours; five test item concentrations plus a dilution water control; 5 daphnids per test vessel (loading: 0.05 animals/mL), four replicates (test vessels) per treatment; assessment of immobility and other symptoms of toxicity 24 hours and 48 hours after start of exposure.

Endpoints: EC_{50} / EL_{50} related to immobility and assessment of abnormal behaviour or appearance of daphnids.

Test concentrations: Dilution water control; test item concentrations: 3.4, 7.5, 16.5, 36 and 80 mg test item/L (nominal loading rates) The term “loading rate” (mass to volume ratio of the test chemical to test medium) is used as recommended in OECD GD 23 to express exposures to poorly water-soluble test chemicals dosed above water solubility that may not form a stable dispersion or emulsion over the tested range (OECD GD 23, 2019).

A stock solution of 80 mg test item/L was prepared by stirring for 24 hours. To remove any suspended undissolved particles and obtain a water-soluble fraction for testing as recommended in OECD GD 23 (2019), the stock solution was filtered. The filtered stock solution was diluted by factor 2.2, 4.84, 10.65 and 23.43 to obtain the nominal loading rates.

Dosage of test item: The test medium was prepared at a loading rate of 80 mg test item/L to obtain maximum dissolved concentrations of the poorly soluble active ingredients. The test item was mixed into test water by intense stirring for 24 hours. The stock solution was then filtered (0.45 µm cellulose acetate filter) to remove any remaining undissolved material (OECD GD 23, 2019) to avoid any potential physical effects of suspended particles on the test organisms. The remaining filtrate contains all toxicologically relevant soluble components of the formulation that would be expected to cause toxicity to aquatic organisms in a

realistic exposure scenario. The solution with dissolved test item was used as the test medium of the highest test concentration and to prepare the desired dilutions.

Test conditions: 150-mL glass beakers; test volume: 100 mL; test medium: "M4" Elendt medium; temperature: 19.2 - 20.0 °C; pH: 7.9 - 8.1; oxygen content: 8.2 - 9.0 mg/L; hardness: 195.8 mg $CaCO_3$/L; alkalinity: 43.6 mg HCO_3^-; conductivity: 625 µS; photoperiod: 16 h light : 8 h dark; light intensity: 800 - 950 lux; no aeration; no feeding.

Analytics: Analytical verification of test item concentrations was conducted using an HPLC-method with MS/MS-detection (method no. L0361/01) and an HPLC-method with UV-detection (method no. L0477/01).

Statistics: Descriptive statistics; probit analysis for determination of the EC_{50} / EL_{50}.

Description of the analytical procedures

The concentrations of mefentrifluconazole (BAS 750 F) and sulfur (BAS 175 F; contained in BAS 768 00 F) in dilution water were determined according to the analytical method L0361/01 and L0477/01, respectively. The analytical methods are fully validated in separate studies (BASF DocID: 2017/1065621 and 2021/2016981, respectively). The validation of the analytical methods is described in the study report.
Method L0361/01 was used for the analysis of mefentrifluconazole in test samples and was adapted to the requirements of the study by using a HPLC column with a similar stationary phase but with a changed inner diameter and from a different supplier. The aqueous test samples were initially diluted with acetonitrile/HPLC water/formic acid (= D1; 400/600/2; v/v/v) by factor 2 and further diluted with D1/test water (= D2; 1/1; v/v). Final determination was accomplished by LC-MS/MS. The limit of quantification (LOQ) was 0.10 µg mefentrifluconazole/L (equiv. to 0.005 mg test item/L), and the limit of detection (LOD) was set to 0.020 µg mefentrifluconazole/L (equiv. to 1.02 µg test item/L).
Method L0477/01 was used for the analysis of sulfur in test samples. The aqueous test samples were initially diluted with acetonitrile by factor 2 and further diluted with acetonitrile/test water (1/1; v/v) as appropriate. Final determination was accomplished by HPLC-UV. The limit of quantification (LOQ) was 0.050 mg sulfur/L (equiv. to 0.11 mg test item/L) and the limit of detection (LOD) was set to 10.0 µg sulfur/L (equiv. to 21.89 µg test item/L).
No peak interferences occurred at the retention time and mass transitions of mefentrifluconazole. No peak interference occurred at the retention time of sulfur. Potential matrix effects were not investigated since matrix-matched standards were used and matrix effect for mefentrifluconazole has already been investigated for comparable matrices within method L0361/01 (see 2017/1065621) and for sulfur in comparable matrices within method L0477/01 (see 2021/2016981). Samples for mefentrifluconazole analysis were stored for a maximum duration of 69 days within this study. The storage stability of sulfur in test water/acetonitrile (1/1; v/v) was shown for up to 80 days when stored frozen (≤ -20 °C). Mean recoveries of the procedural recovery samples were between 90.2% and 94.6% for mefentrifluconazole and between 87.9% and 106% for sulfur. Details on measured fortification samples and obtained procedural recoveries for the formulated product BAS 768 00 F (based on measurements of mefentrifluconazole and sulfur) are provided in Table A 11 and Table A 12.

Table A 11: Procedural recoveries for BAS 768 00 F (based on measurement of mefentrifluconazole)

Matrix	Fortification level [mg test item/L]	n	Mean recovery [%]	RSD [%]
Dilution water	0.005	7	90.2	13.4
Dilution water	100	8	94.6	5.71

Abbreviations: n: number of replicates; RSD: relative standard deviation

Table A 12: Procedural recoveries for BAS 768 00 F (based on measurement of sulfur)

Matrix	Fortification level [mg test item/L]	n	Mean recovery [%]	RSD [%]
Dilution water	0.11	8	87.9	7.19
Dilution water	100	8	106	6.04

Abbreviations: n: number of replicates; RSD: relative standard deviation

Results

Analytical results

Analytical verification of test item concentrations (based on the active substances mefentrifluconazole and sulfur) was conducted in the control and in the test media samples from each test item concentration (filtrate of test item stock solution and the subsequent dilutions of the filtrate) at test initiation and at the end of the test. At test end, samples were taken from unstirred and stirred test media since both dissolved and undissolved test material can be bioavailable to the test organism. Measured concentrations for mefentrifluconazole ranged from 33.1 to 701 µg a.s./L at test initiation and were between 99.7% and 107% of the initial measured concentrations at test termination, demonstrating that concentrations were maintained within ±20% over the duration of the study. At test initiation, measured concentrations for sulfur were 0.0536 and 0.123 mg a.s./L in the nominal loading rates of 36 and 80 mg test item/L and were < LOD / < LOQ in the nominal loading rates of 3.4, 7.5 and 16.5 mg test item/L. At test termination, measured concentrations for sulfur were < LOD / < LOQ. At the highest nominal loading rate, where no significant effect on mortality was observed (see biological results below), the geometric mean measured concentration of sulfur was above the solubility limit. The biological results are based on nominal loading rates of the test item (since a filtrate of a stock solution and subsequent dilutions of the filtrate were tested), according to OECD GD 23 (2019), for application solutions that may contain ingredients above the solubility limit which are partly removed by filtration.

Biological results

After 48 hours of exposure, no immobilization of the test animals was observed in the control and up to and including the nominal loading rate of 36 mg test item/L. At the nominal loading rate of 80 mg test item/L, 5 animals were immobile. At 80 mg test item/L, sublethal effects like slow movement were observed. The results are summarized in Table A 13.

Table A 13: Effects of BAS 768 00 F on the water flea (*Daphnia magna*) after 48 hours of exposure

Loading rate [mg test item/L] (nominal)	**Dilution water control**	**3.4**	**7.5**	**16.5**	**36**	**80**
Total initial number of daphnids	20	20	20	20	20	20
Cumulative number of immobile daphnids after 48 h [individuals]	0	0	0	0	0	5
Cumulative immobility after 48 h [%]	0	0	0	0	0	25
Symptoms after 48 h [1)]	none	none	none	none	none	SL
	Endpoints [mg BAS 768 00 F/L] (nominal)					
EC_{50} (48 h)	> 80 (95% confidence limits: n.d.) (equiv. to > 1.56 mg BAS 750 F/L)					

Abbreviations: n.d.: not determined (could not be calculated due to mathematical reasons)

1) Symptoms after 48 h: SL: slow movement

Table A 14: Validity criteria according to OECD 202 (2004)

Criteria	**Trigger value**	**Study value and page no.**	**Criteria met? Y/N**	**Guideline**
Immobility or signs of disease or stress in the control	≤ 10% (at end of exposure)	0% immobility; no signs of disease or stress (p. 23)	Y	OECD 202
Dissolved oxygen at test termination in all treatments	≥ 3 mg/L	≥ 8.7 mg/L (p. 23)	Y	OECD 202

All validity criteria were met (see Table A 14 above).

Conclusion

In a 48-h static acute toxicity study with water flea (*Daphnia magna*), the EC_{50} / EL_{50} (48 h) for BAS 768 00 F was determined to be > 80 mg test item /L based on nominal loading rates (equivalent to > 1.56 mg BAS 750 F/L).

A 2.2.1.5 Study 5

The following toxicity study on green algae performed with BAS 768 00 F is provided in support of the assessment and has not been previously evaluated on EU level.

Comments of zRMS:	The submitted study was accepted. The validity criteria were met. No deviations were noted. The following endpoints were derived: **Growth rate**: 72h ErC_{50} > 80 mg test item /L based on nominal loading rates (equivalent to > 1.56 mg BAS 750 F/L). 72h ErC_{10} > 80 mg test item /L based on nominal loading rates (equivalent to > 1.56 mg BAS 750 F/L). 72h NOEC = 32 mg test item /L based on nominal loading rates (equivalent to 0.625 mg BAS 750 F/L). The only value of 72h EyC_{10} = 30.4 mg test item /L based on nominal loading rates (equivalent to = 0.594 mg BAS 750 F/L) was lower than EyC_{10} for growth rate. The other endpoint values were the same.

Reference:	CP 10.2.1/5
Report	BAS 768 00 F: Toxicity to Pseudokirchneriella subcapitata in an Algal Growth Inhibition Test, Bebon, R., 2022 Report No 894516, 162361210 BASF DocID 2021/2016979 Authority registration No
Guideline(s):	EPA 850.1000, OECD 201 (2006), OECD Series on Testing and Assessment No. 23 (2000) - Aquatic Toxicity Testing of Difficult Substances and Mixtures, OECD-ENV/JM/MONO/(2007)17, SANTE/2020/12830 rev. 1
Deviations:	No
GLP:	Yes (certified by Hessisches Ministerium fuer Umwelt, Klimaschutz, Landwirtschaft und Verbraucherschutz, Wiesbaden)
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive summary

In a static toxicity laboratory study, the effects of the formulated product BAS 768 00 F on the growth of the green alga *Pseudokirchneriella subcapitata* were investigated. Algae were exposed for 72 hours to a dilution water control and to BAS 768 00 F at nominal loading rates of 2.0, 5.1, 12.8, 32 and 80 mg test

item/L, with 5 replicates for each test item concentration and 10 replicates for the dilution water control. Assessment of growth was conducted 24, 48 and 72 hours after start of exposure.

The biological results are based on the nominal loading rates of the test item. After 72 hours of exposure, statistically significant effects on growth rate and yield were observed at the highest loading rate of 80 mg test item/L. No effects on the morphology of algal cells were observed.

In a 72-h static toxicity study with green algae (*Pseudokirchneriella subcapitata*), the E_rC_{50} / E_rL_{50} (72 h) for BAS 768 00 F was estimated to be > 80 mg test item/L based on nominal loading rates (equivalent to > 1.56 mg BAS 750 F/L).

Materials and methods

Materials

Test item: BAS 768 00 F, Batch no. FD-210120-1029; content of a.s.: mefentrifluconazole (BAS 750 F; Reg. no. 5834378): 26.3 g/L (nominal: 25.0 g/L), sulfur (BAS 175 F, Reg. no.: 240586): 614.9 g/L (nominal: 600.0 g/L); density: 1.346 g/cm^3.

Study design and methods

Test species: Unicellular freshwater green alga, *Pseudokirchneriella subcapitata* (Korshikov) Hindák (syn. *Selenastrum capricornutum* Printz), SAG 61.81; source: in-house culture; stock originally obtained from 'Culture Collection of Algae at Goettingen University (SAG)', Göttingen, Germany.

Test design: Static system; exposure period: 72 hours; 5 test item concentrations plus a dilution water control; 5 replicates for each test item concentration and 10 replicates for the dilution water control; assessment of growth via spectrophotometric measurement, 24, 48 and 72 hours after start of exposure; assessment of cell morphology at test end.

Endpoints: EC_{10}, EC_{20}, EC_{50}, NOEC and LOEC / EL_{10}, EL_{20}, EL_{50}, NOELR and LOELR with respect to growth rate and yield after exposure over 72 hours.

Test concentrations: Dilution water control; test item concentrations: 2.0, 5.1, 12.8, 32 and 80 mg test item/L (nominal loading rates) The term "loading rate" (mass to volume ratio of the test chemical to test medium) is used as recommended in OECD GD 23 to express exposures to poorly water-soluble test chemicals dosed above water solubility that may not form a stable dispersion or emulsion over the tested range (OECD GD 23, 2019).

A stock solution of 80 mg test item/L was prepared by stirring for 24 hours. To remove any suspended undissolved particles and obtain a water-soluble fraction for testing as recommended in OECD GD 23 (2019) the stock solution was filtered. The filtered stock solution was diluted by factor 2.5, 6.25, 15.63 and 39.06 to obtain the nominal loading rates.

Dosage of test item:	The test medium was prepared at a loading rate of 80 mg test item/L to obtain maximum dissolved concentrations of the poorly soluble active ingredients. The test item was mixed into test water by intense stirring for 24 hours. The stock solution was then filtered (0.45 µm cellulose acetate filter) to remove any remaining undissolved material (OECD GD 23, 2019) to avoid any potential physical effects of suspended particles on the test organisms. The remaining filtrate contains all toxicologically relevant soluble components of the formulation that would be expected to cause toxicity to aquatic organisms in a realistic exposure scenario. The solution with dissolved test item was used as the test medium of the highest test concentration and to prepare the desired dilutions.
Test conditions:	50-mL Erlenmeyer flasks; test volume: 50 mL; test medium: OECD medium; temperature: 21.2 - 21.8 °C; pH: 8.0 - 8.1; initial cell density: 5 x 10^3 cells/mL; continuous light at 4670 - 5300 lux; continuous stirring.
Analytics:	Analytical verification of test item concentrations was conducted using an HPLC-method with MS/MS-detection (method no. L0361/01) and an HPLC-method with UV-detection (method no. L0477/01).
Statistics:	Descriptive statistics; determination of ECx / ELx via non-linear regression using the 3-parametric normal cumulative distribution function (CDF); Williams t-test for determination of NOEC/LOEC / NOELR/LOELR values.

Description of the analytical procedures

The concentrations of mefentrifluconazole (BAS 750 F) and sulfur (BAS 175 F; contained in BAS 768 00 F) in dilution water were determined according to the analytical method L0361/01 and L0477/01, respectively. The analytical methods are fully validated in separate studies (BASF DocID: 2017/1065621 and 2021/2016981, respectively). The validation of the analytical methods is described in the study report.
Method L0361/01 was used for the analysis of mefentrifluconazole in test samples and was adapted to the requirements of the study by using a HPLC column with a similar stationary phase but with a changed inner diameter and from a different supplier. The aqueous test samples were initially diluted with acetonitrile/HPLC water/formic acid (= D1; 400/600/2; v/v/v) by factor 2 and further diluted with D1/test water (= D2; 1/1; v/v). Final determination was accomplished by LC-MS/MS. The limit of quantification (LOQ) was 0.10 µg mefentrifluconazole/L (equiv. to 0.005 mg test item/L) and the limit of detection (LOD) was set to 0.020 µg test item/L.
Method L0477/01 was used for the analysis of sulfur in test samples. The aqueous test samples were initially diluted with acetonitrile by factor 2 and further diluted with acetonitrile/test water (1/1; v/v) as appropriate. Final determination was accomplished by HPLC-UV. The limit of quantification (LOQ) was 0.05 mg sulfur/L (equiv. to 0.11 mg test item/L) and the limit of detection (LOD) was set to 0.010 mg sulfur/L (equiv. to 0.022 mg test item/L).
No peak interferences occurred at the retention time and mass transitions of mefentrifluconazole. No peak interference occurred at the retention time of sulfur. Potential matrix effects were not investigated since matrix-matched standards were used and matrix effect for mefentrifluconazole has already been investigated for comparable matrices within method L0361/01 (see 2017/1065621) and for sulfur in comparable matrices within method L0477/01 (see 2021/2016981). Samples for mefentrifluconazole analysis were stored for a maximum duration of 57 days within this study. The storage stability of sulfur in test water/acetonitrile (1/1; v/v) was shown for up to 88 days when stored frozen (≤ -20 °C). Mean recoveries of the procedural recovery samples were between 83.9% and 99.0% for mefentrifluconazole and between 77.2% and 90.6% for sulfur. Details on measured fortification samples and obtained procedural

recoveries for the formulated product BAS 768 00 F (based on measurements of mefentrifluconazole and sulfur) are provided in Table A 15 and Table A 16.

Table A 15: Procedural recoveries for BAS 768 00 F (based on measurement of mefentrifluconazole)

Matrix	Fortification level [mg test item/L]	n	Mean recovery [%]	RSD [%]
Dilution water	0.005	5	83.9	3.74
Dilution water	100	5	99.0	3.85

Abbreviations: n: number of replicates; RSD: relative standard deviation

Table A 16: Procedural recoveries for BAS 768 00 F (based on measurement of sulfur)

Matrix	Fortification level [mg test item/L]	n	Mean recovery [%]	RSD [%]
Dilution water	0.11	8	77.2	10.6
Dilution water	100	8	90.6	9.27

Abbreviations: n: number of replicates; RSD: relative standard deviation

Results

Analytical results

Analytical verification of test item concentrations (based on the active substances mefentrifluconazole and sulfur) was conducted in the control and in the test media samples from each test item concentration (filtrate of test item stock solution and the subsequent dilutions of the filtrate at test initiation and at the end of the test. At test end, samples were taken from unstirred and stirred test media since both dissolved and undissolved test material can be bioavailable to the test organism. Measured concentrations for mefentrifluconazole ranged from 13.3 to 479 µg a.s./L at test initiation and were between 83.4% and 109% of the initial measured concentrations at test termination, demonstrating that concentrations were maintained within ±20% over the duration of the study. At test initiation, measured concentrations for sulfur were 0.0587 and 0.160 mg a.s./L in the nominal loading rates of 32 and 80 mg test item/L and were < LOD / < LOQ in the nominal loading rates of 2.0, 5.1 and 12.8 mg test item/L. At test termination, measured concentrations for sulfur were < LOD. At the highest nominal loading rate, where no significant effect on mortality was observed (see biological results below), the geometric mean measured concentration of sulfur was above the solubility limit. The biological results are based on nominal loading rates of the test item (since a filtrate of a stock solution and subsequent dilutions of the filtrate were tested), according to OECD GD 23 (2019), for application solutions that may contain ingredients above the solubility limit which are partly removed by filtration.

Biological results

After 72 hours of exposure, statistically significant effects on growth rate and yield were observed at the highest loading rate of 80 mg test item/L. No effects on the morphology of algal cells were observed. The results are summarized in Table A 17.

Table A 17: Effects of BAS 768 00 F on the growth of the green alga (*Pseudokirchneriella subcapitata*) after 72 hours of exposure

Loading rate [mg test item/L] (nominal)	**Dilution water control**	**2.0**	**5.1**	**12.8**	**32**	**80**
No. of replicates	10	5	5	5	5	5
Mean growth rate after 72 h [1/day]	1.435	1.450	1.465	1.475	1.405	1.360
Standard deviation	0.0349	0.0690	0.0353	0.0138	0.0198	0.0216
CV [%]	2.4	4.8	2.4	0.9	1.4	1.6
Inhibition of growth rate after 72 h [% compared to control] [1)]	-	-1.1	-2.1	-2.8	2.1	5.2 [2)]
Mean yield after 72 h [10^4 cells/mL]	36.724	38.882	40.245	41.343	33.393	29.153
Standard deviation	4.104	7.273	4.223	1.758	2.032	1.842
CV [%]	11.2	18.7	10.5	4.3	6.1	6.3
Inhibition of yield after 72 h [% compared to control] [1)]	-	-5.9	-9.6	-12.6	9.1	20.6 [2)]
	Endpoints [mg BAS 768 00 F/L] (nominal)					
E_rC_{50} / E_rL_{50} (72 h)	> 80 [3)] (95% confidence limits: n.d.) (equivalent to > 1.56 mg BAS 750 F/L)					
E_rC_{20} / E_rL_{20} (72 h)	> 80 [3)] (95% confidence limits: n.d.)					
E_rC_{10} / E_rL_{10} (72 h)	> 80 (95% confidence limits: n.d.)					
E_yC_{50} / E_yL_{50} (72 h)	> 80 [3)] (95% confidence limits: n.d.) (equivalent to > 1.56 mg BAS 750 F/L)					
E_yC_{20} / E_yL_{20} (72 h)	55.4 (95% confidence limits: 24.8 - > 80)					
E_yC_{10} / E_yL_{10} (72 h)	30.4 (95% confidence limits: 10.1 - > 80)					
NOE_rC / NOE_rLR (72 h)	32					
NOE_yC / NOE_yLR (72 h)	32					

Abbreviations: n.d.: not determined; CV: coefficient of variation

1) Negative values indicate stimulated growth

2) Statistically significant difference compared to control (Williams t-test, $\alpha = 0.05$, one-sided)

3) Determined directly from the raw data

Table A 18: Validity criteria according to OECD 201 (2011)

Criteria	Trigger value	Study value and page no.	Criteria met? Y/N	Guideline
Exponential biomass increase in the control	≥ 16-fold (within 72-h test period)	74.4-fold (p. 26)	Y	OECD 201
Mean coefficient of variation for section-by-section specific growth rates in the control cultures	≤ 35%	16.0% (p. 26)	Y	OECD 201
Coefficient of variation of average specific growth rates in replicate control cultures	≤ 7% (whole test period)	2.4% (p. 26)	Y	OECD 201

All validity criteria were met (see Table A 18 above).

Conclusion

In a 72-h static toxicity study with green algae (*Pseudokirchneriella subcapitata*), the E_rC_{50} / E_rL_{50} (72 h) for BAS 768 00 F was estimated to be > 80 mg test item/L based on nominal loading rates (equivalent to > 1.56 mg BAS 750 F/L).

A 2.2.2 KCP 10.2.2 Additional long-term and chronic toxicity studies on fish, aquatic invertebrates and sediment dwelling organisms

A 2.2.2.1 Study 1

The following chronic toxicity study on *Daphnia magna* performed with Sulfur (tested with the formulation 'Sulfur 80%WG') is provided in support of the assessment and was recently submitted under the Annex I renewal process for sulfur (AIR 4). The study summary was provided in the revised Renewal Assessment Report of sulfur (revised RAR, Vol. 3, B.9 (AS), June 2022).

Comments of zRMS:	The study was evaluated and accepted at the EU level during active substance renewal. 21-d NOEC = 16 µg/L.

Reference:	CP 10.2.2/1
Report	BAS 175 01 F - Daphnia magna reproduction test Salinas, E., 2011 Report No 394135 BASF DocID 2011/1023625 Authority registration No
Guideline(s):	Commission Regulation (EC) No 440/2008 - Part C.20, EPA 850.1300, ISO 10706, OECD 211
Deviations:	No
GLP:	Yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive summary

Groups of 10 daphnids (divided into 10 groups of 1 animal) were exposed to five test concentrations (1.0, 2.6, 6.4, 16 and 40 mg a.s./L as nominal concentration) of BAS 175 01 F (a.s. content: 77.7 % sulfur) for 21 days under semi-static conditions. A control group exposed to test water without test item was run concurrently. The invertebrates were observed daily for immobilisation, abnormal behaviour and reproduction.

Analytical verification of test item concentrations was performed weekly in the fresh and in the old test solutions. Since the measured concentration at the end of the test was outside the acceptable range of ± 20 %, the results were evaluated based on the time-weighted mean measured concentrations.

Under the conditions of this study, the NOEC (21 d) was determined at a time-weighted mean measured concentration of 35.6 mg a.s./L.

The EC_{10} and EC_{20} values could not be calculated since no dose-response curve can be retrieved from the data. However, EC_{10} and EC_{20} can be estimated to be above 35.6 mg a.s./L.

Remark: In the study, also undissolved sulfur in the test solutions was analysed. Thus, it is justified to set the endpoint for risk assessments at the water solubility limit of sulfur, i.e., > 0.016 mg a.s./L as for the other aquatoxicity studies.

Materials and methods

Materials

1. Test material:	BAS 175 01 F
Description:	solid, brown
Lot/Batch no.:	53208747G0
Active ingredient content:	77.7 % sulfur (analysed)
Stability of test compound:	date of expiry: June 01, 2013
2. Control:	test medium without test item
Solvent:	none
Toxic reference:	none
3. Test organisms -	
Species:	*Daphnia magna* STRAUS
Age:	< 24 hours old
Source:	bred in-house (Origin: Institut National de Recherche Chimique Appliquée, France)
Acclimatisation:	cultured under identical conditions as the test
Feeding (during the study):	fed daily a diet of live green algae *Desmodesmus subspicatus*.
No of *Daphnia*:	10 daphnids per concentration, with 1 individual in each vessel
4. Test units and exposure -	
Type and size:	100 mL glass beakers with 50 mL test medium (Elendt M4), covered with glass Petri plates to slow evaporation
Test procedure:	semi-static, renewal 3 times per week
Exposure time:	21 days
5. Test conditions -	
Temperature:	20 ± 1°C
Photoperiod:	16 h
Light intensity:	648 - 666 lux
Total hardness:	2.20 – 3.20 mmol/L
Dissolved oxygen:	> 3 mg/L
pH value:	7.5 – 8.5

Study design and methods

1. In life dates (experimental phase): November 29, 2010 to December 20, 2010

2. Description of test procedures:
   Groups of 10 daphnids (divided into 10 groups of 1 animal) were exposed under semi-static conditions to nominal test concentrations of:
   - 1.3, 3.3, 8.2, 20.6, 51.5 mg/L as nominal concentration based on the whole formulation
   - *or* 1.0, 2.6, 6.4, 16 and 40 mg a.s./L as nominal concentration for the active substance

   A control with test medium without test substance was run concurrently.

   The invertebrates were observed daily for immobilisation, abnormal effects and the number of offspring and then transferred into freshly prepared test medium. The test temperature, pH-value and dissolved oxygen concentration were measured for one interval per week.

3. Analysis of test item concentrations:
   Analytical verification of the test item concentration was performed in one representative test interval per week in each freshly prepared test solution and in the 48 hour or 72 hour old test solutions before renewal. The concentrations of sulfur were determined by means of HPLC.

4. Statistics
   A statistical evaluation was performed to determine LOEC and NOEC analysing data for the following parameters: reproduction as number of living young (Dunnett's test, one-sided analysis), growth as length in mm (Dunnett's test, two-sided analysis), time to first brood (Wilcoxon test, one-sided).

Results

Analytical results

Method validation
The analytical system gave a linear response between 0.25 mg/L and 2 mg/L of BAS 175 01 F. Linearity (r^2) of the calibration curves was > 0.999. To check the applicability of the method for the determination of the test item in M4-medium and its stability in the carrier during the test period, fortified samples with nominal concentrations between 0.2 and 50 mg/L were analysed. Exemplarily two fortified samples were additionally measured after 12 days to check their stability. The directly measured samples showed mean recovery rates of 91 % and 106 % of the nominal concentrations. This confirms the applicability of the method. The stability in the carrier over the test period of 11 days could only be confirmed for the higher concentration sample, for the lower concentrated sample degradation was observed.

Analytical verification of the active substance
The concentrations of sulfur were determined weekly, and the results are summarized in Table A 19. Since the measured concentrations at the end of the test was outside the acceptable range of ± 20 %, the results were evaluated based on the time-weighted mean measured concentrations.

Table A 19: Analytically measured concentrations of 'Sulfur 80% WG' as active ingredient (77.7 %) in the test solutions

Nominal concentration		Analytically measured concentration as active ingredient					
as BAS 175 01 F	as active ingredient	Mean initial		Mean 48-72 h old		Time-weighted mean	
[mg/L]	[mg/L]	[mg a.s./L]	[%] [a]	[mg a.s./L]	[%] [b]	[mg a.s./L] [c]	[%] [a]
0 (control)	0 (control)	< LOD	-	< LOD	-	< LOD	-
1.3	1.0	0.826	83	0.418	51	0.576	58
3.3	2.6	2.25	86	1.42	63	1.76	68
8.2	6.4	6.08	95	4.44	73	5.13	80
20.6	16	15.7	98	11.4	73	13.4	84
51.5	40	40.8	102	31.3	77	35.6	89

[a)] % of nominal as active ingredient
[b)] % of mean initial measured
[c)] based on 7 measured renewal period days

Biological results

Immobilisation: One dead adult daphnid was observed in the highest test group 40 mg/L (10% mortality: see Table A 20). This minor level effect is within the accepted tolerance (OECD no. 211) for control performance (< 20% mortality) and was thus not considered toxicologically relevant. Effect concentrations were not calculated since there was no concentration response relationship observed from the data.

Table A 20: Number of living daphnids per concentration

Test groups		Number of living daphnids
Nominal (loading)	TWM [mg a.s./L]	
0 (control)		10
1.0	0.576	10
2.6	1.76	10
6.4	5.13	10
16.0	13.4	10
40.0	35.6	9

TWM: time-weighted mean

Reproduction: The number of offspring in the test concentrations was not different from the control (see Table A 21).

Table A 21: Reproduction (Number of living young adult daphnids) per concentration

Test groups		Reproduction [mean living young]	Standard deviation	Coefficient of variance [%]
Nominal (loading)	TWM [mg a.s./L]			
0 (control)		117	20.4	17.5
1.0	0.576	121	11.6	9.6
2.6	1.76	128	9.0	7.0
6.4	5.13	125	9.3	7.5
16.0	13.4	125	20.1	16.1
40.0	35.6	129	6.1	4.7

TWM: time-weighted mean

Other observations: No additional adverse effects on growth, immobilized young, time to first brood, aborted eggs or abnormal behaviour were observed in any of the test groups (see Table A 22).

Table A 22: Other biological observations among surviving parent animals

Test groups		Growth [mean length, mm]	Mean immobile young	Mean days to first brood	Mean aborted eggs
Nominal (loading)	TWM [mg a.s./L]				
0 (control)		4.4	1.4	10	1.3
1.0	0.576	4.4	1.4	9	0.8
2.6	1.76	4.4	2.7	9	1.3
6.4	5.13	4.3	1.2	9	1.1
16.0	13.4	4.3	1.7	9	1.6
40.0	35.6	4.3	0.6	9	1.7

TWM: time-weighted mean

Deficiencies: The number of immobilised daphnids in the control group did not exceed 20% at the end of the test (*i.e.*, 0.0%). The mean number of offspring produced per parent animal surviving at the end of the test is ≥ 60 (*i.e.*, 78 - 142). Thus, the test was considered to be valid without restrictions.

Conclusion

Under the conditions of this study, the NOEC (21 d) was determined at a time-weighted mean measured concentration of 35.6 mg a.s./L.
The EC_{10} and EC_{20} values could not be calculated since no dose-response curve can be retrieved from the data. However, EC_{10} and EC_{20} can be estimated to be above 35.6 mg a.s./L.

Remark: In the study, also undissolved sulfur in the test solutions was analysed. Thus, it is justified to set the endpoint for risk assessments at the water solubility limit of sulfur, i.e., > 0.016 mg a.s./L as for the other aquatoxicity studies.

A 2.2.3 KCP 10.2.3 Further testing on aquatic organisms

Not relevant.

A 2.3 KCP 10.3 Effects on arthropods

A 2.3.1 KCP 10.3.1 Effects on bees

A 2.3.1.1 KCP 10.3.1.1 Acute toxicity to bees

A 2.3.1.1.1 KCP 10.3.1.1.1 Acute oral toxicity to bees

A 2.3.1.1.1.1 Study 1

Comments of zRMS:	The study was evaluated and accepted at the EU level during active substance renewal.

Reference:	CP 10.3.1.1.1/1
Report	Acute toxicity of Netzschwefel Stulln (Sulphur 80% WG) to the honeybee Apis mellifera L. under laboratory conditions, Franke, M., 2016 report No EU-161048159B,16 10 48 159 B BASF DocID 2016/1345280 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

30 bees per group (3 replicates/group, 10 bees/replicate) were exposed to Netzschwefel Stulln ('Sulfur 80% WG') via oral or contact exposure at dose rates between 54.2 – 866.4 µg prod./bee. Control groups and a reference item group were run concurrently. Mortality and behaviour of the bees was recorded after 4, 24 and 48 hours.

Under the conditions of this study, the contact and oral LD_{50} after 48 hours was > 866.4 µg prod./bee, which is equivalent to > 700.1 µg a.s./bee. No behavioural effects on honeybees were observed in either the contact or the oral tests within 48 hours after application.

I. Materials and methods

A. Materials

1. Test substance:	Netzschwefel Stulln (alternative name: 'Sulfur 80% WG')
Lot/Batch no.:	51202
Content/Purity:	sulfur: 80.8 ± 0.6 % w/w (analysed)
Control:	two control groups: a deionised water control and a 1 % (v/v) wetting agent control (Tween®80)
Toxic reference:	Dimethoate EC 400 (dimethoate, analysed 420.3 g/L)
2. Test organisms	
Species:	*Apis mellifera* L.
Age:	young adult worker from a healthy colony
No. of organisms:	30 per test group (divided in 3 replicates of 10 bees each)
Feeding:	50 % (w/v) sucrose solution
3. Test units and exposure	
Type and size:	Disposable cardboard cages with holes in the bottom side for ventilation and a glass plate in front; 95 mm x 50 mm x 65 mm (length x width x height)
Test procedure:	acute oral and contact exposure
Test duration:	48 hours
4. Test conditions	
Temperature:	23.5 – 24.2 °C
Relative humidity:	51 – 67 %
Photoperiod:	continuous dark
Light intensity:	not relevant (see above)

B. Study design and method

1. In-life dates (experimental phase): September 27 to September 29, 2016

2. Test design:

Oral test:
The test and reference item, respectively, were administered in 200 µL feeding solution (236.2 mg). The control was fed with untreated feeding solution at the same quantity. Before the feeding solutions were filled into the feeding tubes, the tare of the tubes was determined. Groups of 10 bees per cage were provided with 200 µL test solution in a glass ampoule (half-open on its longitudinal axis, 5 cm long). The feeding tubes were introduced through a hole in the roof of the cage. Due to their social feeding behaviour (trophallaxis), honeybees of a distinct group are assumed to receive approximately the same amount of. food (approximately 20 µL/bee) and consequently the same dose of test item and reference item, respectively. Two hours after application, the feeding tubes were removed and the exact quantity of consumed test solution was determined. The feeding tubes for application were replaced with feeding tubes containing untreated 50 % w/v sucrose solution

Contact test:
Before application, bees in the test cage were anaesthetised with CO_2 for approximately ½ min. Anaesthetised bees were removed from the cages to a large petri dish and turned around with the forceps for thoracal application of a single droplet of the control(s) or test item (4 µL) and reference item solutions (2 µL), respectively. The doses were placed on the dorsal thorax using an Eppendorf Micropipette. After application, bees were carefully transferred one by one into the test cages by means of forceps. BioChem agrar experience has proven that greater volumes (up to 4 µL are suitable) than 1 µL droplets (as recommended in OECD 214) are suitable and no adverse effects on the outcome of the study are to be expected. Tween®80 solution (1 % v/v) was used to ensure good penetration or adhesion of the test solution droplet on the bee body. Tween solution is non-toxic to honey bees at the concentration used.

3. Statistics:
The Multiple sequentially-rejective Fisher Test after Bonferroni-Holm was used for statistical evaluation of the mortality values. The accepted significance level was $p \leq 0.05$ (one-sided greater). The median lethal doses (LD_{50}) along with the 95 % confidence limits were calculated:
   - Contact and oral test item: no LD_{50}-calculation
   - Contact Reference item: Probit analysis (linear maximum likelihood regression)
   - Oral Reference item: Probit analysis (linear maximum likelihood regression)

II. Results and discussion

A. Findings

Contact test

In the control groups either treated with deionised water or tween solution, no mortality was observed after 48 hours. In the test item treatment, no statistically significant mortality occurred after thoracic application of ≤ 866.4 µg prod./bee, after 48 hours. Minor mortality of 13.3 % was observed at the highest dose rate of 866.4 µg prod./bee, whereas no effect on bee mortality within 48 hours occurred at the lower dose rates of ≤ 433.2 µg prod./bee. Throughout the contact toxicity test no effects on behaviour of honeybees were observed when bees were treated with ≤ 866.4 µg prod./bee.

Table A 23: Cumulative mortality in honey bees after contact application

Group	Mean mortality [%] - oral mode		
	4 h	24 h	48 h
Water control	0.0	0.0	0.0
Tween solution control	0.0	0.0	0.0
Test item – 866.4 µg prod./bee	0.0	13.3	13.3
Test item – 433.2 µg prod./bee	0.0	0.0	0.0
Test item – 216.6 µg prod./bee	0.0	0.0	0.0
Test item – 108.3 µg prod./bee	0.0	0.0	0.0
Test item – 54.2 µg prod./bee	0.0	0.0	0.0
Reference item – 0.250 µg a.s./bee	0.0	93.3*	93.3*
Reference item – 0.188 µg a.s./bee	0.0	53.3*	73.3*
Reference item – 0.141 µg a.s./bee	0.0	20.0*	40.0*
Reference item – 0.106 µg a.s./bee	0.0	6.7	13.3

* Significant difference in pairwise comparison between treatment and tween control by multiple sequentially-rejective Fisher Test after Bonferroni-Holm correction for mortality

Oral test

The control group fed with pure 50 % (w/v) sucrose solution demonstrated no mortality after 48 hours. In the test item treatment, no statistically significant mortality occurred after consumption of ≤ 866.4 µg prod./bee, after 48 hours. Minor mortality of 3.3 % was observed at the other dose rates of 866.4 and 54.2 µg prod./bee, whereas the other dose rates revealed no effects on bee mortality within 48 hours. The resulting LD_{50} (48 h) was > 866.4 µg consumed prod./bee that is equivalent to > 700.1 µg consumed a.s./bee. Throughout the oral toxicity test no effects on behaviour of bees were observed when bees were fed ≤ 866.4 µg Netzschwefel Stulln ('Sulfur 80% WG')/bee.

Table A 24: Cumulative mortality in honey bees after oral application

Group	Mean mortality [%] - oral mode		
	4 h	24 h	48 h
Water control	0.0	0.0	0.0
Tween solution control	0.0	0.0	0.0
Test item – 866.4 µg prod./bee	0.0	3.3	3.3
Test item – 433.2 µg prod./bee	0.0	0.0	0.0
Test item – 216.6 µg prod./bee	0.0	0.0	0.0
Test item – 108.3 µg prod./bee	0.0	0.0	0.0
Test item – 54.2 µg prod./bee	0.0	3.3	3.3
Reference item – 0.250 µg a.s./bee	0.0	93.3*	96.7*
Reference item – 0.188 µg a.s./bee	0.0	70.0*	80.0*
Reference item – 0.141 µg a.s./bee	0.0	10.0	16.7*
Reference item – 0.106 µg a.s./bee	0.0	3.3	3.3

* Significant difference in pairwise comparison between treatment and tween control by multiple sequentially-rejective Fisher Test after Bonferroni-Holm correction for mortality

B. Deficiencies

None: The mortality rate in the control was below 10 % at the end of the test (*i.e.* 0.0 %). Furthermore, the oral and contact LD_{50} (24h) of the reference item was calculated to be 0.145 µg a.s./bee (oral) and 0.175 µg a.s./bee (contact), respectively, that is within the recommended range. Thus, the validity criteria of the underlying guidelines were fulfilled and the test was considered to be valid without restrictions.

III. Conclusions

The acute contact and oral toxicity of Netzschwefel Stulln ('Sulfur 80% WG') was tested on honeybees under laboratory conditions over 48 hours. The contact and oral LD_{50} after 48 hours was > 866.4 µg prod./bee, which is equivalent to > 700.1 µg a.s./bee. No behavioural effects on honeybees were observed in either the contact or the oral tests within 48 hours after application, therefore, also the NOEC was set at the highest dose level, *i.e.* NOEC (48 h) = 866.4 µg product/bee (equivalent to 700.1 µg a.s./bee).

A 2.3.1.1.1.2 Study 2

Comments of zRMS:	The study was accepted. The study was conducted in accordance with OECD guideline 213 (acute oral) and 214 (acute contact). In the summary study presented below there are only data considering the oral toxicity test. The acute contact study summary is presented in p. A.2.3.1.1.2.2. The validity criteria were met: The following endpoints were derived Oral: LD_{50} 48 h > 845 µg consumed product/bee (corresponding to > 392 µg consumed total a.s./bee); NOED 48 h = 209 µg formulation/bee

Reference:	CP 10.3.1.1.1/2
Report	BAS 768 00 F - Acute Oral and Contact Toxicity to the Honey Bee Apis mellifera L. (Hymenoptera, Apidae) under Laboratory Conditions, Kling, A., 2021 report No 894520, S21-04684 BASF DocID 2021/2014196 Authority registration No
Guideline(s):	OECD 213 (1998), OECD 214 (1998)
Deviations:	No
GLP:	yes (certified by Landesanstalt fuer Umwelt, Baden-Wuerttemberg, Karlsruhe, Germany),
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive summary

In an acute oral toxicity laboratory study, the effects of the formulated product BAS 768 00 F on honey bees (*Apis mellifera*) were investigated. Adult worker honey bees were orally exposed to an untreated control (sugar solution) and to BAS 768 00 F at nominal doses of 62.5, 125, 250, 500 and 1000 µg product/bee. The actual food uptake resulted in doses of 68.4, 139, 269, 534 and 845 µg consumed product/bee (corresponding to 31.8, 64.5, 125, 248 and 392 µg consumed total a.s./bee). Bees were exposed in groups of 10 animals with 4 replicates per treatment. Assessment of honey bee mortality and behavioural effects was conducted after 4, 24 and 48 hours.

After 48 hours of oral exposure, no mortality was observed in the untreated control, whereas 2.5%, 2.5%, 7.5%, 0% and 12.5% mortality was observed at test item doses of 68.4, 139, 269, 534 and 845 µg consumed product/bee. At the highest test item dose of 845 µg consumed product/bee, the food consumption was slightly reduced and 31.4% of the surviving bees were observed to be apathic. After 48 hours, no behavioural abnormalities or sublethal effects were observed at lower test item doses.

In a 48-h acute oral toxicity study with honey bees (*Apis mellifera*), the LD_{50} (48 h) for BAS 768 00 F was estimated to be > 845 µg consumed product/bee (corresponding to > 392 µg consumed total a.s./bee).

Materials and methods

Materials

Test item:	BAS 768 00 F, Batch no. FD-210120-1029; content of a.s.: mefentrifluconazole (BAS 750 F; Reg. no. 5834378): 26.3 g/L (nominal: 25.0 g/L), sulfur (BAS 175 F, Reg. no. 240586): 614.9 g/L (nominal: 600.0 g/L); density: 1.346 g/cm³.

Study design and methods

Test species:	Honey bee (*Apis mellifera*), young adult worker bees, derived from queen-right and healthy colonies; randomly collected by brushing off the combs of the honey chamber and distributed into test cages one day before exposure; source: in-house colonies.
Test design:	Exposure period: 48 hours; adult worker bees were exposed orally to 5 test item doses in treated food (50% (w/v) aqueous sucrose solution) plus an untreated control; 10 bees per cage; 4 replicates (cages) per treatment; the treated food was offered in feeders, which were weighed before and after introduction into the cages; the duration of uptake was 6 hours for the test item treatments; assessments of honey bee mortality and behavioural effects after 4, 24 and 48 hours of exposure.
Endpoints:	LD_{50} related to mortality and assessment of sub-lethal effects.
Reference item:	BAS 152 65 I (dimethoate, 38.8% w/w (412 g/L) analysed); dose rates: 0.06 - 0.14 µg dimethoate/bee in an aqueous sugar solution (50% (w/v)), resulting in an uptake of 0.07 - 0.15 µg consumed a.s./bee.
Test doses:	Untreated control (50% (w/v) aqueous sugar solution); nominal test item dose rates: 62.5, 125, 250, 500 and 1000 µg product/bee in a 50% (w/v) sugar solution, resulting in actual uptake rates of 68.4, 139, 269, 534 and 845 µg consumed product/bee, corresponding to 31.8, 64.5, 125, 248 and 392 µg total a.s./bee.
Test conditions:	Stainless stell cages (8 x 6 x 4 cm) with a transparent pane and perforated bottom; temperature: 24.6 - 25.1 °C; relative humidity: 57.1 - 61.0%; photoperiod: 24 h darkness; feeding: 50% (w/v) aqueous sugar solution *ad libitum* directly after treatment using feeders.
Analytics:	No analytical verification of the test item is required according to the current test guideline.
Statistics:	Descriptive statistics.

Results

Biological results

After 48 hours of oral exposure, no mortality was observed in the untreated control, whereas 2.5%, 2.5%, 7.5%, 0% and 12.5% mortality was observed at test item doses of 68.4, 139, 269, 534 and 845 µg consumed product/bee. At the highest test item dose of 845 µg consumed product/bee, the food consumption was slightly reduced and 31.4% of the surviving bees were observed to be apathic. After 48 hours, no behavioural abnormalities or sublethal effects were observed at lower test item doses. The results are summarized in Table A 25.

Table A 25: Effects of BAS 768 00 F on honey bees (*Apis mellifera*) after 48 hours of oral exposure

Dose rate			**Mortality [%]**	
Nominal	**Measured actual uptake**		**After 24 hours**	**After 48 hours**
[µg product/bee]	**[µg consumed product/bee]**	**[µg consumed total a.s./bee]#**	**Total**	**Total**
Untreated control	-	-	0.0	0.0
62.5	68.4	31.8	2.5	2.5
125	139	64.5	2.5	2.5
250	269	125	7.5	7.5
500	534	248	0.0	0.0
1000	845	392	10.0	12.5
	Endpoints			
	[µg consumed product/bee]		**[µg consumed total a.s./bee] #**	
LD_{50} (48 h)	> 845 (95% confidence limits: n.d.)		> 392 (95% confidence limits: n.d.)	

Abbreviations: n.d.: not determined (could not be calculated due to mathematical reasons)

Values based on the active substance have been recalculated from the study data considering the nominal content of 625 g total a.s./L and density 1.346 g/cm^3.

The LD_{50} value (24 h) for the reference item dimethoate was determined to be 0.12 µg consumed a.s./bee (95% confidence limits: 0.11 - 0.13 µg a.s./bee).

Table A 26: Validity criteria according to OECD 213 (1998)

Criteria	**Trigger value**	**Study value and page no.**	**Criteria met? Y/N**	**Guideline**
Mortality in the control at end of exposure	≤ 10%	Untreated control: 0% (p. 20)	Y	OECD 213
LD_{50} (24 h) of the reference item	0.1 - 0.35 µg/bee	LD_{50} (24 h) = 0.12 µg consumed dimethoate/bee (p. 20)	Y	OECD 213

All validity criteria were met (see Table A 26 above).

Conclusion

In a 48-h acute oral toxicity study with honey bees (*Apis mellifera*), the LD_{50} (48 h) for BAS 768 00 F was estimated to be > 845 µg consumed product/bee (corresponding to > 392 µg consumed total a.s./bee).

A 2.3.1.1.1.3 Study 3

Comments of zRMS:	The study was accepted. The study was conducted in accordance with OECD guideline 247 (acute oral). The validity criteria were met: • **oral test**: the mean mortality of the control groups was 0% (recommended ≤ 10 %); The following endpoints were derived: LD_{50} 48 h > 976.9 µg formulation/bumblebee (equivalent to > 453.6 µg consumed a.s./bumble bee) NOED 48 h ≥ 976.9 µg formulation/bumblebee No behavioural effects appeared at all tested dose rates of BAS 768 00 F during the oral toxicity test up to 48 hours. In the summary study presented below there are only data considering the oral toxicity test. The acute contact study summary is presented in p. A.2.3.1.1.2.3.

Reference:	CP 10.3.1.1.1/3
Report	Acute toxicity of BAS 768 00 F to the bumblebee Bombus terrestris L. under laboratory conditions, Amsel, K., 2022 report No 919844, 2248BBA0024 BASF DocID 2022/2010697 Authority registration No
Guideline(s):	OECD 246 (2017), OECD 247 (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 an acute oral toxicity laboratory study, the effects of the formulated product BAS 768 00 F on bumble bees (*Bombus terrestris*) were investigated. Adult worker bumble bees were orally exposed to an untreated control (sucrose solution) and to BAS 768 00 F at nominal dose rates of 62.5, 125.0, 250.0, 500.0 and 1000.0 µg product/bumble bee (equivalent to 29.1, 58.0, 116.0, 232.2 and 464.4 µg total a.s./bumble bee), resulting in actual uptake rates of 61.9, 122.9, 243.3, 487.4 and 976.9 µg consumed product/bumble bee (equivalent to 28.7, 57.1, 113.0, 226.4 and 453.6 µg consumed total a.s./bumble bee), with 30 replicates per treatment, each containing 1 bumble bee. Assessments of bumble bee mortality and behavioural effects were conducted after 4, 24 and 48 hours.

After 48 hours of oral exposure, no mortality was observed in the untreated control and at up to and including the highest test item dose rate of 976.9 µg consumed product/bumble bee. No test item induced behavioural effects were observed.

In a 48-h acute oral toxicity study with bumble bees (*Bombus terrestris*), the LD_{50} (48 h) for BAS 768 00 F was estimated to be > 976.9 µg consumed product/bumble bee (equivalent to > 453.6 µg consumed a.s./bumble bee).

Materials and methods

Materials

Test item: BAS 768 00 F, Batch no. FD-210120-1029; content of a.s.: mefentrifluconazole (BAS 750 F; Reg. no. 5834378): 26.3 g/L (nominal: 25.0 g/L), sulfur (BAS 175 F, Reg. no.: 240586): 614.9 g/L (nominal: 600.0 g/L); density: 1.346 g/cm^3.

Study design and methods

Test species: Bumble bee (*Bombus terrestris*), young adult worker bumble bees, weight range: 160 - 340 mg; derived from queenright and healthy bumble bee hives; bumble bees were collected from the bumblebee hives under red light with a starvation period of 4 hours before test begin; source: originally obtained from "Biobest Belgium N.V.", Westerlo, Belgium; delivered by "Katz Biotech AG", Baruth, Germany.

Test design: Exposure period: 48 hours; adult worker bumble bees were exposed orally to 5 test item dose rates in treated food (50% (w/v) aqueous sucrose solution) plus an untreated control; 1 bumble bee per cage; 30 replicates (cages) per treatment; the treated food was offered in syringes, which were weighed before and after introduction into the cages; the duration of uptake was 2 hours for the test item treatments; "non-feeders" (bumblebees which consume less than 80% of the mean consumption) were replaced by feeders; assessments of bumble bee mortality and behavioural effects after 4, 24 and 48 hours of exposure.

Endpoints: LD_{50} and NOED related to mortality and assessment of sub-lethal effects.

Reference item: Dimethoate EC 400 (dimethoate, 400 g/L nominal); dose rate: 1.5 µg a.s./bumble bee in 50% (w/v) aqueous sucrose solution, resulting in an uptake of 1.47 µg consumed a.s./bumble bee.

Test doses: Untreated control (50% (w/v) aqueous sucrose solution); nominal test item dose rates: 62.5, 125.0, 250.0, 500.0 and 1000.0 µg product/bumble bee (equivalent to 29.1, 58.0, 116.0, 232.2 and 464.4 µg total a.s./bumble bee) in a 50% (w/v) aqueous sucrose solution, resulting in actual uptake rates of 61.9, 122.9, 243.3, 487.4 and 976.9 µg consumed product/bumble bee (equivalent to 28.7, 57.1, 113.0, 226.4 and 453.6 µg consumed total a.s./bumble bee).

Test conditions: Nicot cages (length: 7 cm; diameter: 2 cm); temperature: 25.0 - 25.4°C; relative humidity: 60 - 63%; photoperiod: 24 h darkness; feeding: 50% (w/v) aqueous

sucrose solution provided continuously *ad libitum* directly after treatment using syringes.

Analytics: Analytical verification of test item concentrations in the feeding solutions was conducted using an HPLC-method with MS-MS detection (Method No. L0452/02).

Statistics: Descriptive statistics.

Description of the analytical procedures

The concentrations of mefentrifluconazole (contained in BAS 768 00 F) in bumble bee feeding solutions were determined according to the analytical method L0452/02. The analytical method is fully validated in a separate study (BASF DocID: 2021/2022023). The validation of the analytical method is described in the study report. The samples were extracted with 50/50/1 (v/v/v) acetonitrile/water/formic acid and QuEChERS salt mix containing 0.5 g magnesium sulfate, 0.12 g sodium chloride, 0.06 g disodium hydrogen-citrate sesquihydrate and 0.12 g of trisodium citrate dihydrate by shaking. 1 mL of the acetonitrile phases were transferred into a 10 mL-volumetric flask and filled to the mark with 50/50/1 (v/v/v) acetonitrile/water/formic acid and, if necessary, further diluted with dilution medium into the range of the calibration curve. The diluted extracts were injected into the HPLC system. The determination was performed by HPLC-MS/MS. The limit of quantification (LOQ) was set to 0.01 mg a.s./kg food and the limit of detection (LOD) was 0.002 mg a.s./kg food (≤ 30% of LOQ). Matrix effects were taken into account by the addition of the same amount of blank extract to calibration samples as included in the analysis samples. Thus, all measured samples contained the same amount of original sample matrix. The maximum storage period of the samples was 14 days Stability samples were not analysed, as a storage period of 30 days was not exceeded. Mean recoveries of the procedural recovery samples were between 91.3% and 109% of the nominal concentrations. Details on measured fortification samples and obtained procedural recoveries for the formulated product BAS 768 00 F (based on measurements of mefentrifluconazole) are provided in **Table A 27**.

Table A 27: Procedural recoveries for BAS 768 00 F (based on measurements of mefentrifluconazole)

Matrix	**Fortification level [mg a.s./kg food]**	**n**	**Mean recovery [%]**	**RSD [%]**
Bumble bee feeding solutions	0.01	5	91.3	6.30
	519	5	109	2.07

Abbreviations: n: number of replicates; RSD: relative standard deviation

Results

Biological results

After 48 hours of oral exposure, no mortality was observed in the untreated control and at up to and including the highest test item dose rate of 976.9 µg consumed product/bumble bee. No test item induced behavioural effects were observed. The results are summarised in Table A 28.

Table A 28: Effects of BAS 768 00 F on bumble bees (*Bombus terrestris*) after 48 hours of oral exposure

Dose rate [µg product/bumble bee]		**Mortality [%]**	
		After 24 hours	**After 48 hours**
Nominal	**Measured actual uptake**	**Total**	**Total**
Untreated control	-	0.0	0.0
62.5	61.9	0.0	0.0
125.0	122.9	0.0	0.0
250.0	243.3	0.0	0.0
500.0	487.4	0.0	0.0
1000.0	976.9	0.0	0.0
		Endpoints	
		[µg consumed product/ bumble bee]	**[µg consumed a.s./ bumble bee]**
LD_{50} (48 h)		> 976.9 (95% CL: n.d.)	> 453.6 (95% CL: n.d.)
NOED (48 h)		≥ 976.9	≥ 453.6

Abbreviations: CL: confidence limits; n.d.: not determined (could not be calculated due to mathematical reasons)

The mortality rate in the reference item treatment was 93.3% after 48 hours.

Table A 29: Validity criteria according to OECD 247 (2017)

Criteria	**Trigger value**	**Study value and page no.**	**Criteria met? Y/N**	**Guideline**
Mortality in the control(s) at end of exposure	≤ 10%	Untreated control: 0% (p. 22)	Y	OECD 247
Mortality in the reference item treatment at end of exposure	≥ 50%	93.3% mortality at 1.47 µg consumed dimethoate/ bumble bee (p. 22)	Y	OECD 247

All validity criteria were met (see Table A 29 above).

Conclusion

In a 48-h acute oral toxicity study with bumble bees (*Bombus terrestris*), the LD_{50} (48 h) for BAS 768 00 F was estimated to be > 976.9 µg consumed product/bumble bee (equivalent to > 453.6 µg consumed a.s./bumble bee).

A 2.3.1.1.2 KCP 10.3.1.1.2 Acute contact toxicity to bees

A 2.3.1.1.2.1 Study 1

Comments of zRMS:	The study was evaluated and accepted during active substance renewal.

Reference:	CP 10.3.1.1.2/1
Report	Acute toxicity of Netzschwefel Stulln (Sulphur 80% WG) to the honeybee Apis mellifera L. under laboratory conditions, Franke, M., 2016 report No EU-161048159B,16 10 48 159 B BASF DocID 2016/1345280 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

30 bees per group (3 replicates/group, 10 bees/replicate) were exposed to Netzschwefel Stulln ('Sulfur 80% WG') via oral or contact exposure at dose rates between 54.2 – 866.4 µg prod./bee. Control groups and a reference item group were run concurrently. Mortality and behaviour of the bees was recorded after 4, 24 and 48 hours.

Under the conditions of this study, the contact and oral LD_{50} after 48 hours was > 866.4 µg prod./bee, which is equivalent to > 700.1 µg a.s./bee. No behavioural effects on honeybees were observed in either the contact or the oral tests within 48 hours after application.

I. Materials and methods

A. Materials

1.	Test substance:	Netzschwefel Stulln (alternative name: 'Sulfur 80% WG')
	Lot/Batch no.:	51202
	Content/Purity:	sulfur: 80.8 ± 0.6 % w/w (analysed)
	Control:	two control groups: a deionised water control and a 1 % (v/v) wetting agent control (Tween®80)
	Toxic reference:	Dimethoate EC 400 (dimethoate, analysed 420.3 g/L)
2.	Test organisms	
	Species:	*Apis mellifera* L.

Age:	young adult worker from a healthy colony
No. of organisms:	30 per test group (divided in 3 replicates of 10 bees each)
Feeding:	50 % (w/v) sucrose solution

3. Test units and exposure

Type and size:	Disposable cardboard cages with holes in the bottom side for ventilation and a glass plate in front; 95 mm x 50 mm x 65 mm (length x width x height)
Test procedure:	acute oral and contact exposure
Test duration:	48 hours

4. Test conditions

Temperature:	23.5 – 24.2 °C
Relative humidity:	51 – 67 %
Photoperiod:	continuous dark
Light intensity:	not relevant (see above)

B. Study design and method

1. In-life dates (experimental phase): September 27 to September 29, 2016

2. Test design:

Oral test:
The test and reference item, respectively, were administered in 200 µL feeding solution (236.2 mg). The control was fed with untreated feeding solution at the same quantity. Before the feeding solutions were filled into the feeding tubes, the tare of the tubes was determined. Groups of 10 bees per cage were provided with 200 µL test solution in a glass ampoule (half-open on its longitudinal axis, 5 cm long). The feeding tubes were introduced through a hole in the roof of the cage. Due to their social feeding behaviour (trophallaxis), honeybees of a distinct group are assumed to receive approximately the same amount of. food (approximately 20 µL/bee) and consequently the same dose of test item and reference item, respectively. Two hours after application, the feeding tubes were removed and the exact quantity of consumed test solution was determined. The feeding tubes for application were replaced with feeding tubes containing untreated 50 % w/v sucrose solution

Contact test:
Before application, bees in the test cage were anaesthetised with CO_2 for approximately ½ min. Anaesthetised bees were removed from the cages to a large petri dish and turned around with the forceps for thoracal application of a single droplet of the control(s) or test item (4 µL) and reference item solutions (2 µL), respectively. The doses were placed on the dorsal thorax using an Eppendorf Micropipette. After application, bees were carefully transferred one by one into the test cages by means of forceps. BioChem agrar experience has proven that greater volumes (up to 4 µL are suitable) than 1 µL droplets (as recommended in OECD 214) are suitable and no adverse effects on the outcome of the study are to be expected. Tween®80 solution (1 % v/v) was used to ensure good penetration or adhesion of the test solution droplet on the bee body. Tween solution is non-toxic to honey bees at the concentration used.

3. Statistics:
The Multiple sequentially-rejective Fisher Test after Bonferroni-Holm was used for statistical evaluation of the mortality values. The accepted significance level was $p \leq 0.05$ (one-sided greater). The median lethal doses (LD_{50}) along with the 95 % confidence limits were calculated:
   - Contact and oral test item: no LD_{50}-calculation
   - Contact Reference item: Probit analysis (linear maximum likelihood regression)
   - Oral Reference item: Probit analysis (linear maximum likelihood regression)

II. Results and discussion

A. Findings

Contact test

In the control groups either treated with deionised water or tween solution, no mortality was observed after 48 hours. In the test item treatment, no statistically significant mortality occurred after thoracic application of ≤ 866.4 µg prod./bee, after 48 hours. Minor mortality of 13.3 % was observed at the highest dose rate of 866.4 µg prod./bee, whereas no effect on bee mortality within 48 hours occurred at the lower dose rates of ≤ 433.2 µg prod./bee. Throughout the contact toxicity test no effects on behaviour of honeybees were observed when bees were treated with ≤ 866.4 µg prod./bee.

Table A 30: Cumulative mortality in honey bees after contact application

Group	Mean mortality [%] - oral mode		
	4 h	24 h	48 h
Water control	0.0	0.0	0.0
Tween solution control	0.0	0.0	0.0
Test item – 866.4 µg prod./bee	0.0	13.3	13.3
Test item – 433.2 µg prod./bee	0.0	0.0	0.0
Test item – 216.6 µg prod./bee	0.0	0.0	0.0
Test item – 108.3 µg prod./bee	0.0	0.0	0.0
Test item – 54.2 µg prod./bee	0.0	0.0	0.0
Reference item – 0.250 µg a.s./bee	0.0	93.3*	93.3*
Reference item – 0.188 µg a.s./bee	0.0	53.3*	73.3*
Reference item – 0.141 µg a.s./bee	0.0	20.0*	40.0*
Reference item – 0.106 µg a.s./bee	0.0	6.7	13.3

* Significant difference in pairwise comparison between treatment and tween control by multiple sequentially-rejective Fisher Test after Bonferroni-Holm correction for mortality

Oral test

The control group fed with pure 50 % (w/v) sucrose solution demonstrated no mortality after 48 hours.
In the test item treatment, no statistically significant mortality occurred after consumption of ≤ 866.4 µg prod./bee, after 48 hours. Minor mortality of 3.3 % was observed at the other dose rates of 866.4 and 54.2 µg prod./bee, whereas the other dose rates revealed no effects on bee mortality within 48 hours. The resulting LD_{50} (48 h) was > 866.4 µg consumed prod./bee that is equivalent to > 700.1 µg consumed a.s./bee. Throughout the oral toxicity test no effects on behaviour of bees were observed when bees were fed ≤ 866.4 µg Netzschwefel Stulln ('Sulfur 80% WG')/bee.

Table A 31: Cumulative mortality in honey bees after oral application

Group	Mean mortality [%] - oral mode		
	4 h	**24 h**	**48 h**
Water control	0.0	0.0	0.0
Tween solution control	0.0	0.0	0.0
Test item – 866.4 µg prod./bee	0.0	3.3	3.3
Test item – 433.2 µg prod./bee	0.0	0.0	0.0
Test item – 216.6 µg prod./bee	0.0	0.0	0.0
Test item – 108.3 µg prod./bee	0.0	0.0	0.0
Test item – 54.2 µg prod./bee	0.0	3.3	3.3
Reference item – 0.250 µg a.s./bee	0.0	93.3*	96.7*
Reference item – 0.188 µg a.s./bee	0.0	70.0*	80.0*
Reference item – 0.141 µg a.s./bee	0.0	10.0	16.7*
Reference item – 0.106 µg a.s./bee	0.0	3.3	3.3

* Significant difference in pairwise comparison between treatment and tween control by multiple sequentially-rejective Fisher Test after Bonferroni-Holm correction for mortality

B. Deficiencies

None: The mortality rate in the control was below 10 % at the end of the test (*i.e.* 0.0 %). Furthermore, the oral and contact LD_{50} (24h) of the reference item was calculated to be 0.145 µg a.s./bee (oral) and 0.175 µg a.s./bee (contact), respectively, that is within the recommended range. Thus, the validity criteria of the underlying guidelines were fulfilled and the test was considered to be valid without restrictions.

III. Conclusions

The acute contact and oral toxicity of Netzschwefel Stulln ('Sulfur 80% WG') was tested on honeybees under laboratory conditions over 48 hours. The contact and oral LD_{50} after 48 hours was > 866.4 µg prod./bee, which is equivalent to > 700.1 µg a.s./bee. No behavioural effects on honeybees were observed in either the contact or the oral tests within 48 hours after application, therefore, also the NOEC was set at the highest dose level, *i.e.* NOEC (48 h) = 866.4 µg product/bee (equivalent to 700.1 µg a.s./bee).

A 2.3.1.1.2.2 Study 2

Comments of zRMS:	The study was accepted. The study was conducted in accordance with OECD guideline 214 (acute contact). The validity criteria were met: The following endpoints were derived: LD_{50} 48 h > 1000 µg consumed product/bee (corresponding to > 464 µg consumed total a.s./bee). NOED 48 h = 250 µg formulation/bee

Reference:	CP 10.3.1.1.2/2
Report	BAS 768 00 F - Acute Oral and Contact Toxicity to the Honey Bee Apis mellifera L. (Hymenoptera, Apidae) under Laboratory Conditions, Kling, A., 2021 report No 894520, S21-04684 BASF DocID 2021/2014196 Authority registration No
Guideline(s):	OECD 213 (1998), OECD 214 (1998)
Deviations:	No
GLP:	yes (certified by Landesanstalt fuer Umwelt, Baden-Wuerttemberg, Karlsruhe, Germany),
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive summary

In an acute contact toxicity laboratory study, the effects of the formulated product BAS 768 00 F on honey bees (*Apis mellifera*) were investigated. Adult worker honey bees were exposed to a wetting agent control (deionised water containing Triton X-100) and to BAS 768 00 F at nominal dose rates of 62.5, 125, 250, 500 and 1000 µg product/bee (corresponding to 29.0, 58.0, 116, 232 and 464 µg total a.s./bee), in groups of 10 animals with 4 replicates per treatment. All treatments were applied on the dorsal bee thorax. Assessment of honey bee mortality and behavioural effects was conducted after 4, 24 and 48 hours.

After 48 hours of contact exposure, no mortality was observed in the wetting agent control and at the test item dose rates of up to and including 250 µg product/bee, whereas 2.5% mortality was observed at test item dose rates of 500 and 1000 µg product/bee. No behavioural abnormalities and sublethal effects were observed at the any of the test item dose rates.

In a 48-h acute contact toxicity study with honey bees (*Apis mellifera*), the LD_{50} (48 h) for BAS 768 00 F was estimated to be > 1000 µg product/bee (corresponding to > 464 µg total a.s./bee).

Materials and methods

Materials

Test item:	BAS 768 00 F, Batch no. FD-210120-1029; content of a.s.: mefentrifluconazole (BAS 750 F; Reg. no. 5834378): 26.3 g/L (nominal: 25.0 g/L), sulfur (BAS 175 F, Reg. no. 240586): 614.9 g/L (nominal: 600.0 g/L); density: 1.346 g/cm³.

Study design and methods

Test species:	Honey bee (*Apis mellifera*), young adult worker bees, derived from queen-right and healthy colonies; randomly collected by brushing off the combs of the honey chamber and distributed into test cages one day before exposure; source: in-house colonies.
Test design:	Exposure period: 48 hours; adult worker bees were exposed to 5 test item doses plus a wetting agent control; for all treatments a single 2 µL droplet was applied on the dorsal bee thorax after anaesthetisation of the bees with CO_2; 10 bees per cage; 4 replicates (cages) per treatment; assessments of honey bee mortality and behavioural effects after 4, 24 and 48 hours of exposure.
Endpoints:	LD_{50} related to mortality and assessment of sub-lethal effects.
Reference item:	BAS 152 65 I (dimethoate, 38.8% w/w (412 g/L) analysed); dose rates: 0.10 - 0.34 µg dimethoate/bee.
Test doses:	Wetting agent control (deionised water containing 0.1% Triton X-100); nominal test item dose rates: 62.5, 125, 250, 500 and 1000 µg product/bee, corresponding to 29.0, 58.0, 116, 232 and 464 µg total a.s./bee.
Test conditions:	Stainless stell cages (8 x 6 x 4 cm) with transparent pane and perforated bottom; temperature: 24.6 - 25.1°C; relative humidity: 57.1 - 61.0%; photoperiod: 24 h darkness; feeding: 50% (w/v) aqueous sugar solution *ad libitum*.
Analytics:	No analytical verification of the test item is required according to the current test guideline.
Statistics:	Descriptive statistics.

Results

Biological results

After 48 hours of contact exposure, no mortality was observed in the wetting agent control and at the test item dose rates of up to and including 250 µg product/bee, whereas 2.5% mortality was observed at test item dose rates of 500 and 1000 µg product/bee. No behavioural abnormalities and sublethal effects were observed at the any of the test item dose rates. The results are summarized in **Table A 32**.

Table A 32: Effects of BAS 768 00 F on honey bees (*Apis mellifera*) after 48 hours of contact exposure

Dose rate		**Mortality [%]**	
[µg product/bee]	**[µg total a.s./bee] #**	**After 24 hours**	**After 48 hours**
		Total	**Total**
Wetting agent control	-	0.0	0.0
62.5	29.0	0.0	0.0
125	58.0	0.0	0.0
250	116	0.0	0.0
500	232	2.5	2.5
1000	464	2.5	2.5
		Endpoints	
		[µg product/bee]	**[µg total a.s./bee] #**
LD_{50} (48 h)		> 1000 (95% confidence limits: n.d.)	> 464 (95% confidence limits: n.d.)

Abbreviations: n.d.: not determined (could not be calculated due to mathematical reasons)

Values based on the active substance have been recalculated from the study data considering the nominal content of 625 g total a.s./L and density 1.346 g/cm^3.

The LD_{50} value (24 h) for the reference item was determined to be 0.16 µg dimethoate/bee (95% confidence limits: 0.15 - 0.18 µg a.s./bee).

Table A 33: Validity criteria according to OECD 214 (1998)

Criteria	**Trigger value**	**Study value and page no.**	**Criteria met? Y/N**	**Guideline**
Mortality in the control at end of exposure	≤ 10%	Wetting agent control: 0% (p. 20)	Y	OECD 214
LD_{50} (24 h) of the reference item	0.1 - 0.30 µg/bee	LD_{50} (24 h) = 0.16 µg dimethoate/bee (p. 20)	Y	OECD 214

All validity criteria were met (see **Table A 33** above).

Conclusion

In a 48-h acute contact toxicity study with honey bees (*Apis mellifera*), the LD_{50} (48 h) for BAS 768 00 F was estimated to be > 1000 µg product/bee (corresponding to > 464 µg total a.s./bee).

A 2.3.1.1.2.3 Study 3

Comments of zRMS:	The study was accepted. The study was conducted in accordance with OECD guideline 246 (acute contact). The validity criteria were met: • **contact test**: the mean mortality of the control groups was 0% in water and TritonX solution, respectively, (recommended ≤ 10 %); The following endpoints were derived: LD_{50} 48 h > 1000 µg formulation/bumblebee (equivalent to > 464 µg a.s./bumble bee) NOED 48 h ≥ 1000 µg formulation/bumblebee No behavioural effects appeared at all tested dose rates of BAS 768 00 F during the contact toxicity test up to 48 hours.

Reference:	CP 10.3.1.1.2/3
Report	Acute toxicity of BAS 768 00 F to the bumblebee Bombus terrestris L. under laboratory conditions, Amsel, K., 2022 report No 919844, 2248BBA0024 BASF DocID 2022/2010697 Authority registration No
Guideline(s):	OECD 246 (2017), OECD 247 (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 an acute contact toxicity laboratory study, the effects of the formulated product BAS 768 00 F on bumble bees (*Bombus terrestris*) were investigated. Adult worker bumble bees were exposed to an untreated control (deionised water), to a wetting agent control (TritonX solution) and to BAS 768 00 F at nominal dose rates of 62.5, 125.0, 250.0, 500.0 and 1000.0 µg product/bumble bee (equivalent to 29.1, 58.0, 116.0, 232.2 and 464.4 µg total a.s./bumble bee), with 30 replicates per treatment, each containing 1 bumble bee. All treatments were applied on the dorsal bee thorax. Assessments of bumble bee mortality and behavioural effects were conducted after 4, 24 and 48 hours.

After 48 hours of contact exposure, no mortality was observed in the untreated control, the wetting agent control and at up to and including the highest test item dose rate of 1000.0 µg product/bumble bee. No test item induced behavioural effects were observed.

In a 48-h acute contact toxicity study with bumble bees (*Bombus terrestris*), the LD_{50} (48 h) for BAS 768 00 F was estimated to be > 1000.0 µg product/bumble bee (equivalent to > 464.4 µg a.s./bumble bee).

Materials and methods

Materials

Test item:	BAS 768 00 F, Batch no. FD-210120-1029; content of a.s.: mefentrifluconazole (BAS 750 F; Reg. no. 5834378): 26.3 g/L (nominal: 25.0 g/L), sulfur (BAS 175 F, Reg. no.: 240586): 614.9 g/L (nominal: 600.0 g/L); density: 1.346 g/cm^3.

Study design and methods

Test species:	Bumble bee (*Bombus terrestris*), young adult worker bumble bees, weight range: 162 - 352 mg; derived from queenright and healthy bumble bee hives; bumble bees were collected from the bumblebee hives under red light with a starvation period of 4 hours before test begin; source: originally obtained from "Biobest Belgium N.V.", Westerlo, Belgium; delivered by "Katz Biotech AG", Baruth, Germany.
Test design:	Exposure period: 48 hours; adult worker bumble bees were exposed to 5 test item doses (in TritonX solution) plus an untreated control and a wetting agent control; 1 bumble bee per cage; 30 replicates (cages) per treatment; for all treatments a single 4 µL droplet was applied on the dorsal bee thorax after anesthetisation of the bees with CO_2; assessments of bumble bee mortality and behavioural effects after 4, 24 and 48 hours of exposure.
Endpoints:	LD_{50} and NOED related to mortality and assessment of sub-lethal effects.
Reference item:	Dimethoate EC 400 (dimethoate, 400 g/L nominal); dose rate in in 0.5% (v/v) TritonX solution: 10.0 µg a.s./bumble bee.
Test doses:	Untreated control (deionised water); wetting agent control (0.5% (v/v) TritonX solution); nominal test item dose rates in 0.5% (v/v) TritonX solution: 62.5, 125.0, 250.0, 500.0 and 1000.0 µg product/bumble bee (equivalent to 29.1, 58.0, 116.0, 232.2 and 464.4 µg a.s./bumble bee.
Test conditions:	Nicot cages (length: 7 cm; diameter: 2 cm); temperature: 25.0 - 25.4°C; relative humidity: 60 - 63%; photoperiod: 24 h darkness; feeding: 50% (w/v) sucrose solution *ad libitum*.
Analytics:	Analytical verification of test item concentrations in the test solution containing 0.5% (v/v) TritonX was conducted using an HPLC-method with MS-MS detection (Method No. L0452/02).
Statistics:	Descriptive statistics.

Description of the analytical procedures

The concentrations of mefentrifluconazole (contained in BAS 768 00 F) in test solution containing 0.5% (v/v) TritonX were determined according to the analytical method L0452/02. The analytical method is fully validated in this study. The validation of the analytical method is described in the study report. The samples, which were present in water containing 0.5% (v/v) TritonX, did not need to be extracted and were diluted with sample matrix and/or 50/50/1 (v/v/v) acetonitrile/ water/ formic acid as well as dilution medium contact, if necessary, and injected into the HPLC system. The determination was performed by HPLC-MS/MS. The limit of quantification (LOQ) was set to 0.01 mg a.s./kg food and the limit of detection (LOD) was 0.002 mg a.s./kg food (≤ 30% of LOQ). Matrix effects were taken into account by the addition of the same amount of blank extract to calibration samples as included in the analysis samples. Thus, all measured samples contained the same amount of original sample matrix. The maximum storage period of the samples was 13 days. Stability samples were not analysed, as a storage period of 30 days was not exceeded. Mean recoveries of the procedural recovery samples were between 103% and 109% of the nominal concentrations. Details on measured fortification samples and obtained procedural recoveries for the formulated product BAS 768 00 F (based on measurement of mefentrifluconazole) are provided in Table A 34.

Table A 34: Procedural recoveries for BAS 768 00 F (based on measurements of mefentrifluconazole)

Matrix	Fortification level [mg a.s./kg]	n	Mean recovery [%]	RSD [%]
Test solution containing 0.5% (v/v) TritonX	0.01	5	103	3.52
	6170	5	109	1.37

Abbreviations: n: number of replicates; RSD: relative standard deviation

Results

Biological results

After 48 hours of contact exposure, no mortality was observed in the untreated control, the wetting agent control and at up to and including the highest test item dose rate of 1000.0 µg product/bumble bee. No test item induced behavioural effects were observed. The results are summarised in **Table A 35**.

Table A 35: Effects of BAS 768 00 F on bumble bees (*Bombus terrestris*) after 48 hours of contact exposure

Dose rate [µg product/bumble bee] (nominal)	**Mortality [%]**	
	After 24 hours	**After 48 hours**
	Total	**Total**
Untreated control	0.0	0.0
Wetting agent control	0.0	0.0
62.5	0.0	0.0
125.0	0.0	0.0
250.0	0.0	0.0
500.0	0.0	0.0
1000.0	0.0	0.0
	Endpoints	
	[µg product/bumble bee]	**[µg a.s./bumble bee]**
LD_{50} (48 h)	> 1000.0 (95% confidence limits: n.d.)	> 464.4
NOED (48 h)	≥ 1000.0	≥ 464.4

Abbreviations: n.d.: not determined (could not be calculated due to mathematical reasons)

The mortality rate in the reference item treatment was 100% after 48 hours.

Table A 36: Validity criteria according to OECD 246 (2017)

Criteria	**Trigger value**	**Study value and page no.**	**Criteria met? Y/N**	**Guideline**
Mortality in the control(s) at end of exposure	≤ 10%	Untreated control: 0% Wetting agent control: 0% (p. 22)	Y	OECD 246
Mortality in the reference item treatment at end of exposure	≥ 50%	100.0% mortality at 10.0 µg dimethoate/bumble bee (p. 22)	Y	OECD 246

All validity criteria were met (see **Table A 36** above).

Conclusion

In a 48-h acute contact toxicity study with bumble bees (*Bombus terrestris*), the LD_{50} (48 h) for BAS 768 00 F was estimated to be > 1000.0 µg product/bumble bee (equivalent to > 464.4 µg a.s./bumble bee).

A 2.3.1.2 KCP 10.3.1.2. Chronic toxicity to bees

A 2.3.1.2.1 Study 1

Comments of zRMS:	The study was evaluated and accepted during active substance renewal.

Reference:	CP 10.3.1.2/1
Report	Chronic toxicity of Netzschwefel Stulln (Sulphur 80% WG) to the honey bee Apis mellifera L. under laboratory conditions, Ruhland, S., 2017 report No EU-161048160B,16 10 48 160 B BASF DocID 2017/1219185 Authority registration No
Guideline(s):	Revised Proposal for a new OECD: Honey bee (Apis mellifera L.) chronic oral toxicity test (10 day feeding in laboratory) (2014)
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, 2-day old worker honey bees (*Apis mellifera* L. subspecies *iberiensis*) were exposed to a daily application of Netzschwefel Stulln ('Sulfur 80% WG') diluted in the bee food (50 % (w/v) aqueous sucrose solution). The chronic toxicity of the test item was determined at nominal doses of 199.6, 99.8, 49.9, 24.9 and 12.5 µg a.s./bee/day, corresponding to concentrations of 5.082, 2.541, 1.271, 0.635 and 0.318 g a.s./kg food. Effective doses were 149.3, 84.7, 45.0, 23.2 and 10.2 µg consumed a.s./bee/day.

Additionally, honey bees were treated with reference substance dimethoate at a nominal dose of 27.3 ng a.s./bee/day and an untreated diet (50 % (w/v) sucrose solution) served as a control. Assessments of bee mortality and behavioural effects were conducted on a daily basis during the exposure period.
After 10 days, the test item and control treatments caused no mortalities and no treatment related behavioural abnormalities, respectively. The effective reference dosage in the study was 13.2 ng dimethoate/bee/day and caused a mean mortality of 86.7 %. The recovery rate of active substance ranged between 90 % and 101 % in the highest test item dose and between 90 % and 104 % in the lowest test item dose.

Under the conditions of this study, the 10-d LDD_{50} for honeybees was determined to be > 149.3 µg consumed a.s./bee/day. The 10-d NOEDD was determined to be ≥ 149.3 µg consumed a.s./bee/day.

Materials and methods

A. Materials

1. Test substance:	Netzschwefel Stulln ('Sulfur 80% WG')
Lot/Batch no.:	51202
Content/Purity:	80 % w/w (nominal); 80.8 ± 0.6 % w/w (analysed)
Control:	50 % w/v sucrose solution
Toxic reference:	Dimethoate 400 EC (BAS 152 11 l; 420.3 g a.s./L analysed)
2. Test organisms	
Species:	*Apis mellifera* L. subspecies *iberiensis* (honey bee)
Age:	2-days old worker bees
No. of organisms:	3 replicates per test item dose, control and reference item dose with 10 bees per replicate
Feeding:	50 % w/v aqueous sucrose solution
3. Test units and exposure	
Type and size:	Aluminium cages, 95 x 60 x 70 mm; with holes for ventilation and glass plates in the front and the back for observation
Test procedure:	oral exposure
Test duration:	10 days
4. Test conditions	
Temperature:	32.1 – 34.1 °C
Relative humidity:	54.5 – 64.3 %
Photoperiod:	darkness (except during assessments)
Light intensity:	not applicable

B. Study design and method

1. In-life dates (experimental phase): January 17 to January 27, 2017

2. Test design:

The test item was daily administered to the bees in sugar solution at the following nominal doses of 199.6, 99.8, 49.9, 24.9 and 12.5 µg a.s./bee/day, corresponding to concentrations of 5.082, 2.541, 1.271, 0.635 and 0.318 g a.s./kg food. The treated (and untreated) feeding solutions were offered *ad libitum* to each cage in syringes. The syringes were weighed daily before introduction into the cages and after the feeding interval (before replacement with fresh food). In the test item groups, the food consumption ranged between 29.4 and 36.5 mg solution/bee/day, which is 74.8 to 93.0 % of the expected amount (control: on average 35.7 mg/bee/day = 90.9 %). The food consumption per cage was corrected by subtracting the mean evaporation figure of each day of application. The number of dead bees and behavioural abnormalities compared to the control were assessed daily for the whole test duration. Analytical verification of the test item concentration (highest and lowest concentration analysed) was performed *via* HPLV-UV directly after preparation on each day of application.

3. Statistics:

For statistical calculation of the mortality results, the Fisher´s exact binomial test with Bonferroni-Correction was used ($p \leq 0.05$, one-sided greater). All statistical calculations were performed with the computer program ToxRat Professional 3.2.1 (2015).
The amount of the individual food consumption was done by subtracting the evaporation figure from the gravimetrically calculated uptake to give the real uptake accounting for the loss by evaporation (in case a

negative value is produced, the food consumption was set "0"). This amount of food was divided by the number of living bees at the start of the corresponding exposure interval.

Results and discussions

A. Analytical data

The recovery rate of active substance sulfur ranged between 90 and 101 % in the highest and between 90 and 104 % in the lowest test item dose (samples taken on each day of application). No test item has been detected in the control sample. For details on the analytical method validation, refer to M-CA Section 4, Annex point CA 4.1.2 (f).

B. Mortality

After 10 days, a mortality of 0.0 % was observed in the control. Taking into account the actual food uptake and the evaporated amount of feeding solution, the bees effectively consumed doses of 149.3, 84.7, 45.0, 23.2 and 10.2 µg a.s./bee/day, which caused mortalities of 0.0 % in all treatment groups, respectively after 10 days. None of the obtained mortalities was statistically significantly increased compared to the control. The reference dosage tested in the study was 27.3 ng a.s./bee/day (actual consumption on average per day: 13.2 ng a.s./bee), which caused a mean mortality of 86.7 %.

Since the obtained mortalities did not reach 50 %, the respective LDD_{50} is > 149.3 µg consumed a.s./bee/day. The NOEDD was determined to be ≥ 149.3 µg consumed a.s./bee/day (see table below).

Table A 37: Toxicity of 'Sulfur 80% WG' in a chronic toxicity feeding test

	Endpoints	10 d
Test item doses	LDD_{50} [µg consumed a.s./bee/day]	> 149.3
	NOEDD [µg consumed a.s./bee/day][1]	≥ 149.3
Test item concentrations	LC_{50} [g a.s./kg food]	> 5.082
	NOEC [g a.s./kg food][1]	≥ 5.082

[1] No observed effect dietary dose/concentration was calculated by using Fisher´s Exact Binomial Test with Bonferroni-Correction (α = 0.05; one sided greater)

C. Behavioural abnormalities

In the course of the test, no treatment related behavioural abnormalities could be observed in the test item and control groups, respectively.

D. Validity of the study

Not applicable, as no official OECD guideline is available. Because mean mortality was < 15 % (*i.e.* 0.0 %) in the control and > 50 % (*i.e.* 86.7 %) mortality in the reference group after 10 days of exposure, the study can be regarded as valid.

Conclusion

The chronic oral toxicity of 'Sulfur 80% WG' on young, adult honey bees (*Apis mellifera* L.) was investigated in a 10-day chronic, dose-response feeding study under laboratory conditions.

Since the obtained mortalities did not reach 50 % the LDD_{50} is considered > 149.3 µg consumed a.s./bee/day. The NOEDD was determined to be ≥ 149.3 µg consumed a.s./bee/day. The EC_{10} and EC_{20} values could not be calculated since no dose-response curve can be retrieved from the data. However, EC_{10} and EC_{20} can be estimated to be above 149.3 µg consumed a.s./bee/day.

A 2.3.1.2.2 Study 2

Comments of zRMS:	The study was accepted. The validity criteria were met: • 0.0% mean mortality in the blank control group AC and 3.3% mean mortality in the viscosifier control group BC after 10 days of continuous exposure. Hence, the validity criterion was met (≤ 15%); • 86.7% mean mortality after 10 days of continuous exposure. Hence, the validity criterion was met (≥ 50%). No deviations were noted. The following endpoints were calculated: LC_{50} = 18.916 g formulation/kg food LDD_{50} = 367 µg formulation/bee/d NOEC = 1.910 g formulation/kg food NOEDD = 45.5 µg formulation/bee/day

Reference:	CP 10.3.1.2/2
Report	Chronic toxicity of BAS 768 00 F to the honey bee Apis mellifera L. under laboratory conditions, Dressler, K., 2022 report No 894521, 2148BAC0065 BASF DocID 2021/2014218 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 chronic oral toxicity laboratory study, the effects of the formulated product BAS 768 00 F on honey bees (*Apis mellifera*) were investigated after exposure over a period of 10 days. Young adult worker honey bees were orally exposed to an untreated control (sucrose solution), to a viscosifier control (sucrose solution with xanthan) and to BAS 768 00 F at doses of 30.0, 75.0, 188, 469 and 1172 µg product/bee/day (equivalent to 14.0, 34.8, 87.1, 217.7 and 544.8 µg total a.s./bee/day). The corresponding effectively consumed doses were 21.2, 45.5, 124, 291 and 496 µg consumed product/bee/day (equivalent to 9.9, 21.1, 57.5, 135.4 and 230.2 µg consumed total a.s./bee/day) The corresponding test item concentrations in the feeding solutions were 0.764, 1.910, 4.776, 11.939 and 29.849 g product/kg food (equivalent to 355.2, 887.5, 2217.7, 5544.0 and 13860.0 mg total a.s./kg food). All treatment groups were set up with

3 replicates, each containing 10 bees. Assessments of bee mortality, food consumption and behavioural effects were conducted daily during the study. The daily food consumption was corrected by subtracting the daily mean evaporation figure of the respective day of application.

After 10 days of permanent exposure, mortality rates of 0.0% and 3.3% were observed in the untreated control and the viscosifier control, respectively. In the test item treatment groups, mortalities ranged from 0.0 to 73.3%. The resulting corrected mortalities ranged from 0.0 to 72.4%. Statistically significant effects on honey bee survival compared to the viscosifier control were observed at the three highest test item doses of 124, 291 and 496 µg consumed product/bee/day. During the course of the test, no behavioural abnormalities were observed in any of the test item treatment groups (*i.e.*, moribund bees, uncoordinated movements).

In a 10-day chronic toxicity feeding study with honey bees (*Apis mellifera*), the NOEDD (10 d) for BAS 768 00 F was determined to be 45.5 µg consumed product/bee/day (equivalent to 21.1 µg consumed total a.s./bee/day), corresponding to a NOEC 1.910 g product/kg food (equivalent to 887.5 mg total a.s./kg food). The LDD_{50} (10 d) was calculated to be 367 µg consumed product/bee/day (equivalent to 170.4 µg consumed total a.s./bee/day), corresponding to an LC_{50} of 18.916 g product/kg food (equivalent to 8783.4 mg total a.s./kg food).

Materials and methods

Materials

Test item: BAS 768 00 F, Batch no. FD-210120-1029; content of a.s.: mefentrifluconazole (BAS 750 F; Reg. no. 5834378): 26.3 g/L (nominal: 25.0 g/L), sulfur (BAS 175 F, Reg. no.: 240586): 614.9 g/L (nominal: 600.0 g/L); density: 1.346 g/cm^3.

Study design and methods

Test species: Honey bee (*Apis mellifera*), max. 2-day old adult worker bees; derived from healthy and queenright colonies; source: in-house colonies.

Test design: 10-day chronic feeding test; young adult worker bees were exposed daily to 5 test item doses in treated food (50% (w/v) aqueous sucrose solution with 0.1% (w/v) xanthan) plus an untreated control and a viscosifier control; 10 bees per cage, 3 replicates (cages) per treatment; treated or untreated food was provided continuously via plastic syringes (feeders), which were weighed before and after introduction into the cages; the feeders remained in the cages for about 24 h (±2 h); the actual consumption was determined by re-weighing the syringe containing the remaining test solution each day after removal from the test units; food consumption was corrected by accounting the loss due to evaporation; assessments of bee mortality and behavioural effects were done daily during the study.

Endpoints: LC/LDD_x and NOEC/NOEDD related to mortality and assessment of sub-lethal effects.

Reference item: Danadim® Progress (dimethoate, 400 g/L nominal); dose rate: 27.3 ng a.s./bee/day (corresponding to 0.694 mg a.s./kg food).

Test doses: Untreated control: untreated diet (50% (w/v) aqueous sucrose solution); viscosifier control (50% (w/v) aqueous sucrose solution with 0.1% (w/v) xanthan); nominal test item dose rates: 30.0, 75.0, 188, 469 and 1172 µg product/bee/day (equivalent to 14.0, 34.8, 87.1, 217.7 and 544.8 µg total a.s./bee/day); effectively consumed doses: 21.2, 45.5, 124, 291 and 496 µg consumed product/bee/day (equivalent to 9.9, 21.1, 57.5, 135.4 and 230.2 µg consumed total a.s./bee/day); nominal test item concentrations: 0.764, 1.910, 4.776, 11.939 and 29.849 g product/kg food (equivalent to 355.2, 887.5, 2217.7, 5544.0 and 13860.0 mg total a.s./kg food).

Test conditions: Aluminium cages (95 x 70 x 60 mm) with holes in the lateral walls for ventilation and two glass plates; temperature: 32.9 - 33.3°C; relative humidity: 56.5 - 63.3%; photoperiod: 24 h darkness; feeding: 50% (w/v) aqueous sucrose solution provided continuously *ad libitum* in plastic syringes.

Analytics: Analytical verification of test item concentrations in honey bee diet was conducted using an HPLC-method with MS-MS detection (Method No. L0452/01).

Statistics: Descriptive statistics; Step-down Cochran-Armitage Test Procedure for mortality data and determination of NOEDD and NOEC. Weibull analysis using linear maximum likelihood regression for the determination of LDD_x and LC_x values along with their 95% confidence limits.

Description of the analytical procedures

The concentrations of mefentrifluconazole (contained in BAS 768 00 F) in honey bee feeding solutions were determined according to the analytical method L0452/01. The analytical method is fully validated in a separate study (BASF DocID: 2021/2022023). The validation of the analytical method is described in the study report. The samples were extracted with 50/50/1 (v/v/v) acetonitrile / water / formic acid and a QuEChERS salt mix containing 0.5 g magnesium sulfate, 0.12 g sodium chloride, 0.06 g disodium hydrogen-citrate sesquihydrate and 0.12 g of trisodium citrate dihydrate by shaking. 1 mL of the extracts were transferred into a 10 mL-volumetric flask and filled to the mark with 50/50/1 (v/v/v) acetonitrile / water / formic acid and, if necessary, further diluted with dilution medium into the range of the calibration curve. Diluted extracts were injected into the HPLC system. The determination was performed by HPLC-MS/MS. The limit of quantification (LOQ) was set to 0.01 mg a.s./kg food and the limit of detection (LOD) was ≤ 30% of the LOQ, 0.003 mg a.s./kg. Matrix effects were taken into account by the addition of the same amount of blank extract to calibration samples as included in the analysis samples. Thus, all measured samples contained the same amount of original sample matrix. The stability of the analyte in feeding solutions has been proven over a time period of 126 days under deep frozen conditions in the dark. Mean recoveries of the procedural recovery samples were between 91.0% and 102% of the nominal concentrations. Details on measured fortification samples and obtained procedural recoveries for the formulated product BAS 768 00 F (based on measurement of BAS 750 F) are provided in Table A 38.

Table A 38: Procedural recoveries for BAS 768 00 F (based on measurements of mefentrifluconazole)

Matrix	Fortification level [mg a.s./kg food]	n	Mean recovery [%]	RSD [%]
Honey bee feeding solutions	0.01	8	91.0	11.4
	730	8	102	2.37

Abbreviations: n: number of replicates; RSD: relative standard deviation

Results

Biological results

After 10 days of permanent exposure, mortality rates of 0.0% and 3.3% were observed in the untreated control and the viscosifier control, respectively. In the test item treatment groups, mortalities ranged from 0.0 to 73.3%. The resulting corrected mortalities ranged from 0.0 to 72.4%. Statistically significant effects on honey bee survival compared to the viscosifier control were observed at the three highest test item doses of 124, 291 and 496 µg consumed product/bee/day (Step-down Cochran-Armitage Test Procedure, $\alpha = 0.05$, one-sided greater). During the course of the test, no behavioural abnormalities were observed in any of the test item treatment groups (*i.e.*, moribund bees, uncoordinated movements). The results are summarized in **Table A 39**.

Table A 39: Effects of BAS 768 00 F on honey bees (*Apis mellifera*) after 10 days of oral exposure

Dose rate [µg product/bee/day]		**Concentration [g product/kg food]**	**Cumulative mortality after 10 d [%]**	
Nominal	**Measured actual uptake**		abs.	corr. [1]
Untreated control	-	-	0.0	--
Viscosifier control	-	-	3.3	--
30.0	21.2	0.764	0.0	0.0
75.0	45.5	1.910	6.7	3.4
188	124	4.776	13.3*	10.3
469	291	11.939	33.3*	31.0
1172	496	29.849	73.3*	72.4
Endpoints after 10 d			**based on product**	**based on total a.s.**
LDD_{50} [µg consumed/bee/day] (95% CL) [2]			367 (300 - 455)	170.4 (139.3 - 211.3)
LDD_{20} [µg consumed/bee/day] (95% CL) [2]			194 (127 - 245)	90.1 (59.0 - 113.8)
LDD_{10} [µg consumed/bee/day] (95% CL) [2]			127 (66.4 - 175)	59.0 (30.8 - 81.3)
NOEDD [µg consumed/bee/day] [3]			45.5	21.1
LC_{50} [g/kg food] (95% CL) [2]			18.916 (14.625 - 24.906)	8.783 (6.791 - 11.565)
LC_{20} [g/kg food] (95% CL) [2]			8.335 (5.180 - 11.159)	3.870 (2.405 - 5.182)
LC_{10} [g/kg food] (95% CL) [2]			4.844 (2.390 - 7.148)	2.249 (1.110 - 3.319)
NOEC [g/kg food] [3]			1.910	0.887

Abbreviations: abs.: absolute; corr.: corrected; CL: confidence limits; n.d.: not determined (could not be calculated due to mathematical reasons)

Negative values are set to 0

* Statistically significant difference in pairwise comparison between treatment and untreated viscosifier control group (Step-down Rao-Scott-Cochran-Armitage Test Procedure; $\alpha = 0.05$; one sided greater).

[1] Corrected cumulative mortality according to Schneider-Orelli (1947); test item group was corrected for mortality of viscosifier control group.

[2] LDD/LC_x were calculated by Weibull analysis using linear max. likelihood regression.

[3] NOEC/NOEDD were determined using Step-down Cochran-Armitage Test Procedure ($\alpha = 0.05$, one-sided greater).

The reference item caused a mortality of ≥ 50% after 10 days.

Table A 40: Validity criteria according to OECD 245 (2017)

Criteria	Trigger value	Study value and page no.	Criteria met? Y/N	Guideline
Average mortality across replicates in control(s) at test end	≤ 15%	0.0% untreated control 3.3% viscosifier control (p. 23)	Y	OECD 245
Average mortality for reference item at test end	≥ 50%	86.7% (p. 23)	Y	OECD 245

All validity criteria were met (see **Table A 40** above).

Conclusion

In a 10-day chronic toxicity feeding study with honey bees (*Apis mellifera*), the NOEDD (10 d) for BAS 768 00 F was determined to be 45.5 µg consumed product/bee/day (equivalent to 21.1 µg consumed total a.s./bee/day), corresponding to a NOEC 1.910 g product/kg food (equivalent to 887.5 mg total a.s./kg food). The LDD_{50} (10 d) was calculated to be 367 µg consumed product/bee/day (equivalent to 170.4 µg consumed total a.s./bee/day), corresponding to an LC_{50} of 18.916 g product/kg food (equivalent to 8783.4 mg total a.s./kg food).

A 2.3.1.3 KCP 10.3.1.3 Effects on honey bee development and other honey bee life stages

A 2.3.1.3.1 Study 1

Comments of zRMS:	The study was evaluated and accepted during active substance renewal.

Reference:	CP 10.3.1.3/1
Report	Microthiol special disperss (Sulphur 80% WG) - Repeated exposure of honey bee (Apis mellifera L.) larvae under laboratory conditions (in vitro), Scheller, K., 2017 report No EU-161048161B,16 10 48 161 B BASF DocID 2017/1218043 Authority registration No
Guideline(s):	OECD 237 (2013) Honey bee (Apis mellifera) larval toxicity test single exposure, 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

In a chronic toxicity test, honeybee larvae of *Apis mellifera* L. were repeatedly and orally exposed to the test item Microthiol Special Disperss ('Sulfur 80% WG') on four successive days (D3 to D6). In total, three treatment groups were set up: 8 doses of the test item (3.9, 7.0, 12.5, 22.5, 42.3, 75.1, 137.7 and 250.4 µg prod./larva (equivalent to 3.1, 5.6, 10.0, 18.0, 33.0, 60.1, 110.2 and 200.3 µg a.s./larva), one untreated control group and one dose of the reference item (7.4 µg dimethoate/larva) with 3 replicates per dose and 12 larvae per replicate.

During the exposure period, larval mortality was assessed on D4, D5, D6, D7, and D8. Additionally, other observations such as smaller body size or remaining food after 96 and 120 hours (on D7 and D8) were recorded. The concentration of the test item was chemically analysed *via* HPLC-UV in the highest and lowest concentration of the stock solution and the final diets, respectively as well as in the corresponding controls.

Under the conditions of this study, the 120-h LD_{50} was determined to be 149.7 µg a.s./larva. Accordingly, the 120-h NOED was determined to be 60.1 µg a.s./larva.

Materials and methods

A. Materials

1. Test substance:	Microthiol Special Disperss (‘Sulfur 80% WG’)
Lot/Batch no.:	14-131-01
Content/Purity:	Content of sulfur: 80 % (w/w, nominal); 799 g/kg (analysed)
Control:	50 % aqueous sugar solution with 50 % royal jelly
Toxic reference:	Dimethoate tech. (98.8 ± 0.5 % w/w)
2. Test organisms	
Species:	*Apis mellifera* iberiensis ENGEL (Hymenoptera, Apoidea)
Age:	honeybee first instar larvae (one-day-old)
Replicates:	Each of three colonies represented one replicate with 12 larvae
Individuals:	36 larvae per treatment group, 3 x 12 larvae per replicate and single colony
Feeding:	Three different diets were offered to the larvae, depending on the development stage (for details, see “test design” below)
3. Test units and exposure	
Type and size:	to polystyrene grafting cells in 48-well cell culture plates
Treatment groups:	Control, 8 dose levels for the test item, one dose level for the reference item
Test procedure:	oral exposure
Test duration:	5 days following first application (120 hours)
4. Test conditions	
Temperature:	34.1 – 35.0 °C
Relative humidity:	90.1 – 98.7 %
Photoperiod:	darkness (except during assessments)
Light intensity:	not applicable

B. Study design and method

1. In-life dates (experimental phase): March 21 to March 30, 2017

2. Test design:

Larvae of *Apis mellifera* L. were transferred from brood combs to polystyrene grafting cells in 48-well cell culture plates 3 days before start of the treatment. On four successive days (D3 to D6), the larvae were repeatedly exposed to ‘Sulfur 80% WG’ diluted in the larvae’s food (aqueous sugar solution mixed with royal jelly). After the applications, no additional feedings of the larvae took place.

In total, 3 treatment groups with 3 replicates per dose and 12 larvae (from three different colonies) per replicate were set up: 8 doses of the test item, one control (untreated food), and one dose of the reference item. Assessments of larval mortality took place on D4, D5, D6, D7, and D8. Additionally, other observations such as smaller body size or remaining food after 96 and 120 hours (on D7 and D8) were recorded. In an analytical phase of the study, the concentration of the test item was chemically analysed *via* HPLC-UV in the highest and lowest concentration of the stock solution and the final diets, respectively as well as in the corresponding controls.

Three different diets were offered to the larvae, depending on the development stage (for details, see table below).

Table A 41: Feeding scheme

Test day	1[1]	2	3[2]	4[2]	5[2]	6[2]
Artificial diet	**A**	**-**	**B**	**C**	**C**	**C**
Volume of diet	20 µL	-	20 µL	30 µL	40 µL	50 µL
Composition of diets: Royal jelly Sugar solution	 44.25 % w/w 55.75 % w/w	-	 50 % 50 %	50 % 50 %		
Composition of sugar solution: Glucose Fructose Yeast Water	 9.50 % w/w 9.50 % w/w 1.61 % w/w 79.39 % w/w	-	 15 % w/v 15 % w/v 3 % w/v filled up	18 % w/v 18 % w/v 4 % w/v filled up		

[1] day of grafting
[2] days of application

The assessment of the larval mortality took place after 24, 48, 72, 96 and 120 hours.

3. Statistics:

Mortality data was statistically evaluated by using the Step-down Cochran-Armitage test ($\alpha = 0.05$, one-sided greater). The same test was used for the determination of the no observed effect level (NOED/NOEC). The calculation of the LD_{50}/LC_{50} value of the test item along with the 95 % confidence limits was evaluated using Probit analysis with linear maximum likelihood regression. All statistical calculations were performed with the computer program ToxRat Professional 3.2.1 (2015).

Results and discussions

A. Analytical data

For analytical determination of the active substance, samples of the highest and lowest concentration of the stock solution and final diets, respectively, as well as the corresponding controls were taken in duplicate on each day of application (D3, D4, D5, D6). For the stock solution and the final diets, recovery rates ranged from 86 to 109 % and from 80 – 106 %, respectively. For details on the analytical method validation, refer to M-CA Section 4, Annex point CA 4.1.2 (f).

B. Mortality

After 120 hours (D8), a mortality of 2.8 % was observed in the control group, whereas in the reference item group a mortality > 50 % (*i.e.* 82.9 % as corrected for control mortality) across all replicates was observed. In the test item group, mortalities ranged from 0.0 to 61.1 % (0.0 and 60.0 % as corrected for control mortality). Statistically significant increased mortalities occurred at the two highest test item doses (*i.e.* 200.3 and 110.2 µg a.i./larva) if compared to the untreated diet control, which amounted to 61.1 and 47.2 % (corrected for control mortality: 60.0 and 45.7 %). Results are presented in the table below.

Table A 42: Toxicity of 'Sulfur 80% WG' to *Apis mellifera* after repeated exposure

Treatment group	Dosage (total amount fed on D3 – D6) [µg a.s./larva]	Mean mortality after 120 h [%]	Mean mortality corrected [%]
Control	--	2.8	--
Test item	200.3	61.1*	60.0
	110.2	47.2*	45.7
	60.1	5.6	2.9
	33.0	2.8	0.0
	18.0	0.0	0.0
	10.0	0.0	0.0
	5.6	0.0	0.0
	3.1	0.0	0.0
Reference item	7.4	83.3	82.9

Results are averages based on 3 replicates, containing 12 larvae each from three different colonies
corrected: mortality of test item treatments corrected for the control according to Schneider-Orelli 1947 (negative values are set to "0", calculations are performed with non-rounded values)
* Statistically significant different in pairwise comparison between treatment group and control

The endpoints resulting from the observed mortality are shown in the table below.

Table A 43: Toxicity of 'Sulfur 80% WG' towards *Apis mellifera* after repeated exposure

	Endpoints	On D8 (120 h after 1st application)
Test item doses	LD_{50} [µg consumed a.s./larva] [2] (95 %-CL / lower-upper)	> 149.7 (124.8 – 179.4)
	NOED [µg a.s./larva] [1]	60.1
Test item concentrations	LC_{50} [mg a.s./kg food] [2] (95 %-CL / lower-upper)	946 (789 – 1134)
	NOEC [mg a.s./kg food] [1]	380

[1] Step-down Cochran-Armitage test ($\alpha = 0.05$; one sided greater)
[2] Median lethal dose/concentration (LD_{50}/LC_{50}) of test item was calculated with Probit analysis using linear maximum likelihood regression

C. Sublethal effects

Besides the observed mortality, no other observations (e.g. smaller body size or remaining food) were observed for any of the larvae on D8.

D. Validity of the study

The test is considered valid, as the control mortality was ≤ 15 % (*i.e.* 2.8 %) and the control-corrected mortality of the reference item (dimethoate) was ≥ 50 % at the test end (*i.e.* 82.9 %).

Conclusion

Under the conditions of this study, the 120-h LD_{50} was determined to be 149.7 µg a.s./larva. Accordingly, the 120-h NOED was determined to be 60.1 µg a.s./larva. The LD_{10} was determined to be 67.6 µg a.s./larva and the LD_{20} = 88.8 µg a.s./larva.

A 2.3.1.3.2 Study 2

Comments of zRMS:	The study was evaluated and accepted during active substance renewal.

Reference:	CP 10.3.1.3/2
Report	Microthiol Special Disperss – Repeated exposure of honey bee larvae (Apis mellifera L.) under laboratory conditions, Haensel, M., 2021 report No 20 48 BLC 0024 BASF DocID 2023/2005062 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

In a chronic toxicity test, honeybee larvae of *Apis mellifera* L. were repeatedly exposed to the test item Microthiol Special Disperss ('Sulfur 80% WG') on four successive days (D3 to D6). In total, three treatment groups were set up: six doses of the test item (30.54, 10.18, 3.39, 1.13, 0.38 and 0.13 µg product/larva, equivalent to 30.54, 10.18, 3.39, 1.13, 0.38 and 0.13 µg product/larva), one untreated control group and one dose of the reference item (7.6 µg dimethoate/larva) with 3 replicates per dose and 12 larvae per replicate.

During the exposure period, larval mortality was assessed daily on the days D4 to D8. Additionally, other observations such as smaller body size or remaining food after 96 and 120 hours (on D7 and D8) were recorded. The number of dead pupae (larvae which had not transformed into pupae) was assessed on D15. At the end of the test, on D22, the number of successfully emerged bees, alive or dead were assessed. The concentration of the test item was chemically analysed via gas chromatography with MS-detection in the stock solutions, respectively as well as in the corresponding controls.
Under the conditions of this study, the ED_{50} was determined to be 10.40 µg a.s./larva. Accordingly, the NOED was determined to be 0.9 µg a.s./larva.

Materials and methods

A. Materials

1. Test material:	Microthiol Special Disperss (‘Sulfur 80% WG’)
Lot/Batch no.:	190771
Content/Purity:	Content of sulfur: 80 % (w/w, nominal); 813 g/kg (analysed)
Control:	Untreated artificial diet: 50 % aqueous sugar solution with 50 % royal jelly
Toxic reference:	Dimethoate tech. (98.8 ± 0.5 % w/w)
2. Test organisms -	
Species:	*Apis mellifera* L.
Age:	honeybee first instar larvae (one-day-old)
Replicates:	Each of three colonies represented one replicate with 12 larvae
Individuals:	36 larvae per treatment group 3 x 12 larvae per replicate and single colony
Feeding:	Three different diets were offered to the larvae, depending on the development stage (for details, see “test design” below)
3. Test units and exposure –	
Type and size:	Crystal polystyrene grafting cells (internal diameter 9 mm, depth 8 mm) were placed in 48-well culture plates
Treatment groups:	Control, 8 dose levels for the test item, one dose level reference item
Test procedure:	oral exposure, larval toxicity test, repeated exposure
Test duration:	22 days
4. Test conditions –	
Temperature:	34.1 – 34.9 °C
Relative humidity:	from D1 to D8 = 95.7 - 100.0 %, from D8 to D15: 79.3 - 84.9 %, from D15 to D22: 60.0 – 65.9 %
Photoperiod:	constant darkness (diffuse artificial light only during handling processes

B. Study design and method

1. In-life dates:	June 15 to July 20, 2020 (experimental phase)

2. Test design:

Larvae of *Apis mellifera* L. were transferred from brood combs to polystyrene grafting cells in 48-well cell culture plates 3 days before start of the treatment. On four successive days (D3 to D6), the larvae were repeatedly exposed to ‘Sulfur 80% WG’ diluted in the larvae’s food (aqueous sugar solution mixed with royal jelly). After the applications, no additional feedings of the larvae took place.

Table A 44: Applied dosages in the chronic larval toxicity test

Treatment group	Treatment ID	Applied item	Cumulative dose	Concentration
			[µg product/larva]	[mg product/kg food]
Control	AC	Diet B/C	-	-
Test item	AT	Microthiol Special Dispersss [2]	30.54	193.04
	BT		10.18	64.34
	CT		3.39	21.45
	DT		1.13	7.14
	ET		0.38	2.37
	FT		0.13	0.79
Reference item	AR	Dimethoate tech. [1]	[µg a.s./ larva]	[mg a.s./kg food]
			7.6	48

[1] Based on analysed purity
[2] Based on a density of 1.15 g/mL of the aqueous sugar solution and 1.13 g/mL of diet B/C
Calculations are performed with non-rounded values

As shown in the table above, in total, 3 treatment groups with 3 replicates per dose and 12 larvae (from three different colonies) per replicate were set up: 6 doses of the test item, one control (untreated food), and one dose of the reference item. Mortalities were assessed and recorded from D4 to D8 and on D15. An immobile larva or a larva which did not react to the contact of the grafting tool or paintbrush was recorded as dead. On D15 larvae that had not transformed into pupae were recorded as dead and removed. Hatched adults were recorded on D22.

3. Analytical verification:

The test item stock solutions A to F and the control (dilution medium) were sampled in duplicate as specimens for analysis and retention directly after preparation on D3, D4, D5 and D6. Specimens were frozen immediately. The determination was conducted by using gas chromatography with MS-detection of the derivatization product of sulfur with triphenylphophine: triphenylphosphinsulfid (TPPS). The method was validated according to the guidance document SANTE/2020/12830, Rev.1.

4. Statistics:

For statistical calculation of the mortality results and for determination of the NOEC/NOED for D22, the Step-down Cochran-Armitage test was used. The accepted significance level was $\alpha = 0.05$ (one-sided greater). For the calculation of the ED_{50}/EC_{50}) the Probit procedure was conducted. In order to correct for control mortality, any calculations were conducted with mortality data.

Results and discussion

A. Analytical data

The mean recoveries of sulfur in the analysed samples were between 89 % and 106 %, so was demonstrated that the larvae were treated with the corresponding nominal dose of test item and the endpoints were calculated on the basis of the nominal doses of test item (see table below). In the control samples, no active ingredients were detected.

Table A 45: Analytical results for sulfur in feeding solutions

Treatment	Identification	Sampling Time	Nominal conc. of a.s. [mg/kg]	Analysed conc. of a.s. [mg/kg]	Recovery [% of nominal]	Mean recovery [%]	RSD [%]
Test item	StA	D3	313.9	387.9	124	106	11.2
		D4		323.0	103		
		D5		303.0	97		
		D6		319.1	102		
	StB	D3	104.6	118.2	113	103	6.3
		D4		102.9	98		
		D5		105.3	101		
		D6		106.6	102		
	StC	D3	34.9	35.8	103	99	4.1
		D4		32.6	93		
		D5		34.5	99		
		D6		35.3	101		
	StD	D3	11.6	12.32	106	96	7.2
		D4		11.09	96		
		D5		10.60	91		
		D6		10.62	91		
	StE	D3	3.9	4.07	105	97	8.3
		D4		3.61	93		
		D5		3.97	103		
		D6		3.40	88		
	StF	D3	1.3	1.07	83	89	6.8
		D4		1.09	85		
		D5		1.19	92		
		D6		1.24	96		
Control	C	D3	0.00	<LOD	-	-	-
		D4		<LOD	-		
		D5		<LOD	-		
		D6		<LOD	-		

LOQ: 0.64 mg/kg, corresponding to 0.061 mg/L in diluted extracts

B. Mortality

On D8 of the test, a mortality of 0.0 % was observed in the control AC. In the test item treatment group, cumulative mortalities were between 0.0 and 11.1 % (corrected for control mortality: 0.0 and 11.1 %). Cumulative mortality in the reference item group (AR) was above 50 % across all replicates on D8, being 58.3 % (corrected for control mortality: 58.3 %).

Table A 46: Cumulative mortality on D8

Treatment group	Treatment ID	Cumulative dose	Cumulative concentration	On D8		
				Mean mortality of larvae D3-D8 [%]		Mean OO
		[µg product/larva]	[mg product/ kg food]	abs.	corr.	[%]
Control	AC	-	-	0.0		0.0
Test item	AT	30.54	193.04	11.1	11.1	0.0
	BT	10.18	64.34	11.1	11.1	0.0
	CT	3.39	21.45	11.1	11.1	0.0
	DT	1.13	7.14	2.8	2.8	0.0
	ET	0.38	2.37	2.8	2.8	0.0
	FT	0.13	0.79	0.0	0.0	0.0
		[µg a.s./larva]	[mg a.s./kg food]			
Reference item	AR	7.6	48	58.3	58.3	0.0

Results are averages based on 3 replicates, containing 12 larvae each; corr.: corrected mortality
abs.: absolute mortality as counted from the results; Negative values were set to "0"; OO: Other observations (e.g. remaining food, small body size)
Calculations were performed with non-rounded values

Between D8 and D15, pupal mortality was 11.1 % in the control AC, and ranged between 11.1 and 40.6 % (corrected for control mortality: 0.0 and 33.2 %) in the test item treatment group.

Remaining food on D8 or any other observations indicating sublethal effects were not observed in any treatment group.

Table A 47: Cumulative mortality of pupae on D15

Treatment group	Treatment ID	Cumulative dose	Cumulative concentration	On D15	
				Mean cumulative mortality of pupae D8-D15 [%]	
		[µg product/larva]	[mg product/ kg food]	abs.	corr.
Control	AC	-	-	11.1	
Test item	AT	30.54	193.04	40.6	33.2
	BT	10.18	64.34	37.3	29.4
	CT	3.39	21.45	31.5	23.0
	DT	1.13	7.14	14.1	3.4
	ET	0.38	2.37	14.1	3.4
	FT	0.13	0.79	11.1	0.0
		[µg a.s./larva]	[mg a.s./kg food]		
Reference item	AR	7.6	48	71.1	67.5

Results are averages based on 3 replicates, containing 12 larvae each; corr.: corrected mortality
abs.: absolute mortality as counted from the results; Negative values were set to "0"
Calculations were performed with non-rounded values

After 22 days, the adult emergence rate in the untreated control AC was 80.6 % (mortality 19.4 %). In the test item treatment group, adult emergence rate was 27.8, 47.2, 52.8, 77.8, 80.6 and 80.6 % (from the highest to the lowest dose/concentration). The respective cumulative mortality was 72.2, 52.8, 47.2, 22.2, 19.4 and 19.4 % (corrected for control mortality: 65.5, 41.4, 34.5, 3.4, 0.0 and 0.0 %).

Statistically significant differences in adult emergence/cumulative mortality on D22 compared to the control occurred in the three highest test item treatment groups (30.54, 10.18 and 3.39 µg product/larva corresponding to 193.04, 64.34 and 21.45 mg product/kg food).

Table A 48: Cumulative mortality of larvae and pupae and adult emergence rate on D22

Treatment group	Treatment ID	Cumulative dose	Cumulative concentration	On D22		
				Mean cumulative mortality of larvae & pupae D3-D22 [%]		Adult emergence rate [%]
		[µg product/ larva]	[mg product/ kg food]	abs.	corr.	abs.
Control	AC	-	-	19.4		80.6
Test item	AT	30.54	193.04	72.2	65.5	27.8*
	BT	10.18	64.34	52.8	41.4	47.2*
	CT	3.39	21.45	47.2	34.5	52.8*
	DT	1.13	7.14	22.2	3.4	77.8
	ET	0.38	2.37	19.4	0.0	80.6
	FT	0.13	0.79	19.4	0.0	80.6
		[µg a.s./larva]	[mg a.s./kg food]			
Reference item	AR	7.6	48	100.0	100.0	0.0

Results are averages based on 3 replicates, containing 12 larvae each; corr.: corrected mortality
abs.: absolute mortality as counted from the results; Negative values were set to "0"
Calculations were performed with non-rounded values
* Statistically significant different to the control (Step-down Cochran-Armitage Test Procedure, $\alpha = 0.05$, one-sided greater)

In a larval toxicity study with repeated exposure of honeybee larvae to Microthiol Special Disperss, the ED_{50} (successful adult emergence up to D22) was calculated to be 13.00 µg product/larva (10.40 µg a.i./larva), the ED_{20} was calculated to be 3.22 µg product/larva (2.58 µg a.i./larva) and the ED_{10} was calculated to be 1.56 µg product/larva (1.25 µg a.i./larva). The EC_{50} was calculated to be 82.19 mg product/kg food (65.75 mg a.i./kg food), the EC_{20} was calculated to be 20.34 mg product/kg food (16.27 mg a.i./kg food) and the EC_{10} was calculated to be 9.80 mg product/kg food (7.84 mg a.i./kg food).

The respective NOED was determined to be 1.13 µg product/larva (0.90 µg a.i./larva) and the corresponding NOEC was determined to be 7.14 mg product/kg food (5.71 mg a.i./kg food). The results are summarised in the following table:

Table A 49: Statistical outcome of the chronic larval toxicity test

Treatment	Endpoint: Successful adult emergence	On D22	
		Product	Active substance
Test item doses	ED_{50} [µg/larva] (CL) [2]	13.00 (8.87 – 21.46)	10.40 (7.10. – 17.17)
	ED_{20} [µg/larva] (CL) [2]	3.22 (1.96 – 4.72)	2.58 (1.57 – 3.78)
	ED_{10} [µg/larva] (CL) [2]	1.56 (0.78 – 2.46)	1.25 (0.62 – 1.97)
	NOED [µg/larva] [1]	1.13	0.90
Test item concentrations	EC_{50} [mg/kg food] (CL) [2]	82.19 (56.02 – 135.74)	65.75 (44.82 – 108.59)
	EC_{20} [mg/kg food] (CL) [2]	20.34 (12.36 – 29.81)	16.27 (9.89 – 23.85)
	EC_{10} [µg/larva] (CL) [2]	9.80 (4.88 – 15.51)	7.84 (3.90 – 12.41)
	NOEC [mg/kg food] [1]	7.14	5.71

[1] Step-down Cochran-Armitage Test Procedure, $\alpha = 0.05$, one-sided greater
[2] Probit analysis using linear maximum likelihood regression

C. Validity of the test:

The test is considered to be valid, as the control mortality was ≤ 15 % (i.e. 0.0 %) across all control replicates on D8 and the control-corrected mortality of the reference item (dimethoate) was ≥ 50 % on D8 across all replicates (i.e. 58.3 %). The adult emergence rate on D22 was ≥ 70 % across all control replicates (i.e. 80.6 %).

Conclusion

Assessment and conclusion by applicant:

Under the conditions of this study, the ED_{50} for 'Sulfur 80% WG' was determined to be 10.40 µg a.s./larva. Accordingly, the NOED was determined to be 0.90 µg a.s./larva. The ED_{10} was determined to be 1.25 µg a.s./larva and the ED_{20} = 2.58 µg a.s./larva.

A 2.3.1.3.3 Study 3

Comments of zRMS:	The study was accepted. The validity criteria were met: • larvae mortality in the control was below 15%; observed 5.6%; • larvae mortality in the reference item mortality was ≥ 50 %; observed 94.4%; • adult emergence rate at D22 in control replicates was ≥ 70 %; observed 75.0%; No deviations were noted: The following endpoints were calculated: 8-d LD_{50} = 10.8 µg formulation/larva 22-d NOED = 2.7 µg formulation/larva ED_{10} = 4.0 µg formulation/larva NOEC = 17.1 mg formulation/kg food EC_{10} = 25.2 mg formulation/food

Reference:	CP 10.3.1.3/3
Report	Repeated exposure of honey bee (Apis mellifera L.) larvae to BAS 768 00 F under laboratory conditions, Schmidt, K., 2023 report No 894522, 2148BLC0049 BASF DocID 2021/2014219 Authority registration No
Guideline(s):	OECD 239 (2021)
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 larval toxicity laboratory study, the effects of the formulated product BAS 768 00 F on honey bee larvae (*Apis mellifera*) were investigated after repeated exposure over a period of 22 days. Synchronized 1st instar larvae were fed with diet containing BAS 768 00 F at concentrations of 1.9, 5.7, 17.1, 51.4 and 154.3 mg product/kg food (equivalent to 0.9, 2.7, 8.0, 23.9 and 71.7 mg total a.s./kg food), corresponding to total doses of 0.3, 0.9, 2.7, 8.1 and 24.4 µg product/larva (equivalent to 0.1, 0.4, 1.3, 3.8 and 11.3 µg total a.s./larva). Untreated diet served as an untreated control. In addition, a solvent control equivalent to the dose used in the treatment groups was included. Exposure took place on rearing days 3, 4, 5 and 6. All treatment groups contained larvae from at least three different bee colonies and were set up with 3 replicates, each containing 12 larvae from one colony. Assessments of larval mortality were done at the start of the treatment (D3) and 24, 48, 72, 96 and 120 hours (D4 - D8) after start of the treatment.

Additionally, other observations such as small body size or large quantities of remaining food after 120 hours were noted. Pupal mortality was assessed at D15 and emergence of adults was evaluated at D22.

120 hours after start of repeated oral exposure (on D8), cumulative larval mortality was 5.6% in the untreated control. Pupal mortality (between D8 and 15) was 17.4% in the untreated control. The total mortality in the untreated control on D22 was 25.0%. In the test item treatment groups, corrected cumulative larval mortalities at D8 ranged from 0.0 to 94.1%, corrected pupal mortalities (between D8 and D22) ranged from 0.0 to 100.0% and the corrected total mortalities at D22 ranged from 0.0% to 100.0%. On D8, 6.1%, 6.1%, 5.8%, 18.1% and 100.0% of the remaining larvae treated with 0.3, 0.9, 2.7, 8.1 and 24.4 µg product/larva showed remaining food and/or a smaller body size. In the final assessment on D22, an adult emergence rate of 75.0% was determined in the control group.

In the test item treatment groups, the adult honey bees emerged at rates ranged from 0.0 to 77.8%. Adult emergence on D22 was statistically significantly decreased compared to the control at the test item doses of 8.1 and 24.4 µg product/larva.

In a 22-d repeated exposure larval toxicity study with honey bees (*Apis mellifera*), the overall NOED for BAS 768 00 F was 2.7 µg product/larva (equivalent to 1.3 µg total a.s./larva) and the corresponding overall NOEC was 17.1 mg product/kg food (equivalent to 8.0 mg a.s./kg food).

Materials and methods

Materials

Test item: BAS 768 00 F, Batch no. FD-210120-1029; content of a.s.: mefentrifluconazole (BAS 750 F; Reg. no. 5834378): 26.3 g/L (nominal: 25.0 g/L), sulfur (BAS 175 F, Reg. no.: 240586): 614.9 g/L (nominal: 600.0 g/L); density: 1.346 g/cm^3.

Study design and methods

Test species: Honey bee (*Apis mellifera*), synchronized 1st instar larvae (L1 during grafting); derived from at least three queenright and healthy colonies; source: in-house colonies.

Test design: 22-day repeated exposure larval toxicity test; dose-response design; L1 honeybee larvae were transferred from brood combs to grafting cells 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; five test item doses plus an untreated control; 3 replicates per treatment each with 12 larvae; larvae are collected from at least three different colonies, each representing a replicate; assessments of larval mortality at the start of the treatment (D3) and 24, 48, 72, 96 and 120 hours after start of the treatment (respectively D4, D5, D6, D7, D8); other observations such as small body size or large quantities of remaining food after 120 hours (D8); pupal mortality was assessed at D15 and emergence of adults was evaluated at D22.

Endpoints: LD/LC$_x$, ED/EC$_x$ and NOED/NOEC related to mortality, adult emergence and sub-lethal/other effects.

Reference item:	Dimethoate tech. (analysed purity: 99.32% w/w); dose rate: 7.4 µg dimethoate/larva (corresponding to a concentration of 47 mg a.s./kg food).
Test doses:	Untreated control: untreated diet (50% aqueous yeast/sugar solution with 50% royal jelly); nominal test item dose rates: 0.3, 0.9, 2.7, 8.1 and 24.4 µg product/larva (equivalent to 0.1, 0.4, 1.3, 3.8 and 11.3 µg total a.s./larva); corresponding to concentrations of 1.9, 5.7, 17.1, 51.4 and 154.3 mg product/kg food (equivalent to 0.9, 2.7, 8.0, 23.9 and 71.7 mg total a.s./kg food).
Test conditions:	D1 - D15 (larvae + pre-pupae): crystal polystyrene grafting cells (9 mm internal diameter) placed in 48-well plates; D15 - D22 (pupae + adults): emergence boxes; temperature (D1 - D22): 34.0 - 34.9°C; relative humidity: 92.4 - 98.3% (D1 - D8), 81.0 - 84.3% (D8 - D15) and 60.0 - 68.6% (D15 - D22); photoperiod: 24 h darkness; feeding: aqueous yeast/sugar solution with royal jelly on D1 and D3 to D6; no additional feeding of larvae after applications.
Analytics:	Analytical verification of test item concentrations in honeybee larvae diet was conducted using an HPLC-method with MS-MS detection (Method No. L0452/01).
Statistics:	Descriptive statistics; Step-down Cochran-Armitage Test for determination of NOED/NOEC (D8 and D22); Trimmed Spearman Karber Procedure for calculation of LD/LC_{50} values on D8. Weibull analysis using linear max. likelihood regression for calculation of ED/EC_x values on D22.

Description of the analytical procedures

The concentrations of mefentrifluconazole (contained in BAS 768 00 F) in honeybee larvae diet were determined according to the analytical method L0452/01. The analytical method is fully validated in a separate study (BASF DocID: 2021/2022023). The validation of the analytical method is described in the study report. The samples were extracted with acetonitrile/water/formic acid 50/50/1 (v/v/v) and a QuEChERS salt mix containing 0.5 g magnesium sulfate, 0.12 g sodium chloride, 0.06 g disodium hydrogen-citrate sesquihydrate and 0.12 g of trisodium citrate dihydrate by shaking. 1 mL of the extracts were transferred into a 10 mL-volumetric flask and filled to the mark with acetonitrile/water/formic acid 50/50/1 (v/v/v) and, if necessary, further diluted with dilution medium into the range of the calibration curve. Diluted extracts were injected into the HPLC system. The determination was performed by HPLC with MS-MS detection. The limit of quantification (LOQ) was 0.01 mg/kg food, and the limit of detection (LOD) was set to ≤ 30% of LOQ. Matrix effects were taken into account by the addition of the same amount of blank extract to calibration samples as included in the analysis samples. Thus, all measured samples contained the same amount of original sample matrix. The maximum storage period of the samples was 8 days. Stability samples were not analyzed, as a storage period of 30 days was not exceeded. Mean recoveries of the procedural recovery samples were between 98.3% and 94.8%. Details on measured fortification samples and obtained procedural recoveries for the formulated product BAS 768 00 F (based on measurement of mefentrifluconazole) are provided in Table A 50.

Table A 50: Procedural recoveries for BAS 768 00 F (based on measurement of mefentrifluconazole)

Matrix	Fortification level [mg a.s./kg food]	n	Mean recovery [%]	RSD [%]
Honeybee larvae diet	0.01	5	98.3	2.23

Honeybee larvae diet	3.81	5	94.8	5.59

Abbreviations: n: number of replicates; RSD: relative standard deviation

Results

Biological results

120 hours after start of repeated oral exposure (on D8), cumulative larval mortality was 5.6% in the untreated control. Pupal mortality (between D8 and 15) was 17.4% in the untreated control. The total mortality in the untreated control on D22 was 25.0%. In the test item treatment groups, corrected cumulative larval mortalities at D8 ranged from 0.0 to 94.1%, corrected pupal mortalities (between D8 and D22) ranged from 0.0 to 100.0% and the corrected total mortalities at D22 ranged from 0.0% to 100.0%. On D8, 6.1%, 6.1%, 5.8%, 18.1% and 100.0% of the remaining larvae treated with 0.3, 0.9, 2.7, 8.1 and 24.4 µg product/larva showed remaining food and/or a smaller body size. In the final assessment on D22, an adult emergence rate of 75.0% was determined in the control group. In the test item treatment groups, the adult honey bees emerged at rates ranged from 0.0 to 77.8%. Adult emergence on D22 was statistically significantly decreased compared to the control at the test item doses of 8.1 and 24.4 µg product/larva (Step-down Cochran-Armitage Test, one-sided greater, $\alpha = 0.05$).
The results are summarized in Table A 51.

Table A 51: Effects of BAS 768 00 F on honey bee larvae (*Apis mellifera*) after repeated exposure

Dose rate [µg product/ larva] (nominal)	**Concentration mg product/kg food] (nominal) [**	**Larval mortality (D3 - D8) [%]**		**Pupal mortality (D8 - D15) [%**		**Pupal mortality (D8 - D22) [%]**		**Total mortality (D3 - D22) [%]**		**Adult emergence rate on D22 [%]**
		abs.	corr.[1)]	abs.	corr.[1)]	abs.	corr.[1)]	abs.	corr.[1)]	abs.
Untreated control	-	5.6	0.0	17.4	0.0	20.2	0.0	25.0	0.0	75.0
0.3	1.9	8.3	2.9	12.5	0.0	15.8	0.0	22.2	0.0	77.8
0.9	5.7	5.6	0.0	9.1	0.0	21.0	0.9	25.0	0.0	75.0
2.7	17.1	5.6	0.0	17.7	0.3	23.5	4.1	27.8	3.7	72.2
8.1	51.4	30.6*	26.5	37.8	24.6	55.9	44.8	66.7	55.6	33.3*
24.4	154.3	94.4*	94.1	100.0	100.0	100.0	100.0	100.0	100.0	0.0*

Endpoints: Larval mortality on D8	**based on product**	**based on a.s.**
LD_{50} [µg/larva] (95% CL) [2)]	10.9 (9.0 - 13.2)	5.1 (4.2 - 6.1) [#]
LD_{20} [µg/larva] (95% CL) [2)]	--	--
LD_{10} [µg/larva] (95% CL) [2)]	--	--
$NOED_{mortality}$ [µg/larva]	2.7	1.3
LC_{50} [mg/kg food] (95% CL) [2)]	68.8 (56.7 - 83.5)	31.9 (26.3 - 38.8) [#]
LC_{20} [mg/kg food] (95% CL) [2)]	--	--
LC_{10} [mg/kg food] (95% CL) [2)]	--	--
$NOEC_{mortality}$ [mg/kg food]	17.1	8.0
Endpoints: Adult emergence on D22	**based on product**	**based on a.s.**
ED_{50} [µg/larva] (95% CL) [3)]	7.7 (6.6 - 9.0)	3.6 (3.1 - 4.2) [#]
ED_{20} [µg/larva] (95% CL) [3)]	5.2 (4.0 - 6.7)	2.4 (1.9 - 3.1) [#]
ED_{10} [µg/larva] (95% CL) [3)]	4.0 (2.8 - 5.8)	1.9 (1.3 - 2.7) [#]
$NOED_{emergence}$ [µg/larva]	2.7	1.3
EC_{50} [mg/kg food] (95% CL) [3)]	48.6 (41.8 - 56.6)	22.6 (19.4 - 26.3) [#]
EC_{20} [mg/kg food] (95% CL) [3)]	32.8 (25.5 - 42.1)	15.2 (11.8 - 19.5) [#]
EC_{10} [mg/kg food] (95% CL) [3)]	25.2 (17.4 - 36.6)	11.7 (8.1 - 17.0) [#]
$NOEC_{emergence}$ [mg/kg food]	17.1	8.0

Abbreviations: abs.: absolute; corr.: corrected, CL: confidence limits
Negative values are set to 0

* Statistically significant difference compared to the control (Step-down Cochran-Armitage Test Procedure, one-sided greater, $\alpha = 0.05$).

[#] Values based on the active substance have been recalculated from the study data considering the nominal content of 625 g total a.s./L and the product density of 1.346 g/cm^3.

[1)] Corrected mortality according to Schneider-Orelli (1947).

[2)] Calculated using Trimmed Spearman-Karber procedure.

[3)] Calculated using Weibull analysis using linear max. likelihood regression.

The corrected mortality on D8 in the reference item treatment was 94.1%.

Table A 52: Validity criteria according to OECD 239 (2021)

Criteria	Trigger value	Study value and page no.	Criteria met? Y/N	Guideline
Mean cumulative larval mortality in the control(s) from D3 to D8	≤ 15% across all replicates[#]	Untreated control: 5.6% (p. 24)	Y	OECD 239
Mean adult emergence rate in the control(s) on D22	≥ 70% across all replicates[#]	Untreated control: 75.0% (p. 24)	Y	OECD 239
Effects of the reference item:	Dimethoate: larval mortality ≥ 50% on D8 across all replicates[#]	Dimethoate:94.4% (p. 24)	Y	OECD 239

[#] A replicate being defined as the number of larvae originating from the same colony.

All validity criteria were met (see Table A 52 above).

Conclusion

In a 22-d repeated exposure larval toxicity study with honey bees (*Apis mellifera*), the overall NOED for BAS 768 00 F was 2.7 µg product/larva (equivalent to 1.3 µg total a.s./larva) and the corresponding overall NOEC was 17.1 mg product/kg food (equivalent to 8.0 mg a.s./kg food).

A 2.3.1.4 KCP 10.3.1.4 Sub-lethal effects

BAS 768 00 F poses no unacceptable risk to bees. Further studies are not necessary

A 2.3.1.5 KCP 10.3.1.5 Cage and tunnel tests

Comments of zRMS:	The study was evaluated and accepted during active substance renewal In Draft Assessment Report (DAR) of Sulphur Dust (August 2022), Vol. 3CP, B.9 the similar study (with different report number, but with the same study results) was considered.

Reference:	CP 10.3.1.5/1
Report	Effects of Microthiol Special Disperss (Sulphur 80% WG) on the honeybee Apis mellifera L. under semi-field conditions (tunnel test) with additional assessments on colony and brood development, Schnurr, A., 2018 report No EU-1748BTB0004 BASF DocID 2018/1232880 Authority registration No
Guideline(s):	Current ring test protocol of the AG-Bienenschutz (2014), Pistorius et al. (2012), OECD Guidance document No. 75 (2007)
Deviations:	No
GLP:	yes (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany),
Acceptability:	Yes
Duplication (if vertebrate study)	No

Executive summary

The effect of ‘Sulfur 80% WG’ (Microthiol Special Disperss) on the honeybee (*Apis mellifera* L.) was examined after one foliar application on full-flowering Phacelia (*Phacelia tanacetifolia*) during bee flight under semi-field conditions according to OECD 75 (2007). ‘Sulfur 80% WG’ was applied at a single application rate of 10 kg a.s./ha (12.5 kg product/ha) in 400 L/ha. Additionally, a reference item (Insegar 25 WG; nominal: 250 g Fenoxycarb/kg) as well as a control (tap water) were foliar applied using a water volume of 400 L/ha. The trials were conducted with bee colonies acclimated in 93.5 m² tunnels covered with light gauze. The exposure phase lasted 7 days. Thereafter, bee colonies of all treatment groups were removed from the tunnels and placed to a remote site for further 20 days. Main endpoints included mortality, foraging activity, bee behaviour, colony and brood development. Additionally, the levels of residue of the test item on flowers were determined to prove the exposure.

Following the application and during the entire course of the study, no significant differences on adult and pupal bee mortality, foraging activity, behaviour of bees, colony strength and brood development were observed between the test item treatment and the control. In particular, the specific evaluation of the detailed bee brood development of initially labelled eggs revealed no impact of the test item during the entire trial. Therefore, it can be concluded that the test item 'Sulfur 80% WG' applied at a rate of 12.5 kg product/ha has no adverse effects on any of the aforementioned honeybee endpoints in this semi-field study. The sensitivity of the test system was verified by means of the reference item.

Materials and methods

A. Materials

1. Test substance:	'Sulfur 80% WG' (Microthiol Special Disperss)
Lot/Batch no.:	14-131-01
Content/Purity:	nominal: 800 g/kg; analysed: 799 g/kg
Control:	tap water
Toxic reference:	Insegar 25 WG (250 g Fenoxycarb/kg)
2. Test organisms	
Species:	*Apis mellifera* L. Buckfast (Hymenoptera, Apoidea); Healthy, queen-right colonies kept under proper conditions in accordance with good beekeeping practice and comprising adults and juvenile bees of all brood stages; Colonies covered one body with 11 combs, brood combs ranging from 5 - 9 and including 3 - 6 combs with eggs; 6188 - 9000 bees/colony (at experimental start)
Health check:	Yes, for Nosema *apis*, foulbrood, diverse viruses (DWV, SBV, ABPV, KBV, CBPV, BQCV) and Varroa destructor before and after the experimental work
3. Test units and exposure	
Exposure units:	Tunnel: 18 x 6 x 2.5 m (effective crop area of 93.5 m²)
Test design:	The honeybee semi-field/tunnel study comprised 3 treatment groups: control (n = 4), test item (n = 4) and reference item (n = 4 and after loss of one queen bee n = 3) Test substrate (day of application): full-flowering Phacelia (*Phacelia tanacetifolia*), growth stage BBCH 63-65, good condition of crop and only a few weeds visible, 70 cm plant height and 100 % ground cover
Test system:	semi-field test (tunnel test) with fresh-residues
Pre-exposure phase:	Tunnel; 4 *days* from DAT -4 (DAT = days after treatment) to DAT 0 ba (ba = before application)
Exposure phase:	Tunnel; 7 days from DAT 0 aa (aa = after application) to DAT 7
Post-exposure phase:	Remote site; 20 days from DAT 8 to DAT 27
Assessment/ Endpoints:	Mortality, Foraging activity, Behaviour, Colony development (*i.e.* colony strength, brood and food status), detailed brood assessments, meteorological conditions, residue analysis

4. Test conditions

Temperature:	21.9 - 23.4 °C (application); 9.7 - 32.5 °C (DAT-4 – DAT 27)
Relative humidity:	55.7 - 59.3 % (application); 32.0 - 96.0 % (DAT-4 – DAT 27)
Wind:	0.1 - 0.8 m/s (application)
Precipitation:	0.0 mm (application); 0.0 – 55.2 mm (DAT-4 – DAT 27)
Cloud cover:	0 - 10 % (application)
Mean foraging activity:	10.0 - 15.7 bees/m²

B. Study design and method

1. In-life dates (experimental phase): June 20 to July 21, 2017 (*i.e.* DAT-4 – DAT 27)

2. Test design:

Bee hives were set up in the tunnels 5 days before application (acclimatisation). Thereafter, the test item 'Sulfur 80% WG' (Microthiol Special Disperss) was applied once during bee flight to full flowering *Phacelia tanacetifolia* (*i.e.* nectar and pollen source) at an application rate of 10 kg a.s./ha (12.5 kg product/ha). Additionally, a toxic standard at a rate of 1.2 kg Insegar 25 WG/ha (300 g a.s./ha) and a control (tap water) were applied. For all treatments, a water volume of 400 L/ha was used. After a 7 days exposure phase, the bee hives were removed from the tunnels and transported to a remote area, limited in major crops (agriculture main crops). The post-exposure phase started on DAT 8 and lasted for one brood cycle, *i.e.* 3 weeks.

During the pre-exposure, exposure and post-exposure phase, assessments included mortality, foraging activity, behaviour, colony and brood development, weather conditions and chemical residue analysis on flowers. Mortality was daily assessed by counting dead bees in the dead bee trap (DAT-4 to DAT 27) or on linen sheets (DAT-4 to DAT 7). Foraging activity was daily assessed by counting bees per m² in three subplots per tunnel (DAT-4 to DAT 7). Behaviour was daily assessed (DAT-4 to DAT 27). The assessment of the colony development, encompassing colony strength and brood and food status, took place once before and 5-times after application (DAT -2, DAT 2, DAT 9, DAT 13, DAT 20 and DAT 27). Detailed brood assessments were performed via photo documentation of initially labelled eggs (brood fixing day (BFD) 0, BFD 4, BFD 11, BFD 15 and BFD 22) The meteorological conditions were daily monitored (DAT-4 to DAT 27). Finally, flowers for residue analysis were sampled once before (DAT -2 and DAT 0) and 4 hours after the application of the test item.

C. Calculation and statistics

C.1 Mortality

Dead bees from the dead bee trap and the linen sheets were summed up to a total mortality value per replicate. Possible statistical differences during the pre-application phase were analysed via a multiple testing method (*i.e.* Tukey-test, $\alpha = 0.05$). In contrast, post-application data was evaluated using pair-wise statistical comparison methods (*i.e.* Student t-test or Welch t-test for variance homogeneous or inhomogeneous data, respectively; one-sided greater, $p > 0.05$). Because no pupal mortality occurred in the control group during the test, no statistical comparison was performed for this endpoint.

C.2 Foraging activity and bee behaviour

Values of foraging activity based on the three sub-plots per tunnel, which were used to calculated to a mean per replicate. Statistical evaluation of the pre- and post-application phases followed that listed for mortality, with the exception that the option “one-sided smaller” was chosen for the Student t-test or Welch t-tests. The behaviour of bees was assessed in the dead bee trap and on linen sheets, at the hive entrance and in the crop.

C.3 Colony strength, brood and food development

Assessments of colony strength as well as brood and food development were conducted according to methods of Imdorf *et al.* (1987) and Imdorf and Gerig (1999) during BFD 0 - BFD 29 (once before and 5-times after application).

Colony strength was estimated through the number of bees per colony, including the presence of a healthy queen. The assumption that a comb, having an area of 825.1 cm² (+ walls), could be maximally covered by 900 bees per comb side. For simplification, each comb side was separated in 8 equal parts and the number of parts covered by bees (1/8 to 8/8) was assessed. The number per comb in a 11 comb-colony with two bounding hive walls was further calculated. Finally, the counted parts were transferred into the number of bees (rule of thumb: one eighth is covered by ~112.5 bees).

The assessment of the brood and food development encompassed the estimated brood status (*i.e.* area containing eggs, larvae and pupae) as well as the presence of pollen and nectar in combs. The same assumption as described for colony strength (8 equal parts) was used for the estimation. The originally assessed parts of brood or food were transferred in cm^2 (1/8 of a comb is equivalent to 103.1 cm^2). The final evaluation of the brood nest size assessments is based on the “total brood or food covering comb area per colony” [cm^2/colony].

C.4 Detailed brood development of initially labelled cells

Brood termination rate (BTR)

Firstly, the development of individual eggs or larvae is divided into the two following categories:

1. Successful development: (a) bee brood reached the brood stage expected at a certain assessment day or (b) was found empty or (c) contained an egg after hatch of the adult bee on BFD 22

2. Termination of the bee brood development: (a) bee brood did not reach the brood stage expected at a certain assessment day or (b) food was stored in the cell during BFD 4 to BFD 15

For the calculation of the BTR [%], the number of cells of “category 1” are summed up for each treatment and colony, multiplied by 100 and divided by the number of initially marked egg cells. The statistical evaluation of the BTR followed that listed for the post-application phase of the endpoint “mortality”.

Brood index (BI)

The BI is an indicator of the bee brood development (development from egg in initially marked cell to hatching adult) and facilitates a comparison between different treatments. Initially marked cells were divided into categories from 1 to 5 ("egg" to - "successful hatched") or category "0" (brood terminated between BFD 4 and 15 or cell filled with honey or pollen; "0" stays "0" irrespective of the cell where brood terminated was again filled with brood) from BFD 0 up to BFD 22. The BI was then calculated by dividing the sum of values from all cells in each treatment of the same date by the number of totally observed cells. Statistical evaluation of the pre- and post-application phases followed that listed for mortality, with the exception that the option "one-sided smaller" was chosen for the Student t-test or Welch t-tests.

Brood compensation index (BCI)

The BCI is an indicator for colony recovery and was calculated for each assessment day and colony. The cells were classified from 1 to 5 (as described for the BI) and "0" (as long as it was refilled with brood again, reflecting the compensation of bee brood losses) from BFD 0 up to BFD 22. For the final calculation of BCI, the sum of values of all individual cells of a treatment from the same day was divided through the number of observed cells. Statistical evaluation of the pre- and post-application phases followed that listed for mortality, with the exception that the option "one-sided smaller" was chosen for the Student t-test or Welch t-tests.

D. Climatic conditions

The environmental conditions such as air temperature and air humidity were recorded continuously from 20 June – 21 July 2017 under non-GLP conditions by SKW Piesteritz, LAF Cunnersdorf, 04451 Cunnersdorf, Germany.

E. Analytics

Residues of sulfur on flowers has been determined by an in-house developed method using high performance liquid chromatography (HPLC) with diode array detection (DAD-detection). The limit of quantification (LOQ) was defined in the context of this analytical phase as the lowest successfully validated fortification level, *i.e.* 100.6 mg a.i./kg sulfur, regarding DF 1.258 mg/L, since the validation low level with 1.006 mg a.i./kg are not fulfilled all validation criteria. The limit of detection (LOD) was in this analytical phase the lowest calibration standard with 0.808 mg/kg of sulfur, regarding DF 0.101 mg/L.

Validity criteria set during the test

The study is considered valid if:
- Mean brood termination rate in the reference item group is ≥ 50% and preferably ≤ 90% and pupal mortality is distinctly increased compared to the control,
- Brood termination rate in the control not exceeded an average of 40%,
- Mean foraging activity during application ≥ 10 bees/m² in each treatment group.

Results and discussions

A. Mortality

The application of 'Sulfur 80% WG' at a rate of 12.5 kg product/ha (equivalent to 10 kg a.s./ha) had no adverse effects on both, adult bee and pupal mortality at any phase of the study. The same result was obtained for the tap water control. In contrast, the reference item Insegar 25 WG, which is known to threaten larval stages, expectedly caused no adult bee toxicity, but significantly in-creased larval toxicity, starting in the post-exposure phase. Details are given in the table below.

Table A 53: Adult bee and pupal mean mortality during pre-exposure, exposure and post-exposure phase of a semi-field study with the test item 'Sulfur 80% WG'

Phase (DAT)	Adult bee mean mortality ± SD [%] 2)			Pupal mean mortality ± SD [%] 2)		
	Control 1) (tap water)	Test item 1) (10 kg a.s./ha)	Reference 1) (0.25 kg a.s./ha)	Control 1) (tap water)	Test item 1) (10 kg a.s./ha)	Reference 1) (0.25 kg a.s./ha)
Pre-exposure phase (DAT -4 to DAT 0 ba)	9.1 ± 6.5 a	6.7 ± 3.6 a	8.3 ± 5.0 a	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Exposure phase (DAT 0aa to DAT 7)	12.7 ± 7.8	10.8 ± 6.8	11.6 ± 7.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Post-exposure phase (DAT 8 to DAT 27)	4.6 ± 3.8	4.9 ± 2.9	5.8 ± 4.7 *	0.0 ± 0.0	0.0 ± 0.0	4.5 ± 6.5 *
Post-application phase (DAT 0aa to DAT 27)	6.9 ± 6.4	6.6 ± 5.1	7.7 ± 6.2	0.0 ± 0.0	0.0 ± 0.0	3.0 ± 5.7 *

DAT = days after treatment; 1) = application rate in 400 L water/ha; 2) = mean of four replicates; a same letter indicate that groups are not statistically significantly different at pre-application period (Tukey-test, α = 0.05); SD = standard deviation; ba = before application; aa = after application; * = statistically significantly different compared to the control (Student-t test or Welch-t test, one sided greater, p > 0.05); calculations were performed with non-rounded values

B. Foraging activity and behaviour

The application of 'Sulfur 80% WG' at a rate of 12.5 kg product/ha (equivalent to 10 kg a.s./ha) had no adverse effects on the foraging activity of bees. As expected, also the tap water control and the reference item (possessing larval toxicity) had no influence on the foraging activity of the adult worker honey bees. Details are given in the table below.

Table A 54: Foraging activity of worker honey bees during pre-exposure and exposure phase of a semi-field study with the test item 'Sulfur 80% WG'

Phase (DAT)	Number of foraging worker honey bees ± SD [bees/m²] 2)		
	Control 1) (tap water)	Test item 1) (10 kg a.s./ha)	Reference 1) (0.25 kg a.s./ha)
Pre-exposure phase (DAT -4 to DAT 0 ba)	11.6 ± 2.7 a	11.3 ± 2.7 a	10.3 ± 2.8 a
Exposure phase (DAT 0aa to DAT 7)	12.4 ± 2.8	11.5 ± 3.0	11.5 ± 3.1

DAT = days after treatment; 1) = application rate in 400 L water/ha; 2) = mean of four replicates; a same letter indicate that groups are not statistically significantly different at pre-application period (Tukey-test, α = 0.05); SD = standard deviation; ba = before application; aa = after application; calculations were performed with non-rounded values

With regard to the general behaviour of the bees, no abnormalities, e.g. reduced or no foraging activity, signs of apathy, intensified cleaning or intoxication, were observed before application, neither in the crop at the bee hives, nor in the dead bee traps and on the linen sheets in any of the treatments (*i.e.* control, test item and reference item). The application of the test item and reference item did not result in any behavioural abnormalities as well, neither immediately after their application, nor during the respective entire exposure and post-exposure phases when compared to control.

C. Colony strength, brood and food development

The application of 'Sulfur 80% WG' at a rate of 12.5 kg product/ha (equivalent to 10 kg a.s./ha) had no adverse effects on the colony strength. The same was observed for the tap water control. In contrast, at the end of the trial (BFD 29 (DAT 27)) the reference item treatment caused a 7 % decrease in colony strength compared to BFD 0 (DAT -2). Details are given in the table below.

Table A 55: Summary of honeybee colony strength during a semi-field study with the test item 'Sulfur 80% WG'

Assessment day	Control [1] (tap water)			Test item [1] (10 kg a.s./ha)			Reference [1] (0.25 kg a.s./ha)		
	Mean[1]	± SD	%[3]	Mean[1]	± SD	%[3]	Mean[1]	± SD	%[3]
BFD 0 (DAT -2)	8606	507	-	8156	540	-	7509	1024	-
BFD 4 (DAT 2)	8184	515	-5	8213	1043	+1	7116	1216	-5
BFD 11 (DAT 9)	10153	947	+18	10350	1805	+27	8241	1008	+10
BFD 15 (DAT 13)	9309	906	+8	9872	1756	+21	6975	1732	-7
BFD 22 (DAT 20)	10378	444	+21	10181	2243	+25	7088	1952	-6
BFD 29 (DAT 27)	10097	818	+17	10575	1779	+30	6975	1699	-7

DAT = days after treatment; BFD = brood fixing day; [1] = application rate in 400 L water/ha; [2] = mean of four replicates; [3] = relative change [%] in comparison with BFD 0 (DAT -2) calculated from the respective mean values (+ and – indicate an increase or decrease compared to BFD 0); SD = standard deviation

The application of 'Sulfur 80% WG' at a rate of 12.5 kg product/ha (equivalent to 10 kg a.s./ha) had no adverse effects on the brood and food development.

With respect to the colony condition, all colonies had an active egg-laying bee queen and showed an adequate brood nest size with good minimal food storages of nectar and pollen at the start (BFD 0; DAT -2) and during the experimental phase until BFD 29 (DAT 28). Only replicate 1 of the reference item group was found queen-less from BFD 15 on and therefore, it was excluded from the evaluations since this day.

The entire mean brood area (total area occupied by eggs, larvae and pupae) at BFD 0 (DAT -2), amounted to 7477, 8431 and 6627 cm²/colony in the control, test item and reference item treatment, respectively, and thus, was lowest in the reference item group. The initial mean area occupied by eggs was 1650, 1676 and 1444 cm²/colony for the control, test item and reference item treatment, respectively and all colonies revealed a good level for each brood stage area.

The mean areas of the single stages, *i.e.* eggs, larvae and pupae as well as the total mean brood area of the control and test item treatment, respectively developed within the range of natural variability in a comparable manner during the course of the study. Only the reference item revealed adverse effects on the brood nest area, that way supporting the sensitivity of the test system.

Food stores (nectar/honey and pollen) covering the comb areas revealed similar levels for the control, test item and reference item, respectively. Overall, the nectar and pollen stores - with respect to the size of the colonies - were on an acceptable level and had no negative or limiting effects on brood or colony development throughout the entire study.

D. Detailed brood development of initially labelled cells

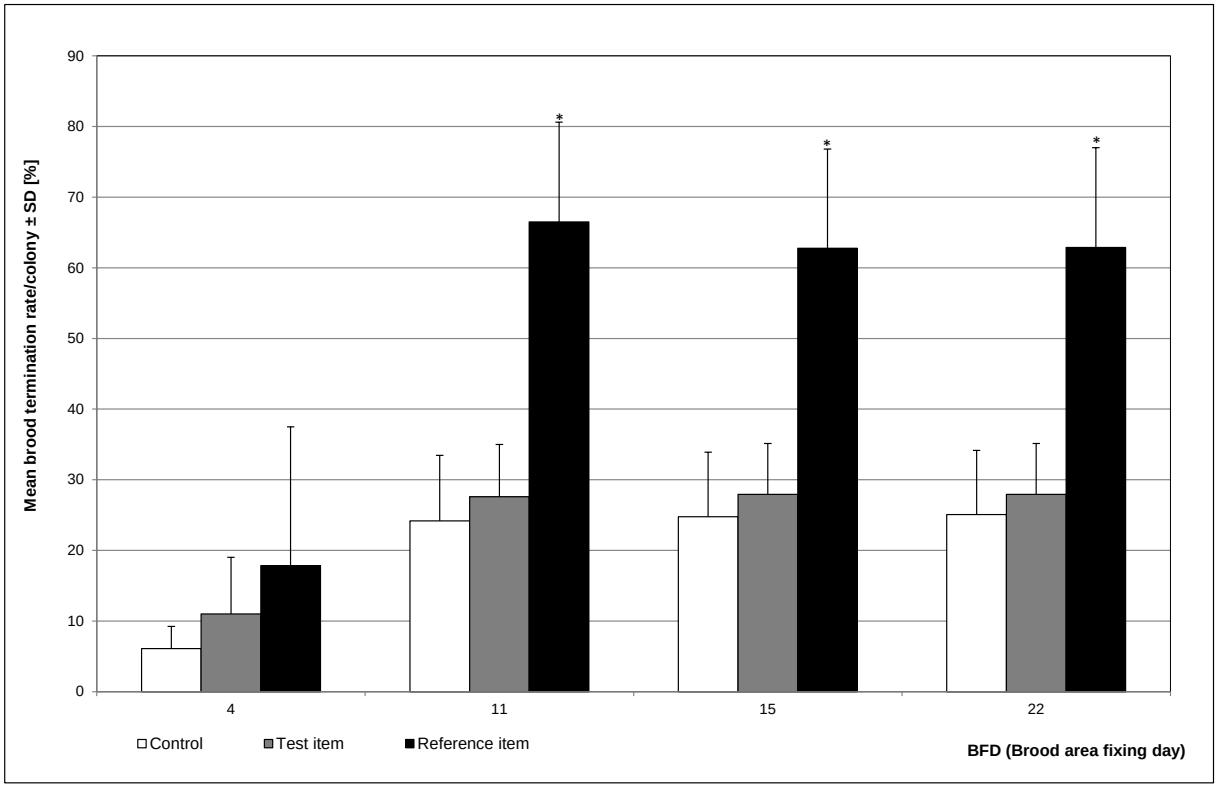
Brood termination rate (BTR)

Both, control and test item treatment displayed a consistently comparable similar pattern of brood termination. In contrast, BTR values determined for the reference item treatment significantly differed from the control, starting with BFD 11 (Student t-test, one-sided greater, $p < 0.05$). The high BTR in the reference item treatment confirmed both the validity and the sensitivity of the test system and thus the capability to evaluate potential effects on the honeybee brood. Details are given in the table and the figure below.

Table A 56: Brood termination rate (BTR) during a semi-field study with the test item 'Sulfur 80% WG'

Assessment day	Mean brood termination rate of initially labelled eggs [%]		
	Control [1) 2)] (tap water)	Test item [1) 2)] (10 kg a.s./ha)	Reference [1) 3)] (0.25 kg a.s./ha)
BFD 4	6.1 ± 3.2	11.0 ± 8.0	17.8 ± 19.6
BFD 11	24.2 ± 9.3	27.6 ± 7.4	66.5 ± 14.1 *
BFD 15	24.8 ± 9.2	27.9 ± 7.2	62.8 ± 14.0 *
BFD 22	25.1 ± 9.1	27.9 ± 7.2	62.9 ± 14.1 *

BFD brood area fixing day; [1)] = application rate in 400 L water/ha; [2)] = mean of four replicates; [3)] = mean of four replicates until BFD 11, thereafter mean of 3 replicates; SD = standard deviation; * = statistically significantly different of the control (Student t-test) one-sided greater, $p<0.05$



***** = statistically significantly different in comparison to the control (STUDENT t-test, one sided greater, $p<0.05$)

Figure A 1: Brood termination rate of eggs (n = 4 (for reference treatment since BFD 15 n = 3))

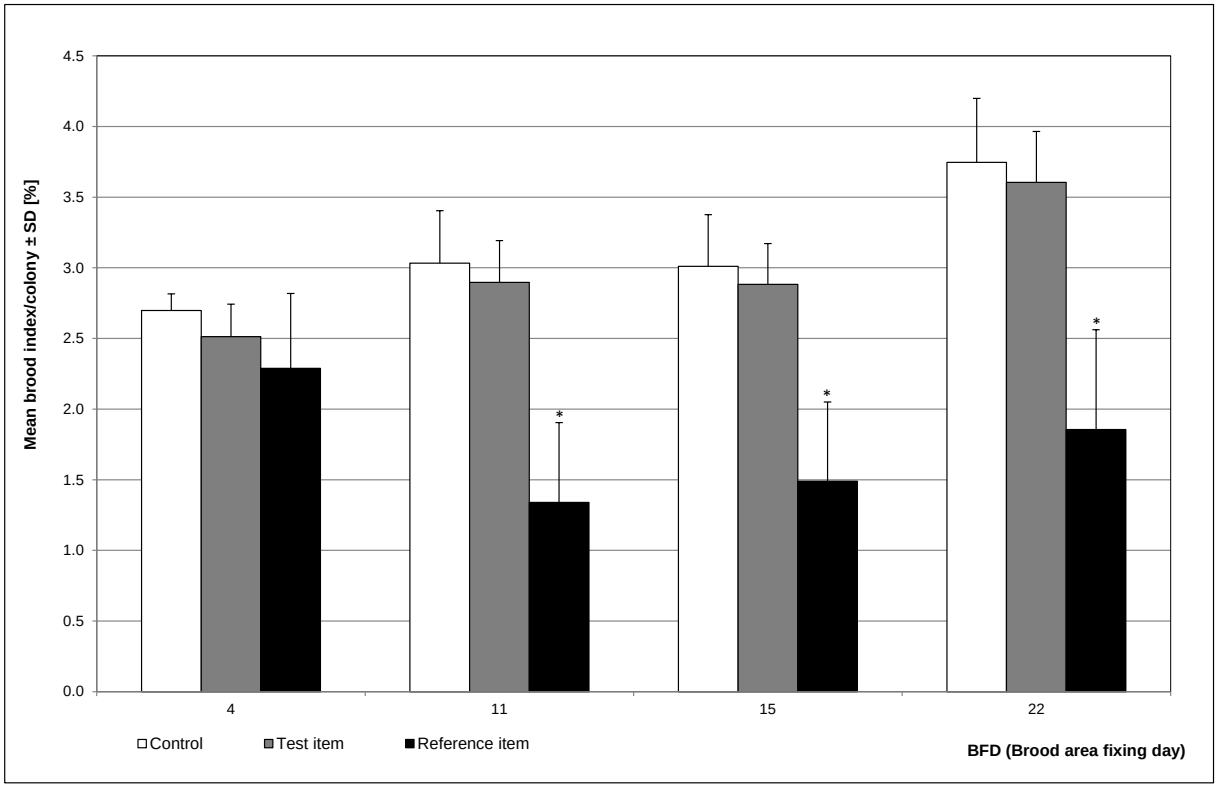
Brood index

The BI displays a negative correlation with the BTR: the higher the BTR, the lower the BI and vice versa. Details are given in the table and the figure below.

Table A 57: Brood index (BI) during a semi-field study with the test 'Sulfur 80% WG'

Assessment day	Mean brood index of initially labelled eggs [%]		
	Control [1) 2)] (tap water)	Test item [1) 2)] (10 kg a.s./ha)	Reference [1) 3)] (0.25 kg a.s./ha)
BFD 4	2.70 ± 0.12	2.51 ± 0.23	2.29 ± 0.53
BFD 11	3.03 ± 0.37	2.90 ± 0.30	1.34 ± 0.56 *
BFD 15	3.01 ± 0.37	2.88 ± 0.29	1.49 ± 0.56 *
BFD 22	3.75 ± 0.45	3.60 ± 0.36	1.86 ± 0.71 *

BFD brood area fixing day; 1) = application rate in 400 L water/ha; 2) = mean of four replicates; 3) = mean of four replicates until BFD 11, thereafter mean of 3 replicates; SD = standard deviation; * = statistically significantly different of the control (Student t-test) one-sided smaller, $p<0.05$



***** = statistically significantly different in comparison to the control (STUDENT t-test, one sided smaller, $p<0.05$)

Figure A 2: Brood index of eggs (n=4 (for reference treatment since BFD 15 n=3))

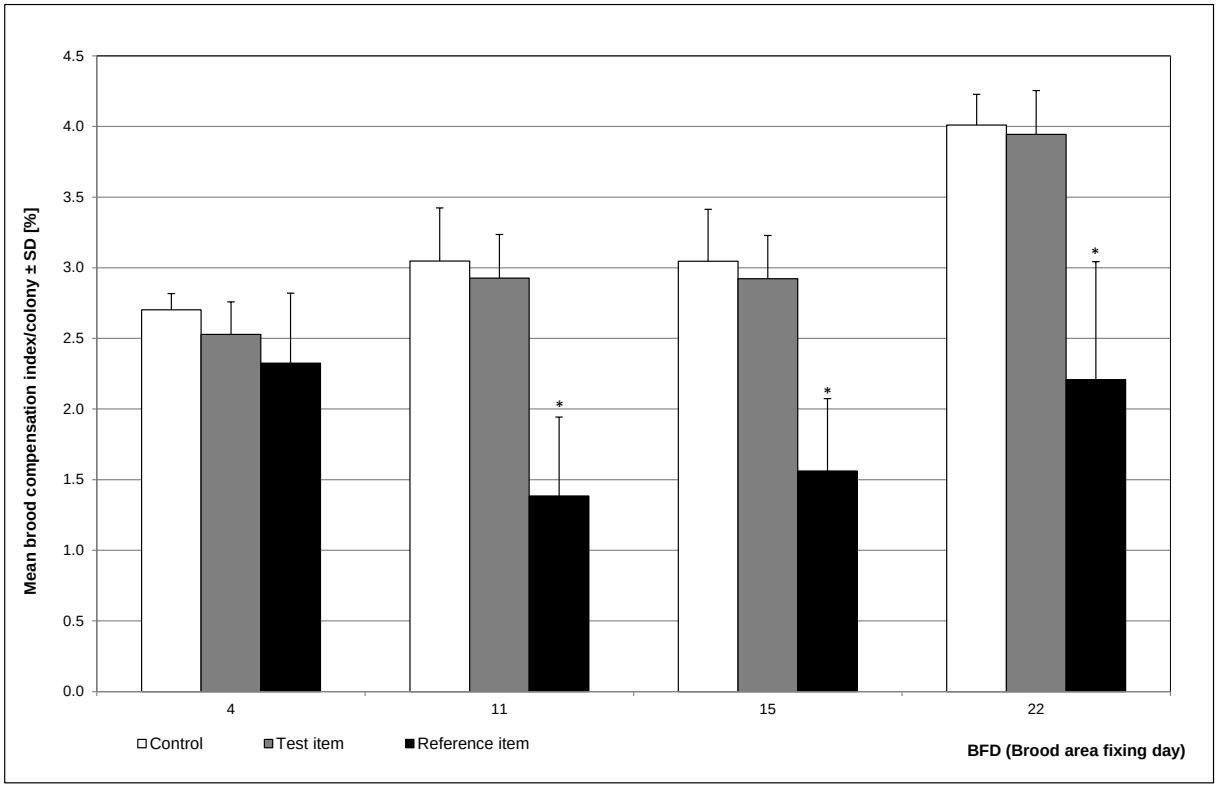
Brood compensation index (BCI)

Mean BCIs of the test item and tap water control were comparable and without any statistically significant differences (Student t-test, one-sided smaller, $p > 0.05$). Although several emptied cells were refilled with eggs which subsequently developed, the BCI of the reference item was statistically significant different to that of the control (Student t-test, one-sided smaller, $p < 0.05$) starting with BFD 11. Details are given in the table and the figure below.

Table A 58: Brood compensation index (BCI) during a semi-field study with the test item 'Sulfur 80% WG'

Assessment day	Mean brood compensation index of initially labelled eggs [%]		
	Control [1) 2)] (tap water)	Test item [1) 2)] (10 kg a.s./ha)	Reference [1) 3)] (0.25 kg a.s./ha)
BFD 4	2.70 ± 0.12	2.53 ± 0.23	2.33 ± 0.49
BFD 11	3.05 ± 0.38	2.93 ± 0.31	1.39 ± 0.56 *
BFD 15	3.05 ± 0.37	2.92 ± 0.31	1.56 ± 0.51 *
BFD 22	4.01 ± 0.22	3.94 ± 0.31	2.21 ± 0.83 *

BFD brood area fixing day; [1)] = application rate in 400 L water/ha; [2)] = mean of four replicates; [3)] = mean of four replicates until BFD 11, thereafter mean of 3 replicates; SD = standard deviation; * = statistically significantly different of the control (Student t-test) one-sided smaller, $p<0.05$



***** = statistically significantly different in comparison to the control (STUDENT-t test, one sided smaller, $p<0.05$)

Figure A 3: Brood compensation index of eggs (n=4 (for reference treatment since BFD 15 n=3))

E. Residue analyses of sulfur on flowers

No residues of sulfur on flowers were found in any of the control specimens sampled before (DAT -2) and in control specimens sampled within 4 hours after application (DAT 0aa), indicating that specimens of flowers were free of contamination during the pre-exposure and exposure phase, respectively. Also, for the test item specimens taken during pre-exposure phase (DAT -2) no residues were found.

After application of 12.5 kg Microthiol Special Disperss ('Sulfur 80% WG')/ha the residue level of sulphur on flowers in the test item specimens generated within 4 hours after application ranged from 935.0 mg a.i./kg to 1281 mg a.i./kg. Based on the low variability, a homogenous application of the test item among the replicates can be concluded.

Table A 59: Results of residues of sulfur on flowers

Specimen identification	Treatment group	Sampling day	Analysed concentration of a.s. [mg/kg]
1748BTB0004-01	Control 1.1	DAT -2	n.d.
1748BTB0004-03			n.d.
1748BTB0004-05			n.d.
1748BTB0004-07			n.d.
1748BTB0004-65		DAT 0aa	n.d.
1748BTB0004-67			n.d.
1748BTB0004-69			n.d.
1748BTB0004-71			n.d.
1748BTB0004-09	Control 1.2	DAT -2	n.d.
1748BTB0004-11			n.d.
1748BTB0004-13			n.d.
1748BTB0004-15			n.d.
1748BTB0004-73		DAT 0aa	n.d.
1748BTB0004-75			n.d.
1748BTB0004-77			n.d.
1748BTB0004-79			n.d.
1748BTB0004-17	Control 1.3	DAT -2	n.d.
1748BTB0004-19			n.d.
1748BTB0004-21			n.d.
1748BTB0004-23			n.d.
1748BTB0004-81		DAT 0aa	n.d.
1748BTB0004-83			n.d.
1748BTB0004-85			n.d.
1748BTB0004-87			n.d.
1748BTB0004-25	Control 1.4	DAT -2	n.d.
1748BTB0004-27			n.d.
1748BTB0004-29			n.d.
1748BTB0004-31			n.d.
1748BTB0004-89		DAT 0aa	n.d.
1748BTB0004-91			n.d.
1748BTB0004-93			n.d.
1748BTB0004-95			n.d.
1748BTB0004-33	Test item 2.1	DAT -2	n.d.
1748BTB0004-35			n.d.
1748BTB0004-37			n.d.
1748BTB0004-39			n.d.
1748BTB0004-97		DAT 0aa	1028
1748BTB0004-99			1122
1748BTB0004-101			1152
1748BTB0004-103			1281
1748BTB0004-41	Test item 2.2	DAT -2	n.d.
1748BTB0004-43			n.d.
1748BTB0004-45			n.d.
1748BTB0004-47			n.d.
1748BTB0004-105		DAT 0aa	990.0
1748BTB0004-107			1040
1748BTB0004-109			1055
1748BTB0004-111			1014

Specimen identification	Treatment group	Sampling day	Analysed concentration of a.s. [mg/kg]
1748BTB0004-49	Test item 2.3	DAT -2	n.d.
1748BTB0004-51			n.d.
1748BTB0004-53			n.d.
1748BTB0004-55			n.d.
1748BTB0004-113		DAT 0aa	988.6
1748BTB0004-115			1078
1748BTB0004-117			1091
1748BTB0004-119			1156
1748BTB0004-57	Test item 2.4	DAT -2	n.d.
1748BTB0004-59			n.d.
1748BTB0004-61			n.d.
1748BTB0004-63			n.d.
1748BTB0004-121		DAT 0aa	1184
1748BTB0004-123			1196
1748BTB0004-125			1024
1748BTB0004-127			935.0

n.d. = not detected or below 3 % of LOQ (LOQ = 100.6 mg/kg, regarding DF 1.258 mg/L)

F. Climatic conditions

The climatic conditions at the test and remote site during the entire course of the (DAT -4 to DAT 27) are given in the table below. No precipitation was observed on the day of application (DAT 0) neither in the morning before nor after the treatment. Precipitation occurred on DAT 4 (4 mm) and DAT 5 (3.5 mm). The temperature was warm during the whole exposure phase. Overall, the observed weather conditions during the exposure phase could be regarded as favourable for the actively foraging of bees and therefore for the exposure of bees to the test item.

Table A 60: Environmental conditions during a semi-field study with the test item 'Sulfur 80% WG'

Parameter	DAT -4 to DAT 27		
	minimum	maximum	average/total
Air temperature	9.7 °C	32.5 °C	19.3 °C
Precipitation	0.0 mm	55.2 mm	122.2 mm
Relative air humidity (daily mean)	32.0 %	96.0 %	66.4 %

The above stated conditions to a large extent correspond to historical long-term weather conditions (year 1977 to year 2008) in Cunnersdorf given as monthly mean (*i.e.* a precipitation of 64 mm and 58 mm in June and July, respectively, and an air temperature of 16.2 °C and 18.3 °C in June and July, respectively).

G. Validity of the study

The mean brood termination rate of initially labelled eggs in the control was < 40 % on BFD 22 (*i.e.* 25.1 %) and was therefore on a low and good level. The mean brood termination rate for initially labelled eggs in the reference item were between ≥ 50 and ≤ 90 % on BFD 22 (*i.e.* 62.9 %) and was distinctly increased compared to the control. The mean pupal mortality in the reference item treatment was distinctly increased compared to the control and predominantly occurred from DAT 9 to DAT 27 (until the end of post-exposure phase on a lower level). The total number of dead pupae in the replicates was in a range of 64 – 115, whereas no dead pupae were found in the control. The mean foraging activity immediately before application was ≥ 10 bees/m² (*i.e.* 11.4 to 13.2 bees/m²) in all treatment groups.

All these data confirm the sensitivity of the test system and therefore, exposure of bees to evaluate potential effects on the bee brood. The study is thus, considered to be valid without restrictions.

The concentrations of sulfur in the specimens were determined to range from 935.0 mg a.i./kg to 1281 mg a.i./kg. The detected concentrations in the control specimens were < 3 % of LOQ.

Conclusions

Assessment and conclusion by applicant:

In a semi-field (tunnel test) study, the test item 'Sulfur 80% WG' (Microthiol Special Disperss) was applied once at a rate of 12.5 kg product/ha (10.0 kg total a.s./ha) to full-flowering *Phacelia tanacetifolia* during active foraging of honeybees. The application of 'Sulfur 80% WG' had no significant difference on adult and pupal bee mortality, foraging activity, behaviour of bees, colony strength and brood development when compared to the control. The sensitivity of the test system was verified as significant effects on bee brood development were shown for the reference item Insegar 25 WG (250 g Fenoxycarb/kg).

Based on the results of this study, the test item 'Sulfur 80% WG' applied at a rate of 12.5 kg product/ha (equivalent to 10.0 kg total a.i./ha) to full-flowering *Phacelia tanacetifolia* during daily bee flight does not adversely affect honeybees including brood or the survival of the honeybee colony.

As all the derived data confirm the sensitivity and adequacy of the test system, the study is considered to be valid without restrictions.

A 2.3.1.6 KCP 10.3.1.6 Field tests with honeybees

BAS 768 00 F poses no unacceptable risk to bees. Further studies are not necessary

A 2.3.2 KCP 10.3.2 Effects on non-target arthropods other than bees

A 2.3.3 KCP 10.3.2.1 Standard laboratory testing for non-target arthropods

A 2.3.3.1 Study 1

Comments of zRMS:	The study was accepted. The validity criteria were met. The following endpoints for *Typhlodromus pyri* were derived: 7-day LR_{50} = 8.0 L formulation/ha; NOER = 4.0 L formulation/ha No ER_{50} was derived. The endpoints were used for risk assessment.

Reference:	CP 10.3.2.1/1
Report	Effects of BAS 768 00 F on the predatory mite Typhlodromus pyri SCHEUTEN in a laboratory test, Roehlig, U., 2021 report No 894524, 2148NTL0007 BASF DocID 2021/2014189 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, the effects of the formulated product BAS 768 00 F on predatory mites (*Typhlodromus pyri*) were investigated. Protonymphs were exposed on glass plates to an untreated control and to dried residues of BAS 768 00 F at application rates of 0.5, 1.0, 2.0, 4.0 and 8.0 L product/ha in groups of 20 mites with 5 replicates for the untreated control and the test item treatments. Assessment of mite mortality was conducted 3 and 7 days after application.

After 7 days of exposure, the mortality in the control was 1.0%. In test item treatment rates of 0.5, 1.0, 2.0, 4.0 and 8.0 L product/ha, corrected mortalities of 0%, 1.0%, 1.0%, 2.0% and 11.1% were observed after 7 days, respectively. In the test item treatment rate of 8.0 L product/ha, a statistically significant effect on mortality was observed in comparison to the control.

In a 7-day worst-case laboratory study on predatory mites (*Typhlodromus pyri*), the LR_{50} (7 d) for BAS 768 00 F was determined to be > 8.0 L product/ha.

Materials and methods

Materials

Test item: BAS 768 00 F, Batch no. FD-210120-1029; content of a.s.: mefentrifluconazole (BAS 750 F; Reg. no. 5834378): 26.3 g/L (nominal: 25.0 g/L), sulfur (BAS 175 F; Reg. no. 240586): 614.9 g/L (nominal: 600.0 g/L); density: 1.346 g/cm^3.

Study design and methods

Test species: Predatory mite (*Typhlodromus pyri*), protonymphs, less than 24 h old, source: "Katz Biotech AG", Baruth, Germany; originally obtained from "Staatliche Lehr- und Versuchsanstalt für Wein- und Obstbau", Weinsberg, Germany.

Test design: Worst-case test; exposure period: 7 days; exposure of the mites via air-dried residues on treated glass plates; 5 test item treatment rates and 1 control group; 5 replicates for the test item treatments and the control; 20 mites per replicate; the treatments were sprayed onto glass plates via calibrated laboratory sprayer and air dried afterwards; after the spray residue had dried, within 1 hour after the application, the test arena was set up and the mites were transferred over; assessment of mortality on day 3 and 7 after application.

Endpoints: LR_{50} related to mortality.

Reference item: DANADIM PROGRESS (dimethoate, 400 g/L nominal); application rate: 0.015 L/ha applied in 200 L water/ha.

Test rates: Untreated control (deionised water); test item treatment rates: 0.5, 1.0, 2.0, 4.0 and 8.0 L product/ha (corresponding to 312.5, 625, 1250, 2500, 5000 g total a.s./ha); all treatments were applied in 200 L water/ha.

Test conditions: Glass cover plates with a barrier of sticky material on moistened filter paper on a sponge placed in a plastic tray; temperature: 23 - 25°C, relative humidity: 65 - 73%; photoperiod: 16 h light : 8 h dark; light intensity: 1980 lux; feeding: regularly, with pollen of 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.

Statistics: Descriptive statistics; Multiple Sequentially-rejective Chi^2 2x2 Table Test after Bonferroni-Holm.

Results

Biological results

After 7 days of exposure, the mortality in the control was 1.0%. In test item treatment rates of 0.5, 1.0, 2.0, 4.0 and 8.0 L product/ha, corrected mortalities of 0%, 1.0%, 1.0%, 2.0% and 11.1% were observed after 7 days, respectively. In the test item treatment rate of 8.0 L product/ha, a statistically significant effect on mortality was observed in comparison to the control (Multiple Sequentially-rejective Chi^2 2x2 Table Test after Bonferroni-Holm; $\alpha = 0.05$). The results are summarized in Table A 61.

Table A 61: Effects of BAS 768 00 F on predatory mites (*Typhlodromus pyri*) after 7 days of exposure

Application rate [L product/ha] [1)]	**Mortality (7 d) [%]**	**Corrected mortality (7 d) [%]** [2)]
Control	1.0	--
0.5	1.0	0
1.0	2.0	1.0
2.0	2.0	1.0
4.0	3.0	2.0
8.0	12.0*	11.1
	Endpoint [L product/ha]	
LR_{50} (7 d)	> 8.0 (95% confidence limits: n.d.)	

Abbreviations: n.d.: not determined (could not be calculated due to mathematical reasons)

1) Application rate in 200 L water/ha.

2) Corrected mortality according to Abbott (1925).

* Statistically significant differences compared to the control (Multiple Sequentially-rejective Chi^2 2x2 Table Test after Bonferroni-Holm, $\alpha = 0.05$).

The reference item produced 77.8% corrected mortality of exposed mites after 7 days.

Table A 62: Validity criteria according to Bluemel *et al.* (2000)

Criteria	**Trigger value**	**Study value and page no.**	**Criteria met? Y/N**	**Guideline**
Control mortality on day 7	≤ 20%	1.0% (p. 23)	Y	Bluemel *et al.* (2000)
Corrected mortality in the reference item group on day 7	50-100%	77.8% (p. 23)	Y	Bluemel *et al.* (2000)

All validity criteria were met (see **Table A 62** above).

Conclusion

In a 7-day worst-case laboratory study on predatory mites (*Typhlodromus pyri*), the LR_{50} (7 d) for BAS 768 00 F was determined to be > 8.0 L product/ha.

A 2.3.3.2 Study 2

Comments of zRMS:	The study was accepted. The validity criteria were met. The following endpoints for *Aphidius rhopalosiphi* were derived: 48 h LR_{50} > 8.0 L formulation/ha; The endpoints were used for risk assessment.

Reference:	CP 10.3.2.1/2
Report	Effects of BAS 768 00 F on the parasitic wasp Aphidius rhopalosiphi (DESTEPHANI-PEREZ) in a laboratory test, Roehlig, U., 2021 report No 894526, 2148NAL0008 BASF DocID 2021/2014190 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, the effects of the formulated product BAS 768 00 F on *Aphidius rhopalosiphi* were investigated. Adult wasps were exposed on glass plates to an untreated control and to dried residues of BAS 768 00 F at application rates of 0.5, 1.0, 2.0, 4.0 and 8.0 L product/ha in groups of 10 wasps with 4 replicates for all treatments. Mortality was assessed after 2, 24 and 48 hours of exposure.

After 48 hours of exposure, 5.0% mortality was observed in the deionised water control. In test item treatment rates of 0.5, 1.0, 2.0, 4.0 and 8.0 L product/ha, corrected mortalities of 0%, 0%, -2.6%, 0% and 2.6% were observed after 48 h. No statistically significant effect on mortality was observed in comparison to the control.

In a 48-hour worst-case laboratory study on parasitic wasps (*Aphidius rhopalosiphi*), the LR_{50} (48 hfor BAS 768 00 F was determined to be > 8.0 L product/ha.

Materials and methods

Materials

Test item: BAS 768 00 F, Batch no. FD-210120-1029; content of a.s.: mefentrifluconazole (BAS 750 F; Reg. no. 5834378): 26.3 g/L (nominal: 25.0 g/L), sulfur (BAS 175 F, Reg. no.: 240586): 614.9 g/L (nominal: 600.0 g/L); density: 1.346 g/cm^3.

Study design and methods

Test species: Parasitic wasp (*Aphidius rhopalosiphi*), adults less than 48 h old; source: "Katz Biotech AG", Baruth, Germany, originally obtained from Rothamsted Experimental Station, UK.

Test design: Worst-case test; exposure period: 48 hours; exposure of the wasps via air dried residues on treated glass plates; 5 test item treatment groups and 1 deionised water control; 4 replicates for the control and the test item treatments; 10 wasps per replicate; the treatments were sprayed onto glass plates via calibrated laboratory sprayer and air dried afterwards; wasps were transferred to the test arenas as soon as possible after the spray residue has dried and the test unit was assembled; assessment of mortality 2, 24 and 48 hours after test initiation.

Endpoints: LR_{50} related to mortality.

Reference item: DANADIM PROGRESS (dimethoate, 400 g/L nominal); application rate: 0.3 mL/ha in 200 L water/ha.

Test rates: Untreated control (deionised water), test item treatment rates: 0.5, 1.0, 2.0, 4.0 and 8.0 L product/ha (corresponding to 312.5, 625, 1250, 2500, 5000 g total a.s./ha); all treatments were applied in 200 L water/ha.

Test conditions: Size of glass plates: 13 x 13 cm (external dimensions); temperature 19 - 22°C, relative humidity 67 - 71%; photoperiod: 16 h light : 8 h dark, light intensity: 2180 lux; food: 25% w/w aqueous fructose solution.

Analytics: No analytical verification of the test item is required according to the current test guideline.

Statistics: Descriptive statistics, Multiple Sequentially-rejective Chi^2 2x2 Table Test after Bonferroni-Holm ($\alpha = 0.05$).

Results

Biological results

After 48 hours of exposure, 5.0% mortality was observed in the deionised water control. In test item treatment rates of 0.5, 1.0, 2.0, 4.0 and 8.0 L product/ha, corrected mortalities of 0%, 0%, -2.6%, 0% and 2.6% were observed after 48 h. No statistically significant effect on mortality was observed in comparison to the control (Multiple Sequentially-rejective Chi^2 2x2 Table Test after Bonferroni-Holm; $\alpha = 0.05$).

The results are summarized **Table A 63**.

Table A 63: Effects of BAS 768 00 F on parasitic wasps (*Aphidius rhopalosiphi*) after 48 hours of exposure

Rate [L product/ha] 1)	**Mortality (48 h) [%] 2)**	**Corrected mortality (48 h) [%] 3)**
Control	5.0	--
0.5	5.0	0
1.0	5.0	0
2.0	2.5	-2.6
4.0	5.0	0
8.0	7.5	2.6
	Endpoint [L product/ha]	
LR_{50}	> 8.0 (95% confidence limits n.d.)	

Abbreviations: n.d.: not determined (could not be calculated due to mathematical reasons)

1) Application rate in 200 L water/ha.
2) Mortality after 48 h of exposure to BAS 768 00 F on glass plates.
3) Corrected mortality according to Abbott.
* Statistically significant differences compared to the control (Multiple Sequentially-rejective Chi^2 2x2 Table Test after Bonferroni-Holm, $\alpha = 0.05$).

The reference item caused a corrected mortality of 100% of exposed wasps after 48 hours.

Table A 64: Validity criteria according to Mead-Briggs *et al.* (2000)

Criteria	**Trigger value**	**Study value and page no.**	**Criteria met? Y/N**	**Guideline**
Control mortality after 48 h	≤ 13%	5.0% (p. 23)	Y	Mead-Briggs *et al.* (2000)
Corrected mortality in the reference item group	50-100%	100% (p. 23)	Y	Mead-Briggs *et al.* (2000)

All validity criteria were met (see **Table A 64** above).

Conclusion

In a 48-hour worst-case laboratory study on parasitic wasps (*Aphidius rhopalosiphi*), the LR_{50} (48 hfor BAS 768 00 F was determined to be > 8.0 L product/ha.

A 2.3.4 KCP 10.3.2.2 Extended laboratory testing, aged residue studies with non-target arthropods

A 2.3.4.1 Study 1

Comments of zRMS:	The study was accepted. The validity criteria were met. The following endpoints for *Aphidius rhopalosiphi* were derived: 48 h LR_{50} > 8.0 L formulation/ha; ER_{50} > 8.0 L formulation/ha The endpoints were used for risk assessment.

Reference:	CP 10.3.2.2/1
Report	Effects of BAS 768 00 F on the parasitic wasp Aphidius rhopalosiphi (DESTEPHANI-PEREZ) in an extended laboratory test, Röhlig,U, 2022 report No 894527, 2248NAE0005 BASF DocID 2022/2010703 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 a dose-response extended laboratory study, the effects of the formulated product BAS 768 00 F on *Aphidius rhopalosiphi* were investigated. Adult wasps were exposed on potted barley plants to an untreated control and to dried residues of BAS 768 00 F at application rates of 1.5, 3, 4, 6 and 8 L product/ha in groups of 5 female wasps with 6 replicates for the untreated control and the treatments. For the reproduction assessment 15 females from each variant were transferred individually to pots with untreated, aphid-infested barley plants for 24 h and then removed. Assessments of the repellence of wasps from the freshly treated plants were done during the first 3 h after their release. Assessment of mortality of wasps was conducted 2, 24 and 48 h after test initiation. The number of parasitized aphid mummies was recorded after an additional 11 days.

After 48 hours of exposure, 6.7% mortality was observed in the deionised water control. In test item treatment rates of 1.5, 3, 4, 6 and 8 L product/ha, corrected mortalities of -3.6%, 0%, -3.6%, 0% and -3.6%, respectively were observed after 48 h. No statistically significant effects on mortality were observed in the test item treatment rates in comparison to the control. The mean number of mummies per female was 17.9 in the control and 18.7, 17.9, 19.0, 17.1 and 18.2 in the test item treatment rates of 1.5, 3, 4, 6 and 8 L product/ha, respectively. Compared to the control, reproduction changed by -4.5%, 0%, -6.1%, 4.5% and -1.7% in the test item treatment rates of 1.5, 3, 4, 6 and 8 L product/ha, respectively. The mean number of mummies in any of the test item groups was not statistically significantly different compared to the control. No statistically significant effects were observed in the repellence of wasps from the treated barley plants in any of the test item groups compared to the control.

In a 14-day dose response extended laboratory study on parasitic wasps (*Aphidius rhopalosiphi*), the LR_{50} (48 hfor BAS 768 00 F was estimated to be > 8 L product/ha. The ER_{50} (14 d) based on reproduction was estimated to be > 8 L BAS 768 00 F/ha.

Materials and methods

Materials

Test item: BAS 768 00 F, Batch no. FD-210120-1029; content of a.s.: mefentrifluconazole (BAS 750 F; Reg. no. 5834378): 26.3 g/L (nominal: 25.0 g/L), sulfur (BAS 175 F, Reg. no.: 240586): 614.9 g/L (nominal: 600.0 g/L); density: 1.346 g/cm^3.

Study design and methods

Test species: Parasitic wasp *(Aphidius rhopalosiphi)*, adults less than 48 h old; source: "Katz Biotech AG", Baruth, Germany originally obtained from Rothamsted Experimental Station, UK.

Test design: Dose response test; exposure period: 48 hours; exposure of wasps via air dried residues on treated potted barley plants. 5 test item treatment groups and 1 deionised water control; with 6 replicates for the control and the test item treatments; 5 individuals (females) per replicate; the treatments were sprayed onto potted barley plants via calibrated laboratory sprayer and air dried afterwards; were transferred to the test arenas as soon as possible after the spray residue has dried, for the reproduction assessment 15 females from each treatment group were transferred individually to pots with untreated, aphid-infested barley plants for 24 h and then removed; host: cereal aphids (*Rhopalosiphum padi*); assessment of the repellence of wasps from the freshly treated plants during the first 3 h after their release; assessment of mortality 2, 24 and 48 h after test initiation; the number of parasitized aphid mummies was recorded 11 days after removal of the adults (i.e. 14 days after test initiation).

Endpoints: LR_{50} related to mortality, ER_{50} related to reproduction.

Reference item: Danadim Progress (dimethoate, 400 g/L nominal); application rate: 10 mL/ha applied in 400 L water/ha.

Test rates:	Untreated control (deionised water), test item treatment rates: 1.5, 3, 4, 6 and 8 L product/ha (corresponding to 937.5, 1875, 2500, 3750 and 5000 g total a.s./ha); all treatments were applied in 400 L water/ha.
Test conditions:	Exposure period: acrylic cylinder with 10 potted barley plants and covered at the top of the cylinder with gauze; temperature: 21 - 22°C; relative humidity: 64 - 72%; photoperiod: 16 h light : 8 h dark, light intensity: 1060 lux (2610 parasitisation); Food: 10% fructose solution (sprayed onto barley plants). Reproduction period: acrylic cylinder with 20 wheat seedlings, 8 days old, infested with > 100 adult and nymphal cereal aphids and covered at the top of the cylinder with gauze; temperature: 20 - 22°C; relative humidity: 65 - 73%, photoperiod: 16 h light : 8 h dark, light intensity: 6490 lux.
Analytics:	No analytical verification of the test item is required according to the current test guideline.
Statistics:	Descriptive statistics, Chi^2 2x2 Table Test with Bonferroni Correction for mortality assessment, Dunnett's-t-test for repellence assessment and Williams-t-test for reproductive capacity assessment.

Results

Biological results

After 48 hours of exposure, 6.7% mortality was observed in the deionised water control. In test item treatment rates of 1.5, 3, 4, 6 and 8 L product/ha, corrected mortalities of -3.6%, 0%, -3.6%, 0% and -3.6%, respectively were observed after 48 h. No statistically significant effects on mortality were observed in the test item treatment rates in comparison to the control (Chi^2 2x2 Table Test with Bonferroni Correction; $\alpha = 0.05$).
The mean number of mummies per female was 17.9 in the control and 18.7, 17.9, 19.0, 17.1 and 18.2 in the test item treatment rates of 1.5, 3, 4, 6 and 8 L product/ha, respectively. Compared to the control, reproduction changed by -4.5%, 0%, -6.1%, 4.5% and -1.7% in the test item treatment rates of 1.5, 3, 4, 6 and 8 L product/ha, respectively. The mean number of mummies in any of the test item groups was not statistically significantly different compared to the control (Williams-t-test; $\alpha = 0.05$). No statistically significant effects were observed in the repellence of wasps from the treated barley plants in any of the test item groups compared to the control. (Dunnett's-t-test, $\alpha = 0.05$).

The results are summarized in Table A 65.

Table A 65: Effects of BAS 768 00 F on parasitic wasps (*Aphidius rhopalosiphi*) in an extended laboratory study

Rate [L product/ha] [1]	Mortality (48 h) [%] [2]	Corrected mortality (48 h) [%] [3]	Reproduction (14 d) [mummies/female] [4]	Effect on Reproduction (14 d) [%] [5]
--	6.7	--	17.9	--
1.5	3.3	-3.6	18.7	-4.5
3	6.7	0	17.9	0
4	3.3	-3.6	19.0	-6.1
6	6.7	0	17.1	4.5
8	3.3	-3.6	18.2	-1.7
	Endpoint [L BAS 768 00 F/ha] [6]			
LR_{50}	> 8 (95% confidence limits: n.d.)			
ER_{50}	> 8 (95% confidence limits: n.d.)			

Abbreviation: n.d.: not determined.
1) Application rate in 400 L water/ha.
2) Mortality after 48 h of exposure to BAS 768 00 F on potted barley plants.
3) Corrected mortality according to Abbott's formula.
4) Reproduction: number of parasitized aphids/female.
5) Percentage effect on reproduction. A negative value indicates an increase, a positive value a decrease, relative to the control.
6) Values estimated in study report based on the raw data.

The reference item caused a corrected mortality of 100% of exposed wasps after 48 hours.

Table A 66: Validity criteria according to Mead-Briggs *et al.* (2009)

Criteria	Trigger value	Study value and page no.	Criteria met? Y/N	Guideline
Control mortality after 48 h	≤ 10%	6.7% (p. 27)	Y	Mead-Briggs *et al.* (2009)
Corrected mortality in the reference item group after 48 h	> 50%	100% (p. 27)	Y	Mead-Briggs *et al.* (2009)
Number of mummies in the control	≥ 5 mummies/female and no more than two zero values	17.9 (mean, two zero values) (p. 27 and 38)	Y	Mead-Briggs *et al.* (2009)

All validity criteria were met (see Table A **66** above).

Conclusion

In a 14-day dose response extended laboratory study on parasitic wasps (*Aphidius rhopalosiphi*), the LR_{50} (48 hfor BAS 768 00 F was estimated to be > 8 L product/ha. The ER_{50} (14 d) based on reproduction was estimated to be > 8 L BAS 768 00 F/ha.

A 2.3.5 KCP 10.3.2.3 Semi-field studies with non-target arthropods

BAS 768 00 F poses no unacceptable risk to non-target arthropods. Further studies are not necessary

A 2.3.6 KCP 10.3.2.4 Field studies with non-target arthropods

BAS 768 00 F poses no unacceptable risk to non-target arthropods. Further studies are not necessary

A 2.3.7 KCP 10.3.2.5 Other routes of exposure for non-target arthropods

BAS 768 00 F poses no unacceptable risk to non-target arthropods. Further studies are not necessary

A 2.4 KCP 10.4 Effects on non-target soil meso- and macrofauna

A 2.4.1 KCP 10.4.1 Earthworms

A 2.4.1.1 KCP 10.4.1.1 Earthworms - sub-lethal effects

A 2.4.1.1.1 Study 1

Comments of zRMS:	The study was evaluated and accepted during active substance renewal. The authors of the study were corrected.

Reference:	CP 10.4.1.1/1
Report	Effects of Sulphur Dust on reproduction of earthworm Eisenia fetida in an artificial soil study, ~~Dini, R.,~~ Tediosi, E., 2018 report No EU-C14DX0115,EU-CH-549/2016 BASF DocID 2018/1241025 Authority registration No
Guideline(s):	OECD 222 (2016)
Deviations:	No
GLP:	yes (certified by Ministero della Sanita, Roma, Italy),
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

1.	Test Material:	Sulphur Dust 98.5 % DP
	Lot/Batch #:	E-GZB
	Purity:	Sulfur: 98.5 % w/w, analytical 98.4 % w/w
	CAS No.:	7704-34-9
	formulated product:	expiry date July 01, 2018
	Stability of test compound:	Stable under normal use and storage conditions
2.	Vehicle and/or positive control:	Artificial soil without test item
	Reference item:	Carbendazim PESTANAL (purity 99.5 %)

3. Test organism:	*Eisenia fetida*
Age:	Adult worms with a completely developed clitellum
Body weight:	300 - 600 mg
Source:	Commercial supplier (Wurmwelten – Dassel, Germany), held in a mixture of soil and cow manure, periodically added water for a correct humidity and food content
Acclimation period:	1 day, in artificial soil with the same characteristics of that used during the test
Housing:	1 L capacity glass containers with a cross-sectional area of approximately 200 cm^2 so that the moist substrate depth of approximately 5-6 cm was achieved when 500 to 600 g dry mass of substrate was added; 750 g wet weight of test medium (artificial soil plus test substance) were placed into each glass container; containers were covered to prevent the test medium from drying and the worms from escaping
Test medium:	the test medium was an artificial soil, composed as follows (expressed as dry weight, dried to a constant weight at 105 °C): - 10 % sphagnum peat - 20 % kaolin clay - 70 % industrial sand (50 % w/w 0.15-0.45 mm; 50 % w/w 0.00-0.20 mm) The test item was thoroughly mixed into the soil.
Loading:	4 replicates per test item, 8 replicates for the negative control; 10 worms per replicate
4. Environmental conditions:	
Temperature:	19.8 to 20.8 °C
Photoperiod:	Daily photoperiod of 16 hours light and 8 hours dark (531 to 651 lux)
pH:	pH ($CaCl_2$, DIN 19684) was checked at the start and end of the study for each test concentration. pH range at experimental start: 5.96 to 6.40 pH range at experimental end: 6.65 to 7.01
Moisture content:	Maximum Water Holding Capacity of the Artificial Soil: 78.5 % of the dry weight Water content (DIN 19683) was checked at the start and termination of the experiment for each test concentration. At experimental start: 33.0 % (mean moisture content) At experimental end: 30.4 % (mean moisture content)

6. In life dates: Experimental Starting Date: April 02, 2017
Experimental Completion Date: January 19, 2018

Treatment: Eight different concentrations in a geometric series (factor 1.8): 16, 29, 53, 95, 171, 309, 556 and 1000 mg/kg dry weight

Test item weight [g]	Nominal concentration in artificial soil [mg/kg d.w.]
0.0433	16
0.0781	29
0.1435	53
0.2570	95
0.4618	171
0.8343	309
1.5012	556
2.7005	1000

Final volume of deionized water for each concentration 953.1 mL

7. Observations: Test duration was 8 weeks
During the test period the following end-points were checked: mortality, body weight change of adult worms and their reproductive output, the exposed adult earthworms were checked for mortality and sub-lethal effects (weight loss, behavioural and pathological symptoms) after 4 weeks of exposure. After that period the surviving worms were removed; the juveniles and cocoons were counted at the end of 8 weeks of exposure

8. Statistics: The NOEC value was determined from the Raw Data by Wilcoxon/Bonferroni adjusted t-Test after testing the normality of distribution of the data and the homogeneity of variance by Shapiro-Wilk W normality test (normal distribution) and Bartlett equality of variance test (equal variances), respectively. EC_{10} and EC_{50} values were determined by a Linear Interpolation analysis.
Both for mortality and reproduction results, nominal concentrations of test item were used as input data (mg/kg dw). The CETIS v.1.8.7.7 software was used to carry out all the statistical analyses

Findings

Mortality

No mortality was observed within the 14 days of experimental time in any of the treatment groups and in the negative control. According to these results the LC_{50} of 'Sulphur Dust' to *Eisenia fetida* after 28 days (4 weeks) in artificial soil was determined to be greater than 1000 mg test item/kg soil. The results are summarised in the following table:

Table A 67: Effect of 'Sulphur Dust' on earthworm mortality and biomass (14 d)

Nominal concentration of test item (mg/kg d.w.)	No. of exposed earthworms	No. of dead earthworms (28 d)	% of dead earthworms
0.0 (negative control)	80	1	1.25
16	40	1	2.50
29	40	1	2.50
53	40	1	2.50
95	40	0	0.00
171	40	0	0.00
309	40	1	2.50
556	40	2	5.00
1000	40	2	5.00

Body weight changes

The body weight changes of the earthworms in the treatment groups were not significantly different compared to the control up to and including the concentration of 1000 mg test item/kg soil (Dunnett test, $\alpha = 0.05$). No behavioural or pathological signs were observed after the period of exposure in the alive worms.

Reproduction of the worms

In the negative control a mean value of 37.0 juveniles was found, according to the validity criterion that provides a minimum number of young worms of 30 for each adult in the negative control. Moreover the % Coefficient of Variation of reproduction (standard deviation/mean) was found to be equal to 16.2 %.

LC_{50}, NOEC, EC_{10}, EC_{20} and EC_{50} values, expressed as nominal concentrations of test item, were as follows.

Table A 68: Toxicity endpoints for *E. fetida* exposed to 'Sulphur Dust'

Endpoint	mg/kg dry soil weight
Survival: LC_{50}	> 1000.0
Reproduction: NOEC	1000
Reproduction: EC_{10}	740
Reproduction: EC_{20}	> 1000
Reproduction: EC_{50}	> 1000

Conclusion

According to the results of this study the LC_{50} for survival and the Effect Concentration EC_{50} for reproduction of 'Sulphur Dust' to earthworms (*Eisenia fetida*) were determined to be greater than 1000 mg test item/kg soil. The EC_{10} for reproduction of 'Sulphur Dust' was determined to be 740 mg/kg soil dw. The No Observed Effect Concentration (NOEC) was determined to be 1000 mg test item/kg soil, *i.e.* the highest concentration tested.

A 2.4.1.1.2 Study 2

Comments of zRMS:	The study was accepted. The validity criteria were met. No deviations were noted. The following endpoints for mortality were derived: NOEC ≥ 2560 mg formulation/kg d.w.; LC_{50} > 2560 mg formulation/kg d.w. and for reproduction the following endpoints were derived: NOEC = 320 mg formulation/kg d.w.; EC_{10} = 404 mg formulation/kg d.w.

Reference:	CP 10.4.1.1/2
Report	Effects of BAS 768 00 F on the reproduction of the earthworm Eisenia andrei in artificial soil, Friedrich, S., 2021 report No 894510, 2148TEC0037 BASF DocID 2021/2014184 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, the effects of the formulated product BAS 768 00 F on adult earthworms (*Eisenia fetida*) were investigated. Earthworms were exposed for 56 days to an untreated control and to BAS 768 00 F at concentrations of 20, 40, 80, 160, 320, 640, 1280 and 2560 mg product/kg dry soil (equivalent to 9.3, 18.6, 37.1, 74.3, 148.6, 297.2, 594.4 and 1189 mg total a.s./kg dry soil) in groups of 10 adult worms with 4 replicates for the test item treatments and 8 replicates for the control. The test item was mixed into artificial soil with an organic content of 10% (as sphagnum peat). Assessments of worm mortality, behavioural effects and weight change were done after 28 days of exposure. After additional 28 days (56 days after application) reproduction (number of juveniles) was assessed.

After 28 days of exposure, no mortality was observed in the untreated control and in all the test item treatments except for the mortality of 2.5% at the test item concentration of 320 mg product/kg dry soil. No statistically significant effects on the survival of the earthworms compared to the control were observed at any test item concentration. The weight change of adult worms after 28 days ranged from 24.8% to 30.1%

in the test item treatment groups compared to 27.4% in the control. The weight change of the earthworms was not statistically significantly different to the control at any test item concentration. The mean number of juveniles at test end was 196.0 in the control and ranged from 22.0 to 208.0 in the test item treatments groups, (corresponding to 11.2% to 106.1% compared to the control). Statistically significant effects on the reproduction of the earthworms compared to the control were observed at the test item concentrations of 640, 1280 and 2560 mg product/kg dry soil. No pathological symptoms and no behavioural abnormalities were observed in any of the treatment groups. The feeding activity of adult worms was comparable to the control.

In a 56-day reproduction study with earthworms (*Eisenia fetida*), the NOEC for BAS 768 00 F based on reproduction was determined to be 320 mg product/kg dry soil (equivalent to 148.6 mg total a.s./kg dry soil). The EC_{10} based on reproduction was 404 mg product/kg dry soil (equivalent to 187.6 mg total a.s./kg dry soil).

Materials and methods

Materials

Test item:	BAS 768 00 F, Batch no. FD-210120-1029; content of a.s.: mefentrifluconazole (BAS 750 F; Reg. no. 5834378): 26.3 g/L (nominal: 25.0 g/L), sulfur (BAS 175 F, Reg. no.: 240586): 614.9 g/L (nominal: 600.0 g/L); density: 1.346 g/cm^3.

Study design and methods

Test species:	Earthworms (*Eisenia fetida*); adult worms with clitellum and weight of 309 - 469 mg/worm, approx. 4 months old; source: in-house culture, originally obtained from 'W. Neudorff GmbH KG', Emmerthal, Germany.
Test design:	56-day exposure in artificial soil, 8 test item concentrations and 1 control group (untreated control) with 4 replicates for the test item treatments and 8 replicates for the control; 10 adult worms per replicate; the test item was homogenously mixed into the artificial soil, which was then filled into vessels, before the earthworms were introduced on the top of the soil surface; assessments of worm mortality, behavioural effects and weight change were done after 28 days of exposure; after an additional 28 days (56 days after application) reproduction (number of juveniles) was assessed.
Endpoints:	LC_x/EC_x and NOEC related to mortality, weight change and reproduction rate.
Reference item:	Maypon Flow (Carbendazim, SC 500). The effects of the reference item were investigated in a separate study.
Test concentrations:	Untreated control; test item concentrations: 20, 40, 80, 160, 320, 640, 1280 and 2560 mg product/kg dry soil (equivalent to 9.3, 18.6, 37.1, 74.3, 148.6, 297.2, 594.4 and 1189 mg total a.s./kg dry soil).
Test conditions:	Plastic vessels (16.5x12x6 cm) covered with plastic lids with perforations; each test vessel contained 810 g (wet weight) moist artificial soil equivalent to 600 g of dry soil mass; artificial soil with 10% peat; pH 6.16 - 6.18 at test initiation,

pH 4.75 - 5.84 at test termination; water content: 55.7 - 55.8% of its max. water holding capacity (WHC) at test initiation, 54.9 - 55.7% of max. WHC at test termination, temperature: 18.0 - 21.1°C; photoperiod: 16 hours light : 8 hours dark, light intensity: 600 lux, feeding: weekly feeding with 5 g dried and finely ground horse manure plus water starting one day after application.

Analytics: No analytical verification of the test item is required according to the current test guideline.

Statistics: Descriptive statistics; Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm for mortality; Dunnett`s Multiple t-test for weight change; Williams Multiple Sequential t-test for reproduction data; Probit analysis for determination of EC_x values.

Results

Biological results

After 28 days of exposure, no mortality was observed in the untreated control and in all the test item treatments except for the mortality of 2.5% at the test item concentration of 320 mg product/kg dry soil. No statistically significant effects on the survival of the earthworms compared to the control were observed at any test item concentration (Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm, $\alpha = 0.05$, one-sided smaller). The weight change of adult worms after 28 days ranged from 24.8% to 30.1% in the test item treatment groups compared to 27.4% in the control. The weight change of the earthworms was not statistically significantly different to the control at any test item concentration (Dunnett`s Multiple t-test, $\alpha = 0.05$, one-sided smaller). The mean number of juveniles at test end was 196.0 in the control and ranged from 22.0 to 208.0 in the test item treatments groups, (corresponding to 11.2% to 106.1% compared to the control). Statistically significant effects on the reproduction of the earthworms compared to the control were observed at the test item concentrations of 640, 1280 and 2560 mg product/kg dry soil (Williams Multiple Sequential t-test, $\alpha = 0.05$, one-sided smaller). No pathological symptoms and no behavioural abnormalities were observed in any of the treatment groups. The feeding activity of adult worms was comparable to the control. The results are summarized in Table A 69.

Table A 69: Effects of BAS 768 00 F on earthworms (*Eisenia fetida*) after 56 days of exposure

Concentration [mg product/kg dry soil]	**Control**	**20**	**40**	**80**	**160**	**320**	**640**	**1280**	**2560**
Mortality (28 d) [%]	0.0	0.0	0.0	0.0	0.0	2.5	0.0	0.0	0.0
Weight change (28 d) [%]	27.4	26.9	28.5	24.8	30.1	25.5	26.3	29.1	26.1
Mean number of juveniles (56 d)	196.0	203.0	208.0	190.0	195.0	192.3	135.0	105.3	22.0
CV [%]	16.0	14.2	12.6	19.4	12.6	17.8	17.3	28.1	35.8
Reproduction (56 d) [% of control]	100	103.6	106.1	96.9	99.5	98.1	68.9*	53.7*	11.2*

	Endpoints	
	[mg product/kg dry soil]	**[mg total a.s./kg dry soil]**
LC_{50} (28 d)	> 2560 (95% CL: n.d.)	> 1189 (95% CL: n.d.)
EC_{10} (56 d)	404 (95% CL: 267 - 612)	187.6 (95% CL: 124.0 - 284.2) #

EC_{20} (56 d)	581 (95% CL: 427 - 790)	269.8 (95% CL: 198.3 - 366.8) #
EC_{50} (56 d)	1162 (95% CL: 955 - 1413)	539.6 (95% CL: 443.4 - 656.1) #
$NOEC_{mortality}$ (28 d)	≥ 2560	≥ 1189
$NOEC_{weight\ change}$ (28 d)	≥ 2560	≥ 1189
$NOEC_{reproduction}$ (56 d)	320	148.6

Abbreviations: CV: Coefficient of variation (recalculated from study data); CL: confidence limits; n.d.: not determined (could not be calculated due to mathematical reasons)

* Statistically significantly different compared to the control (Williams Multiple Sequential t-test, a = 0.05, one-sided smaller).

LC/EC_x values based on the active substance have been recalculated from the study data considering the nominal content of 625 g total a.s./L and the product density of 1.346 g/cm^3.

In a separate study the reference item Maypon Flow (Carbendazim, SC 500) had statistically significant effects on biomass development and reproduction of earthworms. The reproduction rate was clearly inhibited by 56.5% and 99.6% compared to the control at test concentrations of 5 and 10 mg product/kg dry soil.

Table A 70: Validity criteria according to OECD 222 (2016)

Criteria	Trigger value	Study value and page no.	Criteria met? Y/N	Guideline
Mean adult mortality in the control(s)	≤ 10%	control: 0% (p. 21)	Y	OECD 222
Mean number of juveniles per control replicate (with 10 adults per replicate)	≥ 30	control:157 - 244 (p. 21)	Y	OECD 222
Coefficient of variation of reproduction in the control(s)	≤ 30%	control: 16.0%	Y	OECD 222

All validity criteria were met (see Table A 70 above).

Conclusion

In a 56-day reproduction study with earthworms (*Eisenia fetida*), the NOEC for BAS 768 00 F based on reproduction was determined to be 320 mg product/kg dry soil (equivalent to 148.6 mg total a.s./kg dry soil). The EC_{10} based on reproduction was 404 mg product/kg dry soil (equivalent to 187.6 mg total a.s./kg dry soil).

A 2.4.1.2 KCP 10.4.1.2 Earthworms - field studies

BAS 768 00 F poses no unacceptable risk to earthworms. Further studies are not necessary.

A 2.4.2 KCP 10.4.2 Effects on non-target soil meso- and macrofauna (other than earthworms)

A 2.4.2.1 KCP 10.4.2.1 Species level testing

A 2.4.2.1.1 Study 1

Comments of zRMS:	The study was evaluated and accepted during active substance renewal.

Reference:	CP 10.4.2.1/1
Report	Sulphur Dust: Effects on Collembolan reproduction in an artificial soil study, Ponti, B., 2018 report No C14DX0115, CH - 550/2016 BASF DocID 2018/1241024 Authority registration No
Guideline(s):	OECD 232 (2016)
Deviations:	No
GLP:	yes (certified by Ministero della Sanita, Roma, Italy),
Acceptability:	Yes
Duplication (if vertebrate study)	No

I. Materials and methods

A. Materials

1.	Test substance:	Sulphur Dust 98.5 % DP
	Lot/Batch no.:	E-GZB
	Content/Purity:	Sulfur: 98.4 % w/w (analysed)
	Control:	Artificial soil without test item addition
	Toxic reference:	Boric Acid (purity ≥ 99.5 %)
2.	Test organisms	
	Species:	*Folsomia candida*
	Age:	juvenile, 12 days
	No. of organisms:	50 per group (10 springtails per replicate, 4 replicates per test item group; 8 replicates for the control)
	Feeding:	Granule yeast. Food was first provided at the beginning of the test, thereafter it was provided after 14 days of exposure
3.	Test units and exposure	
	Type and size:	50 mL capacity glass bottles were used. The test bottles were loosely closed with a screw cap to prevent the test medium from

	drying; filled with aliquots of 30 g fresh weight of the test medium (artificial soil plus test item)
Test system:	reproductive toxicity test using artificial soil with 5 % peat The test item was thoroughly mixed into the soil
Test duration:	28 days

4. Test conditions

Test substrate:	Artificial soil
Composition:	5 % sphagnum peat 20 % kaolin clay 75 % industrial sand
pH value:	Day 0: 6.50 in the negative control; 6.52 – 7.05 in treated soil Day 28: 6.96 in the negative control; 7.02 – 7.13 in treated soil
Temperature:	19.9 - 20.5°C
Photoperiod:	16 hours light/ 8 hours dark
Light intensity:	730 - 770 lux

B. Study design and method

1. In-life dates (experimental phase): April 27 to August 08, 2017

2. Test design:

The test was performed at eight different concentrations in a geometric series (factor 1.8), namely 16, 29, 53, 95, 171, 309, 556 and 1000 mg/kg dry weight. Moreover, a negative control without test item was tested. There were five replicate vessels counted per test treatment group and the control. Ten juvenile *F. candida* were exposed in each vessel for a period of 28 days, after which the numbers surviving to adulthood and the numbers of juveniles produced in each replicate test vessel were assessed.

3. Statistics

The mortality of adult organisms was assessed by counting the adult organisms after 4 weeks. The 28 d-LC50 was determined by Nonlinear Regression. The effect on reproduction was assessed at the same time by counting the juveniles after the extraction process. The NOEC and LOEC values were determined from the raw data by using the Bonferroni adjusted t-test ($p<0.05$). Nominal test item concentrations were used as input data (mg/kg dw). The CETIS v.1.8.7.7 software was used to carry out all the statistical analyses.

II. Results and discussion

A. Mortality

The observed mortality in the tested concentrations and in the negative control after 28 days (4 weeks) of exposure is reported in the following table.

Table A 71: Lethal effect of 'Sulphur Dust' on *Folsomia candida*

Nominal concentration (mg/kg d.w.)	No. of exposed collembolan	Total No. of dead organisms (28 d)	% of dead organisms
0 (neg. control)	80	13	16.3
16	40	8	20.0
29	40	8	20.0
53	40	8	20.0
95	40	9	22.5
171	40	6	15.0
309	40	9	22.5
556	40	18	45.0
1000	40	21	52.5

In the control 16.3 % mortality was observed after 28 days. This value complies with the validity criterion of the test stating that the test is not acceptable if the mortality in the control exceeds 20 % after 4 weeks of exposure.

The LC_{50} value for survival was assessed to be higher than the highest tested concentration (1000 mg/kg dw). The statistical assessment was carried out by Nonlinear Linear Regression, set by the software CETIS v.1.8.7.7.

B. Reproduction

The reproductive output was evaluated after 4 weeks by counting the juvenile collembolans produced by 12-day old springtails. In the following table the number of juvenile organisms is reported with the mean values and the inhibition % when compared with the negative control.

In the negative control a mean value of 628.4 juveniles were found. This is in accordance with the OECD guideline 222 (2016) validity criterion that requires a minimum number of young organisms of 100. The % Coefficient of Variation of reproduction (standard deviation/mean) was found to be 21.9 % for the negative control. This is in accordance with the OECD guideline criterion of < 30 %.

Table A 72: Reproduction of Collembola after 28 days of exposure to 'Sulphur Dust'

Test concentration [mg prod./kg $soil_{dw}$]	Mean number of juveniles per vessel	Inhibition [%]
0.0 (negative control)	628.4	---
16	611.0	2.8
29	535.8	14.7
53	568.5	9.5
95	572.8	8.9
171	552.5	12.1
309	469.0	25.4
556	404.0	35.7 *
1000	278.3	55.7 *

* Significantly different compared to control ($p < 0.05$)

For reproductive toxicity, the NOEC and LOEC values were determined from the raw data by a Bonferroni adjusted t-test after testing the normality of distribution of the data and the homogeneity of variance by Shapiro-Wilk W normality test (normal distribution) and Bartlett Equality of Variance test (equal variances), respectively.

The EC_{10}, EC_{20} and EC_{50}, NOEC (highest concentration tested without statistically significant effect on reproductive output) and LOEC (lowest concentration tested with statistically significant toxic effect on reproductive output), expressed in terms of nominal test item concentrations, for the two endpoints, are reported in the following table:

Table A 73: Endpoints for reproduction

Endpoint 28 days	EC_{10} (mg/kg)	EC_{20} (mg/kg)	EC_{50} (mg/kg)	NOEC (mg/kg)	LOEC (mg/kg)
Reproduction	144.8	287.1	925.2	309	556

C. Deficiencies

None: In the control, adult mortality was 16.3 % (required: ≤ 20), and the mean number of juveniles in the control was 628.4 (required: ≥ 100) with a coefficient of variation of 21.9 % (required: ≤ 30 %). Thus, the study is considered valid without restrictions.

III. Conclusions

In a 28-day reproduction toxicity test, *Folsomia candida* were exposed to 'Sulphur Dust' in artificial soil with a peat content of 5 %. Under the conditions of this study, the LC_{50} value for survival of the collembolans was established to be higher than the highest test rate, *i.e.* > 1000 mg 'Sulphur Dust'/kg dw. For reproduction the following endpoints were determined: EC_{10} value of 144.8 mg 'Sulphur Dust'/kg dw, EC_{20} of 287.1 mg test item/kg dw and the NOEC of 309.0 mg 'Sulphur Dust'/kg dw (equivalent to 304.4 mg a.s./kg soil dw).

A 2.4.2.1.2 Study 2

Comments of zRMS:	The study was evaluated and accepted during active substance renewal. No robust EC_{10} and EC_{20} could be derived from this study (based on DAR, 2022).

Reference:	CP 10.4.2.1/2
Report	Effects of Sulphur Dust on reproduction of the predatory mite Hypoaspis aculeifer in soil, Rossini, L., 2017 report No EU-BT239/16 BASF DocID 2017/1229983 Authority registration No
Guideline(s):	OECD 226 (2016)
Deviations:	No
GLP:	yes (certified by Ministero della Sanita, Roma, Italy),
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

1. Test Material:	Sulphur Dust DP
Lot/Batch #:	E-GZB
Purity:	Sulfur: 98.4 % w/w
CAS No.:	7704-34-9
Stability of test compound:	formulated product: expiry date July 07, 2018 In test vehicle: test item is considered stable under test conditions.
2. Vehicle and/or positive control:	Untreated (and moistened with deionised water)
Toxic control:	Dimethoate 99.5 %
3. Test organism:	Predatory mite *Hypoaspis aculeifer*
Age:	30 days old female adults, synchronized as described in Annex 4 of OECD 226
Source:	Internal breeding batch HA080517, Biotecnologie BT S.r.l.
Feeding:	*Tyrophagus putresciences*, after test start *ad libitum*
No. of organisms:	4 replicates per test item group, 8 replicates per control, containing 10 mites per replicate
4. Test units and exposure:	100 mL transparent glass container with screw lid (diameter of 3-5 cm)

5. Experimental test conditions:

Artificial soil:	5 % sphagnum-peat (pH 5.5 – 6.5, air dried and finely ground), 20 % kaolin clay, (kaolinite content > 30%), 75 % air dried industrial sand (fine sand with more than 50 % of the particles between 50 and 200 microns)
Temperature:	20.33 to 2.00 °C
Photoperiod:	16 h light + 8 h darkness, light: 475 – 750 Lux
pH:	pH ($CaCl_2$, DIN 19684) was checked at the start and end of the study for each test concentration. pH range at experimental start: 5.96 pH range at experimental end: 5.9 to 6.0
Moisture content:	About 15.65 mL of water/100 g of dry soil Maximum Water Holding Capacity of the Artificial Soil: 50 % of the dry weight; the water content of the soil substrate in the test containers was maintained throughout the test by re-weighing the test containers periodically. Losses were replenished as necessary with deionised water.

6. In life dates:	Experimental Starting Date: June 07, 2017 Experimental Completion Date: June 22, 2017
Treatment:	The test item was mixed with the substrate before introducing it into the test containers (23.13 g wet soil per vessel). The individual mites were carefully transferred into each test vessel (allocated randomly to the test vessel) and placed onto the surface of the soil. The nominal concentrations of the product were: 16.33, 29.40, 52.92, 95.26, 171.47, 308.64, 555.56 and 1000.00 mg test item/kg of dry soil. The control was left untreated.
7. Observations:	The number of the surviving mites was recorded at 14 days after their introduction. At the end of the test, 14 days after treatment, the number of juveniles was determined.
8. Statistics:	The software ToxRat Pro Version 3.2.1 was used to perform the statistical analysis.

Findings

Mortality and reproduction

There were no statistically significant effects on mortality and reproduction of *Hypoaspis aculeifer* up to and including the test concentration of 1000 mg prod./kg soil dry weight.

Table A 74: Effects of 'Sulphur Dust' on *Hypoaspis aculeifer* mortality and reproduction (day 14)

Treatment [mg test item/kg dry soil]	Adult mortality [%]	Mean number of juveniles/vessel	% reduction in reproduction
Control	1.25	239.9	---
16.33	10.00	283.3	0.68
29.40	2.50	269.8	-12.45
52.92	7.50	252.5	-5.26
95.26	2.50	262.5	-9.43
171.47	7.50	242.8	-1.20
308.64	7.50	279.0	-16.31
555.56	2.50	248.0	-3.39
1000.00	7.50	244.8	-2.03

Deficiencies

None. According to the OECD Guideline 226, the test was considered valid because the following criteria were satisfied in the water treated control:
- mean adult female mortality didn't exceed 20% at the end of the test (*i.e.* 1.3 %);
- the mean number of juveniles per vessel was at least 50 at the end of the test (*i.e.* 239.9);
- the coefficient of variation calculated for the number of juvenile mites per replicate was lower than 30% at the end of the test (*i.e.* 11.7 %).

Conclusion

The results of the test showed no adverse effects of the test item 'Sulphur Dust' on reproduction of the predatory mite *Hypoaspis aculeifer* when mixed into an artificial soil at the tested concentrations. For reproduction, no adverse effects were found at all concentrations.

Due to a lack of any dose-response relationship up the highest test level, the EC_{10}, EC_{20} and EC_{50} values were established to be greater 1000 mg 'Sulphur Dust'/kg of dry soil (equivalent to 984 mg of a.i./kg of dry soil). The NOEC based on reproduction as well as the NOEC based on survival were determined to be 1000 mg of test item/kg dry soil (equivalent to 984 mg of a.i./kg of dry soil).

A 2.4.2.1.3 Study 3

Comments of zRMS:	The study was accepted. The validity criteria were met. No deviations werenoted. The following endpoints for mortality were derived: NOEC = 135 mg formulation/kg d.w.; LC_{50} > 836.3 mg formulation/kg d.w. and for reproduction the following endpoints were derived: NOEC = 135 mg formulation/kg d.w.; EC_{10} = 154.2 mg formulation/kg d.w.

Reference:	CP 10.4.2.1/3
Report	Effects of BAS 768 00 F on the reproduction of the collembolan Folsomia candida, Friedrich, S., ~~2020~~ 2022 report No 894511, 2148TCC0024 BASF DocID 2021/2014185 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

In a chronic toxicity study, the effects of the formulated product BAS 768 00 F on the collembolan *Folsomia candida* were investigated. Juvenile collembolans were exposed for 28 days to an untreated control and to BAS 768 00 F at concentrations of 75.0, 135.0, 243.0, 437.4, 787.4, 1417, 2551 and 4592 mg product/kg dry soil (equivalent to 34.8, 62.7, 112.8, 203.1, 365.6, 658.1, 1185 and 2132 mg total a.s./kg dry soil) in groups of 10 animals with 4 replicates for the test item treatments and 8 replicates for the control. The test item was incorporated into artificial soil with an organic content of 5% (as sphagnum peat). Assessments of parent mortality, behavioural effects and reproduction (number of juveniles) were performed after 28 days.

After 28 days of exposure, 2.5% mortality was observed in the untreated control. Mortality rates in the test item treatments ranged from 0% to 97.5%. Statistically significant effects on the survival of the collembolans compared to the control were observed at the test item concentrations of 243 mg product/kg dry soil and above. The mean number of juveniles counted 28 days after introduction of the parental collembolans into the test vessels was 1112 in the control and 1097, 1089, 816, 724, 566, 252, 144 and 59 at the test item concentrations of 75.0, 135.0, 243.0, 437.4, 787.4, 1417, 2551 and 4592 mg product/kg dry soil, respectively. Statistically significant effects on the reproduction compared to the control were recorded

at the test item concentrations of 243 mg product/kg dry soil and above. No effects on the behaviour of the collembolans were observed in any treatment during the test.

In a 28-day reproduction study with collembolans (*Folsomia candida*), the NOEC for BAS 768 00 F based on reproduction was determined to be 135 mg product/kg dry soil (equivalent to 62.7 mg total a.s./kg dry soil). The EC_{10} based on reproduction was 154.2 mg product/kg dry soil (equivalent to 71.6 mg total a.s./kg dry soil).

Materials and methods

Materials

Test item: BAS 768 00 F, Batch no. FD-210120-1029; content of a.s.: mefentrifluconazole (BAS 750 F; Reg. no. 5834378): 26.3 g/L (nominal: 25.0 g/L), sulfur (BAS 175 F, Reg. no.: 240586): 614.9 g/L (nominal: 600.0 g/L); density: 1.346 g/cm^3.

Study design and methods

Test species: Collembolan (*Folsomia candida*, springtails), juveniles, 9 - 12 days old at test initiation; source: in-house culture, originally obtained from "Biologische Bundesanstalt (BBA)", Berlin-Dahlem, Germany.

Test design: 28-day exposure in artificial soil; 8 test item concentrations and 1 control group (untreated control) with 4 replicates for the test item treatments and 8 replicates for the control; 10 juvenile collembolans per replicate; the test item was homogenously mixed into the artificial soil, which was then filled into the test vessels, before the collembolans were introduced on top of the soil; assessment of parent mortality, behavioural effects and reproduction (number of juveniles) was done after 28 days.

Endpoints: LC_x/EC_x and NOEC related to mortality and reproduction rate after 28 days.

Reference item: Boric acid; tested at a concentration of 44, 67, 100, 150 and 225 mg a.s./kg dry soil. The effects were tested in a separate study.

Test concentrations: Untreated control; test item concentrations: 75.0, 135.0, 243.0, 437.4, 787.4, 1417, 2551 and 4592 mg product/kg dry soil (equivalent to 34.8, 62.7, 112.8, 203.1, 365.6, 658.1, 1185 and 2132 mg total a.s./kg dry soil).

Test conditions: 150 mL glass container covered with a lid; each test vessel contained approx. 30 g dry artificial soil according to OECD 232, peat content: 5%; pH 6.08 - 6.15 at test initiation and pH 4.20 - 5.86 at test termination; water content at study initiation 57.6 - 57.9% of the max. water holding capacity (WHC) and 56.0 - 56.7% WHC at test termination; temperature: 19.0 - 21.5°C; photoperiod: 16 h light : 8 h dark, light intensity: 580 lux; feeding: 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.

Statistics: Descriptive statistics; Step-down Cochran-Armitage test for mortality data, Williams Multiple Sequential t-test for reproduction data. The LC/EC_x values were calculated using Probit analysis.

Results

Biological results

After 28 days of exposure, 2.5% mortality was observed in the untreated control. Mortality rates in the test item treatments ranged from 0% to 97.5%. Statistically significant effects on the survival of the collembolans compared to the control were observed at the test item concentrations of 243 mg product/kg dry soil and above (Step-down Cochran-Armitage test, one-sided greater, $\alpha = 0.05$). The mean number of juveniles counted 28 days after introduction of the parental collembolans into the test vessels was 1112 in the control and 1097, 1089, 816, 724, 566, 252, 144 and 59 at the test item concentrations of 75.0, 135.0, 243.0, 437.4, 787.4, 1417, 2551 and 4592 mg product/kg dry soil, respectively. Statistically significant effects on the reproduction compared to the control were recorded at the test item concentrations of 243 mg product/kg dry soil and above (Williams Multiple Sequential t-test, one-sided smaller, $\alpha = 0.05$). No effects on the behaviour of the collembolans were observed in any treatment during the test. The results are summarized in Table A 75.

Table A 75: Effects of BAS 768 00 F on collembolans (*Folsomia candida*) after 28 days of exposure

Concentration [mg product/kg dry soil]	**Untreated control**	**75.0**	**135.0**	**243.0**	**437.4**	**787.4**	**1417**	**2551**	**4592**
Mortality (28 d) [%]	2.5	0.0	0.0	12.5*	25.0*	52.5*	70.0*	90.0*	97.5*
Mean number of juveniles (28 d)	1112	1097	1089	816*	724*	566*	252*	144*	59*
CV [%]	10.8	7.5	10.4	12.7	15.7	25.5	40.2	22.6	35.4
Reproduction (28 d) [% of control] 2)	100.0	98.6	97.9	73.3	65.1	50.9	22.6	12.9	5.3
	Endpoints								
	[mg product/kg dry soil]					**[mg total a.s./kg dry soil]**			
LC_{50} (28 d) 1)	836.3 (95% CL: 707.9 - 988.1)					388.3 (95% CL: 328.7 - 458.8) #			
EC_{10} (28 d) 1)	154.2 (95% CL: 103.3 - 230.0)					71.6 (95% CL: 48.0 - 106.8) #			
EC_{20} (28 d) 1)	256.9 (95% CL: 190.5 - 346.6)					119.3 (95% CL: 88.5 - 160.9) #			
EC_{50} (28 d) 1)	682.5 (95% CL: 563.8 - 826.1)					316.9 (95% CL: 261.8 - 383.6) #			
$NOEC_{mortality}$ (28 d)	135.0					62.7			
$NOEC_{reproduction}$ (56 d)	135.0					62.7			

Abbreviations: CV: Coefficient of variation; CL: confidence limit

* Statistically significantly different compared to the untreated control (Step-down Cochran-Armitage test for mortality, $\alpha = 0.05$, one-sided greater; Williams-t-test for reproduction, $\alpha = 0.05$, one-sided smaller).

\# LC/EC_x values based on the active substance have been recalculated from the study data considering the nominal content of 625 g total a.s./L and the product density of 1.346 g/cm^3.

1) LC/EC_x values were calculated based on Probit analysis

In a separate study, the EC_{50} (reproduction) of the reference item boric acid was calculated to be 111 mg/kg dry soil.

Table A 76: Validity criteria according to OECD 232 (2016)

Criteria	Trigger value	Study value and page no.	Criteria met? Y/N	Guideline
Mean adult mortality in the control(s)	≤ 20%	untreated control: 2.5% (p. 19)	Y	OECD 232
Mean number of juveniles per vessel in the control	≥ 100	untreated control: 1112 (p. 19)	Y	OECD 232
Coefficient of variation of reproduction in the control	≤ 30%	untreated control: 10.8% (p. 19)	Y	OECD 232

All validity criteria were met (see Table A 76 above).

Conclusion

In a 28-day reproduction study with collembolans (*Folsomia candida*), the NOEC for BAS 768 00 F based on reproduction was determined to be 135 mg product/kg dry soil (equivalent to 62.7 mg total a.s./kg dry soil). The EC_{10} based on reproduction was 154.2 mg product/kg dry soil (equivalent to 71.6 mg total a.s./kg dry soil).

A 2.4.2.1.4 Study 4

Comments of zRMS:	The study was accepted. The validity criteria were met. No deviations were noted. The following endpoints for mortality were derived: NOEC ≥ 4592 mg formulation/kg d.w.; LC_{50} > 4592 mg formulation/kg d.w. and for reproduction the following endpoints were derived: NOEC = 1417 mg formulation/kg d.w.; EC_{10} = 3644 mg formulation/kg d.w.

Reference:	CP 10.4.2.1/4
Report	Effects of BAS 768 00 F on the reproduction of the predatory mite Hypoaspis aculeifer, Mann, D., 2021 report No 894512, 2148THC0028 BASF DocID 2021/2014186 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

In a chronic toxicity study, the effects of the formulated product BAS 768 00 F on the predatory soil mite *Hypoaspis aculeifer* were investigated. Adult female soil mites were exposed for 14 days to an untreated control and to BAS 768 00 F at concentrations of 75.0, 135.0, 243.0, 437.4, 787.4, 1417, 2551 and 4592 mg product/kg dry soil (equivalent to 34.8, 62.7, 112.8, 203.1, 365.6, 658.1, 1184.6 and 2132.2 mg total a.s./kg dry soil) in groups of 10 animals per replicate with 8 replicates for the control and 4 replicates for the test item treatments. The test item was incorporated into artificial soil with an organic content of 5% (as sphagnum peat). Assessments of parent mortality, behavioural effects and reproduction (number of juveniles) was performed after 14 days.

After 14 days of exposure, 2.5% mortality was observed in the control. Mortality rates in the test item concentrations ranged from 0.0% to 10.0%. No statistically significant effects on the survival of the mites compared to the control were observed at up to and including the highest test item concentration of 4592 mg product/kg dry soil. The mean number of juveniles counted 14 days after introduction of the parental soil mites into the test vessels was 314.6 in the control and 319.0, 325.5, 320.8, 305.8, 295.0, 314.3, 278.8 and

281.0 at the test item concentrations of 75.0, 135.0, 243.0, 437.4, 787.4, 1417, 2551 and 4592 mg product/kg dry soil, respectively. Statistically significant differences on the number of juveniles compared to the control group were observed at the test item concentrations of 2551 and 4592 mg product/kg dry soil. No differences in the behaviour and the morphology of the mites between the control and the test item treatments were observed.

In a 14-day reproduction study with soil mites (*Hypoaspis aculeifer*), the NOEC for BAS 768 00 F based on reproduction was determined to be 1417 mg product/kg dry soil (equivalent to 658.1 mg total a.s./kg dry soil). The EC_{10} based on reproduction was determined to be 3643.9 mg product/kg dry soil (equivalent to 1692.0 mg total a.s./kg dry soil).

Materials and methods

Materials

Test item: BAS 768 00 F, Batch no. FD-210120-1029; content of a.s.: mefentrifluconazole (BAS 750 F; Reg. no. 5834378): 26.3 g/L (nominal: 25.0 g/L), sulfur (BAS 175 F, Reg. no.: 240586): 614.9 g/L (nominal: 600.0 g/L); density: 1.346 g/cm^3.

Study design and methods

Test species: Predatory soil mite (*Hypoaspis aculeifer*), adult females from a synchronised culture with an age difference of 2 days; source: “Katz Biotech AG”, Baruth, Germany.

Test design: 14-day exposure in artificial soil; 8 test item concentrations and 1 control group (untreated control) with 8 replicates for the control and 4 replicates for the test item treatments; 10 adult female soil mites per replicate. The test item was homogenously mixed into the artificial soil, which was then filled into the test vessels, before the soil mites were introduced on top of the soil surface. Assessment of parent mortality, behavioural effects and reproduction (number of juveniles) was done after 14 days.

Endpoints: LC_x/EC_x and NOEC related to mortality and reproduction rate after 14 days.

Reference item: Dimethoate EC 400 (dimethoate, 400 g/L nominal). The effects of the reference item were investigated in a separate study.

Test concentrations: Untreated control; test item concentrations: 75.0, 135.0, 243.0, 437.4, 787.4, 1417, 2551 and 4592 mg product/kg dry soil (equivalent to 34.8, 62.7, 112.8, 203.1, 365.6, 658.1, 1184.6 and 2132.2 mg total a.s./kg dry soil).

Test conditions: 160 mL WECK-bottle with glass lid (inside dimensions: 4.7 cm in diameter, 8 cm high); each test vessel contained approx. 20 g soil (dry weight; height of soil approx. 1.7 cm); artificial soil according to OECD 226, peat content: 5%; pH 6.0 - 6.2 at test initiation, pH 5.5 - 5.7 at test termination; water content at study initiation 49.17 - 50.57% of the max. water holding capacity (WHC) and 46.67 - 48.78% of the max. WHC at test termination; temperature: 20.6 - 22.4°C;

photoperiod: 16 h light : 8 h dark, light intensity: 494 lux; feeding: every 2 - 3 days before and during the test with food mites (*Tyrophagus putrescentiae*).

Analytics: No analytical verification of the test item is required according to the current test guideline.

Statistics: Descriptive statistics; Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm for mortality data, Williams Multiple Sequential t-test Procedurefor reproduction data. The EC_x values were calculated using Logit analysis using linear maximum likelihood regression.

Results

Biological results

After 14 days of exposure, 2.5% mortality was observed in the control. Mortality rates in the test item concentrations ranged from 0.0% to 10.0%. No statistically significant effects on the survival of the mites compared to the control were observed at up to and including the highest test item concentration of 4592 mg product/kg dry soil (Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm, $\alpha = 0.05$, one-sided greater). The mean number of juveniles counted 14 days after introduction of the parental soil mites into the test vessels was 314.6 in the control and 319.0, 325.5, 320.8, 305.8, 295.0, 314.3, 278.8 and 281.0 at the test item concentrations of 75.0, 135.0, 243.0, 437.4, 787.4, 1417, 2551 and 4592 mg product/kg dry soil, respectively. Statistically significant differences on the number of juveniles compared to the control group were observed at the test item concentration of 2551 and 4592 mg product/kg dry soil (Williams Multiple Sequential t-test Procedure, $\alpha = 0.05$, one-sided smaller). No differences in the behaviour and the morphology of the mites between the control and the test item treatments were observed. The results are summarised in Table A 77.

Table A 77: Effects of BAS 768 00 F on predatory soil mites (*Hypoaspis aculeifer*) after 14 days of exposure

Concentration [mg product/kg dry soil]	**Untreated control**	**75.0**	**135.0**	**243.0**	**437.4**	**787.4**	**1417**	**2551**	**4592**
Mortality (14 d) [%]	2.5	5.0	0.0	2.5	5.0	2.5	5.0	0.0	10.0
Mean number of juveniles (14 d)	314.6	319.0	325.5	320.8	305.8	295.0	314.3	278.8*	281.0*
CV [%]	5.8	5.7	5.9	9.9	11.2	8.1	8.8	4.7	8.2
Reproduction (14 d) [% of control] [2)]	100	101	103	102	97	94	100	89	89

	Endpoints	
	[mg product/kg dry soil]	**[mg total a.s./kg dry soil]**
LC_{50} (14 d) [1)]	> 4592 (95% CL: n.d.)	> 2132.2 (95% CL: n.d.)
EC_{10} (14 d) [2)]	3643.9 (95% CL: 1839.4 - 7218.7)	1692.0 (95% CL: 854.1 - 3351.9) [#]
$EC_{20/50}$ (14 d) [1)]	> 4592 (95% CL: n.d.)	> 2132.2 (95% CL: n.d.)
$NOEC_{mortality}$ (14 d) [1)]	≥ 4592	≥ 2132.2
$NOEC_{reproduction}$ (14 d)	1417	658.1

Abbreviations: CV: Coefficient of variation; CL: confidence limit; n.d.: not determined (could not be calculated due to mathematical reasons)

* Statistically significantly different compared to the untreated control (Williams Multiple Sequential t-test Procedure for reproduction; $\alpha = 0.05$, one-sided smaller).

\# EC_{10} values based on the active substance have been recalculated from the study data considering the nominal content of 625 g total a.s./L and the product density of 1.346 g/cm^3.

1) Due to negligible effects and lacking dose response the values were estimated to be above the highest test concentration.

2) Values were calculated based on Logit analysis using linear maximum likelihood regression.

In a separate study, the EC_{50} (reproduction) of the reference item dimethoate was calculated to be 4.71 mg a.s./kg dry soil.

Table A 78: Validity criteria according to OECD 226 (2016)

Criteria	Trigger value	Study value and page no.	Criteria met? Y/N	Guideline
Mean adult mortality in the control(s)	≤ 20%	untreated control: 2.5% (p. 23)	Y	OECD 226
Mean number of juveniles per control replicate	≥ 50	untreated control: 314.6 (p. 23)	Y	OECD 226
Coefficient of variation of reproduction in the control	≤ 30%	untreated control: 5.8% (p. 23)	Y	OECD 226

All validity criteria were met (see Table A 78 above).

Conclusion

In a 14-day reproduction study with soil mites (*Hypoaspis aculeifer*), the NOEC for BAS 768 00 F based on reproduction was determined to be 1417 mg product/kg dry soil (equivalent to 658.1 mg total a.s./kg dry soil). The EC_{10} based on reproduction was determined to be 3643.9 mg product/kg dry soil (equivalent to 1692.0 mg total a.s./kg dry soil).

A 2.4.2.2 KCP 10.4.2.2 Higher tier testing

BAS 768 00 F poses no unacceptable risk to non-target soil meso- and macro-organisms other than earthworms. Further studies are not necessary.

A 2.5 KCP 10.5 Effects on soil nitrogen transformation

A 2.5.1 Study 1

Comments of zRMS:	The submitted study was accepted. The validity criteria were met. No adverse effects on soil nitrogen transformation (measured as NO_3-N-production) at the end of the 28-day incubation period were observed for 134.6 mg formulation/kg d.w.

Reference:	CP 10.5/1
Report	Effects of BAS 768 00 F on the activity of soil microflora - (Nitrogen transformation test), Schulz, L., 2021 report No 894509, 2148SMN0042 BASF DocID 2021/2014181 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 effects of the formulated product BAS 768 00 F on nitrogen transformation were investigated in a lucerne-enriched loamy sand soil. Untreated soil (control) and soil samples treated with BAS 768 00 F at nominal concentrations of 26.92 and 134.60 mg product/kg dry soil (corresponding to 12.50 and 62.50 mg total a.s./kg dry soil) were incubated with 3 replicates per treatment at 19.4 - 20.5°C in the dark for 42 days. Samples of each treatment were removed for determination of mineral nitrogen transformation (measured as NO_3-N production) 0, 7, 14 and 28 days after application.

At the end of the 28-day incubation period, only slight deviations from the untreated control of +8.2% and +15.8% were measured at the test item concentrations of 26.92 and 134.60 mg product/kg dry soil, respectively. During the study, the coefficients of variation in the untreated control was max. 2.9%.

In a nitrogen transformation study with BAS 768 00 F, no unacceptable long-term effects (deviation from untreated control < 25%) on soil nitrogen transformation (measured as NO_3-N production) were observed after exposure at up to a concentration of 134.60 mg product/kg dry soil (corresponding to 62.50 mg total a.s./kg dry soil) over 28 days in a loamy sand soil.

Materials and methods

Materials

Test item:	BAS 768 00 F, Batch no. FD-210120-1029; content of a.s.: mefentrifluconazole (BAS 750 F; Reg. no. 5834378): 26.3 g/L (nominal: 25.0 g/L), sulfur (BAS 175 F, Reg. no.: 240586): 614.9 g/L (nominal: 600.0 g/L); density: 1.346 g/cm^3.

Study design and methods

Test soil:	Biologically active agricultural soil: loamy sand (DIN) / loam (USDA); pH 6.2; 1.49% C_{org}; water holding capacity (WHC): 38.73 g/100 g dry soil, microbial mass: 1.69% of total C_{org}; enriched with lucerne meal (0.5% of the soil dry weight).
Test design:	42-day exposure in loamy sand soil; 2 test item concentrations and 1 control group (untreated soil), three replicates per treatment; samples of each treatment were removed for determination of mineral nitrogen transformation (measured as NO_3-N production) after 0, 7, 14 and 28 days of incubation using an Autoanalyzer.
Endpoints:	Percentage deviation of nitrate formation rate (< 25%) in treated samples relative to the untreated control after 28 days.
Reference item:	Dinoterb (purity: 99.28% (g/g) analysed). The reference item was tested in a separate study at rates of 6.80, 13.60 and 27.20 mg a.s./kg dry soil.
Test concentrations:	Control (untreated sand), test item concentrations: 26.92 and 134.60 mg product/kg dry soil (corresponding to 12.50 and 62.50 mg total a.s./kg dry soil).
Test conditions:	500 mL wide-mouth glass flasks with screw caps; each bottle was filled with approx. 200 g soil (dry weight); water content in test soil: 41.18 - 42.54% of max. WHC; measured water content: 15.95 - 16.47 g/100 g soil; pH 5.4 - 5.8. Soil samples were incubated at 19.4 - 20.5°C while stored in glass vessels in the dark.
Analytics:	No analytical verification of the test item is required according to the current test guideline.
Statistics:	Descriptive statistics.

Results

Biological results

At the end of the 28-day incubation period, only slight deviations from the untreated control of +8.2% and +15.8% were measured at the test item concentrations of 26.92 and 134.60 mg product/kg dry soil, respectively. During the study, the coefficients of variation in the untreated control was max. 2.9%. The results are summarized in Table A 79.

Table A 79: Effects of BAS 768 00 F on soil micro-organisms (cumulative nitrate formation rate) on intervals 0-7, 0-14 and 0-28

Time interval [days]	Untreated control	26.92 mg/kg dry soil		134.60 mg/kg dry soil	
	NO_3-N [mg/kg dry soil/day]	NO_3-N [mg/kg dry soil/day]	Deviation from the untreated control [%] +	NO_3-N [mg/kg dry soil/day]	Deviation from the untreated control [%] +
0 - 7	2.71	2.51	-7.4	1.06	-60.9
0 - 14	2.92	2.91	-0.4	1.74	-40.4
0 - 28	1.98	2.14	+8.2	2.29	+15.8

+ Negative values indicate inhibition and positive values indicate stimulation of nitrogen transformation compared to the untreated control.

In a separate study the reference item dinoterb produced an excepted stimulation of nitrogen transformation of +26.9 %, +43.2 % and +27.2% at 6.80, 13.60 and 27.20 mg/kg dry soil after 28 days of incubation, respectively.

Table A 80: Validity criteria according to OECD 216 (2000)

Criteria	Trigger value	Study value and page no.	Criteria met? Y/N	Guideline
Coefficient of variation for NO_3-N in the control(s) during whole study duration	≤ 15%	untreated control: max. 2.9% (p. 19)	Y	OECD 216

All validity criteria were met (see Table A 80 above).

Conclusion

In a nitrogen transformation study with BAS 768 00 F, no unacceptable long-term effects (deviation from control < 25%) on soil nitrogen transformation (measured as NO_3-N production) were observed after exposure at up to a concentration of 134.60 mg product/kg dry soil (corresponding to 62.5 mg total a.s./kg dry soil) over 28 days in a loamy sand soil.

A 2.6 KCP 10.6 Effects on terrestrial non-target higher plants

A 2.6.1 KCP 10.6.1 Summary of screening data

The data point is covered by A 2.6.2. (KCP 10.6.2).

A 2.6.2 KCP 10.6.2 Testing on non-target plants

Comments of zRMS:	The submitted study was accepted The validity criteria were met. No deviation was noted. No plant mortality was observed for all tested plant species following the application of 4.0 L BAS 768 00 F/ha at BBCH 12-14. No visual phytotoxicity was observed for all plant species at 21 DAT after application of 4.0 L BAS 768 00 F/ha at BBCH 12-14. Plant length was not significantly reduced for all tested plant species after application of 4.0 L BAS 768 00 F/ha at BBCH stage 12-14. No statistically significant influence of BAS 768 00 F on plant dry biomass was observed for all tested plant species after application of 4.0 L/ha at BBCH 12-14. The NOER for all species tested are ≥ 4.0 Lformulation/ha The ER_{50} for all species tested are > 4.0 Lformulation/ha.

Reference:	CP 10.6.2/1
Report	Effect of BAS 768 00 F on vegetative vigour of several species of terrestrial plants under greenhouse conditions, Maleck, A., 2021 report No 894514, AC/BASF/21/14 BASF DocID 2021/2014237 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 vigour, 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 768 00 F to evaluate the phytotoxic potential. BAS 768 00 F was applied post-emergence at BBCH 12 - 14 at a limit rate of 4.0 L product/ha, with 5 replicates per plant species. After application, the plants were cultivated for 21 days under greenhouse conditions. Assessment of phytotoxicity (visual injury), plant development (BBCH) and plant survival were done 7, 14 and 21 days after treatment (DAT). Assessments of plant length and dry weight were done at study termination 21 DAT.

After 21 days of exposure, no plant mortality and no symptoms of phytotoxicity were observed in the control groups and the test item treatment groups for any test species. In the test item treatment groups, no significant effects on plant length and weight compared to the control were observed in any tested species.

Post-emergence application of up to 4.0 L BAS 768 00 F/ha under worst-case greenhouse conditions resulted in no treatment-related effects on survival, phytotoxicity, plant length and dry weight of all tested plant species. The ER_{50} was estimated to be > 4.0 L product/ha for all tested species.

Materials and methods

Materials

Test item: BAS 768 00 F, Batch no.: FD-210120-1029; content of a.s.: mefentrifluconazole (BAS 750 F, Reg. no.: 5834378): 26.3 g/L (nominal: 25.0 g/L); sulfur (BAS 175 F, Reg. no.: 240586): 614.9 g/L (nominal: 600.0 g/L); density: 1.346 g/cm³.

Study design and methods

Test species: Dicotyledoneae: Carrot (*Daucus carota* L.), lettuce (*Lactuca sativa* L.), oilseed rape (*Brassica napus* L. *ssp. napus*), cabbage (*Brassica oleracea* L. *var. capitata* L. *f. alba*), soybean (*Glycine max* L.) and tomato (*Solanum lycopersicum* L.).
Monocotyledoneae: Onion (*Allium cepa* L.), ryegrass (*Lolium multiflorum* L.), wheat (*Triticum aestivum* L.) and corn (*Zea mays* L.).
All species show emergence rates ≥ 70%.

Test design: Greenhouse trial; limit test; one test item rate plus an untreated control; for replication and no. of plants per pot see table below; post-emergence application at BBCH 12 - 14 using a calibrated laboratory spray chamber at a water volume of 271 L/ha; assessments for survival, phytotoxicity (visual injury) and plant development (BBCH) were done 7, 14 and 21 days after treatment (DAT); plant length and dry weight (shoots above the ground) were determined 21 DAT. Phytotoxicity for treatments was estimated qualitatively as visual injury assigned in distinct classes relative to representative control pots. Visual injury scores per pot were averaged per treatment group.

Species	No. of plants/pot	No. of pots/replicate	No of replicates/treatment	Source
Carrot	6	1	5	Hild
Lettuce	3	2	5	Hild
Oilseed rape	3	2	5	DSV
Cabbage	3	2	5	Hazera
Soybean	2	3	5	Saaten-Union

Tomato	2	3	5	Hild
Onion	6	1	5	bejo
Ryegrass	6	1	5	DSV
Wheat	6	1	5	KWS
Corn	2	3	5	Euralis

Endpoints: Phytotoxicity (visual injury) (NOER); plant survival, length and weight (NOER, ER_{50}).

Test rates: Untreated control (tap water), test item rate: 4.0 L BAS 768 00 F/ha, applied in 271 L/ha for all species.

Test conditions: Greenhouse conditions, pots: plastic containers (15 cm diameter); daily mean temperature: 20.8 - 27.7°C; daily mean relative humidity: 58.1 - 91.8%; photoperiod: day length ≥ 16 h, additional light supply when indoor illumination was < 300 µmol to reach 300 - 400 µmol light intensity; bottom irrigation.

Analytics: Analytical verification of the test item was conducted using a LC-method with MS/MS detection (Method No. L0361/01).

Statistics: Descriptive statistics. The NOER for phytotoxicity was estimated. Phytotoxicity values < 10 % were considered as insignificant.

Description of the analytical procedures

The concentrations of the active substance mefentrifluconazole (contained in BAS 768 00 F) in application solution were determined according to the analytical method L0361/01. The analytical method is fully validated in a separate study (BASF DocID: 2017/1065621). The validation of the analytical method is described in the study report. The aqueous application solutions were diluted by a total factor of 100000 using acetonitrile/water + formic acid (20/80 + 0.1, v/v). The determination was performed by LC with MS/MS detection. The limit of quantification of the original method L0361/01 was 0.1 µg a.s./L for mefentrifluconazole. The lowest concentration level verified within this study was 0.0337 g a.s./L, which represents the LOQ of the method as adapted for this study. Considering the lowest concentration used for the calibration (0.10 µg a.s./L), the limit of detection (LOD) is calculated to be 0.010 g a.s./L. No significant peak interferences occurred at the retention time and mass transition of mefentrifluconazole in the control samples. Due to the high total dilution factor (100000) used, no relevant matrix effects were observed. The actual storage period for the tested application solutions (31 days) was within the maximum specified storage period of 90 days. Thus, no separate analysis of lab made storage stability samples was considered to be necessary. Mean recoveries of the procedural recovery samples were between 90.8% and 94.5%. Details on measured fortification samples and obtained procedural recoveries for the formulated product BAS 768 00 F (based on measurement of mefentrifluconazole) are provided in Table A 81.

Table A 81: Procedural recoveries for BAS 768 00 F (based on measurements of mefentrifluconazole)

Analyte	Fortification level [g/L]	n	Mean recovery [%]	RSD [%]
Mefentrifluconazole in application solution	0.0337	5	90.8	5.85
	0.682 / 0.675	7	94.5	10.0

Abbreviations: n: number of replicates; RSD: relative standard deviation

Results

Biological results

After 21 days of exposure, no plant mortality and no symptoms of phytotoxicity were observed in the control groups and the test item treatment groups for any test species. In the test item treatment groups, no significant effects on plant length and weight compared to the control were observed in any tested species. The results are summarized in Table A 82 and Table A 83.

Table A 82: Effect of BAS 768 00 F on survival, phytotoxicity, plant length and plant dry weight at 21 DAT

Plant species	Treatment [L product/ha]	Plant survival [%]	Visual injury [%]	Plant length [% of control]	Plant weight [% of control]
Carrot	Control	100	0	100.0	100.0
	4.0	100	0	97.3	94.9
Lettuce	Control	100	0	100.0	100.0
	4.0	100	0	101.9	106.5
Oilseed rape	Control	100	0	100.0	100.0
	4.0	100	0	97.5	92.5
Cabbage	Control	100	0	100.0	100.0
	4.0	100	0	99.9	98.7
Soybean	Control	100	0	100.0	100.0
	4.0	100	0	102.8	101.0
Tomato	Control	100	0	100.0	100.0
	4.0	100	0	98.8	98.9
Onion	Control	100	0	100.0	100.0
	4.0	100	0	103.5	97.6
Ryegrass	Control	100	0	100.0	100.0
	4.0	100	0	97.8	98.5
Wheat	Control	100	0	100.0	100.0
	4.0	100	0	99.1	100.0
Corn	Control	100	0	100.0	100.0
	4.0	100	0	101.0	99.3

Table A 83: NOER and ER_{50} of BAS 768 00 F for non-target plants at 21 DAT

Species	Carrot	Lettuce	Oilseed rape	Cabb-age	Soy-bean	Tomato	Onion	Rye-grass	Wheat	Corn
Plant survival [L/ha]										
NOER	≥ 4.0	≥ 4.0	≥ 4.0	≥ 4.0	≥ 4.0	≥ 4.0	≥ 4.0	≥ 4.0	≥ 4.0	≥ 4.0
ER_{50}	> 4.0	> 4.0	> 4.0	> 4.0	> 4.0	> 4.0	> 4.0	> 4.0	> 4.0	> 4.0
Phytotoxicity (visual injury) [L/ha]										
NOER	≥ 4.0	≥ 4.0	≥ 4.0	≥ 4.0	≥ 4.0	≥ 4.0	≥ 4.0	≥ 4.0	≥ 4.0	≥ 4.0
Plant length [L/ha]										
NOER	≥ 4.0	≥ 4.0	≥ 4.0	≥ 4.0	≥ 4.0	≥ 4.0	≥ 4.0	≥ 4.0	≥ 4.0	≥ 4.0
ER50	> 4.0	> 4.0	> 4.0	> 4.0	> 4.0	> 4.0	> 4.0	> 4.0	> 4.0	> 4.0
Plant weight (shoots above ground) [L/ha]										

Species	Carrot	Lettuce	Oilseed rape	Cabb-age	Soy-bean	Tomato	Onion	Rye-grass	Wheat	Corn
NOER	≥ 4.0	≥ 4.0	≥ 4.0	≥ 4.0	≥ 4.0	≥ 4.0	≥ 4.0	≥ 4.0	≥ 4.0	≥ 4.0
ER_{50}	> 4.0	> 4.0	> 4.0	> 4.0	> 4.0	> 4.0	> 4.0	> 4.0	> 4.0	> 4.0

Table A 84: Validity criteria according to OECD 227 (2006)

Criteria	Trigger value	Study value and page no.	Criteria met? Y/N	Guideline
Seedling emergence is at least 70% in the controls	≥ 70%	83 - 98% (p. 20)	Y	OECD 227
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	0%	0% (p. 20)	Y	OECD 227
Mean plant survival in untreated control for the duration of the study	≥ 90%	100% (p. 20)	Y	OECD 227
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	yes (p. 17)	Y	OECD 227

All validity criteria were met (see Table A 84 above).

Conclusion

Post-emergence application of up to 4.0 L BAS 768 00 F/ha under worst-case greenhouse conditions resulted in no treatment-related effects on survival, phytotoxicity, plant length and dry weight of all tested plant species. The ER_{50} was estimated to be > 4.0 L product/ha for all tested species.

A 2.6.2.1 Study 2

Comments of zRMS:	The submitted study was accepted The validity criteria were met. No deviation was noted. None of the tested plant species showed a statistically significant reduction of seedling emergence following the pre-emergence application of 4.0 L BAS 768 00 F/ha. No reduction of plant survival was found for all tested species at 21 DAE after the pre emergence application of 4.0 L/ha BAS 768 00 F/ha. No tested species showed visual phytotoxic symptoms after use of 4.0 L BAS 768 00 F/ha pre-emergence. No statistically significant reduction of plant length was observed for all tested plant species following the application of 4.0 L BAS 768 00 F/ha. The NOER for plant length reduction for all tested plant species is equal or higher than the tested rate of 4.0 L BAS 768 00 F/ha. No statistically significant influence of 4.0 L BAS 768 00 F/ha on plant dry weight was observed for all species. The NOER for all species tested are ≥ 4.0 Lformulation/ha The ER_{50} for all species tested are > 4.0 Lformulation/ha.

Reference:	CP 10.6.2/2
Report	Effect of BAS 768 00 F on seedling emergence and seedling growth of several species of terrestrial plants under greenhouse conditions, Maleck, A., 2021 report No 894513, AC/BASF/21/13 BASF DocID 2021/2014236 Authority registration No
Guideline(s):	EPA 850.4100, OECD 208 (2006)
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 study, 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 768 00 F to evaluate the phytotoxic potential. BAS 768 00 F was applied pre-emergence

at a limit rate of 4.0 L product/ha, with 4 replicates per plant species. After application, the plants were cultivated for 21 days after 50% emergence of the untreated control under greenhouse conditions. Assessment for seedling emergence, survival, phytotoxicity (visual injury) and plant development (BBCH) was done 7, 14 and 21 days after emergence (DAE). Assessments of plant length and plant dry weight was done at study termination 21 DAE.

After 21 days of exposure, no plant mortality and no symptoms of phytotoxicity were observed in the control groups and the test item treatment groups for any test species. In the test item treatment groups, no significant effects on seedling emergence, plant length and plant weight compared to the control were observed in any tested species.

Pre-emergence application of BAS 768 00 F under worst-case greenhouse conditions resulted in no effects on seedling emergence, plant survival, visual phytotoxicity, plant length and dry weight of all tested plant species. The ER_{50} was estimated to be > 4.0 L product/ha for all tested species.

Materials and methods

Materials

Test item: BAS 768 00 F, Batch no.: FD-210120-1029; content of a.s.: mefentrifluconazole (BAS 750 F, Reg. no.: 5834378): 26.3 g/L (nominal: 25.0 g/L); sulfur (BAS 175 F, Reg. no.: 240586): 614.9 g/L (nominal: 600.0 g/L); density: 1.346 g/cm³.

Study design and methods

Test species: Dicotyledoneae: Carrot (*Daucus carota* L.), lettuce (*Lactuca sativa* L.), oilseed rape (*Brassica napus* L. *ssp. napus*), cabbage (*Brassica oleracea* L. *var. capitata* L. *f. alba*), soybean (*Glycine max* L.) and tomato (*Solanum lycopersicum* L.).
Monocotyledoneae: Onion (*Allium cepa* L.), ryegrass (*Lolium multiflorum* L.), wheat (*Triticum aestivum* L.) and corn (*Zea mays* L.).
All species show emergence rates ≥ 70%.

Test design: Greenhouse trial; limit test; one test item rate plus an untreated control; for replication and no. of plants per pot see table below; pre-emergence application shortly after sowing using a calibrated laboratory spray chamber at a water volume of 276 L/ha; assessments for seedling emergence, survival, phytotoxicity (visual injury) and plant development (BBCH) were done 7, 14 and 21 days after emergence (DAE); plant dry weight (shoots above the ground) and plant length was determined 21 DAE. Phytotoxicity for treatments was estimated qualitatively as visual injury assigned in distinct classes relative to representative control pots. Visual injury scores per pot were averaged per treatment group.

Species	No. of seeds/pot	No. of pots/replicate	No of replicates/treatment	Sources
Carrot	10	1	4	Hild
Lettuce	5	2	4	Hild
Oilseed rape	5	2	4	DSV
Cabbage	5	2	4	Hazera
Soybean	5	2	4	Saaten-Union

Species	No. of seeds/pot	No. of pots/replicate	No of replicates/treatment	Sources
Tomato	5	2	4	Hild
Onion	10	1	4	bejo
Ryegrass	10	1	4	DSV
Wheat	10	1	4	KWS
Corn	5	2	4	Euralis

Endpoints: Visual phytotoxicity (NOER), Plant emergence, survival, length and weight (NOER, ER_{50}).

Test rates: Untreated control (tap water), test item rate: 4.0 L BAS 768 00 F/ha, applied in 276 L/ha for all species.

Test conditions: Greenhouse conditions, pots: plastic containers (15 cm diameter); daily mean temperature: 23.1 - 28.2°C; daily mean relative humidity: 52.1 - 63.8%; photoperiod: day length ≥ 16 h, additional light supply automatically for 16 hours in maximum when indoor illumination was < 300 µmol to reach 300 - 400 µmol light intensity; bottom irrigation.

Analytics: Analytical verification of the test item was conducted using a LC-method with MS/MS detection (Method No. L0361/01).

Statistics: Descriptive statistics. The NOER for phytotoxicity was estimated. Phytotoxicity values < 10% were considered as insignificant.

Description of the analytical procedures

The concentrations of the active substance mefentrifluconazole (contained in BAS 768 00 F) in application solution were determined according to the analytical method L0361/01. The analytical method is fully validated in a separate study (BASF DocID: 2017/1065621). The validation of the analytical method is described in the study report. The aqueous application solutions were diluted by a total factor of 100000 using acetonitrile/water + formic acid (20/80 + 0.1, v/v). The determination was performed by LC with MS/MS detection. The limit of quantification of the original method L0361/01 was 0.1 µg a.s./L for mefentrifluconazole. The lowest concentration level verified within this study was 0.0334 g a.s./L, which represents the LOQ of the method as adapted for this study. Considering the lowest concentration used for the calibration (0.10 µg a.s./L), the limit of detection (LOD) is calculated to be 0.010 g a.s./L. No significant peak interferences occurred at the retention time and mass transition of mefentrifluconazole in the control samples. Due to the high total dilution factor (100000) used, no relevant matrix effects were observed. The actual storage period for the tested application solutions (52 days) was within the maximum specified storage period of 90 days. Thus, no separate analysis of lab made storage stability samples was considered to be necessary. Mean recoveries of the procedural recovery samples were between 85.0% and 97.2%. Details on measured fortification samples and obtained procedural recoveries for the formulated product BAS 768 00 F (based on measurement of mefentrifluconazole) are provided in Table A 85.

Table A 85: Procedural recoveries for BAS 768 00 F (based on measurements of mefentrifluconazole)

Analyte	Fortification level [g/L]	n	Mean recovery [%]	RSD [%]
Mefentrifluconazole in application solution	0.0334	5	85.0	2.29
	0.670	5	97.2	0.70

Abbreviations: n: number of replicates; RSD: relative standard deviation

Results

Biological results

After 21 days of exposure, no plant mortality and no symptoms of phytotoxicity were observed in the control groups and the test item treatment groups for any test species. In the test item treatment groups, no significant effects on seedling emergence, plant length and plant weight compared to the control were observed in any tested species. The results are summarized in Table A 86 and Table A 87.

Table A 86: Effect of BAS 768 00 F on seedling emergence, survival, phytotoxicity, plant length and plant dry weight 21 DAE

Plant species	Treatment [L product/ha]	Seedling emergence [% of control]	Plant survival [%]	Visual injury [%]	Plant length [% of control]	Plant weight [% of control]
Carrot	Control	100	100	0	100.0	100.0
	4.0	103	100	0	99.6	101.1
Lettuce	Control	100	100	0	100.0	100.0
	4.0	103	100	0	95.4	100.5
Oilseed rape	Control	100	100	0	100.0	100.0
	4.0	108	100	0	97.2	97.4
Cabbage	Control	100	100	0	100.0	100.0
	4.0	100	100	0	99.6	92.3
Soybean	Control	100	100	0	100.0	100.0
	4.0	100	100	0	100.9	100.3
Tomato	Control	100	100	0	100.0	100.0
	4.0	97	100	0	98.9	97.8
Onion	Control	100	100	0	100.0	100.0
	4.0	103	100	0	96.9	95.6
Ryegrass	Control	100	100	0	100.0	100.0
	4.0	97	100	0	108.1	108.2
Wheat	Control	100	100	0	100.0	100.0
	4.0	100	100	0	99.4	103.0
Corn	Control	100	100	0	100.0	100.0
	4.0	103	100	0	100.0	102.7

Table A 87: NOER and ER_{50} of BAS 768 00 F for non-target plants 21 DAE

Species	Carrot	Lettuce	Oilseed rape	Cabb-age	Soy-bean	Tomato	Onion	Rye-grass	Wheat	Corn
Seedling Emergence [L/ha]										
NOER	≥ 4.0	≥ 4.0	≥ 4.0	≥ 4.0	≥ 4.0	≥ 4.0	≥ 4.0	≥ 4.0	≥ 4.0	≥ 4.0
ER_{50}	> 4.0	> 4.0	> 4.0	> 4.0	> 4.0	> 4.0	> 4.0	> 4.0	> 4.0	> 4.0
Plant survival [L/ha]										
NOER	≥ 4.0	≥ 4.0	≥ 4.0	≥ 4.0	≥ 4.0	≥ 4.0	≥ 4.0	≥ 4.0	≥ 4.0	≥ 4.0
ER_{50}	> 4.0	> 4.0	> 4.0	> 4.0	> 4.0	> 4.0	> 4.0	> 4.0	> 4.0	> 4.0
Phytotoxicity (visual injury) [L/ha]										
NOER	≥ 4.0	≥ 4.0	≥ 4.0	≥ 4.0	≥ 4.0	≥ 4.0	≥ 4.0	≥ 4.0	≥ 4.0	≥ 4.0
Plant length [L/ha]										
NOER	≥ 4.0	≥ 4.0	≥ 4.0	≥ 4.0	≥ 4.0	≥ 4.0	≥ 4.0	≥ 4.0	≥ 4.0	≥ 4.0
ER_{50}	> 4.0	> 4.0	> 4.0	> 4.0	> 4.0	> 4.0	> 4.0	> 4.0	> 4.0	> 4.0
Plant weight (shoots above ground) [L/ha]										
NOER	≥ 4.0	≥ 4.0	≥ 4.0	≥ 4.0	≥ 4.0	≥ 4.0	≥ 4.0	≥ 4.0	≥ 4.0	≥ 4.0
ER_{50}	> 4.0	> 4.0	> 4.0	> 4.0	> 4.0	> 4.0	> 4.0	> 4.0	> 4.0	> 4.0

Table A 88: Validity criteria according to OECD 208 (2006)

Criteria	Trigger value	Study value and page no.	Criteria met? Y/N	Guideline
Seedling emergence of control plants	≥ 70%	83 - 98% (p. 20)	Y	OECD 208 (2006)
Visible phytotoxic effects for the control plants (*e.g.*, chlorosis, necrosis, wilting, leaf and stem deformations). Plants exhibit only normal variation in growth and morphology for that particular species	0%	0% (p. 20)	Y	OECD 208 (2006)
Mean control plant survival for the duration of the study	≥ 90%	100% (p. 20)	Y	OECD 208 (2006)
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	Yes (p. 17)	Y	OECD 208 (2006)

All validity criteria were met (see Table A 88 above).

Conclusion

Pre-emergence application of BAS 768 00 F under worst-case greenhouse conditions resulted in no effects on seedling emergence, plant survival, visual phytotoxicity, plant length and dry weight of all tested plant species. The ER_{50} was estimated to be > 4.0 L product/ha for all tested species.

A 2.6.3 KCP 10.6.3 Extended laboratory studies on non-target plants

Further studies on non-target plants are not triggered.

A 2.6.4 KCP 10.6.4 Semi-field and field tests on non-target plants

Further studies on non-target plants are not triggered.

A 2.7 KCP 10.7 Effects on other terrestrial organisms (flora and fauna)

Not relevant.

A 2.8 KCP 10.8 Monitoring data

According to the knowledge of the applicant, there are currently no monitoring studies available which assess ecotoxicological effects of BAS 768 00 F or of the active substances.