REGISTRATION REPORT

Part B

Section 9

Ecotoxicology

Detailed summary of the risk assessment

Product code: BAS 762 02 F

Product name(s): Revydas

Chemical active substance(s):

Mefentrifluconazole, 100 g/L

Boscalid, 200 g/L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(Authorization)

Applicant: BASF

Submission date: March 2021

MS Finalisation date: November 2021 (initial Core Assessment)

April 2022 (final Core Assessment)

Version history

When	What
03/2021	Initial dRR – BASF DocID 2021/2005815
November 2021	Initial zRMS assessment The report in the dRR format has been prepared by the Applicant, therefore all comments, additional evaluations and conclusions of the zRMS are presented in grey commenting boxes. Minor changes are introduced directly in the text and highlighted in grey. Not agreed or not relevant information are ~~struck through and shaded for transparency~~.
April 2022	Final report (Core Assessment after the commenting period) Additional information/assessments included by the zRMS in the report in response to comments recieved from the cMS and the Applicant are highlighted in yellow, while not agreed use pattern is ~~struck through and shaded~~.

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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	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, 2, 3	Central Zone	Oilseed Rape, winter and spring (BRSNN)	F	See B0 for details	SP	BBCH 57-75	a) 1 b) 1	-	a) 1 b) 1	a) 100* +200** b) 100* + 200**	100-400	F	F is defined by latest application timing. For uses 2 and 3 dose rate range 0.6 - 1.0 L/ha	A	A	A	A	A	A	A
4, 5, 6	Central Zone	Sunflower (HELAN)	F	See B0 for details	SP	BBCH 31-69	a) 2 b) 2	7	a) 1 b) 2	a) 100* +200** b) 200* + 400**	100-400	F	Maximum 2 applications per crop and season. 1st appl. BBCH 31-59 2nd appl. BBCH 61-69. F is defined by latest application timing. For uses 2 and 3 dose rate range 0.6 - 1.0 L/ha	A	A	A	A	A	A	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
7, 8, 9	Central Zone	wheat (winter and spring)	F	See B0 for details	SP	BBCH 30 - 49	a) 1 b) 1	-	a) 1 b) 1	a) 100* +200** b) 100* + 200**	100 - 300	56	For eyespot control, only one application at BBCH 30-32 For use 8 dose rate range 0.6 - 1.0 L/ha	A	A	A	A	A	A	A

\# 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

* Mefentrifluconazole

** Boscalid

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

zRMS comments:
Initially, the GAP table including detailed information on pests in particular cMS has been provided by the Applicant. However, pests are of no relevance for the ecotoxicological risk assessment and GAP table was thus shortened to provide critical GAP, which was considered in the risk assessment covering intended uses of BAS 762 02 F in all concerned Member States.

9.1.1 Overall conclusions

zRMS comments:
Conclusions presented in points 9.1.1.1 to 9.1.1.7 below were checked by the zRMS and amended where necessary.

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
In the screening step and/or tier1 risk assessment, all TER_A values and all TER_{LT} values for mefentrifluconazole and boscalid exceed the trigger set by Commission Regulation (EU) 546/2011 for acceptability of effects.

Exposure to combined active substances
In the screening step of the acute assessment, the TER values for the combined active substances (virtual compound) exceed the trigger value for acceptability of effects. The combined reproductive risk assessment using the concentration addition model result in tier 1 TER values above the trigger of 5 for acceptability of effects.

Drinking water risk assessment
Following EFSA/2009/1438, the puddle scenario is considered relevant for application of BAS 762 02 F according to the proposed use pattern. Since the ratio of the effective application rate to the relevant endpoints is below the value of 3000 for mefentrifluconazole and for boscalid, a quantitative risk assessment for the proposed use pattern of BAS 762 02 F is not necessary.

Secondary poisoning and biomagnification
The log P_{ow} was 3.4 for mefentrifluconazole and 2.96 for boscalid, 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 and boscalid are both above the trigger value of 5 for acceptability of effects. The potential for bioaccumulation of both mefentrifluconazole and boscalid was considered low 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 application of BAS 762 02 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
In the screening step and/or tier1 risk assessment, all TER_A values and all TER_{LT} values for mefentrifluconazole and boscalid exceed the trigger set by Commission Regulation (EU) 546/2011 for acceptability of effects.

Exposure to combined active substances and to formulation

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

Drinking water risk assessment
Following EFSA/2009/1438, the puddle scenario is the one relevant for mammals. Since the ratio of the effective application rate to the relevant endpoints is below the value of 3000 for mefentrifluconazole and for boscalid, a quantitative risk assessment for the proposed use pattern of BAS 762 02 F is not necessary.

Secondary poisoning and biomagnification
The log P_{ow} was 3.4 for mefentrifluconazole and 2.96 for boscalid, which triggers an assessment of the potential risk from secondary poisoning. According to the tier 1 risk assessment for earthworm-eating and fish-eating mammals, the TER values for mefentrifluconazole and boscalid are both above the trigger value of 5 for acceptability of effects. The potential for bioaccumulation of both mefentrifluconazole and boscalid was considered low 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 application of BAS 762 02 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 boscalid, the active substances in the formulation BAS 762 02 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 boscalid indicate an acceptable risk for all groups of aquatic organisms following the intended uses of BAS 762 02 F with no need for any 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 calculation. For boscalid, no major metabolites (> 10% TAR) were formed in a sensibilized water/sediment study; they are thus considered not to be of ecotoxicological relevance and well covered within the assessment of the parent compound.

The formulation risk assessment revealed an acceptable risk to aquatic organisms following the intended uses of BAS 762 02 F with no need for any additional mitigation measures.

The standard risk assessment provided for the fungicidal product BAS 762 02 F, the active substances mefentrifluconazole and boscalid as well as their major metabolites demonstrate that the

proposed applications of BAS 762 02 F according to good agricultural practice are of low risk to aquatic ecosystems.

The following text is added due to agreements during the Central Zone harmonisation meetings. It should be noted that this text has no impact on the outcome of zonal evaluation of formulation BAS 762 02 F, which was performed in line with the EU agreed methodology.

"The endpoint E_rC_{50} is selected in this Core Assessment but there are some uncertainties regarding the level of protection reached for primary producers. This is indicated for macrophytes in the aquatic Guidance Document (EFSA Journal 2013;11(7):3290) that recommends: "... a proper calibration between different tiers (higher and lower tier data) for macrophytes should be performed in the future". Such calibration should be extended to algae. Until available relevant information on the level of protection reached is considered at EU level, it is recommended to address this uncertainty at each Member State level in the National Addendum if considered necessary, although it would be highly appreciated to have a harmonised approach in the Central zone."

9.1.1.3 Effects on bees (KCP 10.3.1)

The risk to honey bees from the use of mefentrifluconazole, boscalid and BAS 762 02 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}) in line with indications of the current guidance document SANCO/10329/2002 rev. 2 final. ~~[*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 762 02 F and the active substances mefentrifluconazole and boscalid for acute oral and acute contact exposure of honey bees are considerably below the Commission Regulation (EU) 546/2011 trigger value of 50. ~~Additionally, the chronic TER for larvae and adult bees exceed the suggested trigger. Considering the very protective assumptions the risk can be considered acceptable.~~

Based on these results it can be concluded that low risk to honey bees is expected from applications of BAS 762 02 F according to the proposed uses. No adverse effects on adult bees, bee brood and bee colonies were observed in the tunnel study performed on flowering winter oilseed rape with BAS 762 02 F applied at 1.1 L/ha during the bee activity, confirming acceptable acute and chronic risk to bees from the intended uses of BAS 762 02 F. ~~**This is confirmed by a worst case assessment following EPPO (2010) for chronic adult and honey bee larvae as well as a honey bee semi-field study with BAS 762 02 F.**~~

9.1.1.4 Effects on arthropods other than bees (KCP 10.3.2)

The testing and risk assessment strategy used here follow the approach recommended in the ESCORT 2 guidance document, ~~ESCORT 3,~~ and the EC Guidance Document on Terrestrial Ecotoxicology (SANCO/10329, 17 October 2002). The risk assessment for BAS 762 02 F is based on Tier I tests with the standard test species *A. rhopalosiphi* and *T. pyri*. 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 762 02 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 762 02 F, mefentrifluconazole, boscalid and the relevant metabolites to earthworms and other non-target soil macro-organisms was assessed by comparing the maximum PEC_{soil} values with NOEC or EC_{10} values, to generate long-term TER values (TER_{lt}).
All TER values for BAS 762 02 F, mefentrifluconazole, boscalid and the relevant metabolites for chronic exposure of earthworms and other non-target soil organisms (meso- and macrofauna) are considerably higher than the Commission Regulation (EU) 546/2011 trigger value of 5. This indicates that BAS 762 02 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 762 02 F, mefentrifluconazole, boscalid and the relevant metabolites to soil micro-organisms was assessed by comparing the maximum PEC_{soil} values with the maximum concentration with effects ≤ 25%.

For the formulation BAS 762 02 F, the active substances mefentrifluconazole and boscalid as well as their relevant metabolites, the maximum concentration with effects < 25% (SANCO/10329/2002 trigger) are all above the maximum PEC_{soil} values. Therefore, it is concluded that the use of BAS 762 02 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 762 02 F to non-target terrestrial plants has been investigated by carrying out vegetative vigor and seedling emergence studies with up to six dicotyledonous and four monocotyledonous non-target plant species. Plants showed similar/higher sensitivity to pre- emergence exposure than to post-emergence exposure. The risk assessment is thus carried out with the respective most sensitive endpoints obtained from the vegetative vigor tests.

The risk assessment is based on the "Guidance Document on Terrestrial Ecotoxicology", (SANCO/10329/2002 rev.2 final, 2002). It is restricted to off-field areas, as non-target plants are non-crop plants located outside the treated area. The amount of spray drift reaching off-crop habitats is calculated using the 90th percentile estimates in Appendix IV of ESCORT 2. For a single application to field crops and vegetables < 50 cm, 2.77% of the application rate was assumed to reach areas at 1 m from the edge of the crop (worst-case scenario). The highest single application rate of BAS 762 02 F is used to calculate the maximum off-field predicted environmental rate ($PER_{off\text{-}field}$). The potential risk of BAS 762 02 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). For convenience of some concerned Member States additional risk assessment was performed with consideration of the cumulative application rate in order to cover multiple applications in sunflower.

Based on the results of the greenhouse trials, the TER values for all tested plant species were above the standard trigger of 5 when single or multiple applications were assumed in performed calculations.

Based on the risk assessment it can be concluded that BAS 762 02 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 762 02 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 762 02 F grouped according to worst-case application

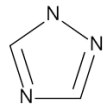
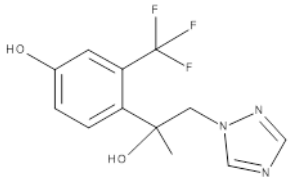
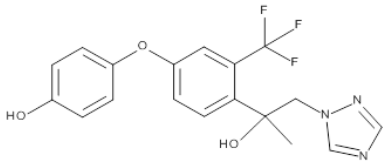
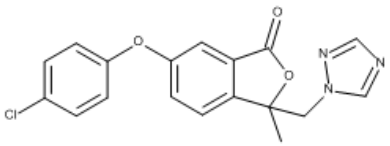
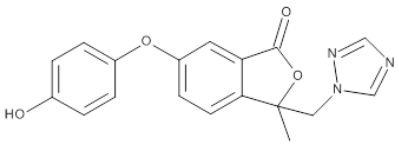
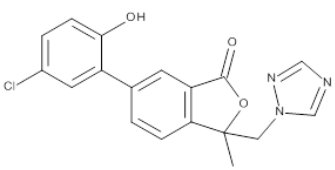
Grouping according to worst-case application				
Area	**Group**	**Intended uses**	**Relevant use parameters for grouping**	**Relevant parameter or value for sorting**
Birds and mammals	Application rate	Oilseed rape	Risk assessments are based on the maximum application rate of 1 x 1.00 L/ha (corresponding to 0.1 kg mefentrifluconazole/ha and 0.2 kg boscalid/ha)	Maximum application rate = 1 x 1.00 L/ha
	Application rate	Sunflower	Risk assessments are based on the maximum application rate of 2 x 1.00 L/ha (corresponding to 0.1 kg mefentrifluconazole/ha and 0.2 kg boscalid/ha)	Maximum application rate = 2 x 1.00 L/ha
	Application rate	Cereals	Risk assessments are based on the maximum application rate of 1 x 1.00 L/ha (corresponding to 0.1 kg mefentrifluconazole/ha and 0.2 kg boscalid/ha)	Maximum application rate = 1 x 1.00 L/ha
Aquatic organisms	Grouping according to Section 8 – Environmental Fate			
Bees, non-target plants	Application rate	All intended uses	Risk assessments are based on the maximum single application rate of 1 x 1.00 L/ha (corresponding to 0.1 kg mefentrifluconazole/ha and 0.2 kg boscalid/ha)	Maximum single application rate = 1.00 L/ha
Non-target arthropods, soil macro- and micro-organisms	Application rate	All intended uses	Risk assessments are based on the maximum application rate of 2 x 1.00 L/ha (corresponding to 2 x 0.1 kg mefentrifluconazole/ha and 2 x 0.2 kg boscalid/ha)	Maximum application rate = 2 x 1.00 L/ha

zRMS comments:
zRMS in general agrees with grouping of intended uses provided in Table9.1-2 above. It is, however, noted that in the GAP table also wheat (spring and winter) is included, which may have impact on the risk assessment for birds and mammals. Respective information has been thus added in table above.

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 762 02 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 observed occurrence in compartments [%]	Exposure assessment required due to
M750F001 (1,2,4-triazole)		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		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		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
M750F006		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		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		355.8	Soil: not detected in soil Water: 7.3 (max. in aqueous photolysis study) Sediment: not detected in sediment Total w/s system: not detected in w/s study	**Terrestrial** Metabolite relevant for RA: no RA conducted: no **Aquatic** Metabolite relevant for RA: yes RA conducted: yes

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

Table 9.1-4 Metabolites of boscalid

Metabolite	Chemical structure	Molar mass (g/mol)	Maximum observed occurrence in compartments	Risk assessment required?
No relevant metabolites	--	--	--	--

zRMS comments:
Information regarding mefentrifluconazole and its metabolites is in line with EU agreed endpoints as reported in EFSA Journal 2018;16(7):5379.

According to EU Review Report SANCO/3919/2007-rev.5, no relevant metabolites of boscalid are formed in soil or aquatic systems.

9.2 Effects on birds (KCP 10.1.1)

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

9.3 Toxicity data

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

Active substances

An overview of the EU agreed endpoints is given in Table 9.3-1 (mefentrifluconazole) and

Table 9.**3-2** (boscalid). In case the selection of studies and endpoints for the risk assessment deviates from the results of the EU review process, justifications are provided below.

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

Species	Substance	Exposure System	Results	Reference
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 9.3-2: Boscalid (BAS 510 F): Endpoints relevant for the risk assessment for birds

Species	**Substance**	**Exposure System**	**Results**	**Reference [BASF DocID]**
Colinus virginianus	Boscalid	Oral, 1 d Acute	LD_{50} > 2000 mg/kg bw	Review Report SANCO/3919/2007-rev. 5 [1999/11115]
Colinus virginianus	Boscalid	Dietary Reproductive toxicity	NOEL = 24.1 mg/kg bw/d	Review Report SANCO/3919/2007-rev. 5 [2000/1017245 and 2000/1017168 (amendment)]
Anas platyrhynchos	Boscalid	Dietary Reproductive toxicity	NOEL = 128.6 mg/kg bw/d	DAR 2002 as NOEC [2000/1018527]
Endpoint used for acute assessment	**Boscalid**	**Oral, 1 d Acute**	**$LD_{50\ (extrapolated)}$ = 3776 mg/kg bw**	**Extrapolation of quail LD50 [1999/11115]**
Endpoint used for reproductive assessment	**Boscalid**	**Dietary Reproductive toxicity – Tier 1**	**NOEL = 24.1 mg/kg bw/d**	**Review Report SANCO/3919/2007-rev. 5 [2000/1017245 and 2000/1017168 (amendment)]**

zRMS comments:

Avian toxicity data for mefentrifluconazole and boscalid are in line with EU agreed endpoints reported in EFSA Journal 2018;16(7):5379 and EU Review Report SANCO/3919/2007-rev. 5, respectively.

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 boscalid

No major metabolites were identified in soil, sediment, or water (see SANCO/3919/2007-rev. 5, January 2008).

The metabolism studies with boscalid in grape leaves and fruits (BASF DocID 2000/1014860) and lettuce (BASF DocID 1999/11240) showed that in all plant matrices, hence also in those that could be used as forage by birds and mammals, no metabolites approached or exceeded 10% TRR. In the metabolism study on green beans (BASF DocID 2000/1014861), M510F47 was present in bean seeds at up to 9.97% TRR but a maximum concentration of only 0.007 mg/kg. M510F47 was detected in the rat metabolism study (BASF DocID 2000/1017220), so it has been tested in mammals, and has also shown no increased toxicity compared to boscalid in an acute rat study (LD_{50} > 2000 mg/kg bw, BASF DocID 1998/10872). Additionally, M510F47 is more hydrophilic than boscalid, so it is reasonable to assume that it would be quickly excreted. The relevant residue is therefore the parent compound boscalid.

In the confined rotational crop study (BASF DocID 2000/1014862) on lettuce, radish, and wheat plants, the unchanged parent compound boscalid remained the major component. In matrices potentially taken by foraging birds and mammals (lettuce and radish leaves, wheat forage), M510F61 was found at ≥ 10% TRR in radish leaves (21.2% TRR) and in wheat forage (18.1% TRR). However, concentrations of these metabolites were relatively low at a maximum of 0.032 mg/kg in radish leaves and 0.102 mg/kg in wheat forage. Additionally, M510F61 is more hydrophilic than boscalid, so it is reasonable to assume that it would be quickly excreted. Therefore, the risk assessment for the active ingredient boscalid covers the potential risk from these minor metabolites.

zRMS comments:

According to information available in the RAR (May 2018), the risk from relevant plant metabolites of mefentrifluconazole is covered by the risk assessment performed for the parent compound. Based on that, no specific risk assessment is deemed necessary.

Information on boscalid metabolites provided above is agreed by the zRMS. In addition to that it is noted that the same conclusion has been taken by the RMS during EU renewal process (see DRAR of 2018). Additional studies with mammals performed for metabolites M510F47 and M510F49 indicated that these metabolites are not more toxic than the parent. Nevertheless, it should be kept in mind that the renewal process has not been finalised yet, so this conclusion will have to be revised once the new LoEP is issued.

Formulation toxicity

No acute bird study with the formulation has been carried out for the following reason. For BAS 762 02 F, the acute oral study in rats resulted in LD_{50} > 5000 mg a.s./kg b.w. (BASF DocID 2019/2034516, see chapter 6.3 and Appendix 2 of chapter 6). No mortality occurred at the tested dose rate of 5000 mg a.s./kg b.w., indicating a low toxicity of the formulation and no increased toxicity compared to the active substances. Consequently, no acute oral tests with birds on the product are considered necessary and toxicity can reliably be predicted on the basis of the data for the active substances.

zRMS comments:

No additional studies with the formulated product were performed which is acceptable for the animal welfare reasons. The combined risk will be addressed with consideration of the data for individual active compounds, in line with EFSA (2009). For details of the performed combined risk assessment, please refer to point 9.3.2.1 below.

9.3.1.1 Justification for new endpoints

Mefentrifluconazole

Acute – Not applicable. Endpoint is EU agreed.

Reproductive – Not applicable. Endpoint is EU agreed.

Boscalid

Acute - Because no mortality or signs of toxicity occurred in the quail acute study (BASF DocID 1999/11115), the endpoint (LD_{50} > 2000 mg/kg b.w.) was extrapolated to LD_{50} = 3776 mg/kg b.w.

Reproductive - Not applicable. The endpoint is EU agreed.

zRMS comments:

Consideration of extrapolated LD_{50} value for boscalid is agreed by the zRMS as no mortalities were observed in performed studies and in such situation extrapolation is possible in line with EFSA (2009). Since 10 birds were tested in the acute toxicity study (Zok, 1999, see boscalid monograph for details) and no mortality was observed up to and including the maximum dose tested (2000 mg/kg bw) extrapolation factor of 1.888 was correctly applied by the Applicant resulting with extrapolated LD_{50} of 3776 mg a.s./kg bw that may be used in the risk assessment.

9.3.2 Risk assessment for spray applications

Proposed use pattern for the risk assessments

The proposed use pattern for the use of BAS 762 02 F is summarized in

Table 9.**3-3**. The detailed use pattern table is presented at the beginning of the ecotoxicology chapter (section 9.1).

Table 9.3-3: Proposed use pattern

Crop	Crop group according to EFSA/2009/1438	Application time (BBCH growth stage)	Number of applications	Interval between applications [d]	Application rate per application		
					Mefentrifluconazole [kg/ha]	Boscalid [kg/ha]	BAS 762 02 H [L/ha]
Wheat (winter, spring)	Cereals	30-49	1	--	0.1	0.2	1.0
OSR (winter, spring) [1)]	OSR	57-75	1	--	0.1	0.2	1.0
Sunflower	Sunflower	31-69	2	7	0.1	0.2	1.0

1) This scenario covers the uses listed in the GAP as "other minor oilseeds".

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).

Dietary risk assessment for the active substances

Acute risk assessment

The TER_A values are presented in Table 9.3-4 (cereals), Table 9.3-6 (oilseed rape) and Table 9.3-8 (sunflowers) for mefentrifluconazole and in
Table 9.3-5 (cereals), Table 9.3-7 (oilseed rape) and Table 9.3-9 (sunflowers) for boscalid. All the TER_A values at the screening step are above the relevant trigger of 10 for acceptability of acute effects.

Table 9.3-4: Mefentrifluconazole: Screening step calculations of the acute risk for birds due to the use of BAS 762 02 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	1	365	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 required
	Small omnivorous bird	158.8	15.88	1.0	15.88	51.4	

Table 9.3-5: Boscalid: Screening step calculations of the acute risk for birds due to the use of BAS 762 02 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	0.2	1	365	10.0	3776.0	
Screening step:							
Acute risk assessment screening step	**Indicator species**	**Shortcut value**	**Daily Dietary Dose (single)**	**MAF (90)**	**Daily Dietary Dose (Multiple)**	**TER**	No refinement required
	Small omnivorous bird	158.8	31.76	1.0	31.76	118.9	

Table 9.3-6: Mefentrifluconazole: Screening step calculations of the acute risk for birds due to the use of BAS 762 02 F for the crop group "oilseed rape"

Data from Data_Entry worksheet	**Crop**	**Application rate (kg a.s./ha)**	**Number of applications**	**Application Interval**	**DT_{50}**	**LD_{50}**	
	Oilseed rape	0.1	1	365	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 required
	Small omnivorous bird	158.8	15.88	1.0	15.88	51.4	

Table 9.3-7: Boscalid: Screening step calculations of the acute risk for birds due to the use of BAS 762 02 F for the crop group "oilseed rape"

Data from Data_Entry worksheet	**Crop**	**Application rate (kg a.s./ha)**	**Number of applications**	**Application Interval**	**DT_{50}**	**LD_{50}**	
	Oilseed rape	0.2	1	365	10.0	3776.0	
Screening step:							
Acute risk assessment screening step	**Indicator species**	**Shortcut value**	**Daily Dietary Dose (single)**	**MAF (90)**	**Daily Dietary Dose (Multiple)**	**TER**	No refinement required
	Small omnivorous bird	158.8	31.76	1.0	31.76	118.9	

Table 9.3-8: Mefentrifluconazole: Screening step calculations of the acute risk for birds due to the use of BAS 762 02 F for the crop group "sunflowers"

Data from Data_Entry worksheet	**Crop**	**Application rate (kg a.s./ha)**	**Number of applications**	**Application Interval**	**DT_{50}**	**LD_{50}**	
	Sunflowers	0.1	2	7	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 required
	Small omnivorous bird	158.8	15.88	1.4	22.23	36.7	

Table 9.3-9: Boscalid: Screening step calculations of the acute risk for birds due to the use of BAS 762 02 F for the crop group "sunflowers"

Data from Data_Entry worksheet	**Crop**	**Application rate (kg a.s./ha)**	**Number of applications**	**Application Interval**	**DT_{50}**	**LD_{50}**	
	Sunflowers	0.2	2	7	10.0	3776.0	
Screening step:							
Acute risk assessment screening step	**Indicator species**	**Shortcut value**	**Daily Dietary Dose (single)**	**MAF (90)**	**Daily Dietary Dose (Multiple)**	**TER**	No refinement required
	Small omnivorous bird	158.8	31.76	1.4	44.46	84.9	

zRMS comments:

Provided above evaluation is agreed by the zRMS. Based on performed calculations, acceptable acute dietary risk to birds from particular active compounds may be concluded.

Reproductive risk assessment

The dietary TER reproductive values for the screening step and tier 1 risk assessments are presented in Table 9.3-10 (cereals), Table 9.3-12 (oilseed rape) and Table 9.3-14 (sunflowers) for mefentrifluconazole and in Table 9.3-11 (cereals), Table 9.3-13 (oilseed rape) and

Table 9.**3-15** (sunflowers) for boscalid.

All the TER_{LT} values for mefentrifluconazole and boscalid, are above the relevant trigger of 5 for acceptability of reproductive effects at the screening step or at tier 1.

Table 9.3-10: Mefentrifluconazole: Screening step and tier 1 calculations of the long-term/reproductive risk for birds due to the use of BAS 762 02 F for the crop group "cereals"

Data from Data_Entry worksheet	**Crop**	**Application rate (kg a.s./ha)**	**Number of applications**	**Application Interval**	**DT_{50}**	**Reproductive End Point (mg/kg b.w./d)**	**Time weighted average (TWA)**
	Cereals	0.1	1	365	10	25.3	0.53
Screening step:							
Reproductive risk assessment screening step	**Indicator species**	**Shortcut value**	**Daily Dietary Dose (single)**	**MAF mean**	**Daily Dietary Dose (Multiple)**	**TER**	No refinement required
	Small omnivorous bird	64.8	6.48	1.0	3.43	7.4	
First Tier Risk Assessment: [1)]							
Calculate TER for each generic focal species	**Crop**	**Generic focal species**			**Short cut value**	**TER**	
	Cereals BBCH 30 - 39	Small omnivorous bird "lark" Combination (invertebrates with interception) 25% crop leaves 25% weed seeds 50% ground arthropods			5.4	88.4	No refinement required
	Cereals BBCH ≥ 40	Small omnivorous bird "lark" Combination (invertebrates with interception) 25% crop leaves 25% weed seeds 50% ground arthropods			3.3	144.7	

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

Table 9.3-11: Boscalid: Screening step and tier 1 calculations of the long-term/reproductive risk for birds due to the use of BAS 762 02 F for the crop group "cereals"

Data from Data_Entry worksheet	**Crop**	**Application rate (kg a.s./ha)**	**Number of applications**	**Application Interval**	**DT_{50}**	**Reproductive End Point (mg/kg b.w./d)**	**Time weighted average (TWA)**
	Cereals	0.2	1	365	10	24.1	0.53
Screening step:							
Reproductive risk assessment screening step	**Indicator species**	**Shortcut value**	**Daily Dietary Dose (single)**	**MAF mean**	**Daily Dietary Dose (Multiple)**	**TER**	**Please perform first tier risk assessment (see below)**
	Small omnivorous bird	64.8	12.96	1.0	6.87	**3.5**	
First Tier Risk Assessment:							
Calculate TER for each generic focal species	**Crop**	**Generic focal species**			**Short cut value**	**TER**	
	Cereals BBCH 30 - 39	Small omnivorous bird "lark" Combination (invertebrates with interception) 25% crop leaves 25% weed seeds 50% ground arthropods			5.4	42.1	No refinement required
	Cereals BBCH ≥ 40	Small omnivorous bird "lark" Combination (invertebrates with interception) 25% crop leaves 25% weed seeds 50% ground arthropods			3.3	68.9	

TER value in **bold** is below the relevant trigger

Table 9.3-12: Mefentrifluconazole: Screening step and tier 1 calculations of the long-term/reproductive risk for birds due to the use of BAS 762 02 F for the crop group "oilseed rape"

Data from Data_Entry worksheet	**Crop**	**Application rate (kg a.s./ha)**	**Number of applications**	**Application Interval**	**DT_{50}**	**Reproductive End Point (mg/kg b.w./d)**	**Time weighted average (TWA)**
	Oilseed rape	0.1	1	365	10	25.3	0.53
Screening step:							
Reproductive risk assessment screening step	**Indicator species**	**Shortcut value**	**Daily Dietary Dose (single)**	**MAF mean**	**Daily Dietary Dose (Multiple)**	**TER**	No refinement required
	Small omnivorous bird	64.8	6.48	1.0	3.43	7.4	
First Tier Risk Assessment: [1)]							
Calculate TER for each generic focal species	**Crop**	**Generic focal species**			**Short cut value**	**TER**	
	Oilseed rape BBCH ≥ 40	medium herbivorous/granivorous bird "pigeon" Comby to be calculated 50 % crop leaves 50 % weed seeds			0.9	530.4	No refinement required
	Oilseed rape BBCH ≥ 40	Small omnivorous bird "lark" Combination (invertebrates without interception) 25% crop leaves 25% weed seeds 50% ground arthropods			2.7	176.8	
	Oilseed rape late – late (with seeds) (BBCH 30-99)	Small insectivorous bird "dunnock) ground invertebrates with interception 100% soil dwelling invertebrates			2.7	176.8	

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

Table 9.3-13: Boscalid: Screening step and tier 1 calculations of the long-term/reproductive risk for birds due to the use of BAS 762 02 F for the crop group "oilseed rape"

Data from Data_Entry worksheet	**Crop**	**Application rate (kg a.s./ha)**	**Number of applications**	**Application Interval**	**DT_{50}**	**Reproductive End Point (mg/kg b.w./d)**	**Time weighted average (TWA)**
	Oilseed rape	0.2	1	365	10	24.1	0.53
Screening step:							
Reproductive risk assessment screening step	**Indicator species**	**Shortcut value**	**Daily Dietary Dose (single)**	**MAF mean**	**Daily Dietary Dose (Multiple)**	**TER**	**Please perform first tier risk assessment (see below)**
	Small omnivorous bird	64.8	12.96	1.0	6.87	**3.5**	
First Tier Risk Assessment:							
Calculate TER for each generic focal species	**Crop**	**Generic focal species**			**Short cut value**	**TER**	
	Oilseed rape BBCH ≥ 40	medium herbivorous/granivorous bird "pigeon" Comby to be calculated 50 % crop leaves 50 % weed seeds			0.9	252.6	No refinement required
	Oilseed rape BBCH ≥ 40	Small omnivorous bird "lark" Combination (invertebrates without interception) 25% crop leaves 25% weed seeds 50% ground arthropods			2.7	84.2	
	Oilseed rape late – late (with seeds) (BBCH 30-99)	Small insectivorous bird "dunnock) ground invertebrates with interception 100% soil dwelling invertebrates			2.7	84.2	

TER value in **bold** is below the relevant trigger

Table 9.3-14: Mefentrifluconazole: Screening step and tier 1 calculations of the long-term/reproductive risk for birds due to the use of BAS 762 02 F for the crop group "sunflowers"

Data from Data_Entry worksheet	**Crop**	**Application rate (kg a.s./ha)**	**Number of applications**	**Application Interval**	**DT_{50}**	**Reproductive End Point (mg/kg b.w./d)**	**Time weighted average (TWA)**
	Sunflowers	0.1	2	7	10	25.3	0.53
Screening step:							
Reproductive risk assessment screening step	**Indicator species**	**Shortcut value**	**Daily Dietary Dose (single)**	**MAF mean**	**Daily Dietary Dose (Multiple)**	**TER**	No refinement required
	Small omnivorous bird	64.8	6.48	1.6	5.50	**4.6**	
First Tier Risk Assessment:							
Calculate TER for each generic focal species	**Crop**	**Generic focal species**			**Short cut value**	**TER**	
	Sunflower Late (Flowering, seed ripening) BBCH 61-92	Small granivorous/insectivorous bird "bunting" Small seeds 100% crop seeds			10.0	29.8	No refinement required

TER value in **bold** is below the relevant trigger

Table 9.3-15: Boscalid: Screening step and tier 1 calculations of the long-term/reproductive risk for birds due to the use of BAS 762 02 F for the crop group "sunflowers"

Data from Data_Entry worksheet	**Crop**	**Application rate (kg a.s./ha)**	**Number of applications**	**Application Interval**	**DT_{50}**	**Reproductive End Point (mg/kg b.w./d)**	**Time weighted average (TWA)**
	Sunflowers	0.2	2	7	10	24.1	0.53
Screening step:							
Reproductive risk assessment screening step	**Indicator species**	**Shortcut value**	**Daily Dietary Dose (single)**	**MAF mean**	**Daily Dietary Dose (Multiple)**	**TER**	**Please perform first tier risk assessment (see below)**
	Small omnivorous bird	64.8	12.96	1.6	10.99	**2.2**	
First Tier Risk Assessment:							
Calculate TER for each generic focal species	**Crop**	**Generic focal species**			**Short cut value**	**TER**	
	Sunflower Late (Flowering, seed ripening) BBCH 61-92	Small granivorous/insectivorous bird "bunting" Small seeds 100% crop seeds			10.0	14.2	No refinement required

TER value in **bold** is below the relevant trigger

The conclusions for the first tier dietary risk assessments for each of the active substances are as follows: Acceptable acute and reproductive risks for birds were shown at the screening and/or tier 1 levels for both mefentrifluconazole and boscalid. No higher tier dietary risk assessments are necessary.

zRMS comments:

Provided above evaluation is agreed by the zRMS. Based on performed calculations, acceptable long-term dietary risk to birds from particular active compounds may be concluded.

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 762 02 F because this formulation contains more than one active substance.

Following Appendix B (step 1) in EFSA/2009/1438 a surrogate LD_{50} = 1718.7 ~~1709.3~~ mg/kg b.w. is calculated based on the assumption of dose additivity (Table 9.3-16). 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 $\leq 10\%$ as in that case the risk is covered by the assessment for that active substance. For BAS 762 02 F this does not apply because the deviation for both active substances is more than 10% (Table 9.3-16).

Table 9.3-16: 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 b.w.]	Tox per fraction (a.s.)	Deviation tox per fraction (a.s.) and tox per fraction (mix) [%]
Mefentrifluconazole	100	0.33	816	0.00041	**1718.7** ~~1709.3~~	2472.7 ~~2448~~	44 ~~43~~
Boscalid	200	0.67	3776	0.00018		5635.8 ~~5664~~	228 ~~231~~

Since there are no experimental data on the acute toxicity of formulation BAS 762 02 F to birds (see justification in point 9.2.1.), the surrogate LD_{50} = 1718.7 ~~1709.3~~ mg/kg b.w. will be the toxicity endpoint used in the acute risk assessment below.

zRMS comments:

The Applicants' calculations could not be reproduced by the zRMS and respective corrections were thus introduced in Table 9.3-16 above. Changes have negligible impact on the outcome of evaluation of the combined risk assessment.

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

The potential exposure to the combined substances follows step 4 of Appendix B of EFSA/2009/1438. The maximum application rate of formulation BAS 762 02 F is 1.0 L product/ha (corresponding to 0.1 kg/ha mefentrifluconazole and 0.2 kg/ha boscalid for the use in cereals, in oilseed rape and in sunflower); applying the concept for dose additivity to the exposure calculations results in a combined application rate of 0.30 kg virtual compound/ha.

The dietary TER acute values for the screening step presented in Table 9.3-17 (cereals),

Table 9.**3-18** (oilseed rape) and
Table 9.**3-19** (sunflower) is above the trigger of 10. Therefore, the acute risk to birds from combined effects of the two active substances in BAS 762 02 F is acceptable.

Table 9.3-17: Screening step of the acute risk for birds due to the use of BAS 762 02 F in the crop group "cereals"- exposure to the combined active substances

Data from Data_Entry worksheet	**Crop**	**Application rate (kg a.s./ha)**	**Number of applications**	**Application Interval**	**DT_{50}**	**LD_{50}**	
	Cereals	0.3	1	365	10.0	1718.7 ~~1709.3~~	
Screening step:							
Acute risk assessment screening step	**Indicator species**	**Shortcut value**	**Daily Dietary Dose (single)**	**MAF (90)**	**Daily Dietary Dose (Multiple)**	**TER**	No refinement step required
	Small omnivorous bird	158.8	47.64	1.0	47.64	36.1 ~~35.9~~	

Table 9.3-18: Screening step of the acute risk for birds due to the use of BAS 762 02 F in the crop group "oilseed rape"- exposure to the combined active substances

Data from Data_Entry worksheet	**Crop**	**Application rate (kg a.s./ha)**	**Number of applications**	**Application Interval**	**DT_{50}**	**LD_{50}**	
	Oilseed rape	0.3	1	365	10.0	1718.7 ~~1709.3~~	
Screening step:							
Acute risk assessment screening step	**Indicator species**	**Shortcut value**	**Daily Dietary Dose (single)**	**MAF (90)**	**Daily Dietary Dose (Multiple)**	**TER**	No refinement step required
	Small omnivorous bird	158.8	47.64	1.0	47.64	36.1 ~~35.9~~	

Table 9.3-19: Screening step of the acute risk for birds due to the use of BAS 762 02 F in the crop group "sunflower"- exposure to the combined active substances

Data from Data_Entry worksheet	**Crop**	**Application rate (kg a.s./ha)**	**Number of applications**	**Application Interval**	**DT_{50}**	**LD_{50}**	
	Sunflowers	0.3	2	7	10.0	1718.7 ~~1709.3~~	
Screening step:							
Acute risk assessment screening step	**Indicator species**	**Shortcut value**	**Daily Dietary Dose (single)**	**MAF (90)**	**Daily Dietary Dose (Multiple)**	**TER**	No refinement step required
	Small omnivorous bird	158.8	47.64	1.4	66.70	25.8 ~~25.6~~	

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

In conclusion, the risk assessment approach for the combined toxicity of the active substances (virtual compound) for the acute exposure resulted in a TER value at the screening step above the trigger of 10 for acceptability of effects. Therefore, the acute dietary risk to birds from the proposed uses of BAS 762 02 F is considered acceptable.

zRMS comments:

Provided above evaluation is agreed by the zRMS with some minor corrections resulting from difference in LD_{50mix} calculated by the zRMS. Based on performed calculations, acceptable acute dietary risk to birds from the mixture may be concluded.

Combined reproductive toxicity

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

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

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

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

The $TER_{LT\ combi}$ values are calculated based on screening step and tier 1 TER values for the active substances. The calculations of the cumulative ecotoxicological effects are summarized in Table 9.3-20 (cereals), Table 9.3-21 (oilseed rape) and Table 9.3-22 (sunflower).

Table 9.3-20: Combined reproductive toxicity risk assessment for birds for the crop group "cereals"

Crop scenario and/or indicator species		TER_{LT} [1)] mefentrifluconazole	TER_{LT} [1)] boscalid	$TER_{LTcombi}$	Trigger
Reproductive (screening step)					
Cereals	Small omnivorous bird	7.4	**3.5**	**2.4**	5
Reproductive (tier 1)					
Cereals BBCH 30 -39	Small omnivorous bird "lark"	88.4	42.1	28.5	5
Cereals BBCH ≥ 40	Small omnivorous bird "lark"	144.7	68.9	46.7	5

TER values shown in **bold** fall below the relevant trigger
1) Reproductive TER values are presented in Table 9.3-10 and Table 9.3-11.

Table 9.3-21: Combined reproductive toxicity risk assessment for birds for the crop group "oilseed rape"

Crop scenario and/or indicator species		TER_{LT} [1)] mefentrifluconazole	TER_{LT} [1)] boscalid	$TER_{LTcombi}$	Trigger
Reproductive (screening step) [1)]					
Oilseed rape	Small omnivorous bird	7.4	**3.5**	**2.4**	5
Reproductive (tier 1) [1)]					
Oilseed rape BBCH ≥ 40	medium herbivorous/granivorous bird "pigeon"	530.4	252.6	171.1	5
Oilseed rape BBCH ≥ 40	Small omnivorous bird "lark"	176.8	84.2	57.0	5
Oilseed rape late – late (with seeds) (BBCH 30-99)	Small insectivorous bird "dunnock"	176.8	84.2	57.0	5

TER values shown in **bold** fall below the relevant trigger
1) Reproductive TER values are presented in Table 9.3-12 and Table 9.3-13.

Table 9.3-22: Combined reproductive toxicity risk assessment for birds for the crop group "sunflower"

Crop scenario and/or indicator species		TER_{LT} [1)] mefentrifluconazole	TER_{LT} [1)] boscalid	$TER_{LTcombi}$	Trigger
Reproductive (screening step) [1)]					
Sunflower	Small omnivorous bird	**4.6**	**2.2**	**1.5**	5
Reproductive (tier 1) [1)]					
Sunflower Late (Flowering, seed ripening) BBCH 61-92	Small granivorous/insectivorous bird "bunting"	29.8	14.2	9.6	5

TER values shown in **bold** fall below the relevant trigger
1) Reproductive TER values are presented in Table 9.3-14 and

Table 9.**3-15**.

The $TER_{LT\ combi}$ values for the relevant scenarios are above the trigger value of 5 at tier I. Thus, it can be concluded that the reproductive risk for birds for the combined exposure to the two active substances in the application of BAS 762 02 F according to good agricultural practice is low and acceptable.

zRMS comments:

Provided above evaluation is agreed by the zRMS. Based on performed calculations, acceptable long-term dietary risk to birds from the mixture may be concluded.

9.3.2.2 Higher-tier risk assessment

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

9.3.2.3 Drinking water exposure

Leaf scenario
Since BAS 762 02 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 b.w./d) does not exceed 50 in the case of less sorptive substances (Koc < 500 L/kg) or 3000 in the case of more sorptive substances (Koc ≥ 500 L/kg).
The ratio calculations for effective application rate to relevant endpoint are detailed in Table 9.3-23 and

Table 9.**3-24** and are based on the use in sunflower as worst-case with regard to the resulting AR_{eff}. The ratios for acute and reproductive endpoints for mefentrifluconazole (0.2 and 7.8, respectively) and for boscalid (0.1 and 16.3, respectively) do not exceed the threshold value of 3000 for both active substances, 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 9.3-23: 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	EFSA Journal 2018; 16(7): 5379
DT_{50} (soil) (normalised geometric mean, field) [days]	200	EFSA Journal 2018; 16(7): 5379
Number of applications	2	Chapter 9.1
Interval [days]	7	Chapter 9.1
MAF_m [1)]	1.98	--
Max use rate [g/ha]	100	Chapter 9.1
AR_{eff} [g/ha] [2)]	198.0	--
LD_{50} [mg/kg b.w.]	816	Chapter 9.2.1
Ratio (acute) [3)]	0.2	--
NO(A)EL [mg/kg b.w./d]	25.3	Chapter 9.2.1
Ratio (repro) [3)]	7.8	--
Trigger	3000	--
Drinking water assessment required [Yes/No]	no	--

1) $MAFm = (1-e^{-nki}) / (1-e^{-ki})$ 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_{mean}
3) Ratio of AR_{eff} and relevant toxicity endpoint

Table 9.3-24: Assessment of the risk for birds due to exposure to boscalid via contaminated drinking water in puddles

Parameter	Boscalid	Reference
K_{oc} (arithmetic mean) [L/kg]	772	BASF DocID 1998/10513
DT_{50} (soil) (normalised geometric mean, lab) [days]	130	Chapter 8.9
Number of applications	2	Chapter 9.1
Interval [days]	7	Chapter 9.1
MAF_m [1)]	1.96	--
Max use rate [g/ha]	200	Chapter 9.1
AR_{eff} [g/ha] [2)]	392.0	--
LD_{50} [mg/kg b.w.]	3776	Chapter 9.2.1
Ratio (acute) [3)]	0.1	--
NO(A)EL [mg/kg b.w./d]	24.1	Chapter 9.2.1
Ratio (repro) [3)]	16.3	--
Trigger	3000	--
Drinking water assessment required [Yes/No]	No	--

1) MAFm = $(1-e^{-nki}) / (1-e^{-ki})$ 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_{mean}
3) Ratio of AR_{eff} and relevant toxicity endpoint

In conclusion, the risk to birds via drinking water from the intended use of BAS 762 02 F according to the proposed use pattern is acceptable.

zRMS comments:

Drinking water risk assessment presented in Tables 9.3-23 and 9.3-24 above is agreed by the zRMS. Since ratios between effective application rates and toxicity endpoints are below the respective trigger, acceptable risk may be concluded with no need for further calculations.

It is noted that the drinking water risk assessment for metabolites was not performed by the Applicant.

Since no relevant soil metabolites are formed from boscalid, the drinking water risk assessment is not triggered.

Mefentrifluconazole forms one relevant soil metabolite (1,2,4-triazole) and respective calculations are provided below. It should be noted that metabolite 1,2,4-triazole was observed in soil at 5.1% at a single time point at 90 d in a single soil. Nevertheless, as no clear decline was observed between 90 d and 120 d (test termination) and this compound is considered to be toxicologically relevant, the drinking water risk assessment was performed for precautionary reason. The pseudo-application rate was calculated with consideration of maximum occurrence of 5.1% and molar ratio of 0.174.

Parameter	Boscalid	Reference
K_{oc} (arithmetic mean) [L/kg]	89	EFSA Journal 2018;16(87):5379
DT_{50} (soil) (geometric mean, lab, slow phase) [days]	67.1	EFSA Journal 2018;16(87):5379
Number of applications	2	Chapter 9.1
Interval [days]	7	Chapter 9.1
MAF_m	1.93	--
Max use rate [g/ha]	0.887	Chapter 9.1
AR_{eff} [g/ha]	1.71	--
LD_{50} [mg/kg b.w.]	377.6	10 times toxicity of the parent
Ratio (acute) [3)]	0.005	--
NO(A)EL [mg/kg b.w./d]	2.41	10 times toxicity of the parent
Ratio (repro) [3)]	0.71	--
Trigger	50	--
Drinking water assessment required [Yes/No]	No	--

Performed above calculations demonstrated acceptable risk from 1,2,4-triazole in drinking water.

9.3.2.4 Effects of secondary poisoning

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

The log P_{ow} of the active substance boscalid is 2.96 (BASF DocID 1998/11082), hence roughly 3.0, which triggers an assessment of the potential risk from secondary poisoning.

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 9.3-26 and Table 9.3-25, the TER_{LT} for mefentrifluconazole and boscalid exceeds the relevant trigger of 5 for acceptability of effects, indicating an acceptable risk to earthworm-eating birds via secondary poisoning.

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

Parameter	Mefentrifluconazole	Reference
PEC_{soil} (accu) [mg/kg soil] [1)]	0.229	Chapter 8.7
K_{ow}	2512 ~~2350~~	BASF DocID 2013/1382370
K_{oc} (geometric mean) [L/kg]	3455.6	EFSA Journal 2018; 16(7): 5379
f_{oc} (default)	0.02	EFSA/2009/1438
BCF [2)]	0.448 ~~0.420~~	--
PEC_{worm} [mg/kg] [3)]	0.103 ~~0.096~~	--
Daily dose [mg/kg b.w./d] [4)]	0.108 ~~0.101~~	--
NO(A)EL [mg/kg b.w./d]	25.3	See chapter 9.2.1
TER_{LT} [5)]	234.3 ~~250.4~~	--

1) Worst case PEC_{soil} (accu) value was calculated for an application scenario of 2 x 100 g a.s./ha with 7-day interval in sunflower. For details see chapter 8.7.
2) Bioconcentration factor (BCF) = (0.84 + 0.012 x K_{ow}) / f_{oc} x K_{oc}
3) PEC_{worm} = PEC_{soil} x BCF
4) Daily dose = 1.05 x PEC_{worm}
5) TER_{LT} = NO(A)EL / Daily dose.

Table 9.3-26: Assessment of the risk for earthworm-eating birds due to exposure to boscalid via bioaccumulation in earthworms (secondary poisoning) for the intended use

Parameter	Boscalid	Reference
PEC_{soil} (accu) [mg/kg soil] [1)]	0.422 ~~0.365~~	Chapter 8.7
K_{ow}	912 ~~915~~	BASF DocID 1998/11082
K_{oc} (arithmetic mean) [L/kg]	772	BASF DocID 1998/10513
f_{oc} (default)	0.02	EFSA/2009/1438
BCF [2)]	0.763 ~~0.766~~	--
PEC_{worm} [mg/kg] [3)]	0.322 ~~0.279~~	--
Daily dose [mg/kg b.w./d] [4)]	0.338 ~~0.293~~	--
NO(A)EL [mg/kg b.w./d]	24.1	See chapter 9.2.1
TER_{LT} [5)]	71.3 ~~82.1~~	--

1) Worst case PEC_{soil} (accu) value was calculated for an application scenario of 2 x 200 g a.s./ha with 7-day interval in sunflower. For details see chapter 8.7.
2) Bioconcentration factor (BCF) = (0.84 + 0.012 x K_{ow}) / f_{oc} x K_{oc}
3) PEC_{worm} = PEC_{soil} x BCF
4) Daily dose = 1.05 x PEC_{worm}
5) TER_{LT} = NO(A)EL / Daily dose.

Risk assessment for fish-eating birds via secondary poisoning

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

As shown in the following Table 9.3-28 and Table 9.3-27, the TER_{LT} for mefentrifluconazole and boscalid exceeds the relevant trigger of 5 for acceptability of effects, indicating an acceptable risk to fish-eating birds via secondary poisoning.

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

Parameter	Mefentrifluconazole	Reference
$PEC_{sw,}$ (twa, 21 days) [mg/L] [1)]	1.395×10^{-3}	Chapter 8.9
BCF fish (max. worst case)	385	EFSA Journal 2018; 16(7): 5379
PEC_{fish} [mg/kg] [2)]	0.537	--
Daily dose [mg/kg b.w./d] [3)]	0.085	--
NO(A)EL [mg/kg b.w./d]	25.3	See Chapter 9.2.1
TER_{LT} [4)]	296.3	--

1) PECsw (21 d twa) value calculated for a multiple application scenario of 2 x 100 g a.s./ha sunflower from FOCUS Step 2 (North Europe) as worst-case. For details see chapter 8.9 and Appendix A 3.2 of Section 8.
2) $PEC_{fish} = PEC_{sw,}$ (twa, 21 days) x BCF
3) Daily dose = 0.159 x PEC_{fish}
4) TER_{LT} = NO(A)EL / Daily dose.

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

Parameter	Boscalid	Reference
$PEC_{sw,}$ (twa, 21 days) [mg/L] [1)]	7.858×10^{-3}	Chapter 8.9
BCF fish (max. worst case)	92	Review Report for the active substance boscalid, SANCO/3919 /2007-rev. 5, 17.01.08
PEC_{fish} [mg/kg] [2)]	0.723	--
Daily dose [mg/kg b.w./d] [3)]	0.115	--
NO(A)EL [mg/kg b.w./d]	24.1	See Chapter 9.2.1
TER_{LT} [4)]	209.7	--

1) The PECsw (21 d twa) value calculated for a multiple application scenario of 2 x 200 g a.s./ha sunflower from FOCUS Step 2 (North Europe) as worst-case. For details see chapter 8.9 and Appendix A 3.2 of Section 8.
2) $PEC_{fish} = PEC_{sw,}$ (twa, 21 days) x BCF
3) Daily dose = 0.159 x PEC_{fish}
4) TER_{LT} = NO(A)EL / Daily dose.

zRMS comments:

The evaluation of the risk of secondary poisoning for earthworm- and fish-eating birds exposed to mefentrifluconazole and boscalid is in general agreed by the zRMS with some minor corrections resulting from different Kow calculated by the zRMS on the basis of log Pow and different PEC_{SOIL} value agreed in area of Section 8 for boscalid. These corrections have no impact on the derived conclusions and are introduced for consistency.

Acceptable risk of secondary poisoning could be concluded on the basis of performed calculations.

Neither of mefentrifluconazole metabolites triggered the evaluation of the risk of secondary poisoning due to log Pow <3 (see EFSA Journal 2018;16(7):5379.

No relevant boscalid metabolites were observed in soil and aquatic systems.

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).

No evidence was found for potential accumulation of boscalid in animal tissue (Review report for the active substance boscalid. Appendix II, endpoints and related information. 1. Toxicology and metabolism 17 January 2008).
Since the bioaccumulation potential of mefentrifluconazole and boscalid is low no further assessment on biomagnification is required**.**

zRMS comments:

Since acceptable risk of secondary poisoning to fish- and earthworm-eating birds could be concluded for both active substances, the potential for biomagnification in terrestrial food chains is expected to be low.

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 birds from the application of BAS 762 02 F according to good agricultural practice is acceptable.

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

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

9.4.1 Toxicity data

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

Active substances

The selection of studies and endpoints for the risk assessment of mefentrifluconazole (Table 9.4-1) and boscalid (

Table 9.**4-2**) is in line with the results of the EU review process. Justifications are provided below.

Table 9.4-1: Mefentrifluconazole (BAS 750 F): Endpoints and effect values relevant for the risk assessment for mammals

Species	Substance	Exposure System	Results	Reference
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 9.4-2: Boscalid (BAS 510 F): Endpoints and effect values relevant for the risk assessment for mammals

Species	Substance	Exposure System	Results	Reference
Rat	Boscalid	Oral, 1 d Acute	LD_{50} > 5000 mg/kg b.w.	EFSA Review Report SANCO/3919/2007-rev. 5 [1998/10643]
Rat	Boscalid	Dietary Reproductive toxicity Two-generation study	$NOEL_{Reproduction}$ = 667 mg/kg b.w./d $NOEL_{Parents}$ = 6.7 mg/kg b.w./d $NOEL_{Offspring}$ = 67 mg/kg b.w./d Ecologically relevant: NOEL = 67 mg/kg b.w./d	EFSA Review Report SANCO/3919/2007-rev. 5 and DAR 2002 amendment 1 [2001/1000117]
Rat	Boscalid	Oral Developmental toxicity	$NOEL_{Maternal}$ = 1000 mg/kg b.w./d $NOEL_{Developmental}$ = 300 mg/kg b.w./d	EFSA Review Report SANCO/3919/2007-rev. 5 and DAR 2002 [2000/1015001]
Rabbit	Boscalid	Oral Developmental toxicity	$NOEL_{Maternal}$ = 100 mg/kg b.w./d $NOEL_{Developmental}$ = 300 mg/kg b.w./d	EFSA Review Report SANCO/3919/2007-rev. 5 and DAR 2002 [2000/1013425]
Endpoint used for acute assessment	**Boscalid**	**Oral, 1 d Acute**	**LD_{50} > 5000 mg/kg b.w.**	**EFSA Review Report SANCO/3919/2007 [1998/10643]**
Endpoint used for reproductive assessment	**Boscalid**	**Oral Developmental toxicity**	**NOEL = 67 mg/kg b.w./d**	**EFSA Review Report SANCO/3919/2007-rev. 5 and DAR 2002 [2000/1013425]**

zRMS comments:

Mammalian toxicity data for mefentrifluconazole and boscalid are in line with the EU agreed endpoints reported in EFSA Journal 2018;16(7):5379 and EU Review Report SANCO/3919/2007-rev. 5, respectively.

Metabolites

Metabolites of mefentrifluconazole

See section 9.2.1 in the bird chapter.

Metabolites of boscalid

See section 9.2.1 in the bird chapter.

zRMS comments:

Information regarding metabolites of both active substances provided in point 9.2.1 of this document has been agreed by the zRMS. Overall, the risk from relevant plant metabolites is considered to be covered by evaluation performed for the particular active compounds.

Formulation toxicity

For toxicological classification and labeling purposes, an acute oral toxicity study with BAS 762 02 F in rats was carried out according the toxic class method described in OECD 423 (BASF DocID 2019/2034516; see chapter 6.3 and Appendix 2 of chapter 6). No mortality occurred, resulting in LD_{50} > 5000 mg formulation/kg b.w. and indicating a low toxicity of the formulation and no increased toxicity compared to the active substances.

zRMS comments:

Study on acute toxicity of BAS 762 02 F to rats has been evaluated and agreed by the zRMS toxicology expert. Based on the derived LD_{50} of >5000 mg product/kg bw no increased toxicity of the product comparing to particular active compounds is anticipated.
For details of the study evaluation, please refer to the Core Assessment, Par B, Section 6.

9.4.2 Justification for new endpoints

Mefentrifluconazole

Acute – Not applicable. Endpoint is EU agreed.

Reproductive – Not applicable. Endpoint is EU agreed.

Boscalid

Acute - Not applicable. Endpoint is EU agreed.

Reproductive - Not applicable. Endpoint is EU agreed.

9.4.3 Risk assessment for spray applications

Proposed use pattern for the risk assessments

The proposed use pattern for the use of BAS 762 02 F is summarized in

Table 9.**3-3**. The detailed use pattern table is presented at the beginning of the ecotoxicology chapter (section 9.1).

Table 9.4-3: Proposed use pattern

Crop	Crop group according to EFSA/2009/1438	Application time (BBCH growth stage)	Number of applications	Interval between applications [d]	Application rate per application		
					Mefentrifluconazole [kg/ha]	Boscalid [kg/ha]	BAS 762 02 H [L/ha]
Wheat (winter, spring)	Cereals	30-49	1	--	0.1	0.2	1.0
OSR (winter, spring) [1)]	OSR	57-75	1	--	0.1	0.2	1.0
Sunflower	Sunflower	31-69	2	7	0.1	0.2	1.0

1) This scenario covers the uses listed in the GAP as “other minor oilseeds”.

9.4.3.1 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).

Dietary risk assessment for the active substances

Acute risk assessment

The TER_A values are presented in Table 9.3-4 (cereals), Table 9.4-6 (oilseed rape) and Table 9.4-8 (sunflowers) for mefentrifluconazole and in Table 9.4-5 (cereals),

Table 9.**4-7** (oilseed rape) and Table 9.4-9 (sunflowers) for boscalid. All the TER_A values at the screening step are above the relevant trigger of 10 for acceptability of acute effects.

Table 9.4-4: Mefentrifluconazole: Screening step calculations of the acute risk for mammals due to the use of BAS 762 02 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	1	365	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 required
	Small herbivorous mammal	118.4	11.84	1.0	11.84	>168.9	

Table 9.4-5: Boscalid: Screening step calculations of the acute risk for mammals due to the use of BAS 762 02 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	0.2	1	365	10.0	>5000	
Screening step:							
Acute risk assessment screening step	**Indicator species**	**Shortcut value**	**Daily Dietary Dose (single)**	**MAF (90)**	**Daily Dietary Dose (Multiple)**	**TER**	No refinement required
	Small herbivorous mammal	118.4	23.68	1.0	23.68	>211.1	

Table 9.4-6: Mefentrifluconazole: Screening step calculations of the acute risk for mammals due to the use of BAS 762 02 F for the crop group "oilseed rape"

Data from Data_Entry worksheet	**Crop**	**Application rate (kg a.s./ha)**	**Number of applications**	**Application Interval**	**DT_{50}**	**LD_{50}**	
	Oilseed rape	0.1	1	365	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 required
	Small herbivorous mammal	118.4	11.84	1.0	11.84	>168.9	

Table 9.4-7: Boscalid: Screening step calculations of the acute risk for mammals due to the use of BAS 762 02 F for the crop group "oilseed rape"

Data from Data_Entry worksheet	**Crop**	**Application rate (kg a.s./ha)**	**Number of applications**	**Application Interval**	**DT_{50}**	**LD_{50}**	
	Oilseed rape	0.2	1	365	10.0	>5000	
Screening step:							
Acute risk assessment screening step	**Indicator species**	**Shortcut value**	**Daily Dietary Dose (single)**	**MAF (90)**	**Daily Dietary Dose (Multiple)**	**TER**	No refinement required
	Small herbivorous mammal	118.4	23.68	1.0	23.68	>211.1	

Table 9.4-8: Mefentrifluconazole: Screening step calculations of the acute risk for mammals due to the use of BAS 762 02 F for the crop group "sunflower"

Data from Data_Entry worksheet	**Crop**	**Application rate (kg a.s./ha)**	**Number of applications**	**Application Interval**	**DT_{50}**	**LD_{50}**	
	Sunflower	0.1	2	7	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 required
	Small herbivorous mammal	118.4	11.84	1.4	16.58	>120.7	

Table 9.4-9: Boscalid: Screening step calculations of the acute risk for mammals due to the use of BAS 762 02 F for the crop group "sunflower"

Data from Data_Entry worksheet	**Crop**	**Application rate (kg a.s./ha)**	**Number of applications**	**Application Interval**	**DT_{50}**	**LD_{50}**	
	Sunflower	0.2	2	7	10.0	>5000.0	
Screening step:							
Acute risk assessment screening step	**Indicator species**	**Shortcut value**	**Daily Dietary Dose (single)**	**MAF (90)**	**Daily Dietary Dose (Multiple)**	**TER**	No refinement required
	Small herbivorous mammal	118.4	23.68	1.4	33.15	>150.8	

zRMS comments:

Provided above evaluation is agreed by the zRMS. Based on performed calculations, acceptable acute dietary risk to mammals from particular active compounds may be concluded.

Reproductive risk assessment

The dietary TER reproductive values for the screening step and tier 1 risk assessments are presented in Table 9.4-10 (cereals), Table 9.4-12 (oilseed rape) and

Table 9.**4-14** (sunflowers) for mefentrifluconazole and in Table 9.4-11 (cereals), Table 9.4-13 (oilseed rape) and Table 9.4-15 (sunflowers) for boscalid.

All the TER_{LT} values for mefentrifluconazole and boscalid, are above the relevant trigger of 5 for acceptability of reproductive effects at the screening step or at tier 1.

Table 9.4-10: Mefentrifluconazole: Screening step and tier 1 calculations of the long-term/reproductive risk for mammals due to the use of BAS 762 02 F for the crop group "cereals"

Data from Data_Entry worksheet	**Crop**	**Application rate (kg a.s./ha)**	**Number of applications**	**Application Interval**	**DT_{50}**	**Reproductive End Point (mg/kg b.w./d)**	**Time weighted average (TWA)**
	Cereals	0.1	1	365	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**	No refinement required
	Small herbivorous mammal	48.3	4.83	1.0	2.56	5.9	
First Tier Risk Assessment: [1)]							
Calculate TER for each generic focal species	**Crop**	**Generic focal species**			**Short cut value**	**TER**	No refinement required
	Cereals BBCH ≥ 20	Small insectivorous mammal "shrew" ground dwelling invertebrates with interception 100% ground arthropods			1.9	149.0	
	Cereals BBCH 30 - 39	Small omnivorous mammal "mouse" Combination (invertebrates with interception) 25% weeds 50% weed seeds 25% ground arthropods			3.9	72.6	
	Cereals BBCH ≥ 40	Small herbivorous mammal "vole Grass + cereals 100% grass			21.7	13.0	
	Cereals BBCH ≥ 40	Small omnivorous mammal "mouse" Combination (invertebrates with interception) 25% weeds 50% weed seeds 25% ground arthropods			2.3	123.1	

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

Table 9.4-11: Boscalid: Screening step and tier 1 calculations of the long-term/reproductive risk for mammals due to the use of BAS 762 02 F for the crop group "cereals"

Data from Data_Entry worksheet	**Crop**	**Application rate (kg a.s./ha)**	**Number of applications**	**Application Interval**	**DT_{50}**	**Reproductive End Point (mg/kg b.w./d)**	**Time weighted average (TWA)**
	Cereals	0.2	1	365	10	67.0	0.53
Screening step:							
Reproductive risk assessment screening step	**Indicator species**	**Shortcut value**	**Daily Dietary Dose (single)**	**MAF mean**	**Daily Dietary Dose (Multiple)**	**TER**	No refinement required
	Small herbivorous mammal	48.3	9.66	1.0	5.12	13.1	
First Tier Risk Assessment: [1)]							
Calculate TER for each generic focal species	**Crop**	**Generic focal species**			**Short cut value**	**TER**	No refinement required
	Cereals BBCH ≥ 20	Small insectivorous mammal "shrew" ground dwelling invertebrates with interception 100% ground arthropods			1.9	332.7	
	Cereals BBCH 30 - 39	Small omnivorous mammal "mouse" Combination (invertebrates with interception) 25% weeds 50% weed seeds 25% ground arthropods			3.9	162.1	
	Cereals BBCH ≥ 40	Small herbivorous mammal "vole Grass + cereals 100% grass			21.7	29.1	

	Cereals BBCH ≥ 40	Small omnivorous mammal "mouse" Combination (invertebrates with interception) 25% weeds 50% weed seeds 25% ground arthropods	2.3	274.8	

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

Table 9.4-12: Mefentrifluconazole: Screening step and tier 1 calculations of the long-term/reproductive risk for mammals due to the use of BAS 762 02 F for the crop group "oilseed rape"

Data from Data_Entry worksheet	**Crop**	**Application rate (kg a.s./ha)**	**Number of applications**	**Application Interval**	**DT_{50}**	**Reproductive End Point (mg/kg b.w./d)**	**Time weighted average (TWA)**
	Oilseed rape	0.1	1	365	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**	No refinement required
	Small herbivorous mammal	48.3	4.83	1.0	2.56	5.9	
First Tier Risk Assessment: 1)							
Calculate TER for each generic focal species	**Crop**	**Generic focal species**			**Short cut value**	**TER**	No refinement required
	Oilseed rape BBCH ≥ 20	Small insectivorous mammal "shrew" ground dwelling invertebrates with interception 100% ground arthropods			1.9	149.0	
	Oilseed rape BBCH ≥ 40	Small herbivorous mammal "vole Grass + cereals 100% grass			18.1	15.6	
	Oilseed rape BBCH ≥ 40	Small omnivorous mammal "mouse" Combination (invertebrates with interception) 25% weeds 50% weed seeds 25% ground arthropods			1.9	149.0	
	Oilseed rape All season	Large herbivorous mammal "lagomorph" Non-grass herbs 100% crop leaves			14.3	19.8	

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

Table 9.4-13: Boscalid: Screening step and tier 1 calculations of the long-term/reproductive risk for mammals due to the use of BAS 762 02 F for the crop group "oilseed rape"

Data from Data_Entry worksheet	**Crop**	**Application rate (kg a.s./ha)**	**Number of applications**	**Application Interval**	**DT_{50}**	**Reproductive End Point (mg/kg b.w./d)**	**Time weighted average (TWA)**
	Oilseed rape	0.2	1	365	10	67.0	0.53
Screening step:							
Reproductive risk assessment screening step	**Indicator species**	**Shortcut value**	**Daily Dietary Dose (single)**	**MAF mean**	**Daily Dietary Dose (Multiple)**	**TER**	No refinement required
	Small herbivorous mammal	48.3	9.66	1.0	5.12	13.1	
First Tier Risk Assessment: 1)							
Calculate TER for each generic focal species	**Crop**	**Generic focal species**			**Short cut value**	**TER**	No refinement required
	Oilseed rape BBCH ≥ 20	Small insectivorous mammal "shrew" ground dwelling invertebrates with interception 100% ground arthropods			1.9	332.7	
	Oilseed rape BBCH ≥ 40	Small herbivorous mammal "vole Grass + cereals 100% grass			18.1	34.9	
	Oilseed rape BBCH ≥ 40	Small omnivorous mammal "mouse" Combination (invertebrates with interception) 25% weeds 50% weed seeds 25% ground arthropods			1.9	332.7	

	Oilseed rape All season	Large herbivorous mammal “lagomorph” Non-grass herbs 100% crop leaves	14.3	44.2	

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

Table 9.4-14: Mefentrifluconazole: Screening step and tier 1 calculations of the long-term/reproductive risk for mammals due to the use of BAS 762 02 F for the crop group "sunflower"

Data from Data_Entry worksheet	Crop	Application rate (kg a.s./ha)	Number of applications	Application Interval	DT_{50}	Reproductive End Point (mg/kg b.w./d)	Time weighted average (TWA)
	Sunflower	0.1	2	7	10	15.0	0.53
Screening step:							
Reproductive risk assessment screening step	**Indicator species**	**Shortcut value**	**Daily Dietary Dose (single)**	**MAF mean**	**Daily Dietary Dose (Multiple)**	**TER**	**Please perform first tier risk assessment (see below)**
	Small herbivorous mammal	48.3	4.83	1.6	4.10	**3.7**	
First Tier Risk Assessment:							
Calculate TER for each generic focal species	**Crop**	**Generic focal species**			**Short cut value**	**TER**	No refinement required
	Sunflower BBCH 20 - 39	Large herbivorous mammal "lagomorph" Non-grass herbs 100% Non-grass herbs			7.2	24.6	
	Sunflower BBCH 20 - 39	Small omnivorous mammal "mouse" Combination (invertebrates without interception) 25% weeds 50% weed seeds 25% ground arthropods			3.9	45.4	
	Sunflower BBCH ≥ 20	Small insectivorous mammal "shrew" ground dwelling invertebrates with interception 100% ground arthropods			1.9	93.1	
	Sunflower BBCH ≥ 40	Large herbivorous mammal "lagomorph" Non-grass herbs 100% Non-grass herbs			3.6	49.1	
	Sunflower BBCH ≥ 40	Small herbivorous mammal "vole Grass + cereals 100% grass			18.1	9.8	
	Sunflower BBCH ≥ 40	Small omnivorous mammal "mouse" Combination (invertebrates without interception) 25% weeds 50% weed seeds 25% ground arthropods			1.9	93.1	

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

Table 9.4-15: Boscalid: Screening step and tier 1 calculations of the long-term/reproductive risk for mammals due to the use of BAS 762 02 F for the crop group "sunflower"

Data from Data_Entry worksheet	Crop	Application rate (kg a.s./ha)	Number of applications	Application Interval	DT_{50}	Reproductive End Point (mg/kg b.w./d)	Time weighted average (TWA)
	Sunflower	0.2	2	7	10	67.0	0.53
Screening step:							
Reproductive risk assessment screening step	**Indicator species**	**Shortcut value**	**Daily Dietary Dose (single)**	**MAF mean**	**Daily Dietary Dose (Multiple)**	**TER**	No refinement required
	Small herbivorous mammal	48.3	9.66	1.6	8.19	8.2	
First Tier Risk Assessment:							
Calculate TER for each generic focal species	**Crop**	**Generic focal species**			**Short cut value**	**TER**	No refinement required
	Sunflower BBCH 20 - 39	Large herbivorous mammal "lagomorph" Non-grass herbs 100% Non-grass herbs			7.2	54.9	
	Sunflower BBCH 20 - 39	Small omnivorous mammal "mouse" Combination (invertebrates without interception) 25% weeds 50% weed seeds 25% ground arthropods			3.9	101.3	
	Sunflower BBCH ≥ 20	Small insectivorous mammal "shrew" ground dwelling invertebrates with interception 100% ground arthropods			1.9	207.9	
	Sunflower BBCH ≥ 40	Large herbivorous mammal "lagomorph" Non-grass herbs 100% Non-grass herbs			3.6	109.7	

	Sunflower BBCH ≥ 40	Small herbivorous mammal "vole Grass + cereals 100% grass	18.1	21.8	
	Sunflower BBCH ≥ 40	Small omnivorous mammal "mouse" Combination (invertebrates without interception) 25% weeds 50% weed seeds 25% ground arthropods	1.9	207.9	

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

The conclusions for the first tier dietary risk assessments for each of the active substances are as follows: Acceptable acute and reproductive risks for mammals were shown at the screening and/or tier 1 levels for both mefentrifluconazole and boscalid. No higher tier dietary risk assessments are necessary.

zRMS comments:

Provided above evaluation is agreed by the zRMS. Based on performed calculations, acceptable long-term dietary risk to mammals from particular active compounds may be concluded.

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 762 02 F because this formulation contains more than one active substance.

Following Appendix B (step 1) in EFSA/2009/1438 a surrogate LD_{50} = 3333.3 mg/kg b.w. is calculated based on the assumption of dose additivity (Table 9.4-16). 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 762 02 F this does not apply because the deviation for both active substances is more than 10% (Table 9.4-16).

Table 9.4-16: 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 b.w.]	**Fraction a.s./ LD_{50} a.s.**	**Surrogate LD_{50}** [mg/kg b.w.]	**Tox per fraction (a.s.)**	**Deviation tox per fraction (a.s.) and tox per fraction (mix) [%]**
Mefentrifluconazole	100	0.33	>2000	0.00017	**3333.3**	6000.0	80
Boscalid	200	0.67	>5000	0.00013		7500.0	125

There is a laboratory study on the acute toxicity of formulation BAS 762 02 F to rats (BASF DocID 2019/2034516). No mortality occurred in the study, which resulted in LD_{50} > 5000 mg/kg b.w. (see 9.3.1).

Appendix B of EFSA/2009/1438 recommends comparing the surrogate LD_{50} with the experimental LD_{50} from formulation testing and to run the risk assessment with the lowest of the two values. However, Appendix B does not provide clear recommendations if for the comparison of the two LD_{50} values and for the calculation of the exposure scenarios only the content of the active substances should be considered as the surrogate LD_{50} is based on toxicity and concentration of active substances while the experimental LD_{50} is based on all components of the formulation. Due to this lack of guidance in Appendix B the most comprehensive approach is adopted by the notifier by presenting the two possible risk assessments, one for the virtual compound and another for the formulation.

zRMS comments:

Calculations performed by the zRMS on unrounded values resulted with slightly higher LD_{50mix} of 3344.5 mg/kg bw. However, Applicants' calculations based on rounded values are confirmed to be correct. Since consideration of rounded values is acceptable, LD_{50mix} provided in Table 9.4-16 is agreed.

The experimentally derived LD_{50} of >5000 mg product/kg bw was agreed in area of Section 6 and demonstrates that no increased toxicity of the product comparing to individual active substances is anticipated.

The approach taken by the Applicant to perform the combined risk assessment with consideration of both, virtual compound and actual formulation toxicity is agreed by the zRMS.

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

The potential exposure to the combined substances follows step 4 of Appendix B of EFSA/2009/1438. The maximum application rate of formulation BAS 762 02 F is 1.0 L product/ha (corresponding to 0.1 kg/ha mefentrifluconazole and 0.2 kg/ha boscalid for the use in cereals, in oilseed rape and in sunflower); applying the concept for dose additivity to the exposure calculations results in a combined application rate of 0.30 kg virtual compound/ha.

The dietary TER acute values for the screening step presented in Table 9.4-17 (cereals), Table 9.4-18 (oilseed rape) and

Table 9.**4-19** (sunflower) are above the trigger of 10. Therefore, the acute risk to mammals from combined effects of the two active substances in BAS 762 02 F is acceptable.

Table 9.4-17: Screening step of the acute risk for mammals due to the use of BAS 762 02 F in "cereals" - exposure to the combined active substances

Data from Data_Entry worksheet	**Crop**	**Application rate (kg virtual compound/ha)**	**Number of applications**	**Application Interval**	**DT_{50}**	**LD_{50}**	
	Cereals	0.3	1	365	10.0	3333.3	
Screening step:							
Acute risk assessment screening step	**Indicator species**	**Shortcut value**	**Daily Dietary Dose (single)**	**MAF (90)**	**Daily Dietary Dose (Multiple)**	**TER**	No refinement required
	Small herbivorous mammal	118.4	35.52	1.0	35.52	93.8	

Table 9.4-18: Screening step of the acute risk for mammals due to the use of BAS 762 02 F in "oilseed rape" - exposure to the combined active substances

Data from Data_Entry worksheet	**Crop**	**Application rate (kg virtual compound/ha)**	**Number of applications**	**Application Interval**	**DT_{50}**	**LD_{50}**	
	Oilseed rape	0.3	1	365	10.0	3333.3	
Screening step:							
Acute risk assessment screening step	**Indicator species**	**Shortcut value**	**Daily Dietary Dose (single)**	**MAF (90)**	**Daily Dietary Dose (Multiple)**	**TER**	No refinement required
	Small herbivorous mammal	118.4	35.52	1.0	35.52	93.8	

Table 9.4-19: Screening step of the acute risk for mammals due to the use of BAS 762 02 F in "sunflower" - exposure to the combined active substances

Data from Data_Entry worksheet	**Crop**	**Application rate (kg virtual compound/ha)**	**Number of applications**	**Application Interval**	**DT_{50}**	**LD_{50}**	
	Sunflower	0.3	2	7	10.0	3333.3	
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	35.52	1.4	49.73	67.0	

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

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

BAS 762 02 F is intended to be used in the crop groups "cereals", "oilseed rape" and "sunflower" with a maximum single application rate of 1.0 L product/ha. Taking into account the density of the formulation of 1.136 g/cm^3, this will result in an application rate of 1.136 kg BAS 762 02 F/ha.

The acute dietary risk assessment for mammals is presented in Table 9.4-20 (cereals), Table 9.4-21(oilseed rape) and in Table 9.4-22 (sunflower). The dietary TER acute values in the screening step risk assessment are above the trigger of 10, therefore the acute risk to mammals from exposure to BAS 762 02 F is acceptable.

Table 9.4-20: BAS 762 02 F: Acute risk assessment mammals for the crop group "cereals"

Data from Data_Entry worksheet	**Crop**	**Application rate (kg formulation/ha)**	**Number of applications**	**Application Interval**	**DT_{50}**	**LD_{50}**	
	Cereals	1.136	1	365	10.0	>5000	
Screening step:							
Acute risk assessment screening step	**Indicator species**	**Shortcut value**	**Daily Dietary Dose (single)**	**MAF (90)**	**Daily Dietary Dose (Multiple)**	**TER**	No refinement required
	Small herbivorous mammal	118.4	134.50	1.0	134.50	>37.2	

Table 9.4-21: BAS 762 02 F: Acute risk assessment mammals for the crop group "oilseed rape"

Data from Data_Entry worksheet	**Crop**	**Application rate (kg formulation/ha)**	**Number of applications**	**Application Interval**	**DT_{50}**	**LD_{50}**	
	Oilseed rape	1.136	1	365	10.0	>5000	
Screening step:							
Acute risk assessment screening step	**Indicator species**	**Shortcut value**	**Daily Dietary Dose (single)**	**MAF (90)**	**Daily Dietary Dose (Multiple)**	**TER**	No refinement required
	Small herbivorous mammal	118.4	134.50	1.0	134.50	>37.2	

Table 9.4-22: BAS 762 02 F: Acute risk assessment mammals for the crop group "sunflower"

Data from Data_Entry worksheet	**Crop**	**Application rate (kg formulation/ha)**	**Number of applications**	**Application Interval**	**DT_{50}**	**LD_{50}**	
	Sunflower	1.136	2	7	10.0	>5000	
Screening step:							
Acute risk assessment screening step	**Indicator species**	**Shortcut value**	**Daily Dietary Dose (single)**	**MAF (90)**	**Daily Dietary Dose (Multiple)**	**TER**	No refinement required
	Small herbivorous mammal	118.4	134.50	1.4	188.30	>26.6	

In conclusion, the two risk assessment approaches (combined toxicity of the active substances and formulation toxicity) have resulted in TER values at the screening level that are above the trigger of 10 for acceptability of effects. Therefore, the acute dietary risk to mammals from BAS 762 02 F is acceptable.

zRMS comments:

Provided above evaluation is agreed by the zRMS. Based on performed calculations, acceptable acute dietary risk to mammals from the mixture may be concluded.

Combined reproductive toxicity

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

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

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

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

The $TER_{LT\ combi}$ values are calculated based on screening step and tier 1 TER values for the active substances. The calculations of the cumulative ecotoxicological effects are summarized in Table 9.4-23 (cereals), Table 9.4-24 (oilseed rape) and Table 9.4-25 (sunflower).

Table 9.4-23: Combined reproductive toxicity risk assessment for mammals for the crop group "cereals"

Crop scenario and/or indicator species		**TER_{LT} [1)] mefentrifluconazole**	**TER_{LT} [1)] boscalid**	**$TER_{LTcombi}$**	**Trigger**
Reproductive (screening step)					
Cereals	Small herbivorous mammal	5.9	13.1	**4.1**	5
Reproductive (tier 1) [1)]					
Cereals BBCH ≥ 20	Small insectivorous mammal "shrew"	149.0	332.7	102.9	5
Cereals BBCH 30 - 39	Small omnivorous mammal "mouse"	72.6	162.1	50.1	5
Cereals BBCH ≥ 40	Small herbivorous mammal "vole	13.0	29.1	9.0	5
Cereals BBCH ≥ 40	Small omnivorous mammal "mouse"	123.1	274.8	85.0	5

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

[1] Reproductive TER values are presented in Table 9.4-10 and Table 9.4-11.

Table 9.4-24: Combined reproductive toxicity risk assessment for mammals for the crop group "oilseed rape"

Crop scenario and/or indicator species		TER_{LT} [1] mefentrifluconazole	TER_{LT} [1] boscalid	$TER_{LTcombi}$	Trigger
Reproductive (screening step)					
Oilseed rape	Small herbivorous mammal	5.9	13.1	**4.1**	5
Reproductive (tier 1) [1]					
Oilseed rape BBCH ≥ 20	Small insectivorous mammal "shrew"	149.0	332.7	102.9	5
Oilseed rape BBCH ≥ 40	Small herbivorous mammal "vole	15.6	34.9	10.8	5
Oilseed rape BBCH ≥ 40	Small omnivorous mammal "mouse"	149.0	332.7	102.9	5
Oilseed rape All season	Large herbivorous mammal "lagomorph"	19.8	44.2	13.7	5

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

[1] Reproductive TER values are presented in Table 9.4-12 and Table 9.4-13.

Table 9.4-25: Combined reproductive toxicity risk assessment for mammals for the crop group "sunflower"

Crop scenario and/or indicator species		TER_{LT} [1] mefentrifluconazole	TER_{LT} [1] boscalid	$TER_{LTcombi}$	Trigger
Reproductive (screening step)					
Sunflower	Small herbivorous mammal	**3.7**	8.2	**2.6**	5
Reproductive (tier 1) [1]					
Sunflower BBCH 20 - 39	Large herbivorous mammal "lagomorph"	24.6	54.9	17.0	5
Sunflower BBCH 20 - 39	Small omnivorous mammal "mouse"	45.4	101.3	31.3	5
Sunflower BBCH ≥ 20	Small insectivorous mammal "shrew"	93.1	207.9	64.3	5
Sunflower BBCH ≥ 40	Large herbivorous mammal "lagomorph"	49.1	109.7	33.9	5
Sunflower BBCH ≥ 40	Small herbivorous mammal "vole	9.8	21.8	6.8	5
Sunflower BBCH ≥ 40	Small omnivorous mammal "mouse"	93.1	207.9	64.3	5

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

[1] Reproductive TER values are presented in

Table 9.**4-14** and Table 9.4-15.

The $TER_{LT\ combi}$ values for the relevant scenarios are above the trigger value of 5 at tier 1. Thus, it can be concluded that the reproductive risk for mammals for the combined exposure to the two active substances in the application of BAS 762 02 F according to good agricultural practice is low and acceptable.

zRMS comments:

Provided above evaluation is agreed by the zRMS. Based on performed calculations, acceptable long-term dietary risk to mammals from the mixture may be concluded.

9.4.3.2 Higher-tier risk assessment

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

9.4.3.3 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 b.w./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 9.4-26 and

Table 9.**4-27** and are based on the use in sunflower as worst-case with regard to the resulting AR_{eff}. The ratios for acute and reproductive endpoints for mefentrifluconazole (< 0.1 and 13.2, respectively) and for boscalid (< 0.8 and 5.9, respectively) do not exceed the threshold values of 3000 for both active substances, thus no specific calculations of exposure for mammals through drinking water for the puddle scenario are necessary. Therefore, a quantitative drinking water risk assessment for the puddle scenario is not triggered.

Table 9.4-26: 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	EFSA Journal 2018; 16(7): 5379
DT_{50} (soil) (normalised geometric mean, field) [days]	200	EFSA Journal 2018; 16(7): 5379
Number of applications	2	Chapter 9.1
Interval [days]	7	Chapter 9.1
MAF_m [1)]	1.98	--
Max use rate [g/ha]	100	Chapter 9.1
AR_{eff} [g/ha] [2)]	198.0	--
LD_{50} [mg/kg b.w.]	>2000	Chapter 9.3.1
Ratio (acute) [3)]	<0.1	--
NO(A)EL [mg/kg b.w./d]	15	Chapter 9.3.1
Ratio (repro) [3)]	13.2	--
Trigger	3000	--
Drinking water assessment required [Yes/No]	no	--

1) $MAFm = (1-e^{-nki}) / (1-e^{-ki})$ 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_{mean}
3) Ratio of AR_{eff} and relevant toxicity endpoint

Table 9.4-27: Assessment of the risk for mammals due to exposure to boscalid via contaminated drinking water in puddles

Parameter	Boscalid	Reference
K_{oc} (arithmetic mean) [L/kg]	772	BASF DocID 1998/10513
DT_{50} (soil) (normalised geometric mean, lab) [days]	130	Chapter 8.9
Number of applications	2	Chapter 9.1
Interval [days]	7	Chapter 9.1
MAF_m [1)]	1.96	--
Max use rate [g/ha]	200	Chapter 9.1
AR_{eff} [g/ha] [2)]	392.0	--
LD_{50} [mg/kg b.w.]	>5000	Chapter 9.3.1
Ratio (acute) [3)]	<0.08	--
NO(A)EL [mg/kg b.w./d]	67	Chapter 9.3.1
Ratio (repro) [3)]	5.9	--
Trigger	3000	--
Drinking water assessment required [Yes/No]	No	--

1) $MAFm = (1-e^{-nki}) / (1-e^{-ki})$ 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_{mean}
3) Ratio of AR_{eff} and relevant toxicity endpoint

In conclusion, the risk to mammals via drinking water from the intended use of BAS 762 02 F according to the proposed use pattern is acceptable.

zRMS comments:

Drinking water risk assessment presented in Tables 9.4-26 and 9.4-27 above is agreed by the zRMS. Since ratios between effective application rates and toxicity endpoints are below the respective trigger, acceptable risk may be concluded with no need for further calculations.

It is noted that the drinking water risk assessment for metabolites was not performed by the Applicant.

Since no relevant soil metabolites are formed from boscalid, the drinking water risk assessment is not triggered.

Mefentrifluconazole forms one relevant soil metabolite (1,2,4-triazole) and respective calculations are provided below. The pseudo-application rate was calculated with consideration of maximum occurrence of 5.1% and molar ratio of 0.174.

Parameter	Boscalid	Reference
K_{oc} (arithmetic mean) [L/kg]	89	EFSA Journal 2018;16(87):5379
DT_{50} (soil) (geometric mean, lab, slow phase) [days]	67.1	EFSA Journal 2018;16(87):5379
Number of applications	2	Chapter 9.1
Interval [days]	7	Chapter 9.1
MAF_m	1.93	--
Max use rate [g/ha]	0.887	Chapter 9.1
AR_{eff} [g/ha]	1.71	--
LD_{50} [mg/kg b.w.]	200	10 times toxicity of the parent
Ratio (acute) [3)]	0.009	--
NO(A)EL [mg/kg b.w./d]	1.5	10 times toxicity of the parent
Ratio (repro) [3)]	1.14	--
Trigger	50	--
Drinking water assessment required [Yes/No]	No	--

Performed above calculations demonstrated acceptable risk from 1,2,4-triazole in drinking water.

9.4.3.4 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.

The log P_{ow} of the active substance boscalid is 2.96 (BASF DocID 1998/11082), hence roughly 3.0, which triggers an assessment of the potential risk from secondary poisoning.

Risk assessment for earthworm-eating mammals via secondary poisoning

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

As shown in the following Table 9.4-28 and Table 9.4-29, the TER_{LT} for mefentrifluconazole and boscalid exceeds the relevant trigger of 5 for acceptability of effects, indicating an acceptable risk to earthworm-eating mammals via secondary poisoning.

Table 9.4-28: Assessment of the risk for earthworm-eating mammals due to exposure to mefentrifluconazole via bioaccumulation in earthworms (secondary poisoning) for the intended uses

Parameter	Mefentrifluconazole	Reference
PEC_{soil} (accu) [mg/kg soil] [1)]	0.229	Chapter 8.7
K_{ow}	2512 ~~2350~~	BASF DocID 2013/1382370
K_{oc} (geometric mean) [L/kg]	3455.6	EFSA Journal 2018; 16(7): 5379
f_{oc} (default)	0.02	EFSA/2009/1438
BCF [2)]	0.448 ~~0.420~~	--
PEC_{worm} [mg/kg] [3)]	0.103 ~~0.096~~	--
Daily dose [mg/kg b.w./d] [4)]	0.132 ~~0.123~~	--
NO(A)EL [mg/kg b.w./d]	15	See chapter 9.3.1
TER_{LT} [5)]	113.6 ~~121.8~~	--

1) Worst case PEC_{soil} (accu) value was calculated for an application scenario of 2 x 100 g a.s./ha with 7-day interval in sunflower. For details see chapter 8.7.
2) Bioconcentration factor (BCF) = (0.84 + 0.012 x K_{ow}) / f_{oc} x K_{oc}
3) PEC_{worm} = PEC_{soil} x BCF
4) Daily dose = 1.28 x PEC_{worm}
5) TER_{lt} = NO(A)EL / Daily dose.

Table 9.4-29: Assessment of the risk for earthworm-eating mammals due to exposure to boscalid via bioaccumulation in earthworms (secondary poisoning) for the intended use

Parameter	Boscalid	Reference
PEC_{soil} (accu) [mg/kg soil] [1)]	0.422 ~~0.365~~	Chapter 8.7
K_{ow}	912 ~~915~~	BASF DocID 1998/11082
K_{oc} (arithmetic mean) [L/kg]	772	BASF DocID 1998/10513
f_{oc} (default)	0.02	EFSA/2009/1438
BCF [2)]	0.763 ~~0.766~~	--
PEC_{worm} [mg/kg] [3)]	0.322 ~~0.279~~	--
Daily dose [mg/kg b.w./d]	0.412 ~~0.358~~	--
NO(A)EL [mg/kg b.w./d]	67.0	See chapter 9.3.1
TER_{LT}	162.6 ~~187.3~~	--

1) Worst case PEC_{soil} (accu) value was calculated for an application scenario of 2 x 200 g a.s./ha with 7-day interval in sunflower. For details see chapter 8.7.
2) Bioconcentration factor (BCF) = (0.84 + 0.012 x K_{ow}) / f_{oc} x K_{oc}
3) PEC_{worm} = PEC_{soil} x BCF
4) Daily dose = 1.28 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 9.4-30 and Table 9.4-31, the TER_{LT} for mefentrifluconazole and boscalid exceeds the relevant trigger of 5 for acceptability of effects, indicating an acceptable risk to fish-eating mammals via secondary poisoning.

Table 9.4-30: 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 days) [mg/L] [1)]	$1.395*10^{-3}$	Chapter 8.9
BCF fish (max. worst case)	385	EFSA Journal 2018; 16(7): 5379
PEC_{fish} [mg/kg] [2)]	0.537	--
Daily dose [mg/kg b.w./d] [3)]	0.076	--
NO(A)EL [mg/kg b.w./d]	15	See chapter 9.3.1
TER_{LT} [4)]	196.7	--

1) The PECsw (21 d twa) value calculated for a multiple application scenario of 2 x 100 g a.s./ha sunflower from FOCUS Step 2 (North Europe) as worst-case. For details see chapter 8.9 and Appendix A 3.2 of Section 8.
2) $PEC_{fish} = PEC_{sw,}$ (twa, 21 days) x BCF
3) Daily dose = 0.142 x PEC_{fish}
4) TER_{lt} = NO(A)EL / Daily dose.

Table 9.4-31: Assessment of the risk for fish-eating mammals due to exposure to boscalid via bioaccumulation in fish (secondary poisoning) for the intended use

Parameter	Boscalid	Reference
$PEC_{sw,}$ (twa, 21 days) [mg/L] [1)]	$7.858*10^{-3}$	Chapter 8.9
BCF fish (max. worst case)	92	Review Report for the active substance boscalid, SANCO/3919 /2007-rev. 5, 17.01.08
PEC_{fish} [mg/kg] [2)]	0.723	--
Daily dose [mg/kg b.w./d] [3)]	0.103	--
NO(A)EL [mg/kg b.w./d]	67.0	See chapter 9.3.1
TER_{LT} [4)]	652.7	--

1) The PECsw (21 d twa) value calculated for a multiple application scenario of 2 x 100 g a.s./ha sunflower from FOCUS Step 2 (North Europe) as worst-case. For details see chapter 8.9 and Appendix A 3.2 of Section 8.
2) $PEC_{fish} = PEC_{sw,}$ (twa, 21 days) x BCF
3) Daily dose = 0.142 x PEC_{fish}
4) TER_{lt} = NO(A)EL / Daily dose.

zRMS comments:

The evaluation of the risk of secondary poisoning for earthworm- and fish-eating birds exposed to mefentrifluconazole and boscalid is in general agreed by the zRMS with some minor corrections resulting from different Kow calculated by the zRMS on the basis of log Pow and different PEC_{SOIL} value agreed in area of Section 8 for boscalid. These corrections have no impact on the derived conclusions and are introduced for consistency.

Acceptable risk of secondary poisoning could be concluded on the basis of performed calculations.

Neither of mefentrifluconazole metabolites triggered the evaluation of the risk of secondary poisoning due to log Pow <3 (see EFSA Journal 2018;16(7):5379.

No relevant boscalid metabolites were observed in soil and aquatic systems.

9.4.3.5 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).

No evidence was found for potential accumulation of boscalid in animal tissue (Review report for the active substance boscalid. Appendix II, endpoints and related information. 1. Toxicology and metabolism 17 January 2008).

Since the bioaccumulation potential of mefentrifluconazole and boscalid is low no further assessment on biomagnification is required**.**

zRMS comments:

Since acceptable risk of secondary poisoning to fish- and earthworm-eating birds could be concluded for both active substances, the potential for biomagnification in terrestrial food chains is expected to be low.

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

Not relevant.

9.4.5 Overall conclusions

It can be concluded that the risk to mammals from the application of BAS 762 02 F according to good agricultural practice is acceptable.

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

~~According to the revised data requirements under regulation 1107/2009 (Commission Regulations (EU) 283/2013 and 284/2013 for the active ingredient and the plant protection products, respectively), the risk to 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**~~

~~Commission Regulation (EU) No 283/2013 setting out data requirements for active substances, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market. Official Journal of the European Union: 1st March 2013.~~

~~Commission Regulation (EU) No 284/2013: setting out the data requirements for plant protection products, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market. Official Journal of the European Union: 1st March 2013.~~

~~Crane, M., Finnegan, M., Weltje, L., Kosmala-Grzechnik, S., Gross, M. and Wheeler, J.R. 2016. Acute oral toxicity of chemicals in terrestrial life stages of amphibians: Comparisons to birds and mammals. Regulatory Toxicology and Pharmacology, 80: 335-341.~~

~~Fryday, S. and Thompson, H. 2009. Compared toxicity of chemicals to reptiles and other vertebrates. EFSA Supporting Publications, 6, EN-14: 169 pp.~~

~~Fryday, S. and Thompson, H. 2012. Toxicity of pesticides to aquatic and terrestrial life stages of amphibians and occurrence, habitat use and exposure of amphibian species in agricultural environments. EFSA Supporting Publications, 9, EN-343: 348 pp.~~

~~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.~~

zRMS comments:

As currently there are no agreed rules or criteria for evaluation of the risk to other terrestrial vertebrates like reptiles and amphibians, this issue should be addressed once respective guidance is available and EU agreed endpoints concluded.

Information provided by the Applicant above has been thus not validated by the zRMS and is struck through and shaded.

9.6 Effects on aquatic organisms (KCP 10.2)

9.6.1 Toxicity data

Studies on the toxicity to aquatic organisms have been carried out using the formulation BAS 762 02 F and the active substances mefentrifluconazole (BAS 750 F) and boscalid (BAS 510 F) and their major metabolites. Full details of these studies are provided in the EFSA conclusion of mefentrifluconazole (EFSA Journal 2018;16(7):5379), the EC Review report of boscalid (SANCO/3919/2007–rev. 5, 2008) and the EU DARs of mefentrifluconazole and boscalid, as well as in Appendix 2 of this document (new studies).

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

In addition to the EU agreed chronic study with boscalid on *Daphnia magna*, a second study is available and can submitted upon request. This second study indicates very similar toxicity (NOEC = 0.8 mg/L) and is considered of limited reliability as it lacks significant details on the analytical method used for analysis of test substance. Therefore, the risk assessment for chronic invertebrates is conducted with the already EU-agreed endpoint. Additionally, for boscalid, a new vertebrate study (i.e. 34-days early life stage test) with *Cyprinodon variegatus* (NOEC = 0.110 mg a.s./L) is available and can be submitted upon request. This study was conducted to fulfill US-EPA requirements and provides an almost identical endpoint to the EU agreed endpoint for rainbow trout (NOEC = 0.125 mg a.s./L). Since the new study does not provide a significantly more critical endpoint, the risk assessment for chronic fish is conducted with the already EU-agreed endpoint. Further studies that were recently submitted for evaluation on EU level under the AIR 3 renewal process are not covered here in detail.

Effects on aquatic organisms of product BAS 762 02 F were not evaluated previously as part of the EU assessment of the active substances. Study summaries for new studies are provided in Appendix 2

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

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

Mefentrifluconazole (BAS 750 F) and metabolites

In

Table 9.6-1 all endpoints relevant for the aquatic risk assessment of mefentrifluconazole and its relevant metabolites are listed.

Table 9.6-1: 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 evaluated on EU level) 2016/1155889~~
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 (FLS 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; 14 d uptake, 7 d depuration)	mefentrifluconazole	BCF_{KLg} (whole fish)	BCF_{KLg} = 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
Crassostrea virginica	mefentrifluconazole	96 h, f	EC_{50} = 0.9472 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 (amendment)

Species	Substance	Exposure system	Results	Reference / BASF DocID
Chironomus dilutus (spiked sediment)	mefentrifluconazole	10 d, ss	NOEC ≥ 7.08 mg a.s./kg dry sediment im,mm EC_{50} > 96 mg a.s./kg dry sediment im,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 nom,mm EC_{50} > 100 mg a.s./kg dry sediment nom,mm	EFSA Journal 2018;16(7):5379 / 2015/7000622
Leptocheirus plumulosus (spiked sediment)	mefentrifluconazole	10 d, s	NOEC ≥ 95 mg a.s./kg dry sediment im,mm EC_{50} > 95 mg a.s./kg dry sediment im,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 (amendment)
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
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-calculations)
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-calculations)
Anabaena flos-aquae [2)]	mefentrifluconazole	72 h, s	E_rC_{50} ~~& E_yC_{50}~~ > 3.08 mg a.s./L mm 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** E_yC_{50} ≥ 2.107 mg a.s./L im	EFSA Journal 2018;16(7):5379 / 2014/1001322 + 2018/1220943 (amendment)
O. mykiss	1,2,4-triazole (Reg. No. 87084; M750F001)	96 h, s	LC_{50} = 498 mg/L mm,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 evaluated on EU level) 2019/1022695
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

Species	Substance	Exposure system	Results	Reference / BASF DocID
D. magna	M750F003	48 h, s	EC_{50} > 100 mg/L mm~~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
C. riparius	M750F003	28 d, s	NOEC ≥ 1.944 mg/kg dry sediment im	EFSA Journal 2018;16(7):5379 / 2015/1003916 + 2017/1044237 (amendment)
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 mm~~nom~~	EFSA Journal 2018;16(7):5379 / 2016/1289875
P. subcapitata 2)	M750F005	72 h, s	E_rC_{50} > 8.572 mg/L mm	EFSA Journal 2018;16(7):5379 / 2015/1184816
P. subcapitata 2)	M750F006	72 h, s	E_rC_{50} = 1.424 mg/L mm	EFSA Journal 2018;16(7):5379 / 2015/1184815
P. subcapitata 2)	M750F007	72 h, s	E_rC_{50} > 10 mg/L mm~~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

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

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

* 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.

zRMS comments:

Aquatic toxicity data for mefentrifluconazole and its metabolites provided in Table 9.6-1 are in general in line with EU agreed endpoints reported in EFSA Journal 2018;16(7):5379.

Some corrections were introduced by the zRMS since information regarding the type of concentration (nominal, measured) provided by the Applicant for some compounds was not fully in line with the LoEP.

It is also noted that in case the endpoint based on mean measured concentration is given in the LoEP it is not necessary to provide additional endpoint based on nominal concentration, even if the test item concentration was maintained at ±20% of nominal throughout the test. Such endpoints were thus struck through in Table 9.6-1 above as being not in line with the EU agreed values.

In support of the zonal evaluation of BAS 762 02 F the Applicant provided two new studies on acute toxicity of mefentrifluconazole and its metabolite M750F005 to fish.
Study performed with the parent does not provide adverse information and is thus not necessary for the risk assessment purposes, since sufficient information was already available from the EU review. Furthermore, no data gap in this area has been identified in EFSA Journal 2018;16(7):5379. Taking this into account, the study was not validated by the zRMS and its results are struck through in Table 9.6-1 above.
No data gap for study on acute toxicity of metabolite M750F005 to fish was identified in EFSA Journal 2018;16(7):5379 and in absence of the relevant endpoint the risk assessment could be performed with assumption of 10 times toxicity of the parent. This approach, however, results with unrealistic toxicity of metabolites leading potentially to unacceptable risk and necessity for calculation of Step 3 exposure. Therefore in order to perform the risk assessment based on the actual endpoint, the study was validated by the zRMS and endpoint reported in Table 9.6-1 is confirmed. Details of the zRMS evaluation and the study summary are provided in Appendix 2 of this document.

Boscalid (BAS 510 F) and metabolites

No major metabolites (> 10% TAR) were formed in a sensibilized water/sediment study (see Monograph of boscalid, Vol. 3, Annex B.9, 2002). For details please refer to 'acceptability of risk for the metabolites of boscalid' below.

The results from toxicity tests on aquatic organisms conducted with boscalid are summarized in Table 9.6-2.

Table 9.6-2: Endpoints and effect values relevant for the risk assessment for aquatic organisms – boscalid

Species	Substance	Exposure system	Results	Reference / BASF DocID
Oncorhynchus mykiss	boscalid	96 h, s	$LC_{50} \cong$ 2.7 mg a.s./L $_{nom}$	EC Review report SANCO/3919/2007–rev. 5, 2008 / 2001/1001726
Oncorhynchus mykiss	boscalid	97 d, f (ELS)	NOEC = 0.125 mg a.s./L $_{nom}$	EC Review report SANCO/3919/2007–rev. 5, 2008 / 1999/11847
Daphnia magna	boscalid	48 h, s	EC_{50} = 5.33 mg a.s./L $_{mm}$	EC Review report SANCO/3919/2007–rev. 5, 2008 / 2000/1018537
Daphnia magna	boscalid	21 d, ss	**NOEC = 1.31 mg a.s./L $_{mm}$**	EC Review report SANCO/3919/2007–rev. 5, 2008 / 2000/1018539
Chironomus riparius	boscalid	28 d, s (spiked water)	NOEC = 1.0 mg a.s./L $_{nom}$	EC Review report SANCO/3919/2007–rev. 5, 2008 / 2000/1018538
Chironomus riparius	boscalid	28 d, s (spiked sediment)	NOEC = 23.26 mg a.s./kg $_{im}$ (dw)	Addendum 2 to the DAR, Vol. 3, Annex B.9, May 2006 / 2005/1022464
Pseudokirchneriella subcapitata [2)]	boscalid	96 h, s	**E_rC_{50} = 3.75 mg a.s./L $_{mm}$** E_yC_{50} = 1.34 mg a.s./L $_{mm}$	EC Review report SANCO/3919/2007–rev. 5, 2008 / 2000/1018524

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

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; dw: dry weight; ELS = early life stage

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

zRMS comments:

Aquatic toxicity data presented in Table 9.6-1 are EU agreed endpoints reported in EU Review Report SANCO/3919/2007-rev.5 or the boscalid monograph.

In the introductory part to point 9.6.1 the Applicant indicated that additional studies on chronic toxicity of boscalid to *Daphnia magna* and fish are available and may be submitted upon request. However, these studies do not provide adverse information and are thus deemed not necessary for finalisation of the risk assessment at the zonal level, which should be based on EU agreed values. Taking this into account, the studies were not requested from the Applicant.

Formulated product (BAS 762 02 F)

In Table 9.6-3 all endpoints relevant for the aquatic risk assessment of the formulated product BAS 762 02 F are listed.

Table 9.6-3: Endpoints and effect values relevant for the risk assessment for aquatic organisms – BAS 762 02 F

Species	Substance	Exposure System	Results	Reference / BASF DocID
Oncorhynchus mykiss	BAS 762 02 F	96 h, s	LC_{50} = 8.12 mg/L $_{nom}$	New study 2019/1050663
Daphnia magna	BAS 762 02 F	48 h, s	EC_{50} = 17.41 mg/L $_{nom}$	New study 2019/1050662
Pseudokirchneriella subcapitata 1)	BAS 762 02 F	72 h, s	**E_rC_{50} = 6.37 mg/L $_{nom}$** E_yC_{50} = 3.69 mg/L $_{nom}$	New study 2019/1050661

Bold figures: Endpoint used in standard tier 1 risk assessment if more than one endpoint is available for the respective group or organism.
Abbreviations: s: static; nom: based on nominal concentrations

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

zRMS comments:

The studies performed with the formulated product were evaluated and agreed by the zRMS (for details, please refer to respective points in Appendix 2). Endpoints reported in Table 9.6-3 are confirmed to be correct.

9.6.1.1 Justification for new endpoints

Mefentrifluconazole

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

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

Boscalid
For boscalid, the EU agreed endpoints are used for the risk assessment.

zRMS comments:

The risk assessment for mefentrifluconazole, boscalid and relevant metabolites was performed with consideration of the EU agreed data with exception of acute endpoint for fish derived from study performed with mefentrifluconazole metabolite M750F005 which was derived from the newly submitted study not evaluated at the EU level. For justification of its use at the zonal level, please refer to zRMS comments in point 9.6.1 above.

9.6.2 Risk assessment

The evaluation of the risk for aquatic and sediment-dwelling 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 in the context of Regulation (EC) No 1107/2009" (EFSA Aquatic GD), as provided by the Commission Services (SANTE-2015-00080, 15 January 2015).

In accordance with the EFSA Aquatic GD, risk assessment for algae and aquatic plants was performed considering only the more relevant endpoint "growth rate" (E_rC_{50}).

Mefentrifluconazole (BAS 750 F) and metabolites

For mefentrifluconazole and its metabolites the EU agreed endpoints are considered for the tier 1 risk assessment.

Acceptability of risk for mefentrifluconazole

The relevant worst-case FOCUS Step 1 – 3 $PEC_{sw, sed}$ values for RAs covering the proposed use pattern and the resulting PEC/RAC ratios (ETR) for the active substance are presented in Table 9.6-4 - Table 9.6-7. For application in 'sunflower', worst-case $PEC_{sw, sed}$ values either from single or multiple application are used in a risk envelope approach. For details please refer to Part B Section 8.

Table 9.6-4: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for mefentrifluconazole for each organism group based on the worst-case FOCUS Step 1 - 2 calculations for single application (1x 100 g a.s./ha) of BAS 762 02 F in 'winter and spring oilseed rape' (BBCH 57 – 75)

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	0.920	0.2	0.4	--	0.6	--	--	15.660	≤ 0.1
S-Europe	0.920	0.2	0.4	--	0.6	--	--	25.747	≤ 0.2

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

For the intended single application of BAS 762 02 F in 'winter and spring oilseed rape' at 1x 100 g a.s./ha, the calculated PEC/RAC ratios for mefentrifluconazole indicate an acceptable risk to all groups of aquatic organisms based on tier 1 toxicity data and worst-case FOCUS Step 1 - 2 $PEC_{sw\ /\ sed}$ values. Therefore, no further assessment is necessary.

Table 9.6-5: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for mefentrifluconazole for each organism group based on the worst-case FOCUS Step 1 - 3 calculations for single and multiple application (1 - 2x 100 g a.s./ha) of BAS 762 02 F in 'sunflower' (BBCH 31 – 69)

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 (µg/L)		LC_{50} 532	NOEC 22	EC_{50} 944	EC_{10} 16.1	E_rC_{50} 679	E_rC_{50} > 2017	**Endpoint (µg/kg)**	NOEC ≥ 1158
AF		100	10	100	10	10	10	**AF**	10
RAC (µg/L)		5.32	2.2	9.44	1.61	67.9	> 201.7	**RAC (µg/kg)**	≥ 115.8
FOCUS Scenario	**PEC $_{gl\text{-}sw\ max}$ (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	1.553	0.3	0.7	0.2	0.96	--	--	49.598	≤ 0.4
S-Europe	2.712	0.5	**1.2**	0.3	**1.7**	--	--	89.461	≤ 0.8
Step 3									
D3 ditch	0.522	--	0.24		0.32	--	--	0.402	--
D4 pond [#]	0.072	--	0.03	--	0.04	--	--	0.615	--
D4 stream [#]	0.463	--	0.2	--	0.3	--	--	0.246	--
D5 pond	0.033	--	0.02	--	0.02	--	--	0.340	--
D5 stream	0.468	--	0.2	--	0.3	--	--	0.075	--
R1 pond	0.151	--	0.07	--	0.09	--	--	2.494	--
R1 stream	0.574	--	0.3	--	0.4	--	--	3.247	--
R3 stream	0.509	--	0.2	--	0.3	--	--	3.250	--
R4 stream	0.648	--	0.3	--	0.4	--	--	2.045	--

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

[#] ~~$PEC_{sw, sed}$ values considering the D4 scenarios are only relevant for Austria and have been calculated using maize as a surrogate (for details please refer to Chapter 8 of this dossier)~~

For the intended single and multiple application of BAS 762 02 F in 'sunflower' at 1-2x 100 g a.s./ha, the calculated PEC/RAC ratios for mefentrifluconazole indicate an acceptable risk to all groups of aquatic organisms based on tier 1 toxicity data and worst-case FOCUS Step 1 - 3 $PEC_{sw, sed}$ values. Therefore, no further assessment is necessary.

Table 9.6-6: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for mefentrifluconazole for each organism group based on the worst-case FOCUS Step 1 - 3 calculations for single application (1x 100 g a.s./ha) of BAS 762 02 F in 'winter cereals' (BBCH 30 – 49)

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	1.166	0.2	0.5	--	0.7	--	--	37.851	≤ 0.3
S-Europe	2.104	0.4	0.96	--	**1.3**	--	--	70.127	≤ 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	0.044	--	--	--	0.03	--	--	0.598	--
R1 stream	0.416	--	--	--	0.3	--	--	0.707	--
R3 stream	0.585	--	--	--	0.4	--	--	0.895	--
R4 stream	0.418	--	--	--	0.3	--	--	0.929	--

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

For the intended single application of BAS 762 02 F in 'winter cereals' at 1x 100 g a.s./ha, the calculated PEC/RAC ratios for mefentrifluconazole indicate an acceptable risk to all groups of aquatic organisms based on tier 1 toxicity data and worst-case FOCUS Step 1 - 3 $PEC_{sw,\ sed}$ values. Therefore, no further assessment is necessary.

Table 9.6-7: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for mefentrifluconazole for each organism group based on the worst-case FOCUS Step 1 - 3 calculations for single application (1x 100 g a.s./ha) of BAS 762 02 F in 'spring cereals' (BBCH 30 – 49)

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	1.166	0.2	0.5	--	0.7	--	--	37.851	≤ 0.3
S-Europe	2.104	0.4	0.96	--	**1.3**	--	--	70.127	≤ 0.6
Step 3									
D3 ditch	0.632	--	--	--	0.4	--	--	0.427	--
D4 pond	0.035	--	--	--	0.02	--	--	0.325	--
D4 stream	0.517	--	--	--	0.3	--	--	0.116	--
D5 pond	0.023	--	--	--	0.01	--	--	0.195	--
D5 stream	0.531	--	--	--	0.3	--	--	0.024	--
~~R1 pond [#]~~	~~0.069~~	~~--~~	~~--~~	~~--~~	~~0.04~~	~~--~~	~~--~~	~~1.207~~	~~--~~
~~R1 stream [#]~~	~~0.415~~	~~--~~	~~--~~	~~--~~	~~0.3~~	~~--~~	~~--~~	~~1.186~~	~~--~~
R4 stream	0.418	--	--	--	0.3	--	--	1.462	--

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

~~[#] $PEC_{sw,\ sed}$ values considering the R1 scenarios are only relevant for Austria and have been calculated using spring oilseed rape as a surrogate (for details please refer to Chapter 8 of this dossier)~~

For the intended single application of BAS 762 02 F in 'spring cereals' at 1x 100 g a.s./ha, the calculated PEC/RAC ratios for mefentrifluconazole indicate an acceptable risk to all groups of aquatic organisms based on tier 1 toxicity data and worst-case FOCUS Step 1 - 3 $PEC_{sw,\ sed}$ values. Therefore, no further assessment is necessary.

Acceptability of risk for the metabolites of mefentrifluconazole

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

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

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

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

In Table 9.6-8 the ETR ratios for aquatic organisms are given for the use of BAS 762 02 F in ‘sunflower’ and for each organism group for the relevant metabolites of mefentrifluconazole. Worst-case $PEC_{sw,\ sed}$ values for single and twofold application in ‘sunflower’ are used for risk assessment covering the proposes uses in ‘oilseed rape’ in a risk envelope approach.

Table 9.6-8: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for metabolites of mefentrifluconazole 1 for each organism group based on worst-case FOCUS Step 1 - 2 calculations following single and twofold application of BAS 762 02 F in 'sunflower' (covering all other intended uses)

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Algae**	**Group**	**Sed. dwell. prolonged**	**Sed. dwell. prolonged**
Test species		***O. mykiss***	***O. mykiss***	***D. magna***	***P. subcapitata***	**Test species**	***C. riparius***	***C. riparius***
AF		100	10	100	10	**AF**	10	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 #	
RAC (µg/L)		4980	320	> 1000	> 2250	**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								
	2.154	0.0004	0.007	< 0.002	< 0.001	1.783	≤ 0.02	
M750F003								
Endpoint (µg/L)		LC_{50} 532 #	NOEC n.a.	EC_{50} > 100000	E_rC_{50} > 100000	**Endpoint (µg/kg)**	NOEC ≥ 1944	
RAC (µ/L)		5.32	--	> 1000	> 10000	**RAC (µg/kg)**	≥ 194.4	
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								
	2.871	0.5	--	< 0.003	< 0.0003	16.858	≤ 0.09	
M750F005								
Endpoint (µg/L)		LC_{50} > 5000	NOEC n.a.	EC_{50} > 8580	E_rC_{50} > 8570	**Endpoint (µg/kg)**	~~NOEC ≥1158 #~~	NOEC ≥ 115.8 *
RAC (µg/L)		>50	--	> 85.8	> 857	**RAC (µg/kg)**	~~≥ 115.8~~	≥ 11.58
FOCUS Scenario	**PEC $_{gl\text{-}max,\ sw}$ (µg/L)**	**PEC/RAC ratio (= ETR)**				**PEC $_{gl\text{-}sed\ max}$ (µg/kg)**	~~**PEC/RAC (= ETR)**~~	**PEC/RAC (= ETR)**
Step 1								
	2.347	< 0.05	--	< 0.03	< 0.003	143.916	~~≤ 1.2~~	**<12.4**
Step 2								
N-Europe	0.282	--	--	--	--	17.044	~~≤ 0.1~~	**<1.5**
S-Europe	0.411	--	--	--	--	30.691	~~≤ 0.3~~	**<2.7**

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Algae**	**Group**	**Sed. dwell. prolonged**	**Sed. dwell. prolonged**
Test species		***O. mykiss***	***O. mykiss***	***D. magna***	***P. subcapitata***	**Test species**	***C. riparius***	***C. riparius***
M750F006								
Endpoint (µg/L)		LC_{50} 6200	NOEC n.a.	EC_{50} 4420	E_rC_{50} 1420	**Endpoint (µg/kg)**	~~NOEC ≥1158 #~~	NOEC ≥ 115.8 *
RAC (µg/L)		62	--	44.2	142	**RAC (µg/kg)**	~~≥ 115.8~~	≥ 11.58
FOCUS Scenario	**PEC $_{gl\text{-}max,\ sw}$ (µg/L)**	**PEC/RAC ratio (= ETR)**				**PEC $_{gl\text{-}sed\ max}$ (µg/kg)**	~~**PEC/RAC (= ETR)**~~	**PEC/RAC (= ETR)**
Step 1								
	2.927	0.05	--	0.07	0.02	122.336	~~≤ 1.1~~	**<10.6**
Step 2								
N-Europe	0.320	--	--	--	--	14.488	~~≤ 0.1~~	**<1.3**
S-Europe	0.556	--	--	--	--	26.088	~~≤ 0.2~~	**<2.3**
M750F007								
Endpoint (µg/L)		LC_{50} > 7200	NOEC n.a.	EC_{50} > 9900 ~~10000~~	E_rC_{50} > 10000	**Endpoint (µg/kg)**	~~NOEC ≥1158 #~~	NOEC ≥ 115.8 *
RAC (µg/L)		> 72	--	> 99 ~~100~~	> 1000	**RAC (µg/kg)**	~~≥ 115.8~~	≥ 11.58
FOCUS Scenario	**PEC $_{gl\text{-}max,\ sw}$ (µg/L)**	**PEC/RAC ratio (= ETR)**				**PEC $_{gl\text{-}sed\ max}$ (µg/kg)**	~~**PEC/RAC (= ETR)**~~	**PEC/RAC (= ETR)**
Step 1								
	4.655	< 0.06	--	< 0.05	< 0.005	160.547	~~≤ 1.4~~	**<13.9**
Step 2								
N-Europe	0.522	--	--	--	--	19.013	~~≤ 0.2~~	**<1.6**
S-Europe	0.909	--	--	--	--	34.237	~~≤ 0.3~~	**<3.0**
M750F008								
Endpoint (µg/L)		LC_{50} 53.2*	NOEC n.a.	EC_{50} > 8070	E_rC_{50} 4080	**Endpoint (µg/kg)**	~~NOEC ≥1158 #~~	NOEC ≥ 115.8 *
RAC (µg/L)		0.532	--	> 80.7	408	**RAC (µg/kg)**	~~≥ 115.8~~	≥ 11.58
FOCUS Scenario	**PEC $_{gl\text{-}max,\ sw}$ (µg/L)**	**PEC/RAC ratio (= ETR)**				**PEC $_{gl\text{-}sed\ max}$ (µg/kg)**	~~**PEC/RAC (= ETR)**~~	**PEC/RAC (= ETR)**
Step 1								
	0.302	0.6	--	< 0.004	0.0007	32.130	~~≤ 0.3~~	**<2.8**
Step 2								

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Algae**	**Group**	**Sed. dwell. prolonged**	**Sed. dwell. prolonged**
Test species		***O. mykiss***	***O. mykiss***	***D. magna***	***P. subcapitata***	**Test species**	***C. riparius***	***C. riparius***
N-Europe	0.060	--	--	--	--	3.805	–	<0.33
S-Europe	0.060	--	--	--	--	6.852	–	<0.59

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**

[#] 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.

* 10-fold higher toxicity compared to the active substance is assumed.

The calculated PEC/RAC ratios for the mefentrifluconazole metabolites indicate an acceptable risk for all groups of aquatic organisms for all proposed uses of BAS 762 02 F based on worst-case FOCUS Step 1 - 2 assumptions. Therefore, no further assessment is necessary.

zRMS comments:

Mefentrifluconazole

Aquatic risk assessment performed by the Applicant for mefentrifluconazole is agreed by the zRMS. Acceptable acute and chronic risk may be concluded for all relevant aquatic species with no need for risk mitigation measures.

It is noted that for intended uses of BAS 762 02 F in spring cereals additional calculations were performed for R1 scenario using spring oilseed rape as surrogate crop in order to fulfil specific AT requirements. It should be, however, noted that evaluations relevant for particular cMS should be presented in the National Addendum and not in the Core Assessment, since the zRMS does not have sufficient knowledge to check if provided calculations fulfil the specific national requirements of the given cMS. In this particular case the zRMS does not know if spring oilseed rape is accepted by AT as surrogate crop for spring cereals. From the zRMS perspective, winter cereals seem to be better surrogate crop and is of the opinion that the evaluation performed for winter cereals covers risk following application of BAS 762 02 F in spring cereals in Central Zone scenarios not defined for this crop. In case AT does not agree with this conclusion - a comment in this area would be appreciated so the zRMS could restore struck through calculations (Step 3 modelling for spring oilseed rape has been validated in area of Section 8 anyway).

It is also not clear on what basis the Applicant concluded that scenario D4 is relevant only for AT (see footnote to Table 9.6-5), while the Central Zone guidance in area of environmental fate and behaviour[1] clearly indicates that D4 scenario is relevant for the whole Central Zone. Furthermore, in the course of evaluation the Applicant was specifically informed that not all Central Zone scenarios are defined for sunflower and for this reason additional surface water modelling using maize as a surrogate crop for sunflower was requested by the zRMS and submitted in October 2021. Taking this into account, risk assessment for sunflower performed with consideration of the surface water exposure calculated for scenarios D3 and D4 using maize as a surrogate crop is considered relevant for the whole Central Zone and not only AT.

Mefentrifluconazole metabolites

Aquatic risk assessment performed by the Applicant for metabolites 1,2,4-triazole and M750F003 is agreed by the zRMS.

[1] Working document of the Central Zone in the authorisation of plant protection products, Section 8, Environmental fate and behaviour. Version 1, rev. 1, June 2018.

The risk assessment performed by the Applicant for aquatic species exposed to metabolites M750F005, M750F006, M750F007 and M750F008 via the water column was based on endpoints agreed in the course of the EU review of mefentrifluconazole and is thus agreed by the zRMS. It is, however, noted that in the risk assessment performed for sediment dwellers exposed to these compounds the Applicant considered the parent endpoint although at the EU level 10 times toxicity of the parent has been assumed in evaluation performed for these compounds. Nevertheless the zRMS agrees with the Applicant, that all available aquatic toxicity data indicate that all mefentrifluconazole metabolites are clearly less toxic than the parent and it is not expected that they would be more toxic to *C. riparius*. Taking this into account, assumption of 10 times toxicity of the parent seems to be overly conservative and consideration of the parent endpoint is agreed by the zRMS.

Although the Applicants' arguments regarding toxicity of metabolites M750F005, M750F006, M750F007 and M750F008 to sediment dwellers were agreed by the zRMS, this approach has been challenged during the commenting period and it was pointed out that the approach agreed at the EU level (i.e. consideration of 10 times toxicity of the parent) should have been followed. The zRMS maintains its opinion that based on the available data consideration of 10 times higher toxicity of metabolites comparing to the parent seems to be overly conservative. However, the risk assessment above has been amended accordingly in order to comply with decisions taken at the EU level.

Amended calculations demonstrated acceptable risk to sediment dwelling organisms exposed to metabolite M750F008 based on Step 2 PEC_{SED}. However, potentially unacceptable risk was demonstrated for metabolites M750F005, M750F006 and M750F007 and for this reason further calculations based on Step 3 PECSED for particular crops were performed by the zRMS and are presented below.

Risk assessment for sediment dwellers from metabolites M750F005, M750F006 and M750F007 (winter oilseed rape, BBCH 57-75, 1x100 g a.s./ha)

Group	Sediment dwellers					
Species	*Chironomus riparius*					
Endpoint (µg/kg dws)	NOEC ≥ 115.8 * (relevant for all metabolites considered below)					
AF	10					
RAC (µg/kg dws)	≥ 11.58					
Compound	**M750F005**		**M750F006**		**M750F007**	
Step 3 FOCUS scenario	**PEC_{SED} (µg/kg dws)**	**PEC/RAC**	**PEC_{SED} (µg/kg dws)**	**PEC/RAC**	**PEC_{SED} (µg/kg dws)**	**PEC/RAC**
D3 ditch	0.034	<0.003	0.029	<0.003	0.027	<0.002
D4 pond	0.078	<0.007	0.068	<0.006	0.062	<0.005
D4 stream	0.004	<0.0003	0.003	<0.0003	0.003	<0.0003
D5 pond	0.101	<0.009	0.088	<0.008	0.080	<0.007
D5 stream	0.008	<0.001	0.007	<0.001	0.006	<0.001
R1 pond	0.188	<0.02	0.163	<0.01	0.149	<0.01
R1 stream	0.425	<0.04	0.381	<0.03	0.352	<0.03
R3 stream	0.315	<0.03	0.281	<0.02	0.261	<0.02

* 10 times toxicity of the parent assumed as a worst case

Risk assessment for sediment dwellers from metabolites M750F005, M750F006 and M750F007 (spring oilseed rape, BBCH 57-75, 1x100 g a.s./ha)

Group	Sediment dwellers					
Species	*Chironomus riparius*					
Endpoint (µg/kg dws)	NOEC ≥ 115.8 * (relevant for all metabolites considered below)					
AF	10					
RAC (µg/kg dws)	≥ 11.58					
Compound	**M750F005**		**M750F006**		**M750F007**	
Step 3 FOCUS scenario	**PEC_{SED} (µg/kg dws)**	**PEC/RAC**	**PEC_{SED} (µg/kg dws)**	**PEC/RAC**	**PEC_{SED} (µg/kg dws)**	**PEC/RAC**
D3 ditch	0.036	<0.003	0.031	<0.003	0.028	<0.002
D4 pond	0.066	<0.006	0.058	<0.005	0.052	<0.004
D4 stream	0.006	<0.0005	0.005	<0.0004	0.005	<0.0004
D5 pond	0.102	<0.009	0.089	<0.008	0.081	<0.007
D5 stream	0.008	<0.001	0.007	<0.001	0.006	<0.001
R1 pond	0.391	<0.03	0.341	<0.03	0.311	<0.03
R1 stream	0.628	<0.05	0.559	<0.05	0.514	<0.04

* 10 times toxicity of the parent assumed as a worst case

Risk assessment for sediment dwellers from metabolites M750F005, M750F006 and M750F007 (sunflower, BBCH 31-69, 2x100 g a.s./ha, 7 d interval)

Group	Sediment dwellers					
Species	*Chironomus riparius*					
Endpoint (µg/kg dws)	NOEC ≥ 115.8 * (relevant for all metabolites considered below)					
AF	10					
RAC (µg/kg dws)	≥ 11.58					
Compound	**M750F005**		**M750F006**		**M750F007**	
Step 3 FOCUS scenario	**PEC_{SED} (µg/kg dws)**	**PEC/RAC**	**PEC_{SED} (µg/kg dws)**	**PEC/RAC**	**PEC_{SED} (µg/kg dws)**	**PEC/RAC**
D3 ditch [1)]	0.030	<0.003	0.026	<0.002	0.024	<0.002
D4 pond [1)]	0.129	<0.011	0.113	<0.01	0.102	<0.009
D4 stream [1)]	0.010	<0.0009	0.009	<0.0008	0.008	<0.0007
D5 pond	0.172	<0.02	0.149	<0.01	0.136	<0.01
D5 stream	0.006	<0.001	0.005	<0.0004	0.005	<0.0004
R1 pond	0.528	<0.05	0.457	<0.04	0.415	<0.04
R1 stream	0.831	<0.07	0.745	<0.06	0.688	<0.06
R3 stream	1.213	<0.10	1.080	<0.09	0.992	<0.09
R4 stream	0.841	<0.07	0.743	<0.06	0.681	<0.06

* 10 times toxicity of the parent assumed as a worst case

[1)] additional calculations performed for D3 and D4 scenarios (relevant for the Central Zone) for sunflower using maize as surrogate crop

Risk assessment for sediment dwellers from metabolites M750F005, M750F006 and M750F007 (winter cereals, BBCH 30-49, 1x100 g a.s./ha)

Group	Sediment dwellers					
Species	*Chironomus riparius*					
Endpoint (µg/kg dws)	NOEC ≥ 115.8 * (relevant for all metabolites considered below)					
AF	10					
RAC (µg/kg dws)	≥ 11.58					
Compound	**M750F005**		**M750F006**		**M750F007**	
Step 3 FOCUS scenario	**PEC_{SED} (µg/kg dws)**	**PEC/RAC**	**PEC_{SED} (µg/kg dws)**	**PEC/RAC**	**PEC_{SED} (µg/kg dws)**	**PEC/RAC**
D3 ditch	0.023	<0.002	0.020	<0.002	0.018	<0.002
D4 pond	0.092	<0.008	0.081	<0.007	0.073	<0.006
D4 stream	0.004	<0.0003	0.003	<0.0003	0.003	<0.0003
D5 pond	0.118	<0.010	0.103	<0.009	0.094	<0.008
D5 stream	0.002	<0.0002	0.002	<0.0002	0.001	<0.0001
R1 pond	0.230	<0.02	0.199	<0.02	0.181	<0.02
R1 stream	0.214	<0.02	0.191	<0.02	0.176	<0.02
R3 stream	0.308	<0.03	0.275	<0.02	0.253	<0.02
R4 stream	0.260	<0.02	0.236	<0.02	0.219	<0.02

* 10 times toxicity of the parent assumed as a worst case

Risk assessment for sediment dwellers from metabolites M750F005, M750F006 and M750F007 (spring cereals, BBCH 30-49, 1x100 g a.s./ha)

Group	Sediment dwellers					
Species	*Chironomus riparius*					
Endpoint (µg/kg dws)	NOEC ≥ 115.8 * (relevant for all metabolites considered below)					
AF	10					
RAC (µg/kg dws)	≥ 11.58					
Compound	**M750F005**		**M750F006**		**M750F007**	
Step 3 FOCUS scenario	**PEC_{SED} (µg/kg dws)**	**PEC/RAC**	**PEC_{SED} (µg/kg dws)**	**PEC/RAC**	**PEC_{SED} (µg/kg dws)**	**PEC/RAC**
D3 ditch	0.026	<0.002	0.023	<0.002	0.021	<0.002
D4 pond	0.087	<0.008	0.076	<0.007	0.069	<0.006
D4 stream	0.005	<0.0004	0.004	<0.0003	0.004	<0.0003
D5 pond	0.116	<0.01	0.102	<0.009	0.093	<0.008
D5 stream	0.002	<0.0002	0.002	<0.0002	0.002	<0.0002
R4 stream	0.456	<0.04	0.407	<0.04	0.374	<0.03

* 10 times toxicity of the parent assumed as a worst case

Overall, acceptable acute and chronic risk may be concluded for all relevant aquatic species exposed to mefentrifluconazole metabolites with no need for risk mitigation measures.

Boscalid (BAS 510 F) and metabolites

For boscalid and its metabolites the EU agreed endpoints are considered for the tier 1 risk assessment.

Acceptability of risk for boscalid

The relevant worst-case FOCUS Step 1 – 3 $PEC_{sw, sed}$ values for RAs covering the proposed use pattern and the resulting PEC/RAC ratios (ETR) for the active substance are presented in **Błąd! Nie można odnaleźć źródła odwołania.**,

and Table 9.6-11. For application in 'sunflower' worst-case $PEC_{sw,\ sed}$ values from either single or multiple application are used in a risk envelope approach. For details please refer to Part B Section 8.

Table 9.6-9: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for boscalid for each organism group based on the worst-case FOCUS Step 1 - 2 calculations for single application (1x 200 g a.s./ha) of BAS 762 02 F in 'winter and spring oilseed rape' (BBCH 57 – 75)

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Sed. dwell. prolonged**		**Sed. dwell. prolonged**
Test species		***O. mykiss***	***O. mykiss*** (ELS study)	***D. magna***	***D. magna***	***P. subcapitata***	***C. riparius***		***C. riparius***
Endpoint		LC_{50}	NOEC	EC_{50}	NOEC	E_rC_{50}	NOEC		NOEC
(µg/L)		2700	125	≥ 5330	1310	> 3750	1000		23260
AF		100	10	100	10	10	10		10
RAC (µg/L)		27	12.5	≥ 53.3	131	> 375	100		2326
FOCUS Scenario	**PEC $_{gl\text{-}sw\text{-}max}$ (µg/L)**	**PEC/RAC (= ETR)**						**PEC $_{gl\text{-}sed\text{-}max}$ (µg/kg)**	**PEC/RAC (= ETR)**
Step 1									
	34.691	**1.3**	**2.8**	< 0.7	0.3	< 0.09	0.3	260.430	0.1
Step 2									
N-Europe	2.696	0.1	0.2	--	--	--	--	19.378	--
S-Europe	4.304	0.2	0.3	--	--	--	--	31.782	--

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

For the intended single application of BAS 762 02 F in 'winter and spring oilseed rape' at 1x 200 g a.s./ha, the calculated PEC/RAC ratios for boscalid indicate an acceptable risk for all groups of aquatic organisms based on the tier 1 toxicity endpoints and FOCUS Step 1 - 2 calculations. Therefore, no further assessment is necessary.

Table 9.6-10: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for boscalid for each organism group based on the worst-case FOCUS Step 1 - 3 calculations for single and multiple application (1 - 2 x 200 g a.s./ha) of BAS 762 02 F in 'sunflower' (BBCH 31-69)

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Sed. dwell. prolonged**		**Sed. dwell. prolonged**
Test species		***O. mykiss***	***O. mykiss*** (ELS study)	***D. magna***	***D. magna***	***P. subcapitata***	***C. riparius***		***C. riparius***
Endpoint		LC_{50}	NOEC	EC_{50}	NOEC	E_rC_{50}	NOEC		NOEC
(µg/L)		2700	125	≥ 5330	1310	> 3750	1000		23260
AF		100	10	100	10	10	10		10
RAC (µg/L)		27	12.5	≥ 53.3	131	> 375	100		2326
FOCUS Scenario	**PEC $_{gl\text{-}sw\text{-}max}$ (µg/L)**	**PEC/RAC (= ETR)**						**PEC $_{gl\text{-}sed\text{-}max}$ (µg/kg)**	**PEC/RAC (= ETR)**
Step 1									
	69.382	**2.6**	**5.6**	**< 1.3**	0.5	< 0.2	0.7	520.861	0.2
Step 2									
N-Europe	8.232	0.3	0.7	< 0.2	--	--	--	61.003	--
S-Europe	14.546	0.5	**1.2**	< 0.3	--	--	--	109.712	--
Step 3									
D3 ditch	0.910	--	0.07	--	--	--	--	0.705	--
D4 pond [#]	0.717	--	0.06	--	--	--	--	6.546	--
D4 pond [#]	1.393	--	0.1	--	--	--	--	2.085	--
D5 pond	0.396	--	0.03	--	--	--	--	4.011	--
D5 stream	0.938	--	0.08	--	--	--	--	0.872	--
R1 pond	0.380	--	0.03	--	--	--	--	4.405	--
R1 stream	2.717	--	0.2	--	--	--	--	3.278	--
R3 stream	2.120	--	0.2	--	--	--	--	3.218	--
R4 stream	2.405	--	0.2	--	--	--	--	2.829	--

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

[#] $PEC_{sw, sed}$ values considering the D4 scenarios are only relevant for Austria and have been calculated using maize as a surrogate (for details please refer to Chapter 8 of this dossier)

For the intended single and multiple application of BAS 762 02 F in 'sunflower' at 1 - 2x 200 g a.s./ha, the calculated PEC/RAC ratios for boscalid indicate an acceptable risk for all groups of aquatic organisms based on the tier 1 toxicity endpoints and FOCUS Step 1 - 3 calculations. Therefore, no further assessment is necessary.

Table 9.6-11: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for boscalid for each organism group based on the worst-case FOCUS Step 1 - 2 calculations for single application (1x 200 g a.s./ha) of BAS 762 02 F in 'winter and spring cereals' (BBCH 30 – 49)

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Sed. dwell. prolonged**		**Sed. dwell. prolonged**
Test species		***O. mykiss***	***O. mykiss*** (ELS study)	***D. magna***	***D. magna***	***P. subcapitata***	***C. riparius***		***C. riparius***
Endpoint		LC_{50}	NOEC	EC_{50}	NOEC	E_rC_{50}	NOEC		NOEC
(µg/L)		2700	125	≥ 5330	1310	> 3750	1000		23260
AF		100	10	100	10	10	10		10
RAC (µg/L)		27	12.5	≥ 53.3	131	> 375	100		2326
FOCUS Scenario	**PEC $_{gl\text{-}sw\text{-}max}$ (µg/L)**	**PEC/RAC (= ETR)**						**PEC $_{gl\text{-}sed\text{-}max}$ (µg/kg)**	**PEC/RAC (= ETR)**
Step 1									
	34.691	**1.3**	**2.8**	< 0.7	0.3	< 0.09	0.3	260.430	0.1
Step 2									
N-Europe	6.234	0.2	0.5	--	--	--	--	46.667	--
S-Europe	11.379	0.4	0.9	--	--	--	--	86.362	--

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

For the intended single application of BAS 762 02 F in 'winter and spring cereals' at 1x 200 g a.s./ha, the calculated PEC/RAC ratios indicate an acceptable risk for all groups of aquatic organisms based on the tier 1 toxicity endpoints and FOCUS Step 1 - 2 calculations. Therefore, no further assessment is necessary.

Acceptability of risk for the metabolites of boscalid

No major metabolites (> 10% TAR) were formed in a sensibilized water/sediment study (see Monograph of boscalid, Vol. 3, Annex B.9, 2002). The metabolite 4-Cl-benzoic acid (M510F64) reached 9.4% TAR after 30 days in the water phase. Literature data show that this metabolite is of no ecotoxicological relevance (see Monograph of boscalid, Vol. 3, Annex B.9, 2002). Therefore, the risk assessment for boscalid as provided above is assumed to cover the potential risk from these minor metabolites.

zRMS comments:

Aquatic risk assessment performed by the Applicant for boscalid is agreed by the zRMS. Acceptable acute and chronic risk may be concluded for all relevant aquatic species with no need for risk mitigation measures. The risk from metabolites was not triggered since no major metabolites were formed in soil and aquatic metabolism studies.

Formulation risk assessment

A mixture toxicity risk assessment for the formulated product BAS 762 02 F was conducted in accordance with the EFSA Aquatic GD (2013) and is presented below. The concentration addition (CA) model is used. To determine the respective formulation effect, EFSA proposes to calculate the model deviation ratio (MDR), which divides the calculated mixture toxicity ($EC_{x\ mix\text{-}CA}$) by the measured mixture toxicity ($EC_{x\ PPP}$). If the MDR is between 0.2 and 5 the observed and calculated mixture toxicities are considered in agreement. Respective MDR calculations are presented in Table 9.6-12.

Table 9.6-12: Comparison of the measured toxicity of the formulated product BAS 762 02 F and the calculated formulation toxicity based on the data for the active substances mefentrifluconazole and boscalid

Test Species	Test system	Endpoint	Measured toxicity of the active substances ($EC_{x\ a.s.}$) [µg a.s./L]		Measured toxicity of BAS 762 02 F (ECx_{ppp}) [µg product/L]	Calculated mixture toxicity ($EC_{x\ mix\text{-}CA}$) [µg product/L] *	MDR ($EC_{x\ mix\text{-}CA}$ / $ECx_{\ PPP}$)
O. mykiss	acute	96 h LC_{50}	mefentrifluconazole	532	8120 (2155.8 µg sum a.s./L)	4312.3 (1144.8 µg sum a.s./L)	0.5
			boscalid	2700			
D. magna	acute	48 h EC_{50}	mefentrifluconazole	944	17410 (4622.1 µg sum a.s./L)	7877.0 (2091.2 µg sum a.s./L)	0.5
			boscalid	5330			
P. subcapitata	--	72 h E_rC_{50}	mefentrifluconazole	1352	6370 (1691.2 µg sum a.s./L)	8876.8 (2356.7 µg sum a.s./L)	1.4
		72 h E_rC_{50}	boscalid	3750			

Abbreviations: PPP = plant protection product; CA = concentration addition; MDR = model deviation ratio

* The theoretical mixture toxicity of the formulation was re-calculated assuming concentration addition based on the measured toxicity data of the active substances, their nominal contents within the formulation (*i.e.* 100 g mefentrifluconazole/L and 200 g boscalid/L) and the product density of 1.130 g/cm^3.

The calculated MDR values are between 0.5 and 1.4 for all organisms, indicating that the formulation does not cause synergistic or antagonistic toxicity compared to the active substances but instead follows the expected toxicity for all groups of aquatic organisms (*i.e.* the CA model provides a reliable estimate of the toxicity of the given mixture). Furthermore, based on the calculations it can be concluded that chronic studies on fish and invertebrates using the formulations are not required, since the product is not by a factor ≥10 acutely more toxic than the active substances.

With regard to the mixture risk assessment, the EFSA Aquatic GD states (section 10.3.7) that "*If no synergistic effects are indicated and the ETR values of the individual a.s. (ETRi) contained in the formulation are below the relevant trigger value, the mixture RA can follow a simplified approach: if all ETRi ≤ ETR trigger/n (n= number of a.s.) the mixture also fulfils the authorisation criteria and the procedure can be stopped."* In order to verify if a simplified approach can be applied, the acceptability of risk (PECi/RACi < 0.5) for mefentrifluconazole and boscalid was assessed by using the lowest RAC*i* and the highest FOCUS Step 3 PEC*i* following the proposed uses of BAS 762 02 F (see in Table 9.6-13). For relevant PEC values please refer to chapter 8.9 of this dossier.

Table 9.6-13: Simplified approach for mixture risk assessment: acceptability of risk ($PEC_i/RAC_i < 0.5$) for mefentrifluconazole and boscalid based on the lowest RAC_i and the highest FOCUS Step 3 PEC_i following the proposed uses of BAS 762 02 F

Test substance		**Mefentrifluconazole**	**Boscalid**
Lowest RAC_{sw} [µg/L]		1.61	12.5
Highest FOCUS Step 3 PEC_{sw} [µg/L]	Winter oilseed rape	0.633	1.269
	Spring oilseed rape	0.634	1.270
	Sunflower	0.648	2.717
	Winter cereal	0.632	1.685
	Spring cereal	0.632	1.553
PEC_i/RAC_i (= ETR*i*) (all uses)		≤ 0.40	≤ 0.22
Lowest RAC_{sed} [µg/kg]		≥ 115.8	2326
Highest FOCUS Step 3 PEC_{sed} [µg/kg]	Winter oilseed rape	1.427	2.315
	Spring oilseed rape	2.205	2.471
	Sunflower	3.250	6.546
	Winter cereal	0.929	2.161
	Spring cereal	1.462	2.361
PEC_i/RAC_i (= ETR*i*) (all uses)		≤ 0.03	≤ 0.003

The ETR_i values of both active substances contained in the formulation BAS 762 02 F are below the relevant trigger value (*i.e.* 0.5) based on the worst-case FOCUS Step 3 values for all proposed uses. Therefore, a simplified approach can be applied, and no further assessment is necessary.

zRMS comments:

The combined risk assessment provided by the Applicant above is agreed by the zRMS.

Based on the MDR calculation it may be concluded that the measured and estimated toxicity of the mixture are in good agreement. As no synergistic or antagonistic effects of the formulation are expected, in line with EFSA (2013) the risk from the mixture could be evaluated using simplified approach which demonstrated acceptable risk from the mixture of both active compounds with no need for risk mitigation measures.

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.

Boscalid

The log P_{ow} of the active substance boscalid was determined to be 2.96 (P_{ow} 915) and a bioaccumulation study in fish was performed (see Monograph, Vol. 3, Annex B.9, 2002). An apparent steady state was reached after 1-4 days of exposure. The bioconcentration factors for whole fish were 57 (low concentration) and 70 (high concentration). The half-lives for elimination varied between 0.4 and 1.0 days. The time for

elimination of 90% of the activity varied between 1.4 and 3.3 days. The nature of radioactivity in fish tissues after 28 days of exposure proved to primarily consist of the parent substance (84.9% - 97.0%). Due to the low accumulation and rapid excretion of boscalid from fish it is concluded that there is no risk of bioaccumulation in food chains.

zRMS comments:

The EU agreed BCF values for both compounds were considered in evaluation of the risk of secondary poisoning to fish-eating birds and mammals. Acceptable risk with large margin of safety was concluded and on this basis bioaccumulation of neither mefentrifluconazole nor boscalid in the food chain is expected.

9.6.3 Overall conclusions

The standard risk assessment provided for the fungicidal product BAS 762 02 F, the active substances mefentrifluconazole and boscalid as well as their major metabolites demonstrates that the application of BAS 762 02 F according to good agricultural practice is of low risk to aquatic ecosystems.

zRMS comments:

The following text is added due to agreements during the Central Zone harmonisation meetings. It should be noted that this text has no impact on the outcome of zonal evaluation of formulation BAS 762 02 F, which was performed in line with the EU agreed methodology.

"The endpoint E_rC_{50} is selected in this Core Assessment but there are some uncertainties regarding the level of protection reached for primary producers. This is indicated for macrophytes in the aquatic Guidance Document (EFSA Journal 2013;11(7):3290) that recommends: "... a proper calibration between different tiers (higher and lower tier data) for macrophytes should be performed in the future". Such calibration should be extended to algae. Until available relevant information on the level of protection reached is considered at EU level, it is recommended to address this uncertainty at each Member State level in the National Addendum if considered necessary, although it would be highly appreciated to have a harmonised approach in the Central zone."

~~**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.~~

~~Fryday, S. and Thompson, H. 2012. Toxicity of pesticides to aquatic and terrestrial life stages of amphibians and occurrence, habitat use and exposure of amphibian species in agricultural environments. EFSA Supporting Publications, 9, EN-343: 348 pp.~~

~~Weltje, L., P. Simpson, M. Gross, M. Crane & J. R. Wheeler (2013) Comparative acute and chronic sensitivity of fish and amphibians: a critical review of data. Environmental Toxicology and Chemistry, 32, 984-994.~~

9.7 Effects on bees (KCP 10.3.1)

9.7.1 Toxicity data

Acute contact and oral toxicity studies on honey bees have been carried out with the active substances mefentrifluconazole (BAS 750 F) and boscalid (BAS 510 F). Furthermore, a chronic oral toxicity study on honey bees, a single exposure and a repeated exposure toxicity study on honey bee larvae as well as bumble bee acute contact and oral toxicity studies were performed with the active substance mefentrifluconazole. Full details of these studies are provided in the respective EU documents.
Additionally, to address the potential chronic and developmental risks to honey bees toxicity studies on honey bee larvae (acute and repeated exposure) have been carried out with the active substance boscalid and a chronic oral toxicity study on adult honey bees has been carried out with the boscalid solo-formulation BAS 510 01 F (50% boscalid).

For BAS 762 02 F, acute oral and contact toxicity studies on honey bees have been carried out. Furthermore, a chronic toxicity study on adult honey bees as well as a repeated exposure study on honey bee larvae are available. Besides the laboratory tests, a honey bee semi-field tunnel test was conducted with BAS 762 02 F.

All studies are listed in Table 9.7-1, Table 9.7-2 and Table 9.7-3.

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 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	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~~ ~~ED50 (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./bumblebee	EFSA Journal 2018;16(7):5379 2014/1275250
Bombus terrestris (adults)	mefentrifluconazole	acute contact	LD_{50} (96 h) > 200.0 µg a.s./bumblebee	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

zRMS comments:

The bee toxicity data for mefentrifluconazole presented in Table 9.7-1 are in general in line with the EU agreed endpoints reported in EFSA Journal 2018;16(7):5379.

In the course of the EU review the larvae toxicity study (XXX, 2015a, Rep. No 2014/1327676) was considered to be not valid and for this reason its results are struck through in table above. The Applicant is kindly reminded that only valid endpoints should be reported in the dRR.

Table 9.7-2: Endpoints and effect values of boscalid relevant for the risk assessment for bees

Species	Substance	Exposure System	Results	Reference
Apis mellifera (adults)	boscalid	acute oral	LD_{50} (48 h) > 166.0 µg a.s./bee	EC Review report, SANCO/3919 /2007-rev.5, 2008 1999/10823
Apis mellifera (adults)	boscalid	acute contact	LD_{50} (48 h) > 200.0 µg a.s./bee	EC Review report, SANCO/3919 /2007-rev.5, 2008 1999/10823
~~*Apis mellifera* (adults)~~	~~boscalid tested as BAS 510 01 F [1)]~~	~~chronic oral~~	~~LDD_{50} (10 d) > 150.9 µg a.s./bee/day NOEDD (10 d) ≥ 150.9 µg a.s./bee/day~~	~~not EU evaluated 2014/1083455~~
~~*Apis mellifera* (larvae)~~	~~boscalid~~	~~single exposure~~	~~LD_{50} (72 h) > 30.0 µg a.s./larva LC_{50} (72 h) > 914.6 mg a.s./kg food~~	~~not EU evaluated 2013/1275399~~
~~*Apis mellifera* (larvae)~~	~~boscalid~~	~~repeated exposure~~	~~ED_{50} (22 d) > 50 µg a.s./larva NOED (22 d) ≥ 50.0 µg a.s./larva~~	~~not EU evaluated 2017/1000161~~

~~1) Study was carried out with BAS 510 01 F, a boscalid solo formulation containing 50% boscalid.~~

zRMS comments:

The acute bee toxicity data for boscalid presented in Table 9.7-2 are in line with EU agreed endpoints reported in EU Review Report SANCO/3919/2007-rev.5.

In addition to the EU agreed data three new studies were submitted in order to address the chronic and larvae toxicity of boscalid to bees. However, the studies with boscalid and its solo formulation are considered relevant for the EU renewal process, while for the zonal evaluation studies performed with the formulation in question are relevant and were submitted by the Applicant fulfilling the data requirements as set by the Commission Regulation (EU) No 284/2013. No further data were deemed necessary and additional studies with boscalid and BAS 510 01 F were not validated for purposes of this zonal evaluation.

Table 9.7-3: Endpoints and effect values of BAS 762 02 F relevant for the risk assessment for bees

Species	Product	Exposure System	Results	Reference
Apis mellifera (adults)	BAS 762 02 F	acute oral	LD_{50} (48 h) > 772 µg/bee (corresponding to > 205 µg total a.s./bee)	not EU evaluated 2019/1061115
Apis mellifera (adults)	BAS 762 02 F	acute contact	LD_{50} (48 h) > 750 µg/bee (corresponding to > 199 µg total a.s./bee)	not EU evaluated 2019/1061115
Apis mellifera (adults)	BAS 762 02 F	chronic oral	LDD_{50} (10 d) = 429 µg product/bee/day NOEDD (10 d) = 80 µg product/bee/day	not EU evaluated 2020/2032682
Apis mellifera (larvae)	BAS 762 02 F	larvae repeated exposure	ED_{50} (22 d) > 250 µg product/larva ED_{10} (22 d) = 54.5 µg product/larva NOED (22 d) = 62.6 µg product/larva	not EU evaluated 2020/2032683
Higher-tier studies (tunnel test, field studies)				
Apis mellifera (all life stages)	BAS 762 02 F	semi-field tunnel test, application on full-flowering oilseed rape during bee flight, eastern Germany	no unacceptable lethal or sublethal effects on honey bee colonies exposed to 1.1 L/ha	not EU evaluated 2021/2001936

zRMS comments:

The studies performed with the formulated product were evaluated and agreed by the zRMS (for details, please refer to respective points in Appendix 2). Endpoints reported in Table 9.7-3 are confirmed to be correct.

Since at the NOED determined in the larvae repeated exposure study (XXX, 2021, 2020/2032683) there was >10% effect on emergence, the ED_{10} values has been also included in Table 9.7-3 as some cMS performing the larvae risk assessment at the national level may prefer to use lower of NOED and ED_{10}.

9.7.1.1 Justification for new endpoints

Effects of the formulation BAS 762 02 F on honey bees were not evaluated as part of the EU assessment of the active substances mefentrifluconazole or boscalid. Hence, all relevant data and assessments considering this formulation are provided here and are considered adequate.

For boscalid a repeated exposure study with honey bee larvae, tested with the active substance, as well as a chronic toxicity study on adult honey bees, tested with the solo-formulation BAS 510 01 F (50% boscalid) as surrogate for active substance, are provided. Comparable toxicity with minor deviation was observed between the acute data of active substance (Table 9.7-2) and BAS 510 01 F (LD_{50} oral (48 h) > 102.64 µg a.s./bee, LD_{50} contact (48 h) > 100.0 µg a.s./bee; DocID 2000/1011492, see SANCO/3919 /2007-rev. 5, Jan. 2008). Therefore, the chronic toxicity data of BAS 510 01 F is considered representative for the active substance boscalid. In addition, an acute toxicity study on honey bee larvae with boscalid is included as additional information.

All chronic studies on bees which were previously not evaluated on EU level, were checked for their potential to calculate $LC/EC_{10/20}$ values in accordance with Commission Regulations (EU) 283/2013 and 284/2013, respectively. If a calculation was possible, the $LC/EC_{10/20}$ 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 9.7.1. Please note that boscalid is currently under re-evaluation on EU level. To prevent a parallel statistical re-evaluation of these studies, they were not checked for their potential to calculate $L/EC_{10/20}$ values and reference is made to the EU process.

zRMS comments:

New studies on toxicity of boscalid or its solo formulation were deemed not necessary for the risk assessment purposes, since respective studies with BAS 762 02 F were submitted, fulfilling the data requirements as set by the Commission Regulation No 284/2013. The new active substance endpoints should be generated in the course of the EU renewal process.

9.7.2 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 at the time of application.

The application of BAS 762 02 F is envisioned in different field crops (*i.e.* oilseed rape, sunflower and cereals). The following risk assessment is based on the worst-case maximum single application rate of 1.0 L BAS 762 02 F/ha (equivalent to 100 g mefentrifluconazole/ha and 200 g boscalid/ha; see Section 9 Chapter 9.1 for details).

9.7.2.1 Hazard quotients for bees

The risk to honey bees from the use of mefentrifluconazole, boscalid and BAS 762 02 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 9.7-4 to Table 9.7-6).

Table 9.7-4: First-tier assessment of the risk for bees due to the use of mefentrifluconazole as contained in BAS 762 02 F according to the proposed use pattern

Intended use	field crops		
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/ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	> 100	100	< 1
Contact toxicity	> 100		< 1

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

Table 9.7-5: First-tier assessment of the risk for bees due to the use of boscalid as contained in BAS 762 02 F according to the proposed use pattern

Intended use	field crops		
Active substance	boscalid		
Application rate (g a.s./ha)	2 x 200		
Test design	**LD_{50} (lab.) (µg a.s./bee)**	**Single application rate (g/ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	> 166.0	200	< 1.2
Contact toxicity	> 200.0		< 1

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

Table 9.7-6: First-tier assessment of the risk for bees due to the use of BAS 762 02 F according to the proposed use pattern

Intended use	field crops		
Product	BAS 762 02 F		
Application rate (L/ha)	2 x 1.0		
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	> 772	1136 [1)]	< 1.5
Contact toxicity	> 750		< 1.5

Q_{HO}, Q_{HC}: Hazard quotients for oral and contact exposure.
1) Taking into account a single application of 1.0 L product/ha and the density of BAS 762 02 F of 1.136 g/cm^3.

zRMS comments:

The risk assessment presented in Tables 9.7-4 to 9.7-6 is agreed by the zRMS.
On its basis acceptable risk to bees may be concluded from all intended Central Zone uses of BAS 762 02 F.

Please note that the evaluation has been performed in line with SANCO/10329/2002 rev 2 final, as according to conclusions of the Central Zone Steering Committee (CZSC), recommendations of EFSA (2013) should not be considered for the zonal evaluations until the guidance is noted at the EU level. Therefore risk assessment based on indications of EFSA (2013) must be performed at the national level by cMS that do require such evaluation.

~~Commission Regulation (EU) No 284/2013 lists conditions under which testing of the formulated product is required. In accordance with the requirements set out in points 8.3.1 and 8.3.2 of Part A of the Annex to Regulation (EU) No 284/2013, formulated product testing is needed if the product contains more than one active substance and if the toxicity of a plant protection product cannot be reliably predicted to be either the same or lower than the toxicity of the active substances. For BAS 762 02 F, acute honey bee endpoints are available for all active substances and formulation. This data can be used to check the second condition, i.e. whether the formulated product shows unexpected toxicity.~~

~~The comparison of the acute endpoint obtained with the formulated product and the active substance endpoints, under consideration of the model deviation ratio (MDR), is shown in Table 9.7-7. If the MDR is between 0.2 and 5, the observed and calculated mixture toxicities are considered in agreement. Comparing the acute toxicity of the active substances with the acute toxicity of the formulated product BAS 762 02 F, no indication for unpredicted product toxicity is given (MDR of 1.09 and 1.2 for acute oral and acute contact data, respectively).~~

~~Furthermore, 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 phys. chem. properties). Therefore, data on the active substances should be used for the risk assessment. Calculated endpoints for the combination of mefentrifluconazole and boscalid in the product BAS 762 02 F are presented in~~

Table 9.7-8. Also for chronic data the calculated mixture toxicity is in the expected range (see Table 9.7-3). Both calculated and measured values of BAS 762 02 F are used for the risk assessment.

Table 9.7-7: Measured acute toxicity of BAS 762 02 F and calculated mixture toxicity comparison and presentation of the model deviation ratio (MDR)

Test organisms (Species)	Test type & endpoint	Measured toxicity of the a.s. [µg a.s./bee]		Measured toxicity of BAS 762 02 F ($LD_{50\ PPP}$) [µg product/bee]	Calculated mixture toxicity ($LD_{50\ mix\ CA}$) [µg mixture/bee] [1)]	MDR ($LD_{x\ mix\ CA}$ / LDx_{PPP})
honey bee (*Apis mellifera*)	acute oral, 48 h LD_{50}	mefentrifluconazole	> 100	> 772.8 (> 205 µg total a.s./bee)	> 512.5 (> 136 µg total a.s./bee)	0.66
		boscalid	> 166			
	acute contact, 48 h LD_{50}	mefentrifluconazole	> 100	> 750 (> 199 µg total a.s./bee)	> 565.0 (> 150 µg total a.s./bee)	0.75
		boscalid	> 200			

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

[1)] The theoretical formulation toxicity of the product was re-calculated based on the measured toxicity data of the active substances and their nominal content within the formulation (i.e. 100 g mefentrifluconazole/L and 200 g boscalid/L) and a product density of 1.130 g/cm^3 from the study.

Nevertheless, additional toxicity data on BAS 762 02 F were produced and chronic adult and larvae information is summarized in

Table 9.7-**8**. The data indicate that the formulated product shows toxicity in the expected range for most of the endpoints. Only the NOEDD in the chronic adult study is lower than expected. This increase in toxicity could be explained by potential synergism of the active substances which is considered a rare case in ecotoxicological testing. Nevertheless, there are some examples in the literature describing situations where concentration addition is not able to explain the increase in toxicity (e.g. Pilling & Jepson, 1995; Johnson et al. 2013, Thompson et al. 2014, Wernecke et al. 2019). However, according to all empirical evidence, joint actions of active substance that indicate clear synergistic effects, are obviously exceptional situations and not at all the rule (cited from Frische et al. 2014 with reference to Altenburger et al. 2012; Kortenkamp et al. 2009). Therefore, there is a high likelihood that the increase in toxicity can be explained by the interaction with the formulants. Data on the phys-chem properties of the formulants indicate that major constituents are readily biodegradable and volatile (i.e. vapor pressure > 0.1 Pa). For further details on the formulants reference is made to Part C of this dossier and the respective SDS. Because of the high volatility, chronic or in hive exposure to the intact formulation can be excluded. Hence, it is proposed to use the information on the active substances for the chronic risk assessment since a) chronic exposure to formulants is not expected and b) additive toxicity is deemed appropriate to assess the chronic risk to bees. Following this approach, the calculated mixture toxicity is proposed as the relevant endpoint for chronic adult bee and larvae.

Table 9.7-8: Measured chronic and larval toxicity of BAS 762 02 F and calculated mixture toxicity comparison and presentation of the model deviation ratio (MDR)

Test organisms, life stage (Species)	Test type & endpoint	Measured toxicity of the a.s. [µg a.s./bee]		Measured toxicity of BAS 762 02 F	Calculated mixture toxicity [1)]	MDR
honey bee, adults (*Apis mellifera*)	chronic oral, 10 d NOED	mefentrifluconazole	≥ 110.5	80.0 µg/bee/day (21.2 µg total a.s./bee/day)	≥ 506.6 µg/bee/day (≥ 134.5 µg total a.s./bee)	≥ 6.3
		boscalid	≥ 150.9			
	chronic oral, 10 d LDD_{50}	mefentrifluconazole	> 110.5	429.0 µg/bee/day (120.0 µg total a.s./bee/day)	> 506.6 µg/bee/day (> 134.5 µg total a.s./bee/day)	> 1.2
		boscalid	> 150.9			
honey bee, larvae (*Apis mellifera*)	repeated exposure, 22 d NOED	mefentrifluconazole	25.0	62.6 µg/larva (16.6 µg total a.s./larva)	≥ 141.3 µg/larva (≥ 37.5 µg total a.s./larva)	> 2.3
		boscalid	≥ 50.0			
	repeated exposure, 22 d ED_{50}	mefentrifluconazole	> 50.0	250 µg/larva (66.4 µg total a.s./larva)	188.3 µg/larva (50.0 µg total a.s./larva)	> 0.75
		boscalid	> 50.0			

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

1) The theoretical formulation toxicity of the product was re-calculated based on the measured toxicity data of the active substances and their nominal content within the formulation (i.e. 100 g mefentrifluconazole/L and 200 g boscalid/L) and a product density of 1.130 g/cm^3 from the study.

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 are available for mefentrifluconazole (BAS 750 F) and boscalid (BAS 510 F) and the mixture BAS 762 02 F. 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.

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 boscalid, RUD residue values reported in the external EFSA supporting publication on residues in bee relevant matrices (EFSA 2017) have been used to estimate the exposure. For mefentrifluconazole, 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 762 02 F (100 g mefentrifluconazole/ha and 200 g boscalid/ha; see

~~Table 9.7 9).~~

Table 9.7-9: 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
boscalid (Application rate 200 g a.s./ha)	63.70 mg a.s./kg [1)]	12.74 mg a.s./kg
Nectar		
mefentrifluconazole (Application rate 100 g a.s./ha)	3.99 mg a.s./kg [1)]	0.4 mg a.s./kg
boscalid (Application rate 200 g a.s./ha)	3.99 mg a.s./kg [1)]	0.8 mg 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 196.7 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), boscalid (BAS 510 F) and BAS 762 02 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 762 02 F (see **Table 9.7-10** to Table 9.7-12). The calculated chronic TER values are given in Table 9.7-13 to Table 9.7-15. As outlined above, the risk assessment for chronic adult bees and larvae should focus on the data of the active substances to reflect a more realistic exposure situation. In Table 9.7-15 both the TER values for the calculated (based on the active substances) and measured values are presented. 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 100, it can be concluded that the risk for chronic adult and developmental exposure to honey bees can be considered acceptable.**

Table 9.7-10: Total residue intake for adult honey bees and larvae following exposure to BAS 750 F according to the proposed uses of BAS 762 02 F

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 mg/larva
Residue intake through pollen	0 µg a.s./bee/day	0.01 µg a.s./larva
Residue in nectar	0.4 mg a.s./kg (= 0.0004 µg a.s./mg)	0.4 mg a.s./kg (= 0.0004 µg a.s./mg)
Nectar consumption	426.7 mg/bee/day	196.7 mg/larva
Residue intake through nectar	0.17 µg a.s./bee/day	0.08 µg a.s./larva
Total residue intake	**0.17 µg a.s./bee/day**	**0.09 µg a.s./larva**

Table 9.7-11: Total residue intake for adult honey bees and larvae following exposure to BAS 510 F according to the proposed uses of BAS 762 02 F

Honey bee stage	Adult	Larva (over 5 days)

Residue in pollen	12.74 mg a.s./kg (= 0.01274 µg a.s./mg)	12.74 mg a.s./kg (= 0.01274 µg a.s./mg)
Pollen consumption	0	2 mg/larva
Residue intake through pollen	0 µg a.s./bee/day	0.03 µg a.s./larva
Residue in nectar	0.8 mg a.s./kg (= 0.0008 µg a.s./mg)	0.8 mg a.s./kg (= 0.0008 µg a.s./mg)
Nectar consumption	426.7 mg/bee/day	196.7 mg/larva
Residue intake through nectar	0.34 µg a.s./bee/day	0.16 µg a.s./larva
Total residue intake	**0.34 µg a.s./bee/day**	**0.19 µg a.s./larva**

Table 9.7-12: Total residue intake for adult honey bees and larvae following exposure to BAS 762 02 F according to the proposed uses

Honey bee stage	**Adult**	**Larva (over 5 days)**
Residue in pollen	19.1 mg total a.s./kg (= 0.0191 µg total a.s./mg)	19.1 mg total a.s./kg (= 0.0191 µg total a.s./mg)
Pollen consumption	0	2 mg/larva
Residue intake through pollen	0 µg total a.s./bee/day	0.04 µg total a.s./larva
Residue in nectar	1.2 mg total a.s./kg (= 0.0012 µg total a.s./mg)	1.2 mg total a.s./kg (= 0.0012 µg total a.s./mg)
Nectar consumption	426.7 mg/bee/day	196.7 mg/larva
Residue intake through nectar	0.51 µg total a.s./bee/day	0.24 µg total a.s./larva
Total residue intake	**0.51 µg total a.s./bee/day**	**0.28 µg total a.s./larva**

Table 9.7-13: Chronic risk to adult bees and larvae following the use of BAS 750 F according to the proposed uses using the TER approach

Honey bee stage	**Exposure route**	**NOED**	**Worst case residue intake**	**TER_{ch}**	**Trigger value**
Adult	Oral	≥ 110.5 µg a.s./bee/day	0.17 µg a.s./bee/day	≥ 650	1
Larvae	Oral	25 µg a.s./larva	0.09 µg a.s./larva	278	1

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

Table 9.7-14: Chronic risk to adult bees and larvae following the use of BAS 510 F according to the proposed uses using the TER approach

Honey bee stage	**Exposure route**	**NOED**	**Worst case residue intake**	**TER_{ch}**	**Trigger value**
Adult	Oral	≥ 150.9 µg a.s./bee/day	0.34 µg a.s./bee/day	≥ 444	1
Larvae	Oral	≥ 50.0 µg a.s./larva	0.19 µg a.s./larva	≥ 263	1

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

Table 9.7-15: Chronic risk to adult bees and larvae following the use of BAS 762 02 F according to the proposed uses using the TER approach

Honey bee stage	**Exposure route**	**NOED**	**Worst case residue intake**	**TER_{ch}**	**Trigger value**
Adult	Oral	21.3 µg total a.s./bee/day [1)] 134.5 µg total a.s./bee/day [2)]	0.51 µg a.s./bee/day	42 264	1
Larvae	Oral	16.6 µg total a.s./larva [1)] ≥ 37.5 µg total a.s./larva [2)]	0.28 µg a.s./larva	59 ≥ 134	1

[1)] Measured toxicity of BAS 762 02 F.

[2)] Calculated value by concentration addition (Finney); considering the portion of the active substance in relation to the sum of substances within the mixture.

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

- in both approaches the chronic adult and larvae endpoints are set into relation to exposure which is based on pollen and nectar consumption.
- In both approaches the chronic risk assessment is focussed on active substances unless a considerable difference between calculated and observed toxicity of the product is shown in the acute oral study.
- in both approaches the assumed amount of pollen and nectar consumption and the relevant time-frame is identical as it is based on the same literature references.

~~The main difference between the chronic risk assessment according to EFSA (2013a) and EPPO (2010) are endpoints and trigger values used. The EPPO scheme (2010) proposes a simple TER approach and the margin of safety in relation to the no effect level is straightforward and transparent. Hence, the proposed assessment considers all available information and is considered adequate until a revised and adopted guidance document for bees exists.~~

zRMS comments:

The Applicant provided comparison of the measured and estimated mixture toxicity, which was, however, not validated by the zRMS since calculation of MDR values in case of bees is indicated neither in the current guidance document (SANCO/10329/2002 rev 2) nor EFSA (2013). In line with both guidance documents, the risk assessment for bees is performed using either formulation or formulation+active substance toxicity data.

The chronic and larvae risk assessment was not evaluated by the zRMS as being not required according to SANCO/10329/2002 rev 2 final. Furthermore, the assessment was performed in line with the revised EPPO scheme of 2010, while in opinion of the zRMS in case the chronic and larvae risk assessment is performed, it should be conducted in line with EFSA (2013).

Nevertheless, the Applicant submitted a tunnel study performed on flowering oilseed rape with BAS 762 02 F applied during the bee activity (XXX, 2021, Rep. No 2021/2001936) which investigated effects on adult bees, bee brood and bee colonies up to 40 days after the treatment. On the basis of results of this semi-field study no unacceptable chronic effects on adult bees, bee brood and bee colonies are expected when BAS 762 02 F is applied up to 1.1 L/ha. For detailed results of the study and study evaluation by the zRMS, please refer to Appendix 2.

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

For BAS 762 02 F and the active substances mefentrifluconazole and boscalid, a full data set of laboratory studies on adult honey bees and other honey bee live stages is available. The risk assessment based on laboratory studies already resulted in no unacceptable risk to honey bees (compare chapter 9.7.2.1).
However, in addition to the laboratory studies a higher-tier semi-field tunnel test with BAS 762 02 F (DocID 2021/2001936) has been performed according to OECD 75 (2007) in eastern Germany. The study was carried out to gain additional information on the potential toxicity of BAS 762 02 F covering effects on honey bee larvae and bee brood development under more realistic conditions. BAS 762 02 F was applied at a rate of 1.1 L/ha during active foraging of the honey bees on full flowering oilseed rape (BBCH 65) enclosed within tunnel tents. A study summary is presented in Appendix 2.

The application of BAS 762 02 F caused no effects on adult and pupal honey bee mortality, foraging activity, behaviour and brood development. Additionally, the specific evaluation of the detailed bee brood development of initially labelled eggs showed no impact of the test item during the entire trial.

The results of the higher tier study, conducted under more realistic conditions, confirm the outcome of the risk assessment based on laboratory studies. The proposed use of BAS 762 02 F, according to good agricultural practice, presents low risk to honey bees and will not adversely affect honey bee colonies.

zRMS comments:

The tunnel study by XXX (2021, Rep. No 2021/2001936) was evaluated and agreed by the zRMS. On the basis of its results, no unacceptable chronic effects on adult bees, bee brood and bee colonies are expected when BAS 762 02 F is applied up to 1.1 L/ha. For detailed results of the study and study evaluation by the zRMS, please refer to Appendix 2.

9.7.3 Effects on bumble bees

For bumblebees no specific data requirement exists under regulation (EC) No 1107/2009. Nevertheless, to support the application an acute oral and contact study was conducted with the active substance mefentrifluconazole. The oral and contact LD_{50} were determined to be > 195.4 µg a.s./bumblebee and > 200.0 µg a.s./bumblebee, respectively. Both endpoints exceed the acute endpoints for honey bees suggesting that mefentrifluconazole poses no unacceptable risk to bumblebees at the proposed use rate.

zRMS comments:

No risk assessment scheme for bumblebees is available in SANCO/10329/2002 rev 2 final and for this reason no specific evaluation for this species is currently required.

9.7.4 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.

zRMS comments:

No risk assessment scheme for solitary bees is available in SANCO/10329/2002 rev 2 final and for this reason no specific evaluation for this species is currently required.

9.7.5 Overall conclusions

The hazard quotients for BAS 762 02 F and the active substances mefentrifluconazole and boscalid 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 762 02 F according to the proposed uses. This is confirmed by results of the tunnel study performed on flowering winter oilseed rape with BAS 762 02 F applied during the bee activity. ~~a risk assessment following EPPO (2010) for chronic exposure to adult honey bees and repeated exposure to honey bee larvae.~~

~~**References**~~

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9.8 Effects on arthropods other than bees (KCP 10.3.2)

9.8.1 Toxicity data

The toxicity of BAS 762 02 F to non-target arthropods has been investigated by carrying out Tier I tests on *Aphidius rhopalosiphi* and *Typhlodromus pyri*. All studies are listed in Table 9.8-1. New data submitted with this application are listed in Appendix 1 and summarized in Appendix 2.

Table 9.8-1: Endpoints and effect values for BAS 762 02 F relevant for the risk assessment for non-target arthropods

Species	Product	Exposure System	Results	Reference
Typhlodromus pyri (protonymphs)	BAS 762 02 F	laboratory test glass plates 2D exposure	LR_{50} > 3.0 L/ha Corrected mortality: -1.0% at 0.1875 L/ha 0% at 0.375 L/ha 0% at 0.75 L/ha 1.0% at 1.5 L/ha -1.0% at 3.0 L/ha	not EU evaluated 2019/1061533
Aphidius rhopalosiphi (adults)	BAS 762 02 F	laboratory test glass plates 2D exposure	LR_{50} > 3.0 L/ha Corrected mortality: 0% at 0.1875 L/ha -2.6% at 0.375 L/ha -2.6% at 0.75 L/ha 0% at 1.5 L/ha 0% at 3.0 L/ha	not EU evaluated 2019/1061532

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

zRMS comments:

The studies performed with the formulated product were evaluated and agreed by the zRMS (for details, please refer to respective points in Appendix 2). Endpoints reported in Table 9.8-1 are confirmed to be correct.

9.8.1.1 Justification for new endpoints

Effects of BAS 762 02 F on non-target arthropods other than bees were not evaluated as part of the EU assessment of the active substances mefentrifluconazole and boscalid. Hence, all relevant data and assessments considering this formulation are provided here and are considered adequate.

9.8.2 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).

9.8.2.1 Risk assessment for in-field exposure

The application of BAS 762 02 F is envisioned in several field crops (i.e. oilseed rape, sunflower and cereals). The following risk assessment is based on the worst-case field application rate of 2 × 1.0 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:

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

Default foliar and soil MAF (Multiple Application Factor) values following multiple applications are given in the ESCORT 2 Guidance Document and are the following for BAS 762 02 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 762 02 F (see Section 9 Chapter 9.1 for details), the MAF (soil) will not be considered in the following risk assessment. Thus, the $PER_{in-field}$ is 1.7 L/ha.

The potential risk for non-target arthropods exposed in-field to BAS 762 02 F was assessed by calculating the hazard quotient (HQ = exposure/toxicity, see Table 9.8-2) for tier I standard laboratory studies according to the formula:

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

Table 9.8-2: First-tier assessment of the in-field risk for non-target arthropods due to the use of BAS 762 02 F according to the proposed use pattern

Intended use	field crops		
Product	BAS 762 02 F		
Application rate (L/ha)	2 x 1.0		
MAF	1.7 (vegetation)		
Test species	**Tier I**		
	LR_{50} (lab.) [L/ha]	**$PER_{in-field}$ [L/ha]**	**$HQ_{in-field}$ criterion: HQ ≤ 2**
Typhlodromus pyri	> 3.0	1.7	< 0.57
Aphidius rhopalosiphi	> 3.0		< 0.57

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

zRMS comments:

The risk assessment presented in Table 9.8-2 is agreed by the zRMS.
Based on calculations performed with consideration of the Tier I laboratory data acceptable in-field risk to non-target arthropods from all intended uses of BAS 762 02 F may be concluded.

9.8.2.2 Risk assessment for off-field exposure

Exposure of non-target arthropods living in off-field areas to BAS 762 02 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:

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

A vegetation distribution or dilution factor is included in the equation when calculating PER values from toxicity endpoints derived from two-dimensional studies (Table 9.8-3). A dilution factor of 10 is recommended by ESCORT 2.

For 2 applications of BAS 762 02 F in field crops, 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 9.8-3: $PER_{off\text{-}field}$ values following application of BAS 762 02 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	1.7	0.0238	10	0.00405
3D			--	0.0405

PER: (corrected) Predicted environmental rate

To assess the potential risk of BAS 762 02 F to off-field non-target arthropods (see Table 9.8-4), the $PER_{off\text{-}field}$ (Table 9.8-3) is compared to the toxicity endpoints of tier I standard laboratory studies according to the following equation:

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

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 9.8-4: First-tier assessment of the off-field risk for non-target arthropods due to the use of BAS 762 02 F according to the proposed use pattern

Intended use	field crops				
Product	BAS 762 02 F				
Application rate (L/ha)	2 x 1.0				
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	> 3.0	2.38	0.00405	10	< 0.013
Aphidius rhopalosiphi	> 3.0				< 0.013
Additional Tier I risk assessment based on VDF of 5 (please note that discussion on consideration of VDF of 5 is not finalised yet and it is uncertain when consideration of VDF of 5 will be reflected in the respective guidance)					
Typhlodromus pyri	> 3.0	2.38	0.0081	10	<0.027
Aphidius rhopalosiphi	> 3.0				<0.027

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

zRMS comments:

The risk assessment presented in Table 9.8-4 is agreed by the zRMS.

During the commenting period it was pointed out that the VDF of 5 was agreed during the CZHW in Brno in 2019 and that this value should have been used for purposes of the off-field exposure calculation.
It should be, however, noted that in line with implementation schedule indicated in the Bullet points in area of ecotoxicology agreed by the CZSC in November 2021, VDF of 5 should be considered since 1st of July 2022.
Furthermore, Bullet point 4 presented in this document indicates that:

> *The majority of MSs agreed to be in line with the EFSA Technical Report (2019) and use a VDF of 5*

It should be pointed out that the EFSA Technical Report (EFSA Supporting publication 2019:EN-1673) does not indicate that currently VDF of 5 must be used in evaluations, but that VDF of 5 should be considered as an interim solution that will be reflected in the SANCO/10329/2002-rev.2 guidance document with its implementation considered further. However, the SANCO guidance document was not amended yet and this is acknowledged in the most recent version of the Working document on Risk Assessment of Plant Protection Products in the Central Zone (May 2021):

The CZSC will make an urgent request to the Commission to adjust this issue in the guidance document as soon as possible.

Therefore, from the formal point of view, VDF of 10 is still applicable and may be used for purposes of calculation of the off-field exposure.

It is also uncertain if consideration of VDF of 5 will be possible after 1st of July 2022 in case it will not be reflected in the terrestrial GD as an interim solution.

Nevertheless, calculations based on VDF of 5 were included in Table 9.8-4 above for convenience of the cMS that prefer to consider VDF of 5 although its use is not yet reflected in the respective guidance document.

Based on calculations performed with consideration of the Tier I laboratory data acceptable off-field risk to non-target arthropods from all intended uses of BAS 762 02 F may be concluded with no need for risk mitigation measures.

9.8.2.3 Additional higher-tier risk assessment

Not relevant.

9.8.2.4 Risk mitigation measures

No risk mitigation needed.

9.8.3 Overall conclusions

Based on the first-tier risk assessments low risk for non-target arthropods is expected from application of BAS 762 02 F according to the proposed use pattern. No unacceptable effects on non-target arthropods are expected in in-field and off-field habitats.

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

9.9.1 Toxicity data

Studies on the toxicity to earthworms and other non-target soil organisms (meso- and macrofauna) have been carried out with mefentrifluconazole and its 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 boscalid and BAS 762 02 F. All studies are listed in Table 9.9-1,

Table 9.**9-2** and

Table 9.**9-3**

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

Table 9.9-1: Endpoints and effect values of mefentrifluconazole and its 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 *~~	~~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 used for the risk assessment

Acute studies listed for reference only but not used in the risk assessment according to Commission Regulation (EU) 283/2013.

* Corrected value derived by dividing the endpoint by a factor of 2 due to a log P_{OW} >2.

zRMS comments:

The toxicity data for soil macro- and meso-fauna given in Table 9.9-1 are in general line with EU agreed endpoints reported in EFSA Journal 2018;16(7):5379. Endpoints not reported in the LoEP are struck through in table above.

As acute toxicity to earthworms is no longer a data requirement, the results of the acute study are struck through as not considered in the risk assessment. Please note also that they were not reported in EFSA Journal 2018;16(7):5379.

Table 9.9-2: Endpoints and effect values of boscalid relevant for the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna)

Species	Substance	Exposure System	Results	Reference
~~**Acute** #~~				
~~*Eisenia fetida*~~	~~boscalid~~	~~Mixed into substrate 14 d 10% peat content~~	~~LC_{50} > 1000 mg a.s./kg dry soil~~ ~~$LC_{50\ CORR}$ > 500 mg/kg dry soil~~ ~~*~~	~~EC Review report, SANCO/3919/2007 rev.5, 2008 1999/10816~~
Chronic				
Eisenia fetida	boscalid	Mixed into substrate 56 d 10% peat content	NOEC = 25 mg/kg dry soil **$NOEC_{CORR}$ = 12.5 mg/kg dry soil** * EC_{10} = 37 mg/kg dry soil $EC_{10\ CORR}$ = 18.5 mg/kg dry soil *	not EU evaluated 2014/1083454
Folsomia candida	boscalid	Mixed into substrate 28 d 5% peat content	NOEC ≥ 1000 mg/kg dry soil **$NOEC_{CORR}$ ≥ 500 mg/kg dry soil** * EC_{10} = n.d.	not EU evaluated 2014/1083456

Values in **bold** are used for the risk assessment.
n.d. = not determinable

Acute studies listed for reference only but not used in the risk assessment according to Commission Regulation (EU) No 283/2013.

* Corrected value derived by dividing the endpoint by a factor of 2 due to a log P_{OW} >2.

zRMS comments:

The acute toxicity value for earthworms given in Table 9.9-2 is in line with EU agreed endpoints reported in EU Review Report SANCO/3919/2007-rev.5. However, as acute toxicity to earthworms is no longer a data requirement, the results of the study are struck through as not considered in the risk assessment.

In addition to that two new studies on long-term toxicity of boscalid to earthworms and *F. candida* were submitted. Both studies were already evaluated during EU renewal of boscalid and considered acceptable. Taking this into account it was decided by the zRMS to retain the derived NOEC values in Table 9.9-2, bearing in mind that the renewal process for boscalid was not yet finalised and all endpoints are still under discussion and may change.

It should be noted that in the current LoEP (SANCO/3919/2007-rev. 5) lower NOEC of 1.197 mg a.s./kg dws is reported for earthworms. It should be, however, pointed out that this endpoint has been derived from study performed with the representative formulation (BAS 510 01 F) and not with BAS 762 02 F, considered in this zonal assessment. In addition to that, the test item in the EU agreed study was sprayed over the soil surface and not mixed with soil, as indicated in the data requirements. For this reason the endpoint from the study was recalculated from the application rate and may be not fully reliable. Furthermore, the impact of co-formulants of the tested formulation cannot be fully ruled out and for this reason value given in the LoEP is considered not relevant for the risk assessment performed for BAS 762 02 F, especially relevant study with the formulated product has been submitted. Therefore, in opinion of the zRMS, it is more appropriate to consider in the risk assessment the results of the study performed with formulation in question (i.e. BAS 762 02 F) as well as results of the new study performed with the active substance, as this gives a better picture of the long-term effects on earthworms following application of BAS 762 02 F. Although the renewal process is not finalised yet and endpoints may be still subject of discussion, the review of the study summary presented in the Draft Renewal Assessment Report for boscalid and its evaluation by the RMS does not indicate any concerns with regard to the endpoint derived for boscalid.

Table 9.9-3: Endpoints and effect values of BAS 762 02 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 762 02 F	Mixed into substrate 56 d 10% peat content	NOEC = 84 mg/kg dry soil (equivalent to 7.4 mg mefentrifluconazole/kg dry soil and 15 mg boscalid/kg dry soil) [1)] **$NOEC_{CORR}$ = 11.2 mg total a.s./kg dry soil [2)] *** EC_{10} = 86 mg/kg dry soil (equivalent to 7.6 mg mefentrifluconazole/kg dry soil and 15.2 mg boscalid/kg dry soil) [1)] $EC_{10\ CORR}$ = 11.4 mg total a.s./kg dry soil [2)] *	not EU evaluated 2020/1000741
Folsomia candida	BAS 762 02 F	Mixed into substrate 28 d 5% peat content	NOEC = 200 mg/kg dry soil (equivalent to 17.7 mg mefentrifluconazole/kg dry soil and 35.4 mg boscalid/kg dry soil) [1)] **$NOEC_{CORR}$ = 26.5 mg total a.s./kg dry soil [2)] *** EC_{10} = 239.9 mg/kg dry soil (equivalent to 21.2 mg mefentrifluconazole/kg dry soil and 42.5 mg boscalid/kg dry soil) [1)] $EC_{10\ CORR}$ = 31.8 mg total a.s./kg dry soil [2)] *	not EU evaluated 2020/1000742
Hypoaspis aculeifer	BAS 762 02 F	Mixed into substrate 14 d 5% peat content	NOEC = 352.9 mg/kg dry soil (equivalent to 31.2 mg mefentrifluconazole/kg dry soil and 62.5 mg boscalid/kg dry soil) [1)] **$NOEC_{CORR}$ = 46.9 mg total a.s./kg dry soil [2)] *** ~~NOEC ≥ 600 mg/kg dry soil (equivalent to ≥ 53.1 mg mefentrifluconazole/kg dry soil and ≥ 106.2 mg boscalid/kg dry soil) [1)]~~ EC_{10} > 600 mg/kg dry soil (equivalent to > 53.1 mg mefentrifluconazole/kg dry soil and > 106.2 mg boscalid/kg dry soil) [1)] $EC_{10\ CORR}$ = 79.6 mg total a.s./kg dry soil [2)] * ~~$NOEC_{CORR}$ ≥ 79.6 mg total a.s./kg dry soil [2)] **~~	not EU evaluated 2020/1000743

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) Based on the content of the active substances (nominal) and taking into account a density of BAS 762 02 F of 1.130 g/cm^3.

2) Endpoint based on sum of active substances (nominal) and taking into account a density of BAS 762 02 F of 1.130 g/cm^3.

zRMS comments:

The studies performed with the formulated product were evaluated and agreed by the zRMS (for details, please refer to respective points in Appendix 2). Endpoints reported in Table 9.9-3 are confirmed to be in general correct with exception of NOEC value for *H. aculeifer*, which should be 352.9 mg product/kg dw soil, in line with results reported in the study report. Respective corrections were made in table above.

9.9.1.1 Justification for new endpoints

Effects of the formulation BAS 762 02 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 boscalid. Similarly, chronic effects in non-target soil macro-organisms exposed to boscalid were not evaluated in the EU assessments of boscalid. Hence, all relevant data and assessments considering the formulation and boscalid 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.

All chronic studies on earthworms, collembolans and soil mites after guidelines OECD 222, OECD 232 and OECD 226, 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 9.9.1.

In the risk assessment, both NOEC and EC_{10} values (if available) are used for TER calculation. The conclusion, however, will be based on the EC_{10} if reliable. If the EC_{10} is not reliable or could not be calculated, the NOEC is considered the relevant endpoint for the risk assessment.

zRMS comments:

As indicated in zRMS comments in point 9.6.1 above, in line with current approach , the lower of EC_{10} and NOEC value should be used in the risk assessment for soil organisms.

Consideration of the EFSA Supporting publication 2015:EN-924 is commonly agreed within the Central Zone and in line with its indications, the endpoint must be corrected when log Pow is greater than 2, irrespective of the peat contend in soil used in the study. In consequence, endpoints obtained from studies performed with 5% peat are also corrected.

The risk assessment presented in point 9.9.2 was thus amended accordingly.

9.9.2 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).

The log P_{ow} value of the mefentrifluconazole metabolite 1,2,4-triazole is < 2. Therefore, the endpoints are not corrected. The endpoints of active substances mefentrifluconazole and boscalid were corrected (for studies with 10% peat content), due to a log P_{ow} >2.

9.9.2.1 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 needs to be considered for mefentrifluconazole, boscalid and the metabolite 1,2,4-triazole.

The potential risk of BAS 762 02 F, mefentrifluconazole, boscalid and relevant metabolites to earthworms and other non-target soil macro-organisms was assessed by comparing the maximum PEC_{soil} values with NOEC or EC_{10} values, to generate long-term TER values (TER_{lt}, Table 9.9-4 to Table 9.9-6).

The TER was calculated as follows:

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

Table 9.9-4: 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 762 02 F according to the proposed use pattern

Intended use	2 x 100 g mefentrifluconazole/ha in sunflower [1)]		
Chronic effects on earthworms			
Active substance/metabolite	**Endpoint (mg/kg dry soil)**	**PEC_{soil} (mg/kg dry soil)**	**TER_{lt} (criterion TER ≥ 5)**
mefentrifluconazole	*$NOEC_{CORR}$ = 4.0* $EC_{10\ CORR}$ = 2.65	0.229 [*]	17 12
Metabolite, Reg. No. 87 084 1,2,4-triazole	NOEC ≥ 1.0	0.001 [*]	≥ 1000
Chronic effects on other soil meso- and macrofauna			
Active substance/metabolite	**Endpoint (mg/kg dry soil)**	**PEC_{soil} (mg/kg dry soil)**	**TER_{lt} (criterion TER ≥ 5)**
Collembola (*Folsomia candida*)			
mefentrifluconazole	NOEC ≥ 400 *$NOEC_{CORR}$ ≥ 200*	0.229 [*]	≥ 1747 ≥ 873
Metabolite, Reg. No. 87 084 1,2,4-triazole	NOEC = 1.8	0.001 [*]	1800
Soil mite (*Hypoaspis aculeifer*)			
mefentrifluconazole	NOEC ≥ 1000 *$NOEC_{CORR}$ ≥ 500*	0.229 [*]	≥ 4367 ≥ 2183
Metabolite, Reg. No. 87 084 1,2,4-triazole	NOEC = 171	0.001 [*]	171000

Underlined endpoints and TER values are relevant for the conclusion of the risk assessment.
[*] $PEC_{soil,\ accu}$. For details please refer to section 8, chapter 8.7, table 8.7-5 and table 8.7-8.
[1)] Use resulting in the worst-case PEC_{soil} values covering all other intended uses (see chapter 9.1.2).

Table 9.9-5: First-tier assessment of the chronic risk for earthworms and other non-target soil organisms (meso- and macrofauna) due to the use of boscalid as contained in BAS 762 02 F according to the proposed use pattern

Intended use	2 x 200 g boscalid/ha in sunflower [1)]		
Chronic effects on earthworms			
Active substance	**Endpoint (mg/kg dry soil)**	**PEC_{soil} (mg/kg dry soil)**	**TER_{lt} (criterion TER ≥ 5)**
boscalid	NOEC = 25 $NOEC_{CORR}$ = 12.5 EC_{10} = 37 $EC_{10\ CORR}$ = 18.5	0.422 [*]	59 30 88 44
Chronic effects on other soil meso- and macrofauna			
Active substance	**Endpoint (mg/kg dry soil)**	**PEC_{soil} (mg/kg dry soil)**	**TER_{lt} (criterion TER ≥ 5)**
Collembola (*Folsomia candida*)			
boscalid	NOEC ≥ 1000 $NOEC_{CORR}$ ≥ 500	0.422 [*]	≥ 2370 ≥ 1185

Underlined endpoints and TER are relevant for the conclusion of the risk assessment.
* $PEC_{soil, accu.}$ For details please refer to section 8, chapter 8.7, table 8.7-11.
1) Use resulting in the worst-case PEC_{soil} value covering all other intended uses (see chapter 9.1.2).

Table 9.9-6: First tier assessment of the chronic risk for earthworms and other non-target soil organisms (meso- and macrofauna) due to the use of BAS 762 02 F according to the proposed use pattern

Intended use	2 x 1.0 L BAS 762 02 F/ha in sunflower 1)		
Chronic effects on earthworms			
Product	**Endpoint (mg a.s./kg dry soil)**	**PEC_{soil} (mg a.s./kg dry soil)**	**TER_{lt} (criterion TER ≥ 5)**
total a.s. in BAS 762 02 F	NOEC = 22.4 2) EC_{10} = 22.8 2) $NOEC_{CORR}$ = 11.2 2) $EC_{10\ CORR}$ = 11.4 2)	0.651 *, 3)	34 35 17 18
Chronic effects on other soil meso- and macrofauna			
Product	**Endpoint (mg a.s./kg dry soil)**	**PEC_{soil} (mg a.s./kg dry soil)**	**TER_{lt} (criterion TER ≥ 5)**
Collembola (*Folsomia candida*)			
total a.s. in BAS 762 02 F	NOEC = 53.1 2) EC_{10} = 63.7 2) $NOEC_{CORR}$ = 26.5 2) $EC_{10\ CORR}$ = 31.8 2)	0.651 *, 3)	82 98 41 49
Soil mites (*Hypoaspis aculeifer*)			
total a.s. in BAS 762 02 F	NOEC ≥ 159.3 2) EC_{10} > 159.3 2) $NOEC_{CORR}$ ≥ 79.6 2) $EC_{10\ CORR}$ > 79.6 2)	0.651 *, 3)	≥ 245 > 245 ≥ 122 > 122

Underlined endpoints and TER are relevant for the conclusion of the risk assessment.
* $PEC_{soil, accu.}$ For details please refer to section 8, chapter 8.7, table 8.7-5 and table 8.7-11.
1) Use resulting in the worst-case PEC_{soil} value covering all other intended uses (see chapter 9.1.2).
2) Endpoint based on sum of active substances (nominal) and taking into account a density of BAS 762 02 F of 1.130 g/cm^3.
3) Based on the sum of the worst-case active substance PEC_{soil} values.

zRMS comments:

In the risk assessment the Applicant used both, NOEC and EC_{10} values. Furthermore, corrected and not corrected endpoints were used. It should be noted that in the Central Zone the requirements in the risk assessment for soil organisms are rather clear: all endpoints are corrected when log Pow of the active substance(s) is >2, irrespective of the peat content in the study, and the lower of EC_{10} and NOEC is used.
As correction of Applicants' calculations above would made the evaluation even less transparent, respective risk assessment based on the relevant endpoints and maximum PEC_{SOIL} agreed in area of Section 8 and covering all intended uses has been performed by the zRMS and is presented below.

Substance	**Endpoint (EC10/NOEC) [mg/kg dws]**	**PEC_{soil} [mg/kg dws]**	**TER**	**Trigger**
Earthworms				
Mefentrifluconazole	2.65 1)	0.229 3)	11.6	5
1,2,4-triazole	1.0	0.0034 3)	294.1	
Boscalid	12.5 1)	0.422 3)	29.6	
BAS 762 02 F	11.2 1) 2)	0.651 4)	17.2	
Folsomia candida				
Mefentrifluconazole	200 1)	0.229 3)	873.4	5
1,2,4-triazole	1.0	0.0034 3)	294.1	
Boscalid	500 1)	0.422 3)	1184.8	
BAS 762 02 F	26.5 1) 2)	0.651 4)	40.7	
Hypoaspis aculeifer				
Mefentrifluconazole	500 1)	0.229 3)	2183.4	5
1,2,4-triazole	171	0.0034 3)	50294.1	
BAS 762 02 F	46.9 1) 2)	0.621 4)	72.0	

1) Corrected endpoint due to log Pow >2

[2)] Expressed in terms of sum of the active compounds
[3)] $PEC_{SOIL,ACCU}$
[4)] Sum of active substances $PEC_{SOIL,ACCU}$

Based on the above calculations, acceptable risk to soil macro- and meso-fauna from mefentrifluconazole, 1,2,4-triazole, boscalid and formulation may be concluded following all intended Central Zone uses of BAS 762 02 F.

9.9.2.2 Higher tier risk assessments

Not relevant.

9.9.3 Overall conclusions

All TER values for BAS 762 02 F, the active substances mefentrifluconazole and boscalid and relevant metabolites for chronic exposure of earthworms and other non-target soil organisms (meso- and macrofauna) are considerably higher than the Commission Regulation (EU) 546/2011 trigger value of 5. This indicates that BAS 762 02 F poses no unacceptable risk to earthworms and other non-target soil organisms (meso- and macrofauna) when applied according to the proposed use pattern.

9.10 Effects on soil microbial activity (KCP 10.5)

9.10.1 Toxicity data

Studies on the effects on soil microorganisms have been carried out with the active substances mefentrifluconazole and boscalid and their relevant metabolites. Full details of these studies are provided in the respective EU documents. Furthermore, a study on the effects on soil microorganisms has been carried out with BAS 762 02 F.

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

All studies are listed in Table 9.10-1, Table 9.10-2 and

Table 9.**10-3**.

Table 9.10-1: 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	<25% effect on 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	<25% effect on 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 9.10-2: Endpoints and effect values of boscalid relevant for the risk assessment for soil micro organisms

Endpoint	Substance	Exposure System	Results	Reference
N-mineralization	boscalid (tested as BAS 510 01 F) [1)]	28 d, aerobic loamy sand	<25% effect on nitrate formation rate at 8.0 mg a.s./kg dry soil +3.1%	EC Review report, SANCO/3919/2007-rev.5, 2008 2000/1018517 Amendment 2001/1014651
C-mineralization [2)]	boscalid (tested as BAS 510 01 F) [1)]	28 d, aerobic loamy sand	Soil respiration at 8.0 mg a.s./kg dry soil -8.4%	EC Review report, SANCO/3919/2007-rev.5, 2008 2000/1018516 Amendment 2001/1014649

+ = stimulation, - = inhibition

1) Study was conducted with the boscalid solo-formulation BAS 510 01 F (50% boscalid).

2) Carbon transformation studies are listed for reference only but not used in the risk assessment according to Commission Regulation (EU) No 283/2013.

Table 9.10-3: Endpoints and effect values of BAS 762 02 F relevant for the risk assessment for soil microorganisms

Endpoint	Product	Exposure System	Results	Reference
N-mineralization	BAS 762 02 F	28 d, aerobic silty-loamy sand	<25% effect on nitrate formation rate at 30.0 mg/kg dry soil (equivalent to 2.65 mg mefentrifluconazole/kg dry soil and 5.31 mg boscalid/kg dry soil) [1)] ~~+7.1%~~	not EU evaluated 2019/1061116

+ = stimulation, - = inhibition

1) Calculated, based on the nominal content of the a.s. and taking into account a density of BAS 762 02 F of 1.130 g/cm^3.

zRMS comments:

The endpoints for mefentrifluconazole and its metabolite presented in Table 9.10-1 and for boscalid presented in Table 9.10-2 are in line with EU agreed data presented in EFSA Journal 2018;16(7):5379 and EU Review Report SANCO/3919/2007-rev.5, respectively.

The study performed with the formulated product was evaluated and agreed by the zRMS (for details, please refer to respective points in Appendix 2). Endpoints reported in Table 9.10-3 are confirmed to be correct.

Information regarding effects on carbon mineralisation is no longer a data requirement and for this reason is struck through in Tables 9.10-1 and 9.10-2.

9.10.1.1 Justification for new endpoints

Effects on soil microbial activity of BAS 762 02 F were not evaluated as part of the EU review of mefentrifluconazole or boscalid. Therefore, all relevant data and assessments are provided here and are considered adequate.

9.10.2 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 762 02 F, mefentrifluconazole, boscalid and the relevant metabolites to soil micro-organisms was assessed by comparing the maximum PEC_{soil} values with the maximum concentration with effects ≤ 25 % (see

Table 9.**10-4**, Table 9.10-5 and Table 9.10-6).

Table 9.10-4: Assessment of the risk for effects on soil micro-organisms due to the use of mefentrifluconazole as contained in BAS 762 02 F according to the proposed use pattern

Intended use	sunflower [1)]		
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)	0.229 *	yes
Metabolite, Reg. No. 87 084 1,2,4-triazole	> 0.333 (at 28 d)	0.0034 ~~0.001~~ *	yes

* $PEC_{soil, accu.}$ For details please refer to section 8, chapter 8.7, table 8.7-5 and table 8.7-8.
1) Use resulting in the worst-case PEC_{soil} values covering all other intended uses (see chapter 9.1.2).

Table 9.10-5: Assessment of the risk for effects on soil micro-organisms due to the use of boscalid as contained in BAS 762 02 F according to the proposed use pattern

Intended use	sunflower [1)]		
Active substance	boscalid		
Application rate (g a.s./ha)	2 x 200		
N-mineralization			
Active substance	**Max. conc. with effects ≤ 25 % (mg/kg dry soil)**	**PEC_{soil} (mg/kg dry soil)**	**Risk acceptable?**
boscalid	> 8.0 (at 28 d)	0.422 *	yes

* $PEC_{soil, accu.}$ For details please refer to section 8, chapter 8.7, table 8.7-11.
1) Use resulting in the worst-case PEC_{soil} values covering all other intended uses (see chapter 9.1.2).

Table 9.10-6: Assessment of the risk for effects on soil micro-organisms due to the use of BAS 762 02 F according to the proposed use pattern

Intended use	sunflower [1)]		
Product	BAS 762 02 F		
Application rate (L/ha)	2 x 1.0		
N-mineralization			
Product	**Max. conc. with effects ≤ 25 % (mg a.s./kg dry soil)**	**PEC_{soil} (mg a.s./kg dry soil)**	**Risk acceptable?**
total a.s. in BAS 762 02 F	>7.96 (at 28 d) [2)]	0.651 * [3)]	yes

* $PEC_{soil, accu.}$ For details please refer to section 8, chapter 8.7, table 8.7-5 and table 8.7-11.
1) Use resulting in the worst-case PEC_{soil} values covering all other intended uses (see chapter 9.1.2).
2) Endpoint based on the sum of the active substances.
3) Based on the sum of the worst-case active substance PEC_{soil} values.

zRMS comments:

The risk assessment presented in Tables 9.10-4 to 9.10-6 is agreed by the zRMS with minor correction of the $PEC_{SOIL,ACCU}$ for 1,2,4-triazole agreed in area of Section 8. Based on the performed evaluation no unacceptable effects on soil microbial activity are expected from all intended uses of BAS 762 02 F.

9.10.3 Overall conclusions

For the formulation BAS 762 02 F, the active substances mefentrifluconazole and boscalid 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 762 02 F will not pose an unacceptable risk to non-target soil micro-organisms if applied according to good agricultural practice.

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

9.11.1 Toxicity data

Vegetative vigor and seedling emergence studies have been conducted with BAS 762 02 F. New data submitted with this application are listed in Appendix 1 and summarized in Appendix 2.

Table 9.11-1: Endpoints and effect values of BAS 762 02 F relevant for the risk assessment for non-target terrestrial plants

Species	Product	Exposure system	Results	Reference
Greenhouse				
Daucus carota d (carrot) *Lactuca sativa* d (lettuce) *Brassica oleracea* d (cabbage) *Brassica napus* d (oilseed rape) *Solanum lycopersicum* d (tomato) *Glycine max* d (soybean) *Allium cepa* m (onion) *Lolium multiflorum* m (ryegrass) *Triticum aestivum* m (wheat) *Zea mays* m (corn)	BAS 762 02 F	21 d (28 days for carrot and onion) Seedling emergence	ER_{50} emergence > 1.0 L/ha ER_{50} plant height > 1.0 L/ha ER_{50} plant weight > 1.0 L/ha ER_{50} phytotoxiciy > 1.0 L/ha	not EU evaluated 2020/1000744
Daucus carota d (carrot) *Lactuca sativa* d (lettuce) *Brassica oleracea* d (cabbage) *Brassica napus* d (oilseed rape) *Solanum lycopersicum* d (tomato) *Glycine max* d (soybean) *Allium cepa* m (onion) *Lolium multiflorum* m (ryegrass) *Triticum aestivum* m (wheat) *Zea mays* m (corn)	BAS 762 02 F	21 d Vegetative vigor	ER_{50} plant height > 1.0 L/ha ER_{50} plant weight > 1.0 L/ha ER_{50} phytotoxiciy > 1.0 L/ha	not EU evaluated 2020/1000745

m: monocotyledonous; d: dicotyledonous

zRMS comments:

The studies performed with the formulated product were evaluated and agreed by the zRMS (for details, please refer to respective points in Appendix 2). Endpoints reported in Table 9.11-1 are confirmed to be correct.

The phytotoxicity endpoint has been added following the commenting period.

9.11.1.1 Justification for new endpoints

Effects on non-target plants of BAS 762 02 F were not evaluated as part of the EU registration process of mefentrifluconazole or boscalid. Hence, all relevant data and assessments considering this formulation are provided here and are considered adequate.

9.11.2 Risk assessment

9.11.2.1 Tier-1 risk assessment (based screening data)

Not relevant.

9.11.2.2 Tier-2 risk assessment (based on dose-response data)

The risk assessment is based on the "Guidance Document on Terrestrial Ecotoxicology", (SANCO/10329/2002 rev.2 final, 2002). It is restricted to off-field areas, as non-target plants are non-crop plants located outside the treated area.

The application of BAS 762 02 F is envisioned in several field crops (i.e. oilseed rape, sunflower). The following risk assessment is based on the worst-case single field application rate of 1.0 L BAS 762 02 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. Only a single application was considered, because factors like plant growth will reduce residues per unit area between multiple applications. 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, 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 762 02 F is 1.0 L product/ha. The maximum off-field predicted environmental rate ($PER_{off\text{-}field}$) is thus calculated to be 27.7 mL product/ha.

The potential risk of BAS 762 02 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.

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

The results of the risk assessment are presented in

Table 9.**11-2**.

Table 9.11-2: Assessment of the risk for non-target plants due to the use of BAS 762 02 F according to the proposed use pattern

Intended use	Field crops			
Product	BAS 762 02 F			
Application rate (L/ha)	2 x 1.0			
MAF	n/a			
Test species	**ER_{50} (mL/ha) [1)]**	**Drift rate (%)**	**$PER_{off\text{-}field}$ (mL/ha)**	**TER criterion: TER ≥ 5**
Daucus carota d (carrot) *Lactuca sativa* d (lettuce) *Brassica oleracea* d (cabbage) *Brassica napus* d (oilseed rape) *Solanum lycopersicum* d (tomato) *Glycine max* d (soybean) *Allium cepa* m (onion) *Lolium multiflorum* m (ryegrass) *Triticum aestivum* m (wheat) *Zea mays* m (corn))	> 1000	2.77	27.7	> 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.

zRMS comments:

Risk assessment presented in Table 9.11-2 is agreed by the zRMS. Based on the performed evaluation acceptable risk to non-target terrestrial plants from all intended uses of BAS 762 02 F may be concluded with no need for risk mitigation measures.

In line with indications of SANCO/10329/2002 rev. 2 final, single application rate was considered in calculations. It is, however, noted that some Member States require consideration of multiple applications. Therefore for convenience of these Member States additional calculation has been performed by the zRMS with consideration of cumulative application rate as covering extremely worst case and being protective for multiple applications of the product in sunflower. Results are presented in table below.

Intended use	Field crops			
Product	BAS 762 02 F			
Application rate (L/ha)	2.0 (cumulative application rate, covering multiple applications)			
MAF	n/a			
Test species	**ER_{50} (mL/ha) [1)]**	**Drift rate (%)**	**$PER_{off\text{-}field}$ (mL/ha)**	**TER criterion: TER ≥ 5**
All tested species	> 1000	2.77	55.4	> 18.1

Even with the worst case assumption of the cumulative application rate of BAS 762 02 F, the TER for non-target terrestrial plants is still considerably above the trigger of 5 demonstrating acceptable risk with no need for risk mitigation measures.

9.11.2.3 Higher-tier risk assessment

Not relevant.

9.11.2.4 Risk mitigation measures

No risk mitigation needed.

9.11.3 Overall conclusions

Based on the risk assessment it can be concluded that BAS 762 02 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 762 02 F applications are not required for the protection of terrestrial non-target plants.

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

Not relevant.

9.13 Monitoring data (KCP 10.8)

Not relevant.

9.14 Classification and Labelling

According to (EC) No 1272/2008 (CLP) plant protection products must be classified for their environmental hazard (acute and chronic). Classification is based on acute and chronic product data if adequate data is available. If sufficient product data is not available, the summation method is carried out instead.

For the product BAS 762 02 F acute data (LC/EC_{50}) are 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 substances. Both active substances have no harmonized classification in Annex IV of (EC) No 1272/2008 (CLP). Therefore, their chronic classification will be based on the lowest chronic endpoint. Table 9.14-1 shows the relevant data for classification purposes.

Table 9.14-1: Ecotoxicology/Environment data relevant for classification of BAS 762 02 F

Substance tested	Study Type (duration)	Findings	Triggered classification and labelling	Reference
Acute (short-term) aquatic hazard				
BAS 762 02 F	*Oncorhynchus mykiss* (96 h)	96 h LC_{50} > 8.12 mg/L	No aquatic acute hazard cat.	BASF DocID 2019/1050663
BAS 762 02 F	*Daphnia magna* (48 h)	48 h EC_{50} > 17.41 mg/L	No aquatic acute hazard cat.	BASF DocID 2019/1050662
BAS 762 02 F	*Pseudokirchneriella subcapitata* (72 h)	72 h E_rC_{50} = 6.37 mg/L	No aquatic acute hazard cat.	BASF DocID 2019/1050661
		72 h E_rC_{10} = 3.3 mg/L	No aquatic chronic hazard cat.	
		NOE_rC = 0.50 mg/L	Aquatic chronic hazard cat. 2 (H411)	
Chronic (long-term) aquatic hazard				
Mefentrifluconazole (BAS 750 F) [1) 3)]	*Daphnia magna* (21 d)	21 d EC_{10} = 0.0175 mg/L	Aquatic chronic hazard cat. 1 (H410); M=1	BASF DocID 2014/1098028
	Biodegradation	not readily biodegradable	--	BASF DocID 2014/1239574
Boscalid (BAS 510 F) [2) 3)]	*Oncorhynchus mykiss* (97 d)	97 d NOEC = 0.125 mg/L	Aquatic chronic hazard cat. 2 (H411)	BASF DocID 1999/11847
	Biodegradation	not readily biodegradable	--	BASF DocID 1999/10290

1) Nominal contents within the formulated product: **100 g mefentrifluconazole/L (8.8% w/w).**
2) Nominal contents within the formulated product: **200 g boscalid/L (17.61% w/w).**
3) Currently, the substance has no harmonized classification in Annex IV of (EC) No 1272/2008.

Based on the lowest acute aquatic toxicity endpoint obtained with BAS 762 02 F no aquatic acute hazard category is given according to (EC) No 1272/2008 (CLP).

Regarding chronic classification, mefentrifluconazole (a.s. content of 8.8% w/w within the product), classified with chronic hazard cat. 1 (M = 1) and boscalid (a.s. content of 17.61% w/w within the product) classified with chronic hazard cat. 2, are considered for the summation method according to CLP. The method yields a value which is above the trigger of 25% after the 2nd equation. Hence, BAS 762 02 F is classified as aquatic chronic hazard category 2 (H411). Chronic classification of BAS 762 02 F using the summation method is summarized in Table 9.14-2.

Table 9.14-2: Chronic classification of BAS 762 02 F using the summation method according to (EC) No 1272/2008

Chronic classification of BAS 762 02 F							
Formulation component				Result (Content x M-Factor)			
Name	Chronic category	M-Factor	Content in BAS 762 02 F [%]				
BAS 750 F	1	1	8.8	8.8			
BAS 510 F	2	none	17.61	-			
1st equation	SUM (*M x Chronic 1*)			8.8	< 25 %	Category 1 not triggered	
BAS 750 F	1	1	8.8 x 10	88			
BAS 510 F	2	none	17.61	17.61			
2nd equation	SUM *(M x 10 x Chronic 1)* + SUM *(Chronic 2)*			105.61	≥ 25 %	**Aquatic Chronic Hazard Category 2**	

The triggered hazard category is indicated in **bold**.

Conclusion

Based on the data obtained with the product and the lowest chronic aquatic toxicity endpoints of the active substances within the formulated product, the following classification is proposed for BAS 762 02 F: **aquatic chronic hazard category 2 (H411)** according to GHS following Regulation (EC) No 1272/2008.

zRMS comments:

CLP classification of BAS 762 02 F provided by the Applicant above is agreed by the zRMS.

It is noted that harmonised classification of mefentrifluconazole is currently available in the Commission Delegated Regulation (EU) 2020/1182 of May 2020 (Acute 1 with M factor of 1 and Chronic 1 with M factor of 1). Although indications of the Regulation will apply from 1st of March 2022, available information confirms Applicants' proposal of mefentrifluconazole classification provided in Table 9.14-1 above.

For boscalid the same classification (Chronic 2) has been proposed by the RMS in the course of the ongoing renewal process. Although this proposal is not reflected in the available legislation, it gives confidence in the Applicants' proposal provided in Table 9.14-1 above.

Following classification and labelling are considered relevant for BAS 762 02 F:

Hazard pictograms:	GHS09 
Signal word:	None
Hazard statement(s):	H411 - Toxic to aquatic life with long lasting effects
Precautionary statement(s):	P391: Collect spillage P501: Dispose of contents/container to hazardous or special waste collection point, in accordance with local, regional, national and/or international regulation

Appendix 1 Lists of data considered in support of the evaluation

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	Remarks
KCP 10.2.1/2	XXX, A.	2019	Reg.No. 6003433 (metabolite of BAS 750 F) - Acute toxicity study in the rainbow trout (Oncorhynchus mykiss) 2019/1022695 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. yes Unpublished	Yes	BASF	-
KCP 10.2.1/3	XXX, B.	2019	BAS 762 02 F - Acute Toxicity to Rainbow trout (Oncorhynchus mykiss) in a static 96-Hour Test 2019/1050663 IES - Innovative Environmental Services Ltd., Witterswil, Switzerland yes Unpublished	No	BASF	-
KCP 10.2.1/4	XXX, H.	2019	BAS 762 02 F - Effect on Daphnia magna in a static 48-Hour Immobilization Test 2019/1050662 IES - Innovative Environmental Services Ltd., Witterswil, Switzerland yes Unpublished	No	BASF	-
KCP 10.2.1/5	XXX, H.	2020	BAS 762 02 F - Effect on Pseudokirchneriella subcapitata in a 72-hour algal growth Inhibition test 2019/1050661 IES - Innovative Environmental Services Ltd., Witterswil, Switzerland yes Unpublished	No	BASF	-
KCP 10.3.1.1.1/1	XXX, K.	2019	BAS 762 02 F: Acute Oral and Contact Toxicity to the Honey Bee, Apis mellifera L. under Laboratory Conditions 2019/1061115 Eurofins Agroscience Services 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	Remarks
KCP 10.3.1.1.2/1	XXX, K.	2019	BAS 762 02 F: Acute Oral and Contact Toxicity to the Honey Bee, Apis mellifera L. under Laboratory Conditions 2019/1061115 Eurofins Agroscience Services GmbH, Niefern-Oeschelbronn, Germany Fed.Rep. yes Unpublished	No	BASF	-
KCP 10.3.1.2/2	XXX, K.	2021	Chronic toxicity of BAS 762 02 F to the honey bee Apis mellifera L. under laboratory conditions 2020/2032682 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished	No	BASF	-
KCP 10.3.1.3/3	XXX, K.	2021	Repeated exposure of honey bee (Apis mellifera L.) larvae to BAS 762 02 F under laboratory conditions 2020/2032683 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished	No	BASF	-
KCP 10.3.1.5/1	XXX, A.	2021	Effects of BAS 762 02 F on the honeybee Apis mellifera L. under semi-field conditions (tunnel test) with additional assessments on colony and brood development 2021/2001936 BioChem agrar Labor für biologische und chemische Analytik GmbH yes Unpublished	No	BASF	-
KCP 10.3.2.1/1	XXX, U.	2019	Effects of BAS 762 02 F on the predatory mite Typhlodromus pyri Scheuten in a laboratory test 2019/1061533 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	Remarks
KCP 10.3.2.1/2	XXX, U.	2019	Effects of BAS 762 02 F on the parasitic wasp Aphidius rhopalosiphi (DESTEFANI-PEREZ) in a laboratory test 2019/1061532 BioChem agrar GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished	No	BASF	-
KCP 10.4.1.1/1	XXX, S.	2014	Sublethal toxicity of BAS 510 F (Boscalid) to the earthworm Eisenia fetida in artificial soil 2014/1083454 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished	No	BASF	Study not evaluated by the zRMS, but considered in the risk assessment as already evaluated and accepted in the course of the renewal process
KCP 10.4.1.1/2	XXX, S.	2020	Effects of BAS 762 02 F on the reproduction of the earthworm Eisenia andrei in artificial soil 2020/1000741 BioChem agrar GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished	No	BASF	-
KCP 10.4.2.1/1	XXX, S.	2014	Effects of BAS 510 F (Boscalid) on the reproduction of the collembolan Folsomia candida 2014/1083456 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished	No	BASF	Study not evaluated by the zRMS, but considered in the risk assessment as already evaluated and accepted in the course of the renewal process
KCP 10.4.2.1/2	XXX, S.	2020	Effects of BAS 762 02 F on the reproduction of the collembolan Folsomia candida 2020/1000742 BioChem agrar GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished	No	BASF	-
KCP 10.4.2.1/3	XXX, L.	2020	Effects of BAS 762 02 F on the reproduction of the predatory mite Hypoaspis aculeifer 2020/1000743 BioChem agrar GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished	No	BASF	-

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Vertebrate study Y/N**	**Owner**	**Remarks**
KCP 10.5/1	XXX, M.	2019	Effects of BAS 762 02 F on the activity of soil microflora (Nitrogen transformation test) 2019/1061116 BioChem agrar GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished	No	BASF	-
KCP 10.6.2/1	XXX, A.	2020a	Effect of BAS 762 02 F on vegetative vigour of ten species of terrestrial plants under greenhouse conditions 2020/1000745 Agro-Check Dr. Teresiak & Erdmann GbR, Lentzke, Germany Fed.Rep. yes Unpublished	No	BASF	-
KCP 10.6.2/2	XXX, A.	2020b	Effect of BAS 762 02 F on seedling emergence and seedling growth of ten species of terrestrial plants under greenhouse conditions 2020/1000744 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

zRMS comments:

As most of endpoints for mefentrifluconazole, boscalid and relevant metabolites were taken from the EU review, for the list of respective studies please refer to Volume 2 of the RAR for mefentrifluconazole and the monograph for boscalid.

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	Reason for rejection
KCP 10.2/1	XXX, G.	2015	Report Amendment No.1 - Chronic toxicity of BAS 750 F (Reg.No. 5834378) to Daphnia longispina in a 21 day semi-static test 2015/1251197 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	BASF	The amendments were not submitted as separate report and the risk assessment was based on endpoints as reported in EFSA Journal 2018;16(7):5379
KCP 10.2/2	XXX, K., XXX, B.	2017	Report Amendment 1: Chronic toxicity of Reg.No. 5834378 to the non-biting midge Chironomus riparius - A spiked sediment study 2017/1044236 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	BASF	
KCP 10.2/3	XXX, K., XXX, B.	2017	Amendment No. 1: Chronic toxicity of Reg.No. 5924326 (M750F003; metabolite of BAS 750 F) to the non-biting midge Chironomus riparius - A spiked sediment study 2017/1044237 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	BASF	
KCP 10.2/4	XXX, H.	2018	Amendment No. 1: BAS 750 F (Reg.No. 5834378) - Lemna gibba CPCC 310, Growth inhibition test 2018/1220943 Institute of Industrial Organic Chemistry, Pszczyna, Poland yes Unpublished	No	BASF	
KCP 10.2.1/1	XXX, E.	2016	BAS 750 F - Acute toxicity study in the fathead minnow (Pimephales promelas) 2016/1155889 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. yes Unpublished	Yes	BASF	Study not required for finalisation of the risk assessment since sufficient information is available from the EU review.

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	Reason for rejection
KCP 10.3.1.2/1	XXX, S.	2015	Chronic toxicity of BAS 510 01 F to the honeybee (Apis mellifer L.) under laboratory conditions 2014/1083455 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished	No	BASF	Study not considered in the risk assessment as being not relevant for the zonal evaluation of BAS 762 02 F (studies with the formulation in question were submitted)
KCP 10.3.1.3/1	XXX, S.	2014	Effect of Reg.No. 300355 (BAS 510 F) on survival and development of honey bee brood (Apis mellifera), using an in vitro rearing method 2013/1275399 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	BASF	Study not considered in the risk assessment as being not relevant for the zonal evaluation of BAS 762 02 F (studies with the formulation in question were submitted)
KCP 10.3.1.3/2	XXX, K.	2017	Repeated exposure of honey bee (Apis mellifera) larvae in BAS 510 F (Boscalid) under laboratory conditions (in vitro) 2017/1000161 BioChem agrar GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished	No	BASF	Study not considered in the risk assessment as being not relevant for the zonal evaluation of BAS 762 02 F (studies with the formulation in question were submitted)

List of data relied on not submitted by the applicant but necessary for evaluation

zRMS comments:

There were no studies relied on and not submitted by the Applicant.

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 further studies were conducted.

A 2.1.1.2 KCP 10.1.1.2 Higher tier data on birds

No further studies were 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 further studies were conducted.

A 2.1.2.2 KCP 10.1.2.2 Higher tier data on mammals

No further studies were conducted.

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

No further studies were 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

Comments of zRMS:	Sufficient information on acute toxicity of mefentrifluconazole to fish was available from the EU review and no data gap for additional studies with fish was indicated in EFSA Journal 2018;16(7):5379. Taking this into account the summarised below study was not necessary to finalise the risk assessment for BAS 762 02 F and was thus not validated by the zRMS.

Reference:	CP 10.2.1/1
Report	BAS 750 F - Acute toxicity study in the fathead minnow (Pimephales promelas), XXX E., 2016 Report No EU-805877, EU-18F0741/11E200 BASF DocID 2016/1155889 Authority registration No
Guideline(s):	EC 440/2008 C.1, OECD 203, EPA 72-1, EPA 850.1075
Deviations:	No
GLP:	Yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)
Acceptability:	Not validated, not required for the risk assessment purposes (sufficient information available from the EU review)
Duplication (if vertebrate study)	No

Executive Summary

In a 96-hour static acute toxicity laboratory study, fathead minnows were exposed to a dilution water control and to nominal concentrations of 4.6, 10, 22, 46 and 100% of a saturated solution of BAS 750 F (corresponding to mean measured concentrations of 0.0916, 0.204, 0.462, 0.941 and 2.2 mg a.s./L) in groups of 10 animals in stainless steel aquaria containing 20 L water. Fish were observed for survival and symptoms of toxicity directly after start of exposure and 1, 6, 24, 48, 72 and 96 hours after start of exposure.

The biological results are based on mean measured concentrations of the test item. After 96 hours of exposure, no mortality was observed in the dilution water control and the test item concentrations of up to and including 0.462 mg a.s./L. At the two highest tested concentrations, all fish were dead after 96 hours of exposure. No sub-lethal effects were found at any of the test concentrations after 96 hours.

In a static acute toxicity study with fathead minnow the LC_{50} (96 h) of BAS 750 F was determined to be 0.65 mg a.s./L based on mean measured concentrations. The NOEC (96 h) was determined to be 0.462 mg a.s./L (mean measured).

I. MATERIAL AND METHODS

A. MATERIALS

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

B. STUDY DESIGN

Test species: Fathead minnow (*Pimephales promelas*), approx. 4 month old; mean body length: 2.8 cm (2.4 cm – 3.4 cm); mean wet weight: 0.24 g (0.12 g – 0.40 g); supplied by in-house culture; no feeding from approx. 48 h 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} and NOEC related to mortality and sub-lethal effects.

Test concentrations: Control (dilution water), 4.6, 10, 22, 46 and 100% of a saturated solution of BAS 750 F (nominal), corresponding to mean measured concentrations of 0 (control), 0.0916, 0.204, 0.462, 0.941 and 2.20 mg a.s./L.

Test conditions: 20 L stainless steel aquaria, test volume: 20 L; dilution water: non-chlorinated charcoal filtered drinking water mixed with deionized water; hardness: 1.04 mmol $CaCO_3$/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 LC50.

C. DESCRIPTION OF THE ANALYTICAL PROCEDURES

Concentrations of mefentrifluconazole (BAS 750 F) in test water were determined according to the analytical method APL0500/03. The validation of the analytical method is described in the study report. The analytical method APL0500/03 was slightly modified with respect to the chromatographic conditions to determine BAS 750 F in test water. Stock solutions were prepared by weighing about 50 mg test item into 100 mL acetonitrile. Calibration standards, ranging from 0.0002 mg/L to 0.004 mg/L, were prepared from intermediate solutions in test water/acetonitrile/formic acid mixture (80:20:0.1, v/v/v) by diluting with the same solvent mixture. The determination was performed by reversed phase UHPLC with MS detection. The limit of quantification (LOQ) was 0.001 mg/L and the limit of detection (LOD) was set to 0.002 mg/L. Details on measured fortification samples and obtained procedural recoveries for mefentrifluconazole are given in the table below.

Table A 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

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of BAS 750 F concentrations was conducted in each test item concentration at the beginning of the test, after 48 h and at the end of the exposure. The mean measured concentrations of the test item were < LoQ (Limit of quantification), 0.0916, 0.204, 0.462, 0.941 and 2.20 mg a.s./L. The analyzed contents of BAS 750 F ranged from 97% to 105% of overall mean measured concentrations at test initiation, from 93% to 103% after 48 h and from 95% to 103% of overall mean measured concentrations at test termination. The following biological results are based on mean measured concentrations.

Biological results: After 96 hours of exposure, no mortality was observed in the dilution water control and at test item concentrations of up to and including 0.462 mg a.s./L. At the two highest tested concentrations,

all fish were dead after 96 hours of exposure. No sub-lethal effects were found at any of the test concentrations after 96 hours. The results are summarized in Table A 2.

Table A 2: Acute toxicity (96 h) of BAS 750 F to fathead minnow (*Pimephales promelas*)

Concentration [% saturated solution] (nominal)	**Control**	**4.6**	**10**	**22**	**46**	**100**
Concentration [mg a.s./L] (mean measured)	**0**	**0.0916**	**0.204**	**0.462**	**0.941**	**2.2**
Mortality [%] (96 h)	0	0	0	0	100	100
Symptoms (after 96 h)	none	none	none	none	n.d.	n.d.
Endpoints [mg BAS 750 F/L] (mean measured)						
LC_{50} (96 h)	**0.65** (95% confidence limits: 0.577 – 0.731)					
NOEC (96 h)	0.462					

n.d. = not determined; all fish dead

Validity criteria according to OECD 203 (2019)	**Obtained in this study**
In the control(s) (dilution water control, solvent control), the mortality should not exceed 10% (or one fish, if fewer than 10 control fish are tested) at the end of the exposure	0%
The dissolved oxygen concentration must have been at least 60% of the air saturation value throughout the test	> 60% (6.9 – 8.4 mg/L)
Analytical measurement of test concentrations is compulsory (see § 24)	Analysis of each test concentrations. at 0, 48 and 96 hours after test start.

All validity criteria were met.

III. CONCLUSION

In a static acute toxicity study with fathead minnow the LC_{50} (96 h) of BAS 750 F was determined to be 0.65 mg a.s./L based on mean measured concentrations. The NOEC (96 h) was determined to be 0.462 mg a.s./L (mean measured).

A 2.2.1.2 Study 2

Comments of zRMS:	The study was performed in line with OECD 203 with no major deviations. It is noted that the temperature during the study (11.9-12.3°C) was lower comparing to this recommended by the test guideline (13-17°C). However, as all validity criteria were met, this deviation is considered to have no impact on the obtained results. The test item was stable in test solutions throughout the test with measured concentrations at 88.4 and 88.0% of nominal at 0 and 96 hours, respectively. Endpoints from the study may be thus expressed in terms of nominal concentrations. The study is considered acceptable with following endpoints relevant for the risk assessment: LC_{50} >5.0 mg pm/L (based on nominal concentration)

Reference:	CP 10.2.1/2
Report	Reg.No. 6003433 (metabolite of BAS 750 F) - Acute toxicity study in the rainbow trout (Oncorhynchus mykiss), XXX A., 2019 Report No EU-12F0396/18E020, EU-867193 BASF DocID 2019/1022695 Authority registration No
Guideline(s):	EC 440/2008 C.1 Acute Toxicity for Fish, OECD 203
Deviations:	No
GLP:	Yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)
Acceptability:	Yes
Duplication (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 ≥ 5.0 mg/L based on nominal concentration.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: M750F005, metabolite of BAS 750 F (Reg. No. 6003433), batch no. L87-34, purity: 96.9%;

B. STUDY DESIGN

Test species: Rainbow trout (*Oncorhynchus mykiss*), approx. 3.5 months old, mean body length 4.8 (4.3 – 5.5) cm, mean body weight 0.89 (0.5 – 1.49) g; supplied by ´Forellenzucht Trostadt GbR', Trostadt, Germany.

Test design: Static system (96 hours); 1 replicate per treatment; 10 fish per replicate (loading about 0.45 g fish/L); assessment of survival and symptoms of toxicity after 1, 6, 24, 48, 72 and 96 hours.

Endpoints: LC_{50} and NOEC related to mortality and sub-lethal effects.

Test concentrations: Water control, solvent control (DMF), 5 mg M750F005/L (nominal).

Test conditions: ~24 L stainless steel aquaria (38.5x23.5x29 cm); test volume 20 L, dilution water: non-chlorinated charcoal-filtered municipal water mixed with deionized water; temperature: 11.9 – 12.3°C; pH 7.9 – 8.3; oxygen content: 7.9 – 10.4 mg/L; total hardness about 1 mmol/L (dilution water); acid capacity about 2.5 mmol/L

(dilution water); photoperiod: 16 hours light : 8 hours dark; no aeration; no feeding.

Analytics: Analytical verification of the test item concentrations was performed using an LC-method with MS/MS detection.

Statistics: No statistical analysis was carried out since no lethality was observed up to the highest tested concentration.

C. DESCRIPTION OF THE ANALYTICAL PROCEDURES

Concentrations of M750F005 (metabolite of BAS 750 F) in test water were determined according to the analytical method L0359/01. The validation of the analytical method is described in another study (BASF Doc-ID: 2017/1066523). Fortification solutions for the high residue level (5 mg/L) were prepared by dilution of the stock solution with acetonitrile and solutions for the LOQ and 10 x LOQ fortifications were prepared by further dilution with acetonitrile/water (50/50, v/v) The determination was performed by HPLC-method with MS/MS detection.. The limit of quantification (LOQ) was 0.03 µg/L and the limit of detection (LOD) was set to 0.009 µg/L. To check on potential matrix effects quality control samples were prepared at LOQ measurement concentration level. The sample was prepared routinely with untreated test medium solution and compared to solvent standards. The recovery values of all replicates of the quality control sample were all in an acceptable range, therefore no significant matrix effect has been identified. Details on measured fortification samples and obtained procedural recoveries for M750F005 are given in the table below.

Table A 3: Procedural recoveries for mefentrifluconazole

Matrix	**Fortification level (mg/L)**	**n**	**Mean (%)**	**RSD (%)**
Test water	0.03	3	95.7	1.6
Test water	0.3	3	94.0	0.6
Test water	5000	3	98.3	1.1

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in the test item group at the beginning and at the end of the test. The analytically detected concentration was initially 88.4% of the nominal value and 88.0% at the end of the test. The biological results are based on nominal concentrations.

Biological results: No mortality occurred in the controls and in the treatment. No additional adverse effects or abnormal behavior were observed in the test treatment. The results are summarized in Table A 4.

Table A 4: Acute toxicity (96 h) of M750F005 to rainbow trout (*Oncorhynchus mykiss*)

Concentration [mg/L] (nominal)	**Water Control**	**Solvent Control**	**5**
Mortality [%] (96 h)	0	0	0
Symptoms (after 96 h) #	none	none	none
	Endpoints [mg M750F005/L] (nominal)		
LC_{50} (96 h)	> 5 (confidence interval: n.d.)		
NOEC (96 h)	≥ 5		

n.d. not determined

Validity criteria according to OECD 203 (2019)	Obtained in this study
In the control(s) (dilution water control, solvent control), the mortality should not exceed 10% (or one fish, if fewer than 10 control fish are tested) at the end of the exposure	0%
The dissolved oxygen concentration must have been at least 60% of the air saturation value throughout the test	> 60% (7.9 – 10.4 mg/L)
Analytical measurement of test concentrations is compulsory (see § 24)	Analysis of each test concentrations. at 0 and 96 hours after test start.

All validity criteria were met.

III. CONCLUSION

In a 96-h static acute toxicity study with rainbow trout the LC_{50} (96 h) for M750F005 was determined to be > 5 mg/L based on nominal concentration. The NOEC was determined to be ≥ 5.0 mg/L based on nominal concentration.

A 2.2.1.3 Study 3

Comments of zRMS:

The study was performed in line with OECD 203 with no major deviations.

It was reported in the study that in general, the analysis of both active substance concentrations was based on BASF analytical method L0361/01 with the following adaptations which were considered to have no negative impact on the outcome of the study:

- A different Guard Column was used (Raptor C18, 5 x 2.1 mm).
- The injection volume was increased to 40 µL instead of 10 µL for BAS 750 F and 20 µL for BAS 510 F. (Reason: increase of sensitivity)
- The column temperature was set to 40 °C instead of 35 °C. (Reason: sharper peak shape)
- The upper fortification level was at a level corresponding to 125% of the highest test concentration.
- The lower fortification level for BAS 510 F was at 0.00021 mg/L (0.05% of the lowest test concentration) instead of 0.0001 mg/L.

The analytical measurements demonstrated that at the test termination the concentrations of mefentrifluconazole dropped slightly below required 80% of nominal (see table below).

Nominal concentration of formulation [mg/L]	Nominal concentration of mefentrifluconazole [mg/L]	Measured concentration of mefentrifluconazole			
		Test start		Test end (96 h)	
		[µg/L]	% of nominal	[µg/L]	% of nominal
0.5	0.0426	0.191	90.7	0.159	75.6
1.1	0.0936	0.238	102	0.186	79.7
2.5	0.213	0.257	96.9	0.209	78.8
5.5	0.47	0.315	97.1	0.267	82.2
12	1.02	0.223	92.8	0.189	78.5

However, based on the results provided in the table above, the geometric mean measured concentration over the whole study period was determined to be 86.97% of nominal and for this reason the endpoints may be expressed in terms of the nominal concentrations of the test item.

Boscalid was stable in the test solutions over the study period.

It was noted that the LC_{50} was calculated as a geometric mean from concentrations with 0 and 100% mortality, i.e. 5.5 and 12 mg/L, respectively (nominal). Although the zRMS is of the opinion that LC_{50} calculated this way is not fully reliable, this procedure is recommended by the test guideline and is thus accepted

	Overall, the study is considered acceptable with following endpoints relevant for the risk assessment: LC_{50} = 8.12 mg product/L (based on nominal concentration) In the course of the commenting period it was pointed out that The LC50 was determined by using the geometric mean from concentrations with 0 and 100% mortality. According to the revised OECD test guideline 203 this approach is not recommended. It is stated that other techniques such as Spearman-Karber method, the binomial method or the moving average method should be used. It should be noted that OECD 203 (2019) recommends to use other techniques such as the Spearman-Karber method, binomial method, or moving average method to calculate the LC_{50} for experiments that result in only one concentration with partial mortality (>0 and <100%) or no concentrations with partial mortality. In this study, no partial mortality was observed, as 0% mortality occurred at 5.5 mg/L and 100% mortality occurred at 12 mg/L, the next higher (and the highest) concentration. The binomial method is recommended for data where one concentration results in 0% effect and the next higher concentration results in 100% effect (Environment Canada, 2005[2]), Section 4.5.7). For tests with no partial effects, the binomial method approximates the LC_{50} as the geometric average of the concentrations causing no effect and complete effect (see Environment Canada, 2005, Equation 3, Section 4.5.7, p. 69). $EC50 = \sqrt{(C_L)(C_U)}$ [*Equation 3*] where: C_L = the arithmetic value of the "lower" concentration with no effect C_U = the arithmetic value of the "upper" concentration causing complete effect Therefore, the binomial method (i.e. geometric mean) was correctly used to obtain the LC_{50} for this study. Nevertheless, the LC_{50} may be also expressed as >5.5 mg product/L, although the zRMS is of the opinion that LC_{50} of 8.12 mg product/L was correctly calculated given the obtained results.

Reference:	CP 10.2.1/3
Report	BAS 762 02 F - Acute Toxicity to Rainbow trout (Oncorhynchus mykiss) in a static 96-Hour Test, XXX, B., 2019 Report No 834654, 20190145 BASF DocID 2019/1050663 Authority registration No
Guideline(s):	Commission Regulation (EC) No 440/2008 - Part C.1, OECD 203 (2019)
Deviations:	No
GLP:	Yes (certified by Swiss Federal Office of Public Health, Berne, Switzerland)
Acceptability:	Acceptable
Duplication (if vertebrate study)	No

[2] Environment Canada. Guidance Document on Statistical Methods for Environmental Toxicity Tests. Report EPS 1/RM/46. March 2005 (with June 2007 amendments).

Executive Summary

In a 96-hour static acute toxicity laboratory study, rainbow trout (*Oncorhynchus mykiss*) were exposed to a dilution water control and to nominal concentrations of BAS 762 02 F of 0.50, 1.1, 2.5, 5.5 and 12 mg product/L in groups of 7 animals in mono-block glass aquaria containing 14 ~~20~~ L test water. Observations for mortality and toxic signs were conducted after 2, 4, 24, 48, 72 and 96 hours of exposure. The percentage mortality was calculated for each test concentration.

The biological results are based on nominal concentrations of the test item. After 96 hours of exposure, no mortality or other symptoms of toxicity were observed in the control and at any test item concentration, except for the highest concentration of 12 mg/L where 100% mortality was observed. At the test item concentration of 5.5 mg/L, fish showed abnormal swimming behaviour (*i.e.* hypoactivity and abnormal bottom distribution).

In a static acute toxicity study with rainbow trout, the LC_{50} (96 h) of BAS 762 02 F was 8.12 mg/L based on nominal concentrations. The NOEC (96 h) was determined to be 5.5 mg/L (nominal).

I. MATERIAL AND METHODS

A. MATERIALS

Test item:	BAS 762 02 F, batch no. FD-190207-0001; content of a.s.: mefentrifluconazole (BAS 750 F, reg. no. 5834378): 96.2 g/L (nominal: 100 g/L), boscalid (BAS 510 F, reg. no. 300355): 205.2 g/L (nominal: 200 g/L); density: 1.130 g/cm^3.

B. STUDY DESIGN

Test species:	Rainbow trout (*Oncorhynchus mykiss*); age: juveniles; mean body length: 4.7 cm (± 0.17 cm); mean body weight: 0.98 g (± 0.13 g); obtained from Störk, Bad Saulgau, Germany.
Test design:	Static system (96 hours); 5 test item concentrations plus a control in one replicate, 7 fish per replicate (loading: 0.49 g fish/L), assessments of mortality and symptoms of toxicity within 2 and 4 hours after start of exposure and twice daily thereafter.
Endpoints:	LC_{50} and NOEC related to mortality and sub-lethal effects.
Test concentrations:	0 (control), 0.50, 1.1, 2.5, 5.5 and 12 mg BAS 762 02 F/L (nominal).
Test conditions:	Test vessel: 20 L mono-block glass aquarium; test volume: 14 L; test medium: reconstituted water; temperature: 13 °C; pH: 7.3 – 7.4; oxygen content: 9.4 – 9.9 mg/L; total hardness: 125 $CaCO_3$ mg/L (dilution water); photoperiod: 16 h light : 8 h dark with 30 min transition; light intensity: 945 – 985 lux; slight aeration, no feeding.
Analytics:	Analytical verification of test item concentrations was conducted using an HPLC-method with MS/MS-detection (method no. L0361/01).
Statistics:	Descriptive statistics; Fishers Exact Binomial Test with Bonferroni Correction for determination of NOEC values; geomean of LC_0 and LC_{100} for determination of LC_{50}.

C. DESCRIPTION OF THE ANALYTICAL PRODECDURES

Concentrations of BAS 750 F and BAS 510 F (contained in BAS 762 02 F) in test water were determined according to the analytical method L0361/01. The validation of the analytical method is described in the study report. A 5 g test water aliquot is extracted by shaking with Acetonitrile/Water/HCOOH, 400/600/2, v/v/v. An aliquot of 40 µL of the extract is then used for analysis. The determination was performed by HPLC-MS/MS. However, only one mass transition has been reported for quantification of each active substance. The limit of quantification (LOQ) was 0.100 and 0.214 µg/L for BAS 750 F and BAS 510 F, respectively. The limit of detection (LOD) was set to 0.02 µg/L for both active substances. For the assessment of potential matrix effects, matrix matched calibration standards were used. The storage stability of BAS 750 F and BAS 510 F in different test media from ecotoxicological tests was proven to be 90 days. Details on measured fortification samples and obtained procedural recoveries for BAS 750 F and BAS 510 F are given in the tables below.

Table A 5: Procedural recoveries for BAS 750 F (quantifier mass transition 398 → 70)

Matrix	Fortification level (µg/L)	n	Accuracy (mean recovery) (%)	±SD	RSD* (%)
test water	0.1	3	92.1 92.40	0.00293	3.2 2.93
test water	4.17	3	101 28	0.03215	0.7 6
test water	1280	3	104 103.91	10.00000	0.4 0.75

* relative standard deviation

Table A 6: Procedural recoveries for BAS 510 F (quantifier mass transition 343 → 307)

Matrix	Fortification level (µg/L)	n	Accuracy (mean recovery) (%)	±SD	RSD * (%)
test water	0.214	3	100 31	0.00404	1.8 8
test water	8.89	3	103 49	0.06557	0.7 1
test water	2740	3	105 104.76	1.00000	0.3 5

* relative standard deviation

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each concentration at the beginning and at the end of the test and for the highest concentration additionally after 48 h. The measured concentration of mefentrifluconazole (BAS 750 F) ranged from 90.7 to 102% of nominal at test initiation and from 75.6 to 82.2% of nominal at test termination. The measured concentration of boscalid (BAS 510 F) ranged from 93.0 to 98.2% of nominal at test initiation and from 81.2 to 92.0% of nominal at test termination. Since substance concentrations have been generally maintained, the following biological results are based on nominal concentrations.

Biological results: After 96 hours of exposure, no mortality or other symptoms of toxicity were observed in the control and at any test item concentration, except for the highest concentration of 12 mg/L where 100% mortality was observed. At the test item concentration of 5.5 mg/L, fish showed abnormal swimming behaviour (*i.e.* hypoactivity and abnormal bottom distribution). The results are summarized in Table A 9.

Table A 9: The effects of BAS 762 02 F on the mortality of the rainbow trout (*Oncorhynchus mykiss*) in a 96-h acute toxicity study

Concentration [mg/L] (nominal)	**Control**	**0.50**	**1.1**	**2.5**	**5.5**	**12**
Mortality (96 h) [%]	0	0	0	0	0	100
Symptoms (96 h)	none	none	none	none	7 S	n.d.
Endpoints [mg BAS 762 02 F/L] (nominal)						
LC_{50} (96 h)	8.12 (95% confidence limits: n.d.) [1)]					
NOEC (96 h)	5.5					

Abbreviations: n.d. = not determined;
Symptoms after 96 h: S = abnormal swimming behavior (hypoactivity and abnormal bottom distribution)
1) Endpoint has been calculated as geometric mean from concentrations with 0 and 100% effect (*i.e.* 5.5 and 12 mg/L, respectively).

Validity criteria according to OECD 203 (2019)	**Obtained in this study**
In the control(s) (dilution water control, solvent control), the mortality should not exceed 10% (or one fish, if fewer than 10 control fish are tested) at the end of the exposure	0%
The dissolved oxygen concentration must have been at least 60% of the air saturation value throughout the test	> 60% (9.4 – 9.9 mg/L)
Analytical measurement of test concentrations is compulsory (see § 24)	Analysis of each test conc. at test initiation (0 h) and after 96 h; 75.6 – 102% and 81.2 – 98.2% of nominal for BAS 750 F and BAS 510 F throughout the test.

All validity criteria were met.

III. CONCLUSION

In a static acute toxicity study with rainbow trout, the LC_{50} (96 h) of BAS 762 02 F was 8.12 mg/L based on nominal concentrations. The NOEC (96 h) was determined to be 5.5 mg/L (nominal).

A 2.2.1.4 Study 4

Comments of zRMS:	The study was performed in line with OECD 202 with no major deviations. It was reported in the study that in general, the analysis of both active substance concentrations measurements was based on BASF analytical method L0361/01 with the following adaptations which were considered not to have any negative impact on the outcome of the study: – A different Guard Column was used (Raptor C18, 5 x 2.1 mm). – The injection volume was increased to 40 µL instead of 10 µL for BAS 750 F and 20 µL for BAS 510 F. (Reason: increase of sensitivity) – The column temperature was set to 40 °C instead of 35 °C. (Reason: sharper peak shape) – The upper fortification level was at a level corresponding to 120% of the highest test concentration. – The lower fortification level for BAS 510 F was at 0.21 µg/L (0.05% of the lowest test concentration) instead of 0.1 µg/L. The analytical measurements demonstrated that at the test termination the concentrations of mefentrifluconazole dropped below required 80% of nominal in some test groups (see table below).

Nominal concentration of formulation [mg/L]	Nominal concentration of mefentrifluconazole [mg/L]	Measured concentration of mefentrifluconazole			
		Test start		Test end (48 h)	
		[µg/L]	% of nominal	[µg/L]	% of nominal
2.5	0.213	0.183	86.2	0.183	86.1
5.0	0.426	0.382	89.9	0.393	92.4
10	0.851	0.744	87.4	0.76	89.0
20	1.7	1.56	91.8	1.34 (b)	78.6 (b)
20 (a)	1.7	-	-	1.46	86.0
20 (a)	1.7	-	-	1.48	86.9
				Mean of 2: 1.47	Mean of 2: 86.4
40	3.4	3.25	95.5	2.15	63.3
40 (a)	3.4	-	-	2.24	65.8
40 (a)	3.4	-	-	2.21	65.0
				Mean of 3: 2.20	Mean of 3: 64.7

(a) retain samples
(b) considered as an outlier based on Grubbs outlier test (as indicated in the study report)

Based on the results provided in the table above, the geometric mean measured concentration over the whole study period was determined to be 83.1% of nominal.

The analytical measurements demonstrated that at the test termination the concentrations of boscalid dropped below required 80% of nominal in some test groups (see table below).

Nominal concentration of formulation [mg/L]	Nominal concentration of boscalid [mg/L]	Measured concentration of boscalid			
		Test start		Test end (48 h)	
		[µg/L]	% of nominal	[µg/L]	% of nominal
2.5	0.454	0.413	90.9	0.414	91.3
5.0	0.908	0.842	92.7	0.890	98.1
10	1.82	1.64	90.1	1.50	82.7
20	3.63	3.29	90.6	2.61 (b)	71.8 (b)
20 (a)	3.63	-	-	2.93	80.7
20 (a)	3.63	-	-	2.90	79.8
				Mean of 2: 2.91	Mean of 2: 80.3
40	7.26	6.98	96.1	5.00	68.8
40 (a)	7.26	-	-	5.21	71.8
40 (a)	7.26	-	-	5.21	71.7
				Mean of 3: 5.14	Mean of 3: 70.8

(a) retain samples
(b) considered as an outlier based on Grubbs outlier test (as indicated in the study report)

Based on the results provided in the table above, the geometric mean measured concentration over the whole study period was determined to be 84.1% of nominal.

Although at test termination concentrations of active substances dropped below 80% of nominal in some test groups, the geometric mean measured concentrations of both active compounds over the whole study period were >80% of nominal and the endpoints may be thus expressed in terms of nominal concentrations.

Overall, the study is considered acceptable with following endpoint is relevant for the risk assessment:

48h EC_{50} = 17.41 mg product/L (based on nominal concentration)

Reference:	CP 10.2.1/4
Report	BAS 762 02 F - Effect on Daphnia magna in a static 48-Hour Immobilization Test, XXX, H., 2019 Report No 834653, 20190144 BASF DocID 2019/1050662 Authority registration No
Guideline(s):	OECD 202 (2004), SANCO/3029/99 Rev.4 (2000)
Deviations:	No
GLP:	Yes (certified by Swiss Federal Office of Public Health, Berne, Switzerland)
Acceptability:	Acceptable
Duplication (if vertebrate study)	No

Executive Summary

In a 48-hour static acute toxicity laboratory study, the effects of BAS 762 02 F on the mobility of neonates of the water flea *Daphnia magna* were investigated. Daphnids were exposed to a dilution water control and to BAS 762 02 F at nominal concentration of 2.5, 5.0, 10, 20 and 40 mg product/L with 4 replicates per treatment and 5 daphnids per replicate. Assessment of immobility was conducted 24 and 48 hours after test initiation. The percentage of immobility relative to the control was calculated for the test item group from mean immobility.

The biological results are based on the nominal concentrations of the test item. After 48 hours of exposure, no immobility of daphnids was observed in the control and at test item concentrations of up to and including 5.0 mg/L, whereas 15%, 80% and 75% of the daphnids were immobile at the three highest test item concentrations of 10, 20 and 40 mg/L, respectively. Statistically significant differences in the mobility of the animals compared to the control were observed at the three highest test item concentrations of 10, 20 and 40 mg/L. In addition, adverse effects e.g. daphnids with reduced swimming activity compared to the control animals or discoloration of the daphnids, were observed at these concentrations.

In a 48-hour static acute toxicity study with *Daphnia magna,* the EC_{50} (48 h) of BAS 762 02 F was 17.41 mg/L based on nominal concentrations. The NOEC was determined to be 5.0 mg/L (nominal).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 762 02 F, batch no. FD-190207-0001; content of a.s.: mefentrifluconazole (BAS 750 F, reg. no. 5834378): 96.2 g/L (nominal: 100 g/L), boscalid (BAS 510 F, reg. no. 300355): 205.2 g/L (nominal: 200 g/L); density: 1.130 g/cm^3.

Test species: Water flea (*Daphnia magna* STRAUS), neonates; no first brood progeny; age at test initiation: < 24 hours; source: in-house; originally obtained from the Daphnia Collection of the University of Basel/Switzerland.

Test design: Static system; test duration: 48 hours; five test concentrations plus control with 4 replicates per treatment and 5 daphnids per replicate; assessments of immobility after 24 and 48 hours.

Endpoints: EC_{50} and NOEC based on immobility of daphnids.

Test concentrations: 0 (control), 2.5, 5.0, 10, 20 and 40 mg BAS 762 02 F/L (nominal).

Test conditions: Test vessel: 100 mL glass beakers; test volume: 50 mL; test medium: Elendt "M7" medium; pH: 7.8 – 7.9; oxygen concentration: 8.3 mg/L – 8.5 mg/L (94 – 96% of air saturation value); temperature: 22 °C; total hardness: 250 $CaCO_3$ mg/L (dilution water); photoperiod: 16 hours light: 8 hours dark; light intensity: 1070 – 1160 lux; no feeding; no aeration.

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

Statistics: Descriptive statistics; trimmed Spearmen-Karber procedure for calculation of EC_{50}; step-down Cochran-Armitage Test procedure for determination of the NOEC ($\alpha = 0.05$).

C. DESCRIPTION OF THE ANALYTICAL PRODECDURES

Concentrations of BAS 750 F and BAS 510 F (contained in BAS 762 02 F) in test water were determined according to the analytical method L0361/01. The validation of the analytical method is described in the study report.. A 5 g test water aliquot is extracted by shaking with Acetonitrile/Water/HCOOH, 400/600/2, v/v/v. An aliquot of 40 µL of the extract is then used for analysis. The determination was performed by HPLC-MS/MS. However, only one mass transition has been reported for quantification of each active substance. The limit of quantification (LOQ) was 0.0988 and 0.211 µg/L for BAS 750 F and BAS 510 F, respectively. The limit of detection (LOD) was set to 0.02 µg/L for both active substances. For the assessment of potential matrix effects, matrix matched calibration standards were used. The storage stability of BAS 750 F and BAS 510 F in different test media from ecotoxicological tests was proven to be 90 days. Details on measured fortification samples and obtained procedural recoveries for BAS 750 F and BAS 510 F are given in the tables below.

Table A 6: Procedural recoveries for BAS 750 F (quantifier mass transition 398 → 70) [1)]

Matrix	Fortification level (µg/L)	n	Accuracy (mean recovery) (%)	±SD	RSD* (%)
1st analysis					
test water	0.0988	3	79.6 79.66	0.00831	10.6 10.56
test water	20.5	3	91.5 91.20	0.36056	2.0 1.93
test water	4090	3	94.54	55.07571	1.4 1.42
2nd analysis					
test water	0.0987	2	76.5	0.00170	2.2 2.25
test water	20.4	2	98.0 98.039	0.98995	5.0 4.95
test water	4090	2	97.0 96.94	134.35029	3.4 3.39

1) For 1st and 2nd analysis, fortifications are made from different stock solutions.
* relative standard deviation

Table A 6: Procedural recoveries for BAS 510 F (quantifier mass transition 343 → 307) [1)]

Matrix	Fortification level (µg/L)	n	Accuracy (mean recovery) (%)	±SD	RSD* (%)
1st analysis					
test water	0.211	3	97.63	0.02689	13.05
test water	43.7	3	94.8 94.737	0.62450	1.51
test water	8730	3	97.37	155.24175	1.9 1.83
2nd analysis					
test water	0.211	2	94.1 93.84	0.00849	4.3 4.29
test water	43.6	2	101.03	0.19092	4.4 4.33
test water	8720	2	98.91	120.20815	1.4 1.39

1) For 1st and 2nd analysis, fortifications are made from different stock solutions.
* relative standard deviation

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each concentration at the beginning and at the end of the test. The measured concentration of mefentrifluconazole (BAS 750 F) ranged from 86.2 to 95.5% of nominal at test initiation and from 64.7 to 92.4% of nominal at test termination. The measured concentration of boscalid (BAS 510 F) ranged from 90.1 to 96.1% of nominal at test initiation and from 70.8 to 98.1% of nominal at test termination. Since initial test concentrations demonstrate correct application of the test substance, the following biological results are based on nominal concentrations.

Biological results: After 48 hours of exposure, no immobility of daphnids was observed in the control and at test item concentrations of up to and including 5.0 mg/L, whereas 15%, 80% and 75% of the daphnids were immobile at the three highest test item concentrations of 10, 20 and 40 mg/L, respectively. Statistically significant differences in the mobility of the animals compared to the control were observed at the three highest test item concentrations of 10, 20 and 40 mg/L (Step-down Cochran-Armitage Test, $\alpha = 0.05$). In addition, adverse effects e.g. daphnids with reduced swimming activity compared to the control animals or discoloration of the daphnids, were observed at these concentrations. The results are summarized in Table A 10.

Table A 10: Effect of BAS 762 02 F on the mobility of the water flea *Daphnia magna*

Concentration [mg/L] (nominal)	**Control**	**2.5**	**5.0**	**10**	**20**	**40**
Immobility (24 h) [%]	0	0	0	0	45	70
Immobility (48 h) [%]	0	0	0	15*	80*	75*
Endpoints [mg BAS 762 02 F/L] (nominal)						
EC_{50} (48 h)	17.41 (95% confidence limits: 14.06 – 21.56)					
NOEC (48 h)	5.0					

***** Statistically significant different compared to the control (step-down Cochran-Armitage Test; $\alpha = 0.05$)

Validity criteria according to OECD 202	**Obtained in this study**
In the control, including the control containing the solubilising agent, not more than 10 per cent of the daphnids should have been immobilised	0%
The dissolved oxygen concentration at the end of the test should be ≥ 3 mg/L in control and test vessels	8.3 mg/L – 8.5 mg/L

All validity criteria were met.

III. CONCLUSION

In a 48-hour static acute toxicity study with *Daphnia magna*, the EC_{50} (48 h) of BAS 762 02 F was 17.41 mg/L based on nominal concentrations. The NOEC was determined to be 5.0 mg/L (nominal).

A 2.2.1.5 Study 5

Comments of zRMS:	The study was performed in line with OECD 201 with no deviations. It was reported in the study that in general, the analysis of both active substance concentrations measurements was based on BASF analytical method L0361/01 with the following adaptations which were considered not to have any negative impact on the outcome of the study: – A different Guard Column was used (Raptor C18, 5 x 2.1 mm). – The injection volume was increased to 40 µL instead of 10 µL for BAS 750 F and 20 µL for BAS 510 F. (Reason: increase of sensitivity) – The column temperature was set to 40 °C instead of 35 °C. (Reason: sharper peak shape) – The upper fortification level was at a level corresponding to 100% of the highest

	test concentration. – The lower fortification level for BAS 510 F was at 0.00021 mg/L (0.05% of the lowest test concentration) instead of 0.0001 mg/L. The endpoints are expressed as nominal concentrations since the measured concentrations of both active substances were maintained at 80-120% of nominal during the study period. All validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment: E_rC_{50} = 6.37 mg product/L (based on nominal concentration) E_rC_{20} = 4.14 mg product/L (based on nominal concentration) E_rC_{10} = 3.30 mg product/L (based on nominal concentration) NOE_rC = 0.50 mg product/L (based on nominal concentration) E_yC_{50} = 3.69 mg product/L (based on nominal concentration) E_yC_{20} = 2.39 mg product/L (based on nominal concentration) E_yC_{10} = 1.90 mg product/L (based on nominal concentration) NOE_yC = 0.50 mg product/L (based on nominal concentration)

Reference:	CP 10.2.1/5
Report	BAS 762 02 F - Effect on Pseudokirchneriella subcapitata in a 72-hour algal growth Inhibition test, XXX, H., 2020 Report No 834652, 20190143 BASF DocID 2019/1050661 Authority registration No
Guideline(s):	OECD 201 (2011), SANCO 3029/99 Rev.4
Deviations:	Yes
GLP:	Yes (certified by Swiss Federal Office of Public Health, Berne, Switzerland)
Acceptability:	Acceptable
Duplication (if vertebrate study)	No

Executive Summary

In a 72-hour static toxicity laboratory study, the effects of the BAS 762 02 F on the growth of the green alga *Pseudokirchneriella subcapitata* were investigated. Algae were exposed to BAS 762 02 F at nominal concentrations of 0.5, 1.3, 3.2, 8.0 and 20 mg product/L with 5 replicates per concentration and a dilution control with 10 replicates. Assessment of growth was conducted 24, 48 and 72 hours after test initiation. The percentage inhibition relative to the control was calculated for each test concentration from mean growth rate and yield based on the number of cells.

The biological results are based on the nominal concentrations of the test item. Statistically significant differences in algal growth rate and yield compared to control were observed in the four highest concentrations. No morphological effects on the algae were observed in the control and up to and including 8.0 mg/L. Due to low cell densities, the highest concentration has not been examined.

In a 72-hour algae test with *Pseudokirchneriella subcapitata*, the E_rC_{50} of BAS 762 02 F was determined to be 6.37 mg/L and the E_yC_{50} was 3.69 mg/L based on nominal concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 762 02 F, batch no. FD-190207-0001; content of a.s.: mefentrifluconazole (BAS 750 F, reg. no. 5834378): 96.2 g/L (nominal: 100 g/L), boscalid (BAS 510 F, reg. no. 300355): 205.2 g/L (nominal: 200 g/L); density: 1.130 g/cm^3.

Test species: Unicellular fresh water green alga, *Pseudokirchneriella subcapitata* (Reinsch) Korshikov (syn. *Selenastrum capricornutum* Prinz, *Raphidocelis subcapitata*), SAG 61.81; stock obtained from "Culture Collection of Algae "Göttingen, Germany.

Test design: Static system; test duration: 72 hours; 5 test item concentrations with 5 replicates and an untreated control with 10 replicates; daily assessments of growth.

Endpoints: EC_{10}, EC_{20} and EC_{50} as well as NOEC with respect to growth rate and yield after exposure over 72 hours.

Test concentrations: 0 (control), 0.5, 1.3, 3.2, 8.0 and 20 mg BAS 762 02 F/L (nominal)

Test conditions: Test vessel: 75-mL Erlenmeyer flasks covered with glass lid; test volume: 30 mL; test medium: AAP medium (OECD 201); pH: 7.5 – 8.2 (control), test media pH 7.4 – 7.5 (at test start), pH 8.0 – 8.2 (at test end); temperature: 22.3 – 22.5 °C; initial cell densities: 5 x 10^3 cells/mL; light intensity: 4470 – 4700 lux; continuous illumination; continuous shaking.

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

Statistics: Descriptive statistics, probit analysis with linear maximum likelihood regression for determination of EC_x values of growth rate and yield; Welch-t test (one-sided smaller) for determination of NOE_rC and NOE_yC values after 72 h.

C. DESCRIPTION OF THE ANALYTICAL PRODECDURES

Concentrations of BAS 750 F and BAS 510 F (contained in BAS 762 02 F) in test water were determined according to the analytical method L0361/01. The validation of the analytical method is described in the study report. A 5 g test water aliquot is extracted by shaking with Acetonitrile/Water/HCOOH, 400/600/2, v/v/v. An aliquot of 40 µL of the extract is then used for analysis. The determination was performed by HPLC-MS/MS. However, only one mass transition has been reported for quantification of each active substance. The limit of quantification (LOQ) was 0.100 and 0.213 µg/L for BAS 750 F and BAS 510 F, respectively. The limit of detection (LOD) was set to 0.02 µg/L for both active substances. For the assessment of potential matrix effects, matrix matched calibration standards were used. The storage stability of BAS 750 F and BAS 510 F in different test media from ecotoxicological tests was proven to be 90 days. Details on measured fortification samples and obtained procedural recoveries for BAS 750 F and BAS 510 F are given in the tables below.

Table A 7: Procedural recoveries for BAS 750 F (quantifier mass transition 398 → 70)

Matrix	Fortification level (µg/L)	n	Accuracy (mean recovery) (%)	~~±SD~~	RSD* (%)
test water	0.100	3	79.8 ~~79.77~~	~~0.00384~~	4.8~~2~~
test water	42.6	3	94.9~~1~~	~~0.75056~~	1.9 ~~1.86~~
test water	1710	3	95.9 ~~95.52~~	~~15.27525~~	0.9~~4~~

*relative standard deviation

Table A 6: Procedural recoveries for BAS 510 F (quantifier mass transition 343 → 307)

Matrix	Fortification level (µg/L)	n	Accuracy (mean recovery) (%)	±SD	RSD* (%)
test water	0.213	3	90.891.08	0.00721	3.83.72
test water	90.9	3	96.26	1.57162	1.89
test water	3640	3	97.897.71	35.11885	0.99

*relative standard deviation

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each concentration at the beginning and at the end of the test. The measured concentration of mefentrifluconazole (BAS 750 F) ranged from 96.2 to 97.9% of nominal at test initiation and from 83.5 to 92.8% of nominal at test termination. The measured concentration of boscalid (BAS 510 F) ranged from 98.0 to 102.1% of nominal at test initiation and from 95.5 to 98.3% of nominal at test termination. Since substance concentrations have been maintained, the following biological results are based on nominal concentrations.

Biological results: Statistically significant differences in algal growth rate and yield compared to control were observed in the four highest concentrations (Welch t-test, one-sided smaller, $\alpha = 0.05$). No morphological effects on the algae were observed in the control and up to and including 8.0 mg/L. Due to low cell densities, the highest concentration has not been examined. The effects on algal growth rate and yield are summarized in Table A 11.

Table A 11: Effect of BAS 762 02 F on the growth of the green alga *Pseudokirchneriella subcapitata*

Concentration [mg/L] (nominal)	**0.5**	**1.3**	**3.2**	**8.0**	**20**
Inhibition in 72 h (growth rate) [%]	1.2	2.5*	9.1*	67.0*	138.3*
Inhibition in 72 h (yield) [%]	5.9	11.9*	36.5*	97.0*	100.6101*
Endpoints [mg BAS 762 02 F/L] (nominal)					
E_rC_{50} (72 h)	6.37 (95% confidential limits: 5.93 – 6.80)				
E_rC_{20} (72 h)	4.14 (95% confidential limits: 3.61 – 4.58)				
E_rC_{10} (72 h)	3.30 (95% confidential limits: 2.75 – 3.77)				
NOE_rC	0.50				
E_yC_{50} (72 h)	3.69 (95% confidential limits: 3.57 – 3.83)				
E_yC_{20} (72 h)	2.39 (95% confidential limits: 2.25 – 2.50)				
E_yC_{10} (72 h)	1.90 (95% confidential limits: 1.75 – 2.03)				
NOE_yC	0.50				

* Statistically significant difference compared to the control (Welch t-test, one-sided smaller, $\alpha = 0.05$)

Validity criteria according to OECD 202	**Obtained in this study**
Exponential biomass increase in the control. ≥ 16 (within 72-h test period); growth rate of ≥0.92 day^{-1}	138-fold (growth rate 1.642 day^{-1})
Mean coefficient of variation for section-by-section specific growth rates in the control cultures. ≤ 35%.	24.3%
Coefficient of variation of average specific growth rates in replicate control cultures ≤ 7% (whole test period)	1.4%

All validity criteria were met.

III. CONCLUSION

In a 72-hour algae test with *Pseudokirchneriella subcapitata*, the E_rC_{50} of BAS 762 02 F was determined to be 6.37 mg/L and the E_yC_{50} was 3.69 mg/L based on nominal concentrations.

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

Not applicable.

A 2.2.3 KCP 10.2.3 Further testing on aquatic organisms

No further studies conducted.

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 performed in line with OECD 213 with no deviations. All validity criteria were met and the study is considered acceptable with the following endpoint relevant for the risk assessment: 48h LD_{50} >772 µg product/bee (based on actual food uptake)

Reference:	CP 10.3.1.1.1/1
Report	BAS 762 02 F: Acute Oral and Contact Toxicity to the Honey Bee, Apis mellifera L. under Laboratory Conditions, XXX, K., 2019 Report No 834655, S19-02329 BASF DocID 2019/1061115 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:	Acceptable
Duplication (if vertebrate study)	No

Executive Summary

In an oral toxicity dose-response test, honey bees (worker bees of *Apis mellifera* L.) were exposed to BAS 762 02 F. The toxicity of the test item was determined at nominal concentrations of 46.9, 93.8, 188, 375 and 750 µg BAS 762 02 F/bee which were equivalent to an actual uptake of 36.5, 95.8, 184, 383 and 772 µg BAS 762 02 F/bee (corresponding to 9.69, 25.4, 48.8, 102 and 205 µg total a.s./bee). Additionally, honey bees were treated with BAS 152 11 I (dimethoate) as reference item at nominal concentrations of 0.06, 0.08, 0.11 and 0.14 µg dimethoate/bee or with an aqueous sucrose solution as control. Assessment of bee mortality and behavioral effects was done after 4, 24 and 48 hours.

After 48 hours, 2.5% mortality occurred in the control group fed with pure sucrose solution. In the test item treatments, mortalities ranged from 0% to 7.5% and were not dose-response related. One single moribund bee was observed 24 hours after application in the test item treatment group of 36.5 µg BAS 762 02 F/bee.

In an oral toxicity study with BAS 762 02 F on honey bees, the LD_{50} value (48 h) was estimated to be > 772 µg BAS 762 02 F/bee, corresponding to > 205 µg total a.s./bee.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 762 02 F, batch no. FD-190207-0001, content of a.s.: mefentrifluconazole (BAS 750 F, Reg. No. 5 834 378): 96.2 g/L analyzed (nominal 100.0 g/L) and boscalid (BAS 510 F, Reg. No. 300 355): 205.2 g/L analyzed (nominal 200.0 g/L), density: 1.130 g/cm^3.

B. STUDY DESIGN

Test species: *Apis mellifera* L. (honey bee), adult worker bees; derived from a healthy and queen-right colony; brushed off the combs of the honey chamber and distributed into test cages one day before test start; source: in-house hives.

Test design: In a 48-hour dose-response test, adult worker bees of *Apis mellifera* were exposed orally to BAS 762 02 F via food (50% (w/v) aqueous sucrose solution). The following treatment groups were set up: 5 doses of the test item, 1 untreated control and 4 doses of the reference item with 4 replicates per treatment and 10 bees per replicate. Assessment of bee mortality and behavioral effects was done after 4, 24 and 48 hours.

Endpoint: Mortality (LD_{50})

Reference item: BAS 152 11 I (dimethoate, 429.0 g/L analyzed (nominal 400 g/L)).

Test concentrations: Untreated control: sucrose solution (50% (w/v)).
Test item:

Nominal doses of BAS 762 02 F [µg/bee]		Consumed doses of BAS 762 02 F [µg/bee]	
based on product	based on total a.s.	based on product	based on total a.s.
46.9	12.5	36.5	9.69
93.8	24.9	95.8	25.4
188	49.9	184	48.8
375	99.6	383	102
750	199	772	205

Reference item (nominal): 0.06, 0.08, 0.11 and 0.14 µg dimethoate/bee in an aqueous sucrose solution (50% (w/v)).

Test conditions: Temperature: 23.6°C - 25.9°C; relative humidity: 53.9% - 62.7%, photoperiod: 24 h darkness; food: 50% (w/v) aqueous sucrose solution.

Analytics: No analytical verification of the test item is required according to current data test guideline. Hence, no analytical verification of the product was conducted.

Statistics: Descriptive statistics.

II. RESULTS AND DISCUSSION

After 48 hours, 2.5% mortality occurred in the control group fed with pure sucrose solution. In the test item treatments, mortalities ranged from 0% to 7.5% and were not dose-response related. One single moribund bee was observed 24 hours after application in the test item treatment group of 36.5 µg BAS 762 02 F/bee. The results are summarized in Table A 8.

Table A 8 Toxicity of BAS 762 02 F to *Apis mellifera* (honey bee) in an oral toxicity test

Treatment group	Uptake of test item		Mortality [%]			
	[µg product/bee]	[µg a.s./bee] [1)]	24 h		48 h	
			absolute	corrected [2)]	absolute	corrected [2)]
Control	--	--	0.0	--	2.5	--
BAS 762 02 F	36.5	9.69	2.5	2.5	7.5	5.1
	95.8	25.4	0.0	0.0	0.0	-2.6
	184	48.8	2.5	2.5	5.0	2.6
	383	102	0.0	0.0	0.0	-2.6
	772	205	0.0	0.0	2.5	0.0
	Endpoint (nominal)					
	[µg a.s./bee] [1)]		[µg BAS 762 02 F/bee]			
LD_{50} (48 h)	> 205		> 772			

1) Based on the sum of both active substances.
2) Corrected mortality was calculated according to the formula of ABBOTT (1925), modified by SCHNEIDER-ORELLI (1947).

The LD_{50} value (24 h) for the reference item in the oral toxicity test was determined to be 0.11 µg a.s./bee (95% confidence limits: 0.10 – 0.12 µg a.s./bee).

Validity criteria:

Validity criteria according to OECD 213 (1998)	Obtained in this study
Control mortality ≤ 10%	2.5%
LD_{50} (24 h) of the reference item should be in the specified range 0.10 - 0.35 µg a.s./bee	0.11 µg a.s./bee

All validity criteria were met.

III. CONCLUSION

In an oral toxicity study with BAS 762 02 F on honey bees, the LD_{50} value (48 h) was estimated to be > 772 µg BAS 762 02 F/bee, corresponding to > 205 µg total a.s./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 performed in line with OECD 214 with no deviations. All validity criteria were met and the study is considered acceptable with the following endpoint relevant for the risk assessment: 48h LD_{50} >750 µg product/bee

Reference:	CP 10.3.1.1.2/1
Report	BAS 762 02 F: Acute Oral and Contact Toxicity to the Honey Bee, Apis mellifera L. under Laboratory Conditions, XXX, K., 2019 Report No 834655, S19-02329 BASF DocID 2019/1061115 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:	Acceptable
Duplication (if vertebrate study)	No

Executive Summary

In a contact toxicity dose-response test, honey bees (worker bees of *Apis mellifera* L.) were exposed to BAS 762 02 F. The toxicity of the test item was determined at nominal concentrations of 46.9, 93.8, 188, 375 and 750 µg BAS 762 02 F/bee (corresponding to 12.5, 24.9, 49.9, 99.6 and 199 µg total a.s./bee). Additionally, honey bees were treated with BAS 152 11 I (dimethoate) as reference item at concentrations of 0.10, 0.15, 0.23 and 0.34 µg dimethoate/bee (nominal). Furthermore, bees were treated with deionized water containing 0.1% Triton X-100 as control. Assessment of bee mortality and behavioral effects was done after 4, 24, and 48 hours.

After 48 hours of contact exposure, no mortality occurred in the control group. In the test item treatment groups, mortalities ranged from 0.0 to 10% and were not dose-response related. After 48 hours, affected bees were observed in all test item treatment groups.

In a contact toxicity study with BAS 762 02 F on honey bees, the LD_{50} value (48 h) was estimated to be > 750 µg BAS 762 02 F/bee, corresponding to > 199 µg total a.s./bee.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 762 02 F, batch no. FD-190207-0001, content of a.s.: mefentrifluconazole (BAS 750 F, Reg. No. 5 834 378): 96.2 g/L analyzed (nominal 100.0 g/L) and boscalid (BAS 510 F, Reg. No. 300 355): 205.2 g/L analyzed (nominal 200.0 g/L), density: 1.130 g/cm^3.

B. STUDY DESIGN

Test species: *Apis mellifera* L. (honey bee), adult worker bees; derived from a healthy and queen-right colony; brushed off the combs of the honey chamber and distributed into test cages one day before test start; source: in-house hives.

Test design: In a 48-hour dose-response test, adult worker bees of *Apis mellifera* were exposed to BAS 762 02 F in an appropriate carrier (deionized water + 0.1% Triton X-100) placed on the dorsal bee thorax. The following treatment groups were set up: 5 concentrations of the test item, 1 untreated control (deionized water + 0.1% Triton X-100) and 4 doses of a reference item with 4 replicates per treatment and 10 bees per replicate. Assessment of bee mortality and behavioral effects was done after 4, 24, and 48 hours.

Endpoint: Mortality (LD_{50}).

Reference item: BAS 152 11 I (dimethoate, 429.0 g/L analyzed (nominal 400 g/L)).

Test concentrations: Untreated control (deionized water + 0.1% Triton X-100).
Test item:

Nominal doses of BAS 762 02 F [µg/bee]	
based on product	based on total a.s.
46.9	12.5
93.8	24.9
188	49.9
375	99.6
750	199

Reference item: 0.10, 0.15, 0.23 and 0.34 µg dimethoate/bee.

Test conditions: Temperature: 23.6°C - 25.9°C; relative humidity: 53.9% - 62.7%, photoperiod: 24 h darkness; food: 50% (w/v) aqueous sucrose solution.

Analytics: No analytical verification of the test item is required according to current data test guideline. Hence, no analytical verification of the product was conducted.

Statistics: Descriptive statistics.

II. RESULTS AND DISCUSSION

After 48 hours of contact exposure, no mortality occurred in the control group. In the test item treatment groups, mortalities ranged from 0.0 to 10% and were not dose-response related. Regarding behavioral abnormalities, affected bees were observed in all test item treatment groups after 48 hours. The results are summarized in Table A 9.

Table A 9 Toxicity of BAS 762 02 F to *Apis mellifera* (honey bee) in a contact toxicity test

Treatment		**Mortality [%]**		**Behavioral abnormalities [no. of affected bees] [2)]**
[µg BAS 762 02 F/bee]	**[µg a.s./bee] [1)]**	**24 h**	**48 h**	**48 h**
Control	--	0.0	0.0	0
46.9	12.5	2.5	7.5	4 a
93.8	24.9	0.0	10.0	3 a
188	49.9	0.0	0.0	3 a
375	99.6	0.0	5.0	8 a
750	199	0.0	2.5	8 a
	Endpoint (nominal)			
LD_{50} (48 h)	**[µg a.s./bee] [1)]**		**[µg BAS 762 02 F/bee]**	
	> 199		> 750	

1) Based on sum of both active substances.
2) Total number of affected bees per treatment group (40 bees per treatment group). Symptoms: a = affected.

The LD_{50} value (24 h) for the reference item in the contact toxicity test was determined to be 0.21 µg a.s./bee (95% confidence limits: 0.18 – 0.24 µg a.s./bee).

Validity criteria:

Validity criteria according to OECD 214 (1998)	**Obtained in this study**
Control mortality ≤ 10%	0.0%
LD_{50} (24 h) of the reference item should be in the specified range 0.10 - 0.30 µg a.s./bee	0.21 µg a.s./bee

All validity criteria were met.

III. CONCLUSION

In a contact toxicity study with BAS 762 02 F on honey bees, the LD_{50} value (48 h) was estimated to be > 750 µg BAS 762 02 F/bee, corresponding to > 199 µg total a.s./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 submitted by the Applicant in order to address the chronic toxicity to adult bees exposed to boscalid. However, the study was not validated for purposes of the zonal evaluation of BAS 762 02 F since respective study with the formulated product was submitted while active substance endpoints should be generated in the course of the EU renewal process. The study summary is struck through and shaded as being not evaluated at the zonal level.

Reference:	CP 10.3.1.2/1
Report	Chronic toxicity of BAS 510 01 F to the honey bee (Apis mellifera L.) under laboratory conditions, XXX S., 2015 Report No EU-429179, EU-141048044B BASF DocID 2014/1083455 Authority registration No
Guideline(s):	OECD 213 (1998), Decourty et al. (2005), Suchail et al. (2001), CEB No. 230 (2012), Current ring test protocol of the AG-Bienenschutz (2014)
Deviations:	No
GLP:	Yes (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)
Acceptability:	Not validated since not relevant for the zonal evaluation of BAS 762 02 F (studies with the formulation in question were submitted)
Duplication (if vertebrate study)	No

~~**Executive Summary**~~

~~In a 10-day chronic oral toxicity test, 1-4 day old worker honey bees (*Apis mellifera carnica* P.) were exposed to a daily application of BAS 510 01 F diluted in the bee food (50% (w/v) aqueous sucrose solution). The chronic toxicity of the test item was determined at nominal doses of 12.5, 25.0, 50.0, 100.0 and 200.0 µg a.s./bee/day (effective doses were 12.0, 22.4, 48.1, 95.9 and 150.9 µg a.s./bee/day), corresponding to concentrations of 0.321, 0.642, 1.248, 2.568 and 5.136 g a.s./kg, respectively. Additionally, honey bees were treated with Dimethoate EC 400 as a reference item at nominal doses ranging from 5.9 to 27.3 ng a.s./bee/day. Untreated diet served as a control.~~

~~After 10 days of testing, the control showed a mean mortality of 3.3%. In the test item group, bees showed mortalities between 1.7% and 15.0%, which are not statistically significantly increased compared to the control group.~~

~~In the course of the study only one bee in the highest test item dosage (150.9 µg consumed a.s./bee/day) showed behavioral abnormalities. It was described as moribund on day 9 of the test.~~
~~In the course of the study, behavior of the treated bees in all treatment groups was on level with the control.~~

~~**In a 10-day chronic toxicity feeding study with BAS 510 01 F, the LDD_{50} and LC_{50} were determined to be > 150.9 µg consumed a.s./bee/day and > 5.136 g a.s./kg food, respectively. The NOEDD was determined to be ≥ 150.9 µg consumed a.s./bee/day, corresponding to a NOEC of ≥ 5.136 g a.s./kg food.**~~

~~**I. MATERIAL AND METHODS**~~

A. MATERIALS

Test item: BAS 510 01 F, batch no. FRE 001071, content of a.s.: boscalid (BAS 510 F, Reg. No. 300 355): 50.3% (50.0% nominal).

B. STUDY DESIGN

Test species: Honey bee (*Apis mellifera* L. spp. *carnica*); 1-4 day old bees; obtained from healthy and queen right colonies; source: Bienenfarm Kern GmbH, Leipzig, Germany.

Test design: 10-day chronic oral feeding test in the laboratory (dose response test). The honey bees were provided daily with 5 doses of test item treated sugar solutions (50% (w/v) aqueous sucrose solution). 10 treatment groups were set up: 5 doses of the test item, 1 untreated control group (50% (w/v) aqueous sucrose solution) and 4 doses of the reference item with 3 replicates per dose, each consisting of 20 bees per replicate. Assessments of bee mortality and behavioral effects were done daily over the 10 days test period.

Endpoint: Mortality (LD_{50}).

Reference item: Dimethoate 400 EC (BAS 152 11 I), 400.0 g/L dimethoate (nominal).

Test concentrations: Control: untreated diet (50% (w/v) aqueous sucrose solution),
Test item: 0.321, 0.642, 1.284, 2.568 and 5.136 g a.s./kg food (corresponding to nominal doses of 12.5, 25.0, 50.0, 100.0 and 200.0 µg a.s./bee/day).
Reference item: 0.152, 0.253, 0.421 and 0.702 mg a.s./kg food (corresponding to nominal doses of 5.9, 9.8, 16.4 and 27.3 ng dimethoate/bee/day).

Test conditions: Temperature: 32.7° C – 33.4° C, mean relative humidity: 63.0% – 66.0%, photoperiod: constant darkness (except during assessments), food: 50% (w/v) aqueous sucrose solution.

Analytics: No analytical verification of the test item is required according to current data test guideline. Hence, no analytical verification of the product was conducted.

Statistics: Descriptive statistics; Fisher's Exact Binomial test with Bonferroni Correction for mortality data (one-sided greater, $\alpha = 0.05$). Probit analysis using linear maximum likelihood regression for calculation of the LD_{50}/LC_{50} value for the reference item.

II. RESULTS AND DISCUSSION

In the chronic toxicity test, the control group showed a mean mortality of 3.3% after 10 days of testing. In the test item group, bees showed mortalities between 1.7% and 15.0%, which are not statistically significantly increased compared to the control group (Fisher's Exact Binomial test with Bonferroni Correction, $\alpha = 0.05$, one-sided greater)

In the course of the study, only one bee in the highest test item dosage (150.9 µg consumed a.s./bee/day) showed behavioral abnormalities. It was described as moribund on day 9 of the test.

The results are summarized in

~~Table A 10.~~

Table A 10: Mean cumulative mortality of honey bees exposed to BAS 510 01 F in a 10-day chronic oral toxicity test

Nominal doses [µg a.s./bee/day]	Consumed doses [µg a.s./bee/day]	Concentration [g a.s./kg food]	Cumulative mortality after 10 days [%]	
			absolute	corrected
Control	–	–	3.3	–
12.5	12.0	0.321	5.0	1.8
25.0	22.4	0.642	1.7	0.0
50.0	48.1	1.284	5.0	1.8
100.0	95.9	2.568	3.3	0.0
200.0	150.9	5.136	15.0	12.1

Endpoints		10 days
Test item doses [µg consumed a.s./bee/day]	NOEDD [1)]	≥ 150.9
	LDD_{50}	> 150.9
Test item concentrations [g a.s./kg food]	NOEC [1)]	≥ 5.136
	LC_{50}	> 150.9

corrected: corrected mortality (according to SCHNEIDER-ORELLI 1947), negative values are treated as "0"
[1)] Fisher's Exact Binomial Test with Bonferroni Correction (one-sided greater, α = 0.05).

The reference item dimethoate caused a mean mortality of 88.3% at day 10 at a concentration of 0.702 mg dimethoate/kg food, corresponding to a nominal dose of 27.3 ng dimethoate/bee/day.

Validity criteria:

Validity criteria according to OECD 245 (2017)	Obtained in this study
Control mortality from ≤ 15% at D10 across all replicates	3.3% untreated control
Reference item mortality ≥ 50% on D10	88.3%

All validity criteria were met.

III. CONCLUSION

In a 10-day chronic toxicity feeding study with BAS 510 01 F, the LDD_{50} and LC_{50} were determined to be > 150.9 µg consumed a.s./bee/day and > 5.136 g a.s./kg food, respectively. The NOEDD was determined to be ≥ 150.9 µg consumed a.s./bee/day, corresponding to a NOEC of ≥ 5.136 g a.s./kg food.

A 2.3.1.2.2 Study 2

Comments of zRMS:	The study was performed in line with OECD 245 with no deviations. The endpoints are expressed as nominal concentrations since the measured concentrations of both active substances were maintained at 80-120% of nominal during the study period. All validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment: 10-d LDD_{50} = 429 µg consumed product/bee/day 10-d LC_{50} = 14.792 g product/kg food NOEDD = 80.0 µg consumed product/bee/day NOEC = 1.910 g product/kg food

Reference:	CP 10.3.1.2/2
Report	Chronic toxicity of BAS 762 02 F to the honey bee Apis mellifera L. under laboratory conditions, XXX, K., 2021

	report No 887728, 2048BAC0048 BASF DocID 2020/2032682 Authority registration No
Guideline(s):	OECD 245 (2017)
Deviations:	No
GLP:	yes (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany),
Acceptability:	Acceptable
Duplication (if vertebrate study)	No

Executive Summary

In a 10-day chronic oral toxicity test, max. two 1-4 days old worker honey bees (*Apis mellifera carnica* P.) were exposed to a daily application of BAS 762 02 F diluted in the bee food (50% (w/v) aqueous sucrose solution + 0.1% (w/v) xanthan). The chronic toxicity of the test item was determined at nominal doses of 37.5, 75.0, 150, 300 and 600 µg product/bee/day (effective doses were 41.8, 80.0, 159, 280 and 425 µg a.s./bee/day), corresponding to concentrations of 0.955, 1.910, 3.819, 7.639 and 15.277 g product/kg food, respectively. Additionally, honey bees were treated with Danadim® Progress as a reference item at a nominal dose of 27.3 ng a.s./bee/day. Untreated diet served as a control and untreated diet containing 0.1% (w/v) xanthan served as solvent control.

In the chronic toxicity test, the control groups showed no mortality after 10 days of testing. In the test item groups, bees showed mortalities between 0.0% and 53.3%. Mortalities in the three highest test item doses (159, 280 and 425 µg consumed product/bee/day) were statistically significantly increased compared to the solvent control group. No behavioral abnormalities were observed in any test item treatment group on any assessment day.

In a 10-day chronic toxicity feeding study with BAS 762 02 F, the LDD_{50} and LC_{50} were determined to be 429 µg consumed product/bee/day and 14.792 g product/kg food, respectively. The NOEDD was determined to be 80.0 µg consumed product/bee/day, corresponding to a NOEC of 1.910 g product/kg food.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 762 02 F, batch no. FD-190207-0001, content of a.s.: mefentrifluconazole (BAS 750 F, Reg. No. 5 834 378): 96.2 g/L analyzed (100 g/L nominal); boscalid (BAS 510 F, Reg. No. 300 355): 205.2 g/L analysed (200 g/L nominal); density: 1.130 g/cm³.

B. STUDY DESIGN

Test species: Honey bee (*Apis mellifera* L. spp. Buckfast); max. 2 day old bees; obtained from healthy and queen-right colonies; source: in-house colonies.

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Test design: 10-day chronic oral feeding test in the laboratory (dose response test). The honey bees were provided daily with 5 doses of test item treated sugar solutions (50% (w/v) aqueous sucrose solution + 0.1% (w/v) xanthan). The following treatment groups were set up: 5 doses of the test item, 1 untreated control group (50% (w/v) aqueous sucrose solution), 1 solvent control group (50% (w/v) aqueous sucrose solution + 0.1% (w/v) xanthan) and 1 dose of the reference item with 3 replicates per dose, each consisting of 10 bees per replicate. Assessments of bee mortality,

food consumption and behavioral effects were done daily over the 10 days test period.

Endpoint: Mortality (LD_{50}).

Reference item: Danadim® Progress, 400.0 g/L dimethoate (nominal).

Test concentrations: Untreated control: untreated diet (50% (w/v) aqueous sucrose solution),
Solvent control: untreated diet (50% (w/v) aqueous sucrose solution) containing 0.1% (w/v) xanthan,
Test item:

Concentration [g/kg food]	Nominal dose [µg/bee/day]			Consumed dose [µg/bee/day]		
BAS 762 02 F	BAS 762 02 F	BAS 750 F	BAS 510 F	BAS 762 02 F	BAS 750 F	BAS 510 F
0.955	37.5	3.32	6.64	41.8	3.7	7.4
1.910	75.0	6.64	13.3	80.0	7.1	14.2
3.819	150	13.3	26.5	159	14.0	28.1
7.639	300	26.5	53.1	280	24.8	49.5
15.277	600	53.1	106.2	425	37.7	75.3

Reference item: 0.694 mg a.s./kg food (corresponding to a nominal dose of 27.3 ng dimethoate/bee/day).

Test conditions: Temperature: 31.8 C – 33.5 C, mean relative humidity: 56.1% – 65.1%, photoperiod: constant darkness (except during assessments), food: 50% (w/v) aqueous sucrose solution.

Analytics: Analytical verification of the test item was conducted according to BASF method L0372/02 using HPLC with MS/MS.

Statistics: Descriptive statistics; Step-down Cochran-Armitage Test Procedure for mortality data (one-sided greater, $\alpha = 0.05$). Weibull analysis using linear maximum likelihood regression for calculation of the LDD_x/LC_x values.

C. DESCRIPTION OF THE ANALYTICAL PROCEDURES

Concentrations of BAS 750 F and BAS 510 F (contained in BAS 762 02 F) in honey bee food were determined according to the analytical method L0372/02. The validation of the analytical method is described in the study report. The samples were extracted with 75/25 (v/v) methanol/water by shaking. Aliquots of the extracts were cleaned-up with 150 mg $MgSO_4$, 50 mg C18-EC and 50 mg PSA and diluted with 75/25 (v/v) methanol/water. The determination was performed by HPLC-MS/MS. The limit of quantification (LOQ) was 0.01 mg/kg and the limit of detection (LOD) was set to ≤ 30% of LOQ mg/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. Details on measured fortification samples and obtained procedural recoveries for BAS 750 F and BAS 510 F are given in

Table A **11**.

Table A 11 Procedural recoveries for mefentrifluconazole and boscalid in aqueous sugar solution

Substance	Matrix	Fortification level (mg/kg)	n	Mean Recovery (%)	RSD [%]
BAS 750 F	Aqueous sugar solution	0.10	5	90.2	6.23
	Aqueous sugar solution	1774	5	83.8	2.37
	Mean		10	87.0	4.30
BAS 510 F	Aqueous sugar solution	0.20	5	101	5.90
	Aqueous sugar solution	3548	5	89.2	6.36
	Mean		10	94.9	6.13

RSD = relative standard deviation

The recoveries of BAS 750 F in treated honey bee feeding solution samples were in the range of 97.6 % to 110 % of nominal and of BAS 510 F in the range of 107 % to 119 % of nominal. The analysed untreated control samples showed no residues at or above the LOD (≤ 30% of LOQ).

II. RESULTS AND DISCUSSION

In the chronic toxicity test, the control groups showed no mortality after 10 days of testing. In the test item groups, bees showed mortalities between 0.0% and 53.3%. Mortalities in the three highest test item doses (159, 280 and 425 µg consumed product/bee/day) were statistically significantly increased compared to the solvent control group (Step-down Cochran-Armitage Test Procedure, $\alpha = 0.05$, one-sided greater).

No behavioural abnormalities were observed in any test item treatment group on any assessment day.

The results are summarized in
Table A **12**.

Table A 12: Mean cumulative mortality of honey bees exposed to BAS 762 02 F in a 10-day chronic oral toxicity test

Doses [µg product/bee/day]		**Concentration [g product /kg food]**	**Cumulative mortality after 10 days [%]**	
Nominal	**Consumed [1)]**		**absolute**	**corrected**
Untreated control	--	--	0.0	--
Solvent control	--	--	0.0	--
37.5	41.8	0.955	0.0	--
75.0	80.0	1.910	3.3	--
150	159	3.819	10.0 *	--
300	280	7.639	16.7 *	--
600	425	15.277	53.3 *	--

Endpoints		**10 days**
Test item doses [µg consumed product/bee/day]	NOEDD	80.0
	LDD_{50}	429 (95% CL: 355 – 596)
	LDD_{20}	253 (95% CL: 185 – 305)
	LDD_{10}	179 (95% CL: 103 – 228)
Test item concentrations [g product/kg food]	NOEC	1.910
	LC_{50}	14.792 (95% CL: 11.536 – 22.025)
	LC_{20}	7.370 (95% CL: 5.018 – 9.433)
	LC_{10}	4.647 (95% CL: 2.444 – 6.366)

corrected: corrected mortality (according to SCHNEIDER-ORELLI 1947), 95% CL = 95% confidence limits

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

1) Taking into account the actual food uptake and evaporation.

The reference item dimethoate caused a mean mortality of 100% after day 10 at a concentration of 0.694 mg dimethoate/kg food, corresponding to a nominal dose of 27.3 ng dimethoate/bee/day.

Validity criteria:

Validity criteria according to OECD 245 (2017)	**Obtained in this study**
Control mortality ~~from~~ ≤ 15% at D10 across all replicates	0.0% untreated control 0.0% solvent control
Reference item mortality ≥ 50% on D10	100%

All validity criteria were met.

III. CONCLUSION

In a 10-day chronic toxicity feeding study with BAS 762 02 F, the LDD_{50} and LC_{50} were determined to be 429 µg consumed product/bee/day and 14.792 g product/kg food, respectively. The NOEDD was determined to be 80.0 µg consumed product/bee/day, corresponding to a NOEC of 1.910 g product/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 submitted by the Applicant in order to address the toxicity to bee larvae exposed to boscalid. However, the study was not validated for purposes of the zonal evaluation of BAS 762 02 F since respective study with the formulated product was submitted while active substance endpoints should be generated in the course of the EU renewal process. The study summary is struck through and shaded as being not evaluated at the zonal level.

Reference:	CP 10.3.1.3/1
Report	Effect of Reg.No. 300355 (BAS 510 F) on survival and development of honey bee brood (Apis mellifera), using an in vitro rearing method, XXX S., 2014 Report No 428347 BASF DocID 2013/1275399 Authority registration No
Guideline(s):	OECD 237 (2013) Honey bee (Apis mellifera) larval toxicity test single exposure
Deviations:	No
GLP:	Yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)
Acceptability:	Not validated since not relevant for the zonal evaluation of BAS 762 02 F (studies with the formulation in question were submitted)
Duplication (if vertebrate study)	No

~~**Executive Summary**~~

~~In a single feeding toxicity test, four day old (D4) honey bee larvae (*Apis mellifera carnica* P.) were exposed to one application of boscalid diluted in the larvae food. The toxicity of the test item was determined at doses of 1.875, 3.75, 7.5, 15.0 and 30.0 µg a.s./larva. The concentrations of test item in the diet were 57.2, 114.3, 228.6, 457.3 and 914.6 mg a.s./kg food. Additionally, honey bee larvae were treated with dimethoate tech. as a reference item at a dose of 8.8 µg dimethoate/larva. Untreated diet served as control, in addition a solvent control with acetone (2% v/v) was used.~~

The untreated control and solvent control group showed a mortality of 5.56% and 0.0% after 72 hours (D7). In the test item group, the larvae fed with 1.875, 3.75, 7.5, 15.0 and 30.0 µg a.s. revealed mortality, which was not statistically significant in comparison to the solvent control after 72 hours (D7).

In an acute larval toxicity test with boscalid, the NOED was ≥ 30.0 µg a.s./larva and the corresponding NOEC was ≥ 914.6 mg a.s./kg food. No LD_{50}/LC_{50} (72 h) could be determined.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Boscalid (BAS 510 F, Reg. No. 300 355); batch no. COD 001035; purity: 99.4 % analyzed purity (tolerance ± 1.0%).

B. STUDY DESIGN

Test species: *Apis mellifera carnica* P. (honey bee), synchronized first instar larvae; collected from three healthy and queen-right colonies; source: BASF-owned colonies.

Test design: One day old honey bee larvae (D1) of *Apis mellifera carnica* P. were transferred from brood combs to plastic queen cups in 48-well cell culture plates 3 days before start of the treatment. After this, in a 72-hour (D7) acute test, the 4-day old (D4) larvae were exposed to a single application of boscalid diluted in the larvae food (aqueous sugar solution mixed with royal jelly). In total, 8 treatment groups were set up: 5 doses of the test item, 2 controls: 1 untreated control and 1 acetone solvent control, and 1 dose of the reference item, all with 3 replicates per dose and 12 larvae per replicate. Assessments of larval mortality were done 24 hours prior to (D3) and 24, 48, 72 and 96 hours (respectively D4, D5, D6, D7) after dosing. Additionally, body condition of the larvae was noted daily from D3 to D7. The presence of uneaten food was documented after 72 hours (D7).

Endpoints: Mortality (LD_{50}/LC_{50} and NOED/NOEC).

Reference item: Dimethoate (99.8% purity analyzed, tolerance ± 1.0%).

Test doses/concentrations: Control (50% aqueous sugar solution with 50% royal jelly); solvent control (control solution with 2% acetone); test item: 1.875, 3.75, 7.5, 15.0 and 30.0 µg a.s./larva, the concentrations of test item in the diet were 57.2, 114.3, 228.6, 457.3 and 914.6 mg a.s./kg food; reference item: 8.8 µg dimethoate/larva.

Test conditions: Temperature: 33.1 °C – 34.9 °C (mean 34.7 °C), relative humidity: 51.9% - 97.6% (mean: 96.4%), food: 50% aqueous sugar solution and 50% royal jelly.

Analytics: Analytical verification of the test item in the feeding solution was conducted according to the method APL0500/03 using HPLC/MS.

Statistics: Descriptive statistics; Fisher's Exact Binomial Test with Bonferroni Correction (one-sided greater, $\alpha = 0.05$), Probit analysis.

C. DESCRIPTION OF THE ANALYTICAL PROCEDURES

Concentrations of boscalid in larval food were determined according to the analytical method APL0500/03. The validation of the analytical method is described in the study report. Test samples were diluted based on their intended concentration within the calibration range, using an acetonitrile/test-medium-mixture and

acidified with formic acid before injection into the HPLC/MS-system. The determination was performed by HPLC-MS. Details on measured fortification samples and obtained procedural recoveries for BAS 510 F are given in Table A 13.

Table A 13 Procedural recoveries for boscalid in aqueous sugar solution

Matrix	Fortification level (mg/L)	n	Mean (%)
Aqueous sugar solution	37384	1	102.93
Aqueous sugar solution	37786	1	96.29

II. RESULTS AND DISCUSSION

The untreated control (AC) showed a mortality of 5.56% after 72 hours (D7). The solvent control showed no mortality after 72 hours (D7). In the test item group, larvae fed with 1.875, 3.75, 7.5, 15.0 and 30.0 µg a.s./larva revealed mortality, which was not statistically significant in comparison to the solvent control group after 72 hours (D7) (Fisher's Exact Binomial Test with Bonferroni Correction, one-sided greater, a = 0.05). The results are summarized in Table A 14.

Table A 14: Toxicity of boscalid (BAS 510 F) to *Apis mellifera carnica* P. (honey bee) in an acute larval toxicity test

Treatment		Mortality after 72 hours (D7)	
dosage [µg a.s./larva]	concentration [g a.s./kg food]	mean mortality [%]	
		absolute	corrected [1)]
Control	Control	5.56	–
Solvent control	Solvent control	0.0	–
1.875	57.2	8.33	8.33
3.75	114.3	2.78	2.78
7.5	228.6	5.56	5.56
15.0	457.3	2.78	2.78
30.0	914.6	0.0	0.0
Endpoints		72 hours (D7)	
Test item doses	LD_{50}	> 30.0 µg a.s./larva	
	NOED	≥ 30.0 µg a.s./larva	
Test item concentrations	LC_{50}	> 914.6 mg a.s./kg food	
	NOEC	≥ 914.6 mg a.s./kg food	

[1)] Test item corrected for solvent control mortality, reference item corrected for control mortality (according to Schneider-Orelli 1947).

Validity criteria:

Validity criteria according to OECD 237 (2013)	Obtained in this study
Control mortality from D4 to D7 ≤ 15% across all replicates	5.56% untreated control 0.0% solvent control
Effects of the reference item: Dimethoate: corrected larval mortality ≥ 50% on D7 across all replicates	dimethoate: 91.2%

All validity criteria were met.

III. CONCLUSION

In an acute larval toxicity test with boscalid, the NOED was ≥ 30.0 µg a.s./larva and the corresponding NOEC was ≥ 914.6 mg a.s./kg food. No LD_{50}/LC_{50} (72 h) could be determined

A 2.3.1.3.2 Study 2

Comments of zRMS:	The study was submitted by the Applicant in order to address the toxicity to bee larvae exposed to boscalid. However, the study was not validated for purposes of the zonal evaluation of BAS 762 02 F since respective study with the formulated product was submitted while active substance endpoints should be generated in the course of the EU renewal process. The study summary is struck through and shaded as being not evaluated at the zonal level.

Reference:	CP 10.3.1.3/2
Report	Repeated exposure of honey bee (Apis mellifera) larvae in BAS 510 F (Boscalid) under laboratory conditions (in vitro), XXX K., 2017 Report No EU-808789 BASF DocID 2017/1000161 Authority registration No
Guideline(s):	OECD 239 (2016)
Deviations:	No
GLP:	Yes (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)
Acceptability:	Not validated since not relevant for the zonal evaluation of BAS 762 02 F (studies with the formulation in question were submitted)
Duplication (if vertebrate study)	No

~~**EXECUTIVE SUMMARY**~~

~~The effects of the test item boscalid (BAS 510 F, Reg. No. 300 355) on survival and adult emergence of honey bee larvae (*Apis mellifera*) were investigated in a laboratory test with repeated exposure over a time period of 22 days. Synchronized 1st larval stage (L1) honey bee larvae were fed with artificial diet for 5 days (day 1,3,4,5 and 6). On days 3, 4, 5 and 6, larvae were fed with diet containing five different concentrations of boscalid (BAS 510 F, Reg. No. 300 355) resulting in concentrations of 63, 94, 141, 211 and 317 mg a.s./kg food, corresponding to total doses of 9.9, 14.8, 22.2, 33.4 and 50.0 µg a.s./larva. Untreated diet served as a control, in addition to a solvent control with acetone equivalent to the dose used in the treatment groups. Furthermore, dimethoate at a dose rate of 7.6 µg/larva served as reference item treatment. All treatment groups and controls contained larvae from three different bee colonies. Assessments of larval mortality were done 24, 48, 72, 96 and 120 hours after start of the treatment. Additionally, other observations such as small body size or large quantities of remaining food after 120 hours was noted. Pupal mortality was assessed at D15 and emergence of adults was evaluated at D22.~~

~~After 120 hours of repeated oral exposure (on D8) larval mortalities of 0.0 and 5.6% were observed in the water control and the acetone control, respectively. Pupal mortality (between D8 and D22) was 11.1% in the untreated control and 20.6% in the solvent control. The control groups showed a total mortality of 11.1 and 25.0%, respectively, at D22. In the test item group, larval mortalities at D8 ranged between 0.0 and 8.3%. Pupal mortalities ranged between 8.6 and 18.2% in the test item treatment groups. Total mortalities at D22 ranged between 11.1 and 25.0%. On D8, no remaining larva treated with BAS 510 F showed any abnormalities such as remaining food. In the final assessment at D22, adult emergence rates of 88.9 and 75.0% were determined for the honey bees in the control groups. In the test item group, the adult honey bees emerged at rates ranging between 75.0 and 88.9% following an application of 9.9, 14.8, 22.2, 33.4 and 50.0 µg a.s./larva, respectively, during the larval stages.~~

~~No statistically significant effect occurred in any treatment group of larvae fed with the test item.~~

In a repeated exposure larval toxicity study with boscalid, the LD_{50} (larval mortality on D8) was estimated to be > 50.0 µg a.s./larva, which is equivalent to a LC_{50} of > 317 mg a.s./kg food. The respective NOED was ≥ 50.0 µg a.s./larva and the corresponding NOEC was ≥ 317 mg a.s./kg food. The ED_{50} (successful adult emergence up to D22) was estimated to be > 50.0 µg a.s./larva, which is equivalent to a EC_{50} of > 317 mg a.s./kg food. The respective NOED was ≥ 50.0 µg a.s./larva and the corresponding NOEC was ≥ 317 mg a.s./kg food.

I. MATERIALS AND METHODS

A. MATERIALS

Test item: Boscalid (BAS 510 F, Reg. No. 300 355); batch no.: COD-001415; analyzed purity: 98.9% ±1%.

B. STUDY DESIGN

Test species: Larvae of *Apis mellifera iberiensis* ENGEL (Hymenoptera, Apoidae); first larval stage (L1); derived from healthy and queen-right colonies; source: beekeeper, Cazalla (Sevilla), Spain.

Test design: 22-day chronic feeding test according to OECD 239 (2016). L1 honey bee larvae of *Apis mellifera* were transferred from brood combs to polystyrene grafting cells in 48-well cell culture plates 2 days before start of the treatment (on D1). After this, the larvae were fed during larval development with artificial diet, containing the test item and aqueous yeast/sugar solution mixed with royal jelly 1:1 (w/w) on rearing days 3, 4, 5 and 6. In total, 8 treatment groups were set up: 5 doses of the test item, 2 untreated control groups and 1 dose of the reference item, each with 3 replicates and 12 larvae per replicate. Assessments of larval mortality were done 24, 48, 72, 96 and 120 hours after start of the treatment (respectively D4, D5, D6, D7, D8). Additionally, other observations such as small body size or large quantities of remaining food on D8 were noted. Pupal mortality was assessed at D15 and emergence of adults was evaluated at D22.

Endpoints: Successful adult emergence (dose-effect relationship), Mortality, qualitative observations: body size, remaining food.

Reference item: Dimethoate tech. (analyzed purity: 98.8% w/w).

Test doses: Control 1: untreated diet containing 0.5% water (50% aqueous yeast/sugar solution with 50% royal jelly)
Control 2: untreated diet containing 0.5% acetone (50% aqueous yeast/sugar solution with 50% royal jelly)
Test item treatments including 0.5% w/w acetone:

Nominal dose/concentration of boscalid	
Doses [µg a.s./larva]	Concentrations [mg a.s./kg food]
9.9	63
14.8	94
22.2	141
33.4	211
50.0	317

Reference item: treated diet with a dose of 7.6 µg dimethoate/larva (corresponding concentration: 48 mg a.s./kg food

Test conditions: Temperature (D1-D22): 34.0°C – 35.0°C

Relative humidity:
90.0 – 100.0% (D1–D8)
78.4 – 84.4% (D8 – D15)
57.2 – 64.2% (D15 – D22)
Photoperiod: darkness (except during assessments)
Food: 50% aqueous yeast/sugar solution and 50% royal jelly.

Analytics: Analytical verification of the test item in the feeding solutions was conducted using HPLC/MS.

Statistics: Descriptive statistics; The Chi² Table Test with Bonferroni Correction (one-sided greater, $\alpha = 0.05$) for determination of NOED/NOEC (D8 and D22).

C. DESCRIPTION OF THE ANALYTICAL PROCEDURES

Concentrations of boscalid (BAS 510 F) in feeding solution were determined using the method described within the study report. The validation of the analytical method is described in the study report. For the extraction, 5 mL of water and 5 mL of acetonitrile as well as QuEChERS citrate extraction mix containing 0.5 g magnesium sulfate, 0.12 g sodium chloride were added to a sample aliquot of 0.2 g. The mixture was shaken vigorously for 3 minutes with a Multitube Vortexer and centrifuged for 2 minutes at 3000 rcf. Aliquots of the acetonitrile phase were diluted. The aliquots of the acetonitrile phase were diluted as follows and injected into the HPLC. The determination was performed by HPLC-MS-MS. The limit of quantification (LOQ) was 24.7 mg/kg and the limit of detection (LOD) was set to 5 mg/kg. Matrix effects were taken into account by spiking the calibration solutions with 11% of QuEChERS blank extract obtained from extraction of 0.2 g of untreated sample matrix. Thus, all measuring samples contained the same amount of original sample matrix. Details on measured fortification samples and obtained procedural recoveries for BAS 510 F are given in Table A 15.

Table A 15 Procedural recoveries for BAS 510 F in feeding solution

Matrix	Fortification level (mg/L)	n	Mean (%)	RSD (%)
Summary of the validation results (343→307) for the quantifier				
feeding solution	24.7	2	101	16
Summary of the validation results (343→307) for the qualifier				
feeding solution	24.7	2	104	17

II. RESULTS AND DISCUSSION

After 120 hours of repeated oral exposure (on D8) larval mortalities of 0.0 and 5.6% were observed in the water control and the acetone control, respectively. Pupal mortality (between D8 and D22) was 11.1% in the untreated control and 20.6% in the solvent control. The control groups showed a total mortality of 11.1% and 25.0%, respectively, at D22. In the test item group, larval mortalities at D8 ranged between 0.0 and 8.3%. Pupal mortalities ranged between 8.6 and 18.2% in the test item treatment groups. Total mortalities at D22 ranged between 11.1 and 25.0%. On D8, no remaining larva treated with BAS 510 F showed any abnormalities such as remaining food. In the final assessment at D22, adult emergence rates of 88.9 and 75.0 % were determined for the honey bees in the control groups. In the test item group, the adult honey bees emerged at rates ranging between 75.0 and 88.9% following an application of 9.9, 14.8, 22.2, 33.4 and 50.0 µg a.s./larva, respectively, during the larval stages. No statistically significant effect occurred in any treatment group of larvae fed with the test item (Chi² Table Test with Bonferroni Correction, one-sided greater, $\alpha = 0.05$). The results are summarized in

~~Table A 16.~~

Table A 16: Toxicity of BAS 510 F to *Apis mellifera* (honey bee) in a chronic oral larval toxicity test after 22 days

Dosage [µg a.s./larva]	Concentration [mg a.s./kg food]	D8 mortality		D22 mortality [%]		D22 adult emergence [%] [2]
		absolute	corrected [1]	absolute	corrected [1]	
Control	Control	0.0	–	11.1	0.0	88.9
Acetone solvent control	Acetone solvent control	5.6	0.0	25.0	0.0	75.0
9.9	63	0.0	0.0	13.9	0.0	86.1
14.8	94	0.0	0.0	13.9	0.0	86.1
22.2	141	2.8	0.0	11.1	0.0	88.9
33.4	211	2.8	0.0	16.7	0.0	83.3
50.0	317	8.3	2.9	25.0	0.0	75.0
Endpoints [D22]						
LD_{50} [µg a.s./larva] [2]		> 50.0				
$NOED_{mortality}$ [µg a.s./larva] [3]		≥ 50.0				
LC_{50} [mg a.s./kg food] [2]		> 317				
$NOEC_{mortality}$ [mg a.s./kg food] [3]		≥ 317				
ED_{50} [µg a.s./larva] [2]		> 50.0				
$NOED_{emergence}$ [µg a.s./larva] [3]		≥ 50.0				
EC_{50} [mg a.s./kg food] [2]		> 317				
$NOEC_{emergence}$ [mg a.s./kg food] [3]		≥ 317				

[1] – Corrected for solvent control mortality according to Schneider-Orelli (1947).
[2] – Estimated value.
[3] – Chi^2 Table Test with Bonferroni Correction, one-sided greater, $\alpha = 0.05$.

Validity criteria:

Validity criteria according to OECD 239 (2016)	Obtained in this study
Control mortality from D3 to D8 ≤ 15% across all replicates	0.0% untreated control 5.6% solvent control
Adult emergence in the control group ≥ 70% at D22 across all replicates	88.9% untreated control 75.0% solvent control
Effects of the reference item: Dimethoate: larval mortality ≥ 50% on D8 across all replicates Fenoxycarb: emergence rate ≤ 20% on D22 across all replicates	dimethoate: 83.3% at D8

All validity criteria were met.

III. CONCLUSION

In a repeated exposure larval toxicity study with BAS 510 F, the LD_{50} (larval mortality on D8) was estimated to be > 50.0 µg a.s./larva, which is equivalent to a LC_{50} of > 317 mg a.s./kg food. The respective NOED was ≥ 50.0 µg a.s./larva and the corresponding NOEC was ≥ 317 mg a.s./kg food. The ED_{50} (successful adult emergence up to D22) was estimated to be > 50.0 µg a.s./larva, which is equivalent to a EC_{50} of > 317 mg a.s./kg food. The respective NOED was ≥ 50.0 µg a.s./larva and the corresponding NOEC was ≥ 317 mg a.s./kg food.

A 2.3.1.3.3 Study 3

Comments of zRMS:	The study was performed in line with OECD 239 with a minor deviation. It was noted that the temperature and humidity were out of range on D8 for three hours due to malfunction of the climatic chamber. During that time the temperature ranged between 30.2 and 35.2°C instead of 34.5 ± 0.5°C. The relative humidity ranged between 19.1 and 27.4%. Thereafter, the relative humidity was slightly decreased/increased until D15 (71.5 – 89.6% instead of 80 ± 5%). However, since all validity criteria were met and no effects on development of larvae in the untreated control was observed, this deviation is considered to have no impact on the test results. The endpoints are expressed as nominal concentrations since the measured concentrations of both active substances were maintained at 80-120% of nominal. Recoveries in final diets

	over all applied concentration levels ranged from 80.2 % to 113 % for BAS 750 F and from 86.5 % to 119 % for BAS 510 F of nominal. All validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment: ED_{50} > 250 µg product/larva ED_{10} = 54.5 µg product/larva NOED = 62.6 µg product/larva EC_{50} > 1583 mg product/kg food EC_{10} = 344.3 µg product/kg food NOEC = 395.7 mg product/kg food

Reference:	KCP 10.3.1.3/3 ~~CP 10.3.1.2/2~~
Report	Repeated exposure of honey bee (*Apis mellifera* L.) larvae to BAS 762 02 F under laboratory conditions, XXX, K., 2021 Report No 2020/2032683 BASF DocID 2020/2032683 ~~Chronic toxicity of BAS 762 02 F to the honey bee Apis mellifera L. under laboratory conditions,~~ ~~XXX, K., 2021~~ ~~report No 887728, 2048BAC0048~~ ~~BASF DocID 2020/2032682~~ ~~Authority registration No~~
Guideline(s):	OECD 239 (2016) ~~245 (2017)~~
Deviations:	Minor deviations (see commenting box above) ~~No~~
GLP:	yes (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany),
Acceptability:	Yes
Duplication (if vertebrate study)	No

EXECUTIVE SUMMARY

The effects of the test item BAS 762 02 F on survival and adult emergence of honey bee larvae (*Apis mellifera*) were investigated in a laboratory test with repeated exposure over a time period of 22 days. Synchronized 1st larval stage (L1) honey bee larvae were fed with artificial diet for 5 days (day 1, 3, 4, 5 and 6). On days 3, 4, 5 and 6, larvae were fed with diet containing five different concentrations of BAS 762 02 F of 98.9, 197.8, 395.7, 791 and 1583 mg product/kg food, corresponding to doses of 15.6, 31..3, 62.6, 125 and 250 µg product/larva (equivalent to 4.2, 8.3, 16.6, 33 and 66 µg total a.s./larva). Untreated diet served as a control. Furthermore, dimethoate at a dose rate of 7.6 µg/larva served as reference item treatment. All treatment groups and controls contained larvae from three different bee colonies. Assessments of larval mortality were done 24, 48, 72, 96 and 120 hours after start of the treatment. Additionally, other observations such as small body size or large quantities of remaining food after 120 hours was noted. Pupal mortality was assessed at D15 and emergence of adults was evaluated at D22.

After 120 hours of repeated oral exposure (on D8) larval mortality of 0.0% was observed in the untreated control. Pupal mortality in the untreated control (between D8 and D15) was 22.2% which is equal to the total mortality on D22. In the test item treated groups, cumulated larval mortalities at D8 ranged between 2.8 and 16.7%. Pupal mortalities (D8-D15) ranged between 8.6 and 25.8% in the test item treatment groups. Total mortalities at D22 ranged between 16.7 and 47.2%. On D8, one of the remaining larvae treated with 31.3 µg product/larva showed remaining food. In the final assessment on D22, an adult emergence rate of 77.8% was determined for the honey bees in the control group. In the test item treated groups the adult

honey bees emerged at rates ranging between 52.8% and 83.3% following an application of 15.6, 31.3, 62.6, 125 and 250 µg product/larva during the larval stages. On D22, larvae treated with 125 and 250 µg product/larva, ~~respectively,~~ showed mortality, which was statistically significantly increased if compared to the control.

In a repeated exposure larval toxicity study with BAS 762 02 F, the LD_{50} (larval mortality on D8) was estimated to be > 250.0 µg product/larva, which is equivalent to a LC_{50} of > 1583 mg product/kg food. The respective NOED was ≥ 250.0 µg product/larva and the corresponding NOEC was ≥ 1583 mg product/kg food. The ED_{50} (successful adult emergence up to D22) was estimated to be > 250.0 µg product/larva, which is equivalent to a EC_{50} of > 1583 mg product/kg food. The respective NOED was 62.6 µg product/larva and the corresponding NOEC was 395.7 mg product/kg food.

I. MATERIALS AND METHODS

A. MATERIALS

Test item: BAS 762 02 F, batch no. FD-190207-0001, content of a.s.: mefentrifluconazole (BAS 750 F, Reg. No. 5 834 378): 96.2 g/L analyzed (100 g/L nominal); boscalid (BAS 510 F, Reg. No. 300 355): 205.2 g/L analysed (200 g/L nominal); density: 1.130 g/cm³.

B. STUDY DESIGN

Test species: Larvae of *Apis mellifera* L. ssp. Buckfast (Hymenoptera, Apoidae); first larval stage (L1); derived from at least three healthy and queen-right colonies; each colony represents a replicate; source: in-house colonies.

Test design: 22-day chronic feeding test according to OECD 239 (2016). L1 honey bee larvae of *Apis mellifera* were transferred from brood combs to polystyrene grafting cells in 48-well cell culture plates 2 days before start of the treatment (on D1). After this, the larvae were fed during larval development with artificial diet, containing the test item and aqueous yeast/sugar solution mixed with royal jelly 1:1 (w/w) on rearing days 3, 4, 5 and 6. The following treatment groups were set up: 5 doses of the test item, 1 untreated control group and 1 dose of the reference item, each with 3 replicates and 12 larvae per replicate. Assessments of larval mortality were done 24, 48, 72, 96 and 120 hours after start of the treatment (respectively D4, D5, D6, D7, D8). Additionally, other observations such as small body size or large quantities of remaining food on D8 were noted. Pupal mortality was assessed at D15 and emergence of adults was evaluated at D22.

Endpoints: Successful adult emergence (dose-effect relationship), Mortality, qualitative observations: body size, remaining food.

Reference item: Dimethoate tech. (analyzed purity: 98.8% ±0.5%).

Test doses: Control: untreated diet (50% aqueous yeast/sugar solution with 50% royal jelly)
Test item treatments:

Nominal dose/concentration of BAS 762 02 F			
Doses [µg/larva]		Concentrations [mg/kg food]	
BAS 762 02 F	Total a.s.	BAS 762 02 F	Total a.s.
15.6	4.2	98.9	26.3
31.3	8.3	197.8	52.5
62.6	16.6	395.7	105.0
125	33	791	210
250	66	1583	420

Reference item: treated diet with a dose of 7.6 µg dimethoate/larva (corresponding concentration: 48 mg a.s./kg food

Test conditions: Temperature (D1-D22): 30.2°C – 35.2°C
Relative humidity:
91.8 – 99.9% (D1-D8)
71.5 – 89.6% (D8 – D15); due to a malfunction of the climatic chamber 19.1 – 27.4% for 3 hours at D8
59.1 – 67.7% (D15 – D22)
Photoperiod: darkness (except during assessments)
Food: 50% aqueous yeast/sugar solution and 50% royal jelly.

Analytics: Analytical verification of the test item in the feeding solutions was conducted according to BASF method L0372/02 using HPLC-MS/MS.

Statistics: Descriptive statistics; Multiple Sequentially-rejective Fisher Test After Bonferroni-Holm (D8) and Step-down Cochran-Armitage Test Procedure (D22) were used for determination of NOED/NOEC (one-sided greater, $\alpha = 0.05$). $LD/LC_{10/20/50}$ calculations on D8 were performed with the Logit analysis with lin. max. likelihood regression. $ED/EC_{10/20/50}$ calculations on D22 were performed with the Probit analysis using linear weighted regression. Mortalities of control and reference item treated groups on D8 and D22 were compared using Fisher's Exact Binomial Test.

C. DESCRIPTION OF THE ANALYTICAL PROCEDURES

Concentrations of BAS 750 F and BAS 510 F (contained in BAS 762 02 F) in larval food were determined according to the analytical method L0372/02. The validation of the analytical method is described in the study report. The samples were extracted with 75/25 (v/v) methanol/water by shaking. Aliquots of the extracts were cleaned-up with 150 mg $MgSO_4$, 50 mg C18-EC and 50 mg PSA and diluted with 75/25 (v/v) methanol/water. The determination was performed by HPLC-MS/MS. The limit of quantification (LOQ) was 0.01 mg/kg and the limit of detection (LOD) was set to ≤ 30% of LOQ mg/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. Details on measured fortification samples and obtained procedural recoveries for BAS 750 F and BAS 510 F are given in Table A 17.

Table A 17 Procedural recoveries for mefentrifluconazole and boscalid in feeding solution

Substance	Matrix	Fortification level (mg/L)	n	Mean Recovery (%)	RSD (%)
BAS 750 F	feeding solution	0.10	5	98.2	4.20
	feeding solution	187	5	101	7.69
	Mean		10	99.7	5.94
BAS 510 F	feeding solution	0.20	5	97.7	2.08
	feeding solution	373	5	108	6.09
	Mean		10	103	4.08

RSD = relative standard deviation

II. RESULTS AND DISCUSSION

After 120 hours of repeated oral exposure (on D8) cumulated larval mortality of 0.0% was observed in the untreated control. Pupal mortality in the untreated control (between D8 and D15) was 22.2% which is equal to the total mortality on D22. In the test item treated groups, cumulated larval mortalities at D8 ranged between 2.8 and 16.7%. Pupal mortalities (D8-D15) ranged between 8.6 and 25.8% in the test item treatment groups. Total mortalities at D22 ranged between 16.7 and 47.2%. On D8, one of the remaining larvae treated with 31.3 µg product/larva showed remaining food.

In the final assessment on D22, an adult emergence rate of 77.8% was determined for the honey bees in the control group. In the test item treated groups the adult honey bees emerged at rates ranging between 52.8% and 83.3% following an application of 15.6, 31.3, 62.6, 125 and 250 µg product/larva during the larval stages. On D22, larvae treated with 125 and 250 µg product/larva, ~~respectively,~~ showed reduced adult emergence which was statistically significantly increased if compared to the control (Step-down Cochran-Armitage Test Procedure, one-sided greater, $\alpha = 0.05$). The results are summarized in Table A **18**.

Table A 18: Toxicity of BAS 762 02 F to *Apis mellifera* (honey bee) in a repeated exposure larval toxicity test after 22 days

Dosage [µg product/larva]	**Concentration [mg product/kg food]**	**D8 larval mortality [%]**		**D15 pupal mortality [%]**		**D22 total mortality [%]**		**D22 adult emergence [%] rate**
		abs.	**corr.**	**abs.**	**corr.**	**abs.**	**corr.**	**abs.**
Control	Control	0.0	--	22.2	0.0	22.2	0.0	77.8
15.6	98.9	2.8	--	8.6	0.0	16.7	0.0	83.3
31.3	197.8	2.8	--	19.7	0.0	27.8	7.1	72.2
62.6	395.7	2.8	--	25.8	4.5	33.3	14.3	66.7
125	791	8.3	--	12.4	0.0	38.9	21.4	61.1 *
250	1583	16.7	--	23.1	1.2	47.2	32.1	52.8 *

Endpoints [D8]		**Based on BAS 762 02 F**	**Based on total a.s.**
Doses [µg/larva]	LD_{50} 1)	> 250.0	> 66
	LD_{20} 1)	> 250.0	> 66
	LD_{10} 1)	145.9 (95% CL: 72.7 – 293.1)	38.7 (19.3 – 77.8)
	$NOED_{mortality}$ 2)	≥ 250.0	≥ 66
Concentrations [mg/kg food]	LC_{50} 1)	> 1583	> 420
	LC_{20} 1)	> 1583	> 420
	LC_{10} 1)	922.5 (95% CL: 459.4 – 1852.4)	244.9 (95% CL: 122.0 – 491.8)
	$NOEC_{mortality}$ 2)	≥ 1583	≥ 420
Endpoints [D22]			
Doses [µg/larva]	ED_{50} 3)	> 250.0	> 66
	ED_{20} 3)	116.4 (95% CL: 76.2 – 177.8)	30.9 (95% CL: 20.2 – 47.2)
	ED_{10} 3)	54.5 (95% CL: 32.9 – 90.2)	14.5 (95% CL: 8.7 – 23.9)
	$NOED_{emergence}$ 4)	62.6	16.6
Concentrations [mg/kg food]	EC_{50} 3)	> 1583	> 420
	EC_{20} 3)	736.0 (95% CL: 481.9 – 1124.0)	195.4 (95% CL: 127.9 – 298.4)
	EC_{10} 3)	344.3 (95% CL: 207.9 – 570.0)	91.4 (95% CL: 55.2 – 151.3)
	$NOEC_{emergence}$ 4)	395.7	105.0

abs. = absolute mortality; corr. = corrected mortality (according to Schneider-Orelli 1947); negative values were set to "0"; 95% CL = 95% confidence limits

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

1) Multiple Sequentially-rejective Fisher Test After Bonferroni-Holm; α=0.05; one sided greater.

2) Logit analysis using linear max. likelihood regression.

3) Step-down Cochran-Armitage Test Procedure; α=0.05; one sided greater.

4) Probit analysis using linear max. likelihood regression.

Validity criteria:

Validity criteria according to OECD 239 (2016)	Obtained in this study
Control mortality from D3 to D8 ≤ 15% across all replicates	0.0% untreated control
Adult emergence in the control group ≥ 70% at D22 across all replicates	77.8% untreated control
Effects of the reference item: Dimethoate: larval mortality ≥ 50% on D8 across all replicates Fenoxycarb: emergence rate ≤ 20% on D22 across all replicates	dimethoate: 97.2% at D8

All validity criteria were met.

III. CONCLUSION

In a repeated exposure larval toxicity study with BAS 762 02 F, the LD_{50} (larval mortality on D8) was estimated to be > 250.0 µg product/larva, which is equivalent to a LC_{50} of > 1583 mg product/kg food. The respective NOED was ≥ 250.0 µg product/larva and the corresponding NOEC was ≥ 1583 mg product/kg food. The ED_{50} (successful adult emergence up to D22) was estimated to be > 250.0 µg product/larva, which is equivalent to a EC_{50} of > 1583 mg product/kg food. The respective NOED was 62.6 µg product/larva and the corresponding NOEC was 395.7 mg product/kg food.

A 2.3.1.4 KCP 10.3.1.4 Sub-lethal effects

As BAS 762 02 F poses no unacceptable risk to honey bees, further studies are not necessary.

A 2.3.1.5 KCP 10.3.1.5 Cage and tunnel tests

A 2.3.1.5.1 Study 1

Comments of zRMS:	In general, the study was not required to finalise the risk assessment for BAS 762 02 F since acceptable risk could be concluded based on laboratory studies. Nevertheless, the study was validated by the zRMS as being submitted and providing supporting information on potential acute and chronic effects of the formulation on adult bees and bee colonies. In opinion of the zRMS the study is of high quality and covers multiple parameters which are not necessarily investigated in the tunnel tests (e.g. all relevant brood indices or residue analysis in flower, nectar and pollen). Furthermore, in order to demonstrate sufficient sensitivity of the test system, 2 reference items were used: fenoxycarb (relevant for evaluation of effects on bee brood) and dimethoate (relevant for evaluation of effects on mortality and foraging activity). Detailed brood assessments were performed over one brood cycle, while colony assessments were carried out over almost two brood cycles. The weather conditions during application and exposure phase were favourable with no precipitation. First rainfall (only 1 mm) was observed at DAT 9, when bees were already at the monitoring site. Then rainfall was observed on single days only. BS 762 02 F had no effects on any of the investigated parameters (mortality, foraging activity, behaviour, colony strength, colony development, broo indices) which were at level comparable with controls. Clear effects were observed in the toxic standard groups confirming sufficient sensitivity of the test system. Overall, the study is considered acceptable. Based on its results it may be concluded that under semi-field conditions BAS 762 02 F had no adverse effects on bees and bee colonies when applied up to 1.1 L/ha. During the commenting period it was pointed out that at DAT 4 adverse effects on larvae were observed (compared to both, the number of larvae at DAT -2 and the control at DAT 4). Also at the last observation date (DAT 40) a reduction of larvae compared to DAT -2 was observed, while in the control an increase of larvae could be shown at DAT 49.

	The zRMS agrees that the area of larval brood stages in the treatment groups was lower on BFD 6 (4 DAT) comparing to controls. It is noted that the area of larval brood stages decreased also in control groups, but it was not so pronounced as in the treatment groups on DAT 4. However, at the next brood assessments on DAT 7 and DAT 13 the reduction in the larvae area was comparable in test item and control groups. At the next assessment interval (DAT 20) slight increase in larvae area was observed in test item groups (+2%), while in controls the larvae area was still clearly reduced (-29%). Then on DAT 26 it slightly increased on controls (+5%) and decreased in test item groups (-12%). Reduction was comparable in both groups on DAT 33 (-36 and -34% in control and test item groups, respectively) and at the last assessment (DAT 40) there was a slight increase in controls (+2%) and decrease in treatment groups (-14%). In general, no clear pattern may be observed based on these results - on some days there was increase in controls and decrease in treatment groups, while on other days it was the opposite. At some time points the reduction in both groups was comparable. Based on that it seems that changes in the larvae area were random and resulted from natural variation. It should be also kept in mind that during visual observation and estimation early bee brood stages (i.e. eggs and larvae) are difficult to distinguish at the early stage and can fluctuate significantly between observations. Latter can be observed when just looking at change in the area of eggs and larvae on different observation days in both control and treatment. It is thus important not to put too much weight on the separate brood stages at one individual time point but rather consider the overall colony development including general development of brood stages over time. Looking at the overall area covered by all developmental stages (eggs+larvae+pupae), a continuous increase of number of brood stages could be observed in the treatment groups throughout almost the entire study period. The trend was similar as in control groups, but when the entire brood is considered, the performance in the test item groups was actually better than in controls. The strength of the colonies also continuously increased in treatment groups, similarly as in controls, and by the end of the study has more than doubled compared to DAT -2. The detailed brood assessments resulting with the main quantitative endpoints from the study (i.e. brood termination rate, brood index and brood compensation index) do not indicate any adverse effects of the test item on the bee brood. Brood indices and brood compensation indices in controls and test item groups were at comparable level, while the brood termination rates were clearly lower in test item groups comparing to controls. Taking into account that changes in the area of larval brood stages in the study were not reflected in the detailed brood assessments (and especially in brood termination rates) and they were statistically not significant, the zRMS is of the opinion that they resulted from the natural fluctuations and were not due to the exposure to the test item. Lack of the effects caused by the treatment is also supported by results on other parameters (e.g. total brood area, colony strength).

Reference:	CP 10.3.1.5/1
Report	Effects of BAS 762 02 F on the honeybee Apis mellifera L. under semi-field conditions (tunnel test) with additional assessments on colony and brood development, XXX, A., 2021 report No 834656, 2048BTB0003 BASF DocID 2021/2001936 Authority registration No
Guideline(s):	Current recommendations of the German AG Bienenschutz (2011), Pistorius et al. (2012), EPA 850.3040, EPPO PP 1/170 (4) (2010), OECD Guidance document No. 75 (2007), SANCO/3029/99 rev. 4 (11 July 2000), ICPPR (2014)
Deviations:	No
GLP:	yes (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany),
Acceptability:	Acceptable

Duplication (if vertebrate study)	No

Executive summary

A tunnel test was carried out to determine the effects of BAS 762 02 F on honey bee colonies under semi-field conditions. For this purpose, BAS 762 02 F was applied once at a rate of 1100 mL BAS 762 02 F/ha (equivalent to 110g BAS 750 F/ha and 220g BAS 510 F/ha) to full-flowering *Brassica napus* L. (BBCH 65). Additionally, an untreated control and two reference items were included in the study. Each of the four treatment groups was replicated four times (except for reference item II, which was replicated two times), with one honey bee colony per tunnel set up 4 days before application. Mortality of the honey bees was assessed daily from 3 days before to 41 days after treatment (DAT), respectively. Foraging activity was assessed daily during the pre, - and exposure phase in tunnels. Sub-lethal effects were recorded daily during the entire study period. Colony development (colony strength and brood and food status) was assessed 2 days prior the exposure phase and on DAT 4, DAT 7, DAS 13, DAT 20, DAT 26, DAT 33 and DAT 40.

Furthermore, a detailed assessment of the brood development of single brood cells was performed on DAT -2, DAT 4, DAT 7, DAT 13 and DAT 20, which is equal to BFD 0, BFD 6, BFD 9, BFD 15 and BFD 22.
During pre-exposure phase the overall mean mortality of adult honey bees was 28.3, 24.9, 26.0 and 26.1 dead bees/colony/day in the control, test item, reference item I and reference item II, respectively. No statistically significant differences occurred in the overall mean mortality as well as of the daily mean mortality during pre-exposure phase by comparing the control, test item and reference items against each other. On day of application and during the following days of the exposure phase the overall mortality was on a comparable level and not significantly different throughout the treatment groups of control, test item and reference item I. In contrast, the overall mean mortality was distinctly higher in reference item II treatment group and increased during the exposure phase. During the post-exposure phase and the entire post-application phase low levels of mortality were observed between the control and the test item treatment group. No statistically significant difference was detected comparing the overall means during post-exposure and the entire post-application phase in the control and test item treatment groups, respectively. Overall lower mortality level was observed compared to the pre-exposure phase. With respect to the pupal mortality, no dead pupae were found in the control and test item treatment during the exposure and post-exposure phase, respectively. Therefore, the application of BAS 762 02 F resulted in no adverse effect on pupal mortality during the entire course of the study until DAT 41. Overall mean mortalities of the reference item I during post-exposure and post-application phase were similar in comparison with the control group and not statistically significant different. The overall mean pupal mortality for the entire post-application phase was increased in comparison with the control. For the post-exposure phase, distinctly increased bee mortality was observed in the reference item II group compared to the control on DAT 8 to DAT 10. From DAT 11 until DAT 41 the mean mortality level was comparable in both treatment groups. During the entire post-application phase, mean mortality of 11.5 and 43.7 dead bees/colony/day was observed in the control and reference item II, respectively. As reference item II consisted of only two replicates, no statistical analysis was performed.

During the pre-exposure phase, the overall mean foraging activity was 5.5, 5.2, 5.0 and 5.0 bees/m²/day in the control, test item, reference item I and reference item II treatment group, respectively. Hence, foraging activity was on a similar level among all treatment groups indicating that the colonies had well adapted to the new environmental conditions. Statistical analysis revealed no differences between all treatment groups based on overall comparison.
Shortly before the application foraging activity amounted to 5-10 (Ø 7.2), 3-10 (Ø 7.3), 5-9(Ø 6.6) and 5-7 (Ø 6.3) in the control, test item, reference item I and reference item II treatment, respectively, indicating an appropriate exposure during application.
Foraging activity in the test item was similar when compared to the control on the day of application and at any of the following assessment days. The overall mean number of foraging bees during the exposure phase were 7.5, 7.9 and 7.6 bees/m²/day in the control, test item and reference item I, respectively. Statistical analyses revealed no statistically significant differences between the control and test item/reference item I treatment group. In contrast, the application of reference item II revealed a distinct reduction of foraging

activity during the exposure phase (0.4 bees/m²/day) compared to the control (7.5 bees/m²/day).

The application and subsequent exposure of bees to the test item and the reference item I did not result in behavioural abnormalities compared to the bees in the control group. No symptoms of apathy, intoxication or any deviations to the normal behaviour of bees occurred in comparison to the control. Bees were calm and actively foraging nectar and pollen on the treated crop. In contrast to this, the application of reference item II resulted in behavioural abnormalities compared to the control on DAT 0. This behaviour was characterized by hyperactivity and impaired locomotion.

The mean estimated colony strength on DAT -2 = BFD 0 (brood fixing day) amounted to 9788, 9928, 9563 and 9619 bees/colony in the control, test item reference item I and reference item II, respectively, and thus was on a comparable and appropriate level in relation to the available crop area. Bee colonies confirmed an adequate and good strength for the conduction of the tunnel study.

During the course of the study a positive and similar development of colony strength occurred in the control and test item group which amounted at BFD 42 to 18928 (+93%) and 20363 bees/colony (+105%), respectively. The reference item I and reference item II group revealed lower increases by 51 - 52%, which resulted in a mean colony strength of 14513 and 14569 bees/colony, respectively.

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 groups developed within the range of natural variability in a comparable manner over the course of the study. During the first investigated brood cycle until BFD 22, the mean comb area covered with brood stages increased on a similar level in the control and test item treatment group and developed within the range of natural variability, amounting to 9798 (+10%) and 11319 cm²/colony (+26%) for the control and test item, respectively. In contrast, the mean brood area at BFD 22 amounted to 9360 and 9076cm²/colony for the reference item I and reference item II, respectively, meaning a similar level compared to the pre-application level for reference item I and an increase of +2% for reference item II.
At the last assessment on BFD 42 the mean brood area amounted to 10262, 11629, 11242 and 12789 cm²/colony for the control, test item reference item I and reference item II, respectively. Compared to the pre-application level, the mean brood area increased by 16%, 30%, 20% and 44% in the control, test item, reference item I and reference item II, respectively. The comb area covered with food stores (nectar/honey and pollen) revealed similar levels for the control, test item and reference item groups. Overall, the nectar and pollen stores were on an acceptable level when compared to the size of the colonies and had no negative or limiting effect on brood or colony development throughout the study.

The mean BTR of initially labelled eggs amounted to 20.5 and 9.0% for the control and test item groups, respectively, at final evaluation on BFD 22. Therefore, the termination of labelled eggs was on a similar level and within a natural range of variability, without any statistically significant differences between control and test item treatment. In contrast, the reference item I treatment group revealed a high brood termination rate of 56.4%, which was statistically significantly different when compared to the control group.

The BI displays a negative correlation with the BTR: the higher the BTR the lower the BI and vice versa. Therefore, the BI of initially labelled eggs at BFD 22 was slightly lower in the test item treatment and amounted to 4.0 and 4.6 for the control and test item groups, respectively, without any statistically significant differences during the study. In contrast, the reference item I treatment revealed a much lower brood index of 2.2, which is statistically significantly different compared to the control.

The BCI was on a similar level for both control and test item treatment and amounted to 4.3 and 4.8 in the control and test item treatment, respectively and therefore without any statistically significant difference, indicating that most of the terminated brood-cells were refilled with new eggs. In contrast, the reference item I revealed a statistically significant lower brood compensation index of 3.6, which means that only few emptied cells were refilled with new eggs.

Under semi-field conditions (tunnel test), BAS 762 02 F was applied in a single application at a rate of 1100 ml/ha (equivalent to 110 g BAS 750 F/ha and 220 g BAS 510 F/ha) to flowering *Brassica napus* L. during active foraging conditions. No unacceptable effects on mortality, foraging activity, colony development, colony strength or bee brood were observed after application. Overall, based on the results of this study, BAS 762 02 F does not adversely affect honey bee colonies.

I. MATERIALS AND METHODS

A. MATERIALS

Test item:	BAS 762 02 F, batch no. FD-190207-0001, content of a.s.: mefentrifluconazole (BAS 750 F, Reg. No. 5 834 378): 96.2 g/L analysed (nominal 100.0 g/L) and boscalid (BAS 510 F, Reg. No. 300 355): 205.2 g/L analysed (nominal 200.0 g/L), density: 1.130 g/cm^3.
Reference item I:	Fenoxycarb (Insegar 25 WG), nominal content of a.s. 250 g/kg (analysed 24%).
Reference item II:	Dimethoate (Danadim Progress), nominal content of a.s. 400 g/L (analysed 411.2 g/L)

B. STUDY DESIGN

Test species: Honey bees (*Apis mellifera* L. Buckfast); healthy and queen-right bee colonies with 6863 to 11588 bees/colony and sufficient food supply at start of exposure, colonies consisted of two hive bodies containing 11 combs each (”Deutsch-Normal-Maß”, German standard size of 37 cm x 22.3 cm), including 5982 – 12273 cm² nest area per colony with all brood stages present. Source: in-house hives.

Test plots: The test site was located in Hirschfeld near Leipzig, Germany; separate tunnels for the different groups and replicates; tunnel size: 24 m × 6 m × 2.5 m (length × width × height); effective crop area: 126.5 m^2; for the post-exposure phase (DAT 8 to 41), the colonies were moved to a monitoring site without main crops and intensive agriculture in Altenbach near Leipzig, Germany, where further assessments were performed.

Test design: Honey bee semi-field test in winter oilseed rape *Brassica napus* L.; four treatment groups (untreated control, test item group, two reference items) with four replicates (tunnels) for the control, the test item and reference item I, respectively and two replicates for reference item II; additionally, a fifth replicate for residue analysis was set up for the control and the test item treatment, respectively; application of test item and of the reference items once during bee flight at BBCH 65 (full-flowering) of oilseed rape in separate tunnels; honey bee colonies were introduced to the tunnels in the evening at DAT -4 (DAT = days after treatment); pre-exposure phase was 3 days (from DAT -3 to DAT 0ba (ba = before application)); exposure phase was 7 days (from DAT 0aa (aa = after application) to DAT 7); post-exposure phase was 34 days (from DAT 8 to DAT 41); daily assessments of mortality during entire study period until DAT 41 in dead bee traps and on linen sheets during the exposure phase until DAT 7, additional assessments of mortality were conducted 2 hours and 6 hours after application and in the evening after bee flight at nightfall; daily assessment of foraging activity during pre-exposure and exposure phase on three 1 m^2 plots/tunnel, additional assessments were carried out two times within the 1st hour after application and about 2, 4 and 6 hours after application; daily assessments on behaviour until DAT 41; assessments on colony development: colony strength, general brood and food status on DAT -2, DAT 4, DAT 7, DAS 13, DAT 20, DAT 26, DAT 33 and DAT 40. Furthermore, a detailed assessment of the brood development of single brood cells was performed on DAT -2, DAT 4, DAT 7, DAT 13 and DAT 20, which is equal to BFD 0, BFD 6, BFD 9, BFD 15

and BFD 22.

Endpoints: Mortality: daily assessment until DAT 41, linen sheets until DAT 41;
Foraging activity: daily assessment (on 3 m² per tunnel during tunnel phase);
Sublethal effects: behavioral changes were monitored daily until test end;
Colony assessments: (general food and brood status, colony strength): on DAT -2, DAT 4, DAT 7, DAS 13, DAT 20, DAT 26, DAT 33 and DAT 40;
Detailed brood assessments: development of initially labelled eggs were evaluated by calculating the mean brood termination rate (BTR) on BFD 0, BFD 6, BFD 9, BFD 15 and BFD 22.

Reference items: reference item I: Fenoxycarb (250 g/L nominal); reference item II: Dimethoate (400 g/L).

Application rates:

Date (growth stage)	Treatment	Application rate	
		[mL product/ha]	[g total a.s./ha]
23.04.2020 (BBCH 65)	Control	--	--
	Test item (BAS 762 02 F; equivalent to 110g BAS 750 F and 220g BAS 510 F)	1100	330
	Reference item I	1200 g product/ha	300
	Reference item II	1200	480

The control group was treated with tap water only. All treatments were applied in 400 L water/ha using a calibrated plot-sprayer.

Test conditions: Natural field conditions. Good weather conditions during applications;
application of test item: cloud coverage: 0%; wind: 0.0 - 0.7 m/s^2; temperature: 17.9 - 19.2°C, relative humidity: 31.9 - 34.8%, no rainfall during or until at least 24 hours after application;
application (reference items only): cloud coverage: 0%; wind: 0.0 - 0.4 m/s^2; temperature: 19.2 - 20.3°C, relative humidity: 32.6 - 34.7%, no rainfall during or until at least 24 hours after application;
No precipitation during the exposure phase except.

Analytics: Analytical determination of test item residues in plant matrices (flowers, pollen and nectar, analytical method L0372/02) as well as analytical verification of the test item concentration in the spray solution (analytical method L0361/01) was conducted using an LC-method with MS/MS detection.

Statistics: Descriptive statistics; for pre-treatment data evaluation, Tukey-test (two-sided) for comparisons between control, test item and reference item treatments. Please note that reference item II consisted of two replicates only and was therefore excluded from the statistical evaluation.
Post-treatment data evaluation: pair-wise testing for comparisons between treatments (test item or reference item) separately against control, Student t-test (for variance homogeneous data) or Welch t-test (for variance inhomogeneous data). Mortality and brood termination rate: one-sided greater; foraging activity and brood indices: one-sided smaller. The %-values of the brood termination rate were arcsine-transformed to ensure the homogeneity of the data before conducting the t-test procedure. Significance levels of all tests: $\alpha = 0.05$.

C. DESCRIPTION OF THE ANALYTICAL PROCEDURES

Spray solution analysis:

Concentrations of BAS 750 F and BAS 510 F (contained in BAS 762 02 F) in spray solution were determined according to the analytical method L0361/01. The validation of the analytical method was conducted in a separate study 2017/1065621. A 5g aliquot of spray solution was weighted into a flask and fortified and, if necessary, fortified with spiking solution. 5mL of acetonitrile/water/formic acid (400/600/2, v/v/v) were added and the dilution specimen was shaken. In case spiking solution was added, the volume of acetonitrile/water/formic acid (400/600/2, v/v/v) was reduced about the amount of the volume of the spiking solution. Specimens containing higher residues were further diluted with acetonitrile/water/formic acid (400/600/2, v/v/v) as appropriated before measurement. The dilution specimen was injected into the LC-MS/MS instrument for quantification. For BAS 510 F, mass transitions were used in this study differ from the ones in the analytical method no. L0361/01, as evaluated in the validation study, it was found out that the originally proposed quantifier mass transition showed interferences in the LC-MS/MS chromatogram of pollen specimens. This, the mass transitions described in the validation study were used instead of BAS 510 F, which were 343>271 (quantifier) and 343>307 (qualifier). In case of fortification with spiking solution, the volume of acetonitrile/water/formic acid (400/600/2, v/v//v) added was reduced about the volume of the spiking solution to stay with the same dilution volume of 5 mL. Furthermore, in the original method, an injection volume of 10µL BAS 750 F and 20 µL for BAS 510 F was proposed. To fasten the analysis process and to improve the detection quality of BAS 750 F, the same volumes for both analysis injected, i.e. 20 µL each. The determination was performed by LC-MS/MS, the limit of quantification (LOQ) was set to 0.1 µg/L and the limit of detection (LOD) was set to 0.02 µg/L. No instrument recoveries were used for spray solution specimens, for which no matrix effect was assumed due to the high dilution before injecting.

Flower, nectar surrogate and pollen analysis:
Concentrations of BAS 750 F and BAS 510 F (contained in BAS 762 02 F) in flowers, nectar and pollen were determined according to the analytical method L0372/02. The validation of the analytical method was conducted in separate studies. A 0.2g aliquot of flower, nectar or pollen specimen was weighed into a centrifuge tube and, if necessary, fortified with spiking solution. For extraction, 4mL of methanol/water (75/25, v/v) were add-ed and mixed on a mechanical shaker at 300 rmp for 30min. Subsequently, the specimen was centrifuged at 400 rmp for 5min. The supernant was decanted into 10mL measuring flask. For pollen and flowers, the extraction procedure was repeated as described above, combining the supernants in the end. The 10mL measuring flask was filled up to the marked line with methanol/water (75/25, v/v), before shaking manually. For clean-up, 1mL of the extract was transferred into a QuEChERS dSPE-Kit and shaken for 30 seconds, before it was centrifuged at 1000 rpm for 5 min. The determination was performed by LC-MS/MS. The limit of quantification (LOQ) was 0.01 mg/kg and the limit of detection (LOD) was set to 0.02 mg/kg. In the course of the study, instrument recovery specimens were prepared doing each run to proof the absence of matrix effects and justify the use of solvent-based standards for calibration purposes. No instrument recoveries were used for the matrix flowers, for which no matrix effect was assumed due to the high dilution before injecting and where matrix-matched standards were used due to pre-tests of this study. BAS 510 F and BAS 750 F are stable in the solvent (mixtures) in methanol for 31 days and in MeOH/H_2O (75/25, v/v) for 30 days, when stored at 2-8°C. BAS 510 F and BAS 750 F are stable in extracts and final volumes of nectar surrogate, pollen and flowers in MeOH/H2O (75/25, v/v) over a time period of 10 days in pollen, 11 days in flowers and 16 days in nectar surrogate matrix, respectively, when store at 2-8°C in the dark. Shipping verification (SV) samples were established in order to investigate the stability of the active substances in the nectar surrogate solution (10 g glucose + 10 g fructose + 10 g saccharose in 100 mL ultrapure water) specimens during transport and storage period by the responsible laboratory. The recovery of the BAS 510 F and BAS 750 F standards solution mixture for preparation of the SCs ranged between 99.6% and 101% for both analytes and mass transitions.

Details on measured fortification samples and obtained procedural recoveries for BAS 750 F and BAS 510 F in BAS 762 02 F are given in the table below.

Table A 19: Procedural recoveries for BAS 762 02 F (mefentrifluconazole BAS 750 F and boscalid BAS 510 F) in spray solutions

Matrix	Analyte	Fortification level [mg/kg]	n	Mean recovery [%]	RSD [%]
Untreated spray solution	BAS 510 F	Control	2	<LOD	-
		0.10	1	101	-
		1.0	1	99.7	-
		640.000	3	80.3	1.0
		Overall	5	88.3	13
	BAS 750 F	Control	2	<LOD	-
		0.10	1	101	-
		1.0	1	97.1	-
		245.000	3	104	1.0
		overall	5	102	2.9

Table A 20: Procedural recoveries for BAS 762 02 F (mefentrifluconazole BAS 750 F and boscalid BAS 510 F) in plant matrices

Matrix	Analyte	Fortification level [µg/L]	n	Mean recovery [%]	RSD [%]
Flower [1)]	BAS 510 F	Control	1	<LOD	-
		0.01	3	104	3.6
		0.5	3	107	1.6
		Overall	6	105	2.7
	BAS 750 F	Control	1	<LOD	-
		0.01	3	97.3	2.0
		0.5	3	102	0.8
		Overall	6	100	3.1
Nectar surrogate [2)]	BAS 510 F	Control	1	<LOD	-
		0.01	3	104	3.2
		0.5	3	100	1.3
		Overall	6	102	3.2
	BAS 750 F	Control	1	<LOD	-
		0.01	3	95.9	1.6
		0.5	3	100	1.2
		Overall	6	97.8	2.5
Pollen [3)]	BAS 510 F	Control	1	<LOD	-
		0.01	3	100	3.3
		0.5	3	105	1.9
		Overall	6	102	3.5
	BAS 750 F	Control	1	<LOD	-
		0.01	3	105	1.4
		0.5	3	101	1.0
		Overall	6	103	2.5

1) Originated from the ecotoxicological study
2) Produced in the laboratory. The nectar surrogate (sugar solution) was prepared by diluting 20g of D (-) fructose, D (+) glucose and sucrose, each, in 200 mL of ultrapure water.
3) Mixed pollen originated from the local market

II. RESULTS AND DISCUSSION

Residue analysis
Residues of mefentrifluconazole and boscalid in samples of flower, nectar and pollen are presented in table below.

Residues of BAS 510 F and BAS 750 F in untreated and treated flower, pollen and nectar specimens

Matrix	Treatment group	Sampling day	Mefentrifluconazole [mg/kg]	Boscalid [mg/kg]
Flowers	Control 1.1	DAT 0	<LOQ (0.007)	<LOD
	Control 1.2		<LOD	<LOD
	Control 1.3		<LOD	<LOD
	Control 1.4		<LOD	<LOD
	Control (extra tunnel) 1.5		<LOQ (0.005)	<LOD
	Test item 2.1		35.1	12.2
	Test item 2.2		81.9	27.5
	Test item 2.3		78.0	27.6
	Tet item 2.4		60.5	21.7
	Test item (extra tunnel) 2.5		59.3	20.5
Pollen	Control (extra tunnel) 1.5		<LOQ (0.005)	<LOD
	Test item (extra tunnel) 2.5		11.4	4.66
Nectar	Control (extra tunnel) 1.5		<LOQ (0.006)	<LOD
	Test item (extra tunnel) 2.5		0.450	0.304

LOQ = 0.01 mg/kg; LOD = 0.003 mg/kg

Mortality
During pre-exposure phase (DAT -3 to DAT 0ba) the overall mean mortality of adult honey bees was 28.3, 24.9, 26.0 and 26.1 dead bees/colony/day in the control, test item, reference item I and reference item II, respectively. No statistically significant differences occurred in the overall mean mortality as well as of the daily mean mortality during pre-exposure phase (Tukey-test, two-sided) by comparing the control, test item and reference items against each other.

On day of application (DAT 0aa) and during the following days of the exposure phase (DAT 0aa-DAT 7) the overall mortality was on a comparable level and not significantly different (Student-t test, one sided greater, $p > 0.05$) throughout the treatment groups: control, test item and reference item I with 20.2, 19.8 and 19.4 dead bees/colony/day, respectively. In contrast, the overall mean mortality was distinctly higher in reference item II treatment group and increased to 170.7 dead bees/colony/day during the exposure phase (DAT 0 – DAT 7).

During the post-exposure phase (DAT 8 to DAT 41) and the entire post-application phase (DAT 0aa to DAT 41) low levels of mortality were observed between the control and the test item treatment group. No statistically significant difference was detected (Student-t test, one sided greater, $p > 0.05$) comparing the overall means that amounted 9.5 and 10.1 dead bees/colony/day during post-exposure phase and 11.5 and 11.9 dead bees/colony/day during the entire post-application phase in the control and test item treatment group, respectively. Therefore, an overall lower mortality level was observed compared to the pre-exposure phase. With respect to the pupal mortality, no dead pupae were found in the control and test item treatment during the exposure and post-exposure phase, respectively. Therefore, the application of BAS 762 02 F resulted in no adverse effect on pupal mortality during the entire course of the study until DAT 41.

Overall mean mortalities of the reference item I during post-exposure phase (10.2 dead bees/colony/day) and post-application phase (12.0 dead bees/colony/day) were similar in comparison with the control group (post-exposure phase: 9.5 dead bees/colony/ day and post-application phase: 11.5 dead bees/colony/ day) and therefore not statistically significant different (Student-t test, one sided greater, $p > 0.05$). The overall mean pupal mortality amounted to 21.8 dead pupae/colony for the entire post-application phase, which was increased in comparison with the control.

For the post-exposure phase (DAT 8 to DAT 41), distinctly increased bee mortality was observed in the reference item II group compared to the control on DAT 8 to DAT 10. From DAT 11 until DAT 41 the

mean mortality level was comparable in both treatment groups. Mean mortality of the post-exposure phase amounted to 9.5 and 13.9 dead bees/colony in the control and reference item II group, respectively. During the entire post-application phase (DAT 0aa to DAT 41), mean mortality of 11.5 and 43.7 dead bees/colony/day was observed in the control and reference item II, respectively. As reference item II consisted of only two replicates, no statistical analysis was performed.

Foraging activity
During the pre-exposure phase (DAT -3 to DAT 0ba), the overall mean foraging activity was 5.5, 5.2, 5.0 and 5.0 bees/m²/day in the control, test item, reference item I and reference item II treatment group, respectively. Hence, foraging activity was on a similar level among all treatment groups indicating that the colonies had well adapted to the new environmental conditions. Statistical analysis revealed no differences between all treatment groups based on overall comparison (Tukey test, two-sided, $p > 0.05$).
Shortly before the application (DAT 0ba) foraging activity amounted to 5-10 (Ø 7.2), 3-10 (Ø 7.3), 5-9(Ø 6.6) and 5-7 (Ø 6.3) in the control, test item, reference item I and reference item II treatment, respectively, indicating an appropriate exposure during application.

Foraging activity in the test item was similar when compared to the control on the day of application and at any of the following assessment days (DAT 0aa to DAT 7). The overall mean number of foraging bees during the exposure phase were 7.5, 7.9 and 7.6 bees/m²/day in the control, test item and reference item I, respectively. Statistical analyses revealed no statistically significant differences between the control and test item/reference item I treatment group (Student-t test, one sided smaller, $p > 0.05$). In contrast, the application of reference item II revealed a distinct reduction of foraging activity during the exposure phase (0.4 bees/m²/day) compared to the control (7.5 bees/m²/day). The effects on honey bee mortality and foraging activity are summarized in
Table A **21**.

Table A 21: Effects of BAS 762 02 F on honey bee mortality and foraging activity under semi-field conditions (tunnel test)

Evaluation / Assessment		**Control**		**BAS 762 02 F [1.1 L/ha]**		**Reference item I [0.3 kg/ha]**		**Reference item II [0.48 kg/ha] +**	
		Mean 1)	**± SD**	**Mean 1)**	**± SD**	**Mean 1)**	**± SD**	**Mean 2)**	**± SD**
Adult mortality [bees/colony/day]	Pre-exposure phase DAT -3 to DAT 0ba	28.3a	7.0	24.9a	9.6	26.0a	8.8	26.0	8.8
	Application Sum + mean DAT 0aa (+2h, +6h, after bee flight)	42.0	15.3	34.5	11.0	29.3	8.5	649.0	280.0
	Exposure phase DAT 0aa to DAT 7	20.2	4.2	19.8	10.4	19.4	3.4	170.7	28.4
	Post-exposure phase DAT 8 to DAT 41	9.5	2.8	10.1	2.4	10.2	2.4	13.9	1.4
	Overall after application DAT 0aa to DAT 41	11.5	2.7	11.9	3.1	12.0	2.2	43.7	6.5
Pupal mortality [bees/colony/day]	Pre-exposure phase DAT -3 to DAT 0ba	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Application DAT 0aa	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Exposure phase DAT 0aa to DAT 7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Post-exposure phase DAT 8 to DAT 41	0.0	0.0	0.0	0.0	26.9	10.4	0.0	0.0
	Overall after application DAT 0aa to DAT 41	0.0	0.0	0.0	0.0	21.8	8.4	0.0	0.0
Foraging activity [bees/m²/colony]	Pre-exposure phase DAT -3 to DAT 0ba	5.5a	0.3	5.2a	0.9	5.0a	0.3	5.0	0.5
	Application DAT 0aa (+ ½ h, + 1, 2, 4 and 6h)	7.7	0.5	8.3	1.0	8.2	0.6	1.2	0.2

Evaluation / Assessment		Control		BAS 762 02 F [1.1 L/ha]		Reference item I [0.3 kg/ha]		Reference item II [0.48 kg/ha] +	
		Mean 1)	± SD	Mean 1)	± SD	Mean 1)	± SD	Mean 2)	± SD
	DAT 1	8.9	0.8	9.4	0.4	8.6	0.3	0.0	0.0
	DAT 0aa to DAT 7	7.5	0.2	7.9	0.5	7.6	0.4	0.4	0.0

DAT: day after treatment; ba: before application; aa: after application:
a: Same letters indicate that groups are not statistically significant different (Tukey-test, α=0.05) at pre-application period.
1) Mean of 4 replicates.
2) Mean of two replicates.
* Statically significant different (Student t-test α=0.05, one-sided greater).
+ No statistical analysis performed as reference item II consisted of only two replicates.

Bee behaviour

The application and subsequent exposure of bees to the test item and the reference item I did not result in behavioural abnormalities compared to the bees in the control group. No symptoms of apathy, intoxication or any deviations to the normal behaviour of bees occurred in comparison to the control. Bees were calm and actively foraging nectar and pollen on the treated crop.
In contrast to this, the application of reference item II resulted in behavioural abnormalities compared to the control on DAT 0. This behaviour was characterized by hyperactivity and impaired locomotion.

Colony strength

The mean estimated colony strength on DAT -2 = BFD 0 (brood fixing day) amounted to 9788, 9928, 9563 and 9619 bees/colony in the control, test item reference item I and reference item II, respectively, and thus was on a comparable and appropriate level in relation to the available crop area. Bee colonies confirmed an adequate and good strength for the conduction of the tunnel study.
During the course of the study a positive and similar development of colony strength occurred in the control and test item group which amounted at BFD 42 to 18928 (+93%) and 20363 bees/colony (+105%), respectively. The reference item I and reference item II group revealed lower increases by 51 - 52%, which resulted in a mean colony strength of 14513 and 14569 bees/colony, respectively. The effects on honey bee colony strength are summarized in Table A 22.

Table A 22: Colony strength: estimated average number of bees/colony

Treatment group		BFD 0 (DAT -2)	BFD 6 (DAT 4)	BFD 9 (DAT 7)	BFD 15 (DAT 13)	BFD 22 (DAT 20)	BFD 28 (DAT 26)	BFD 35 (DAT 33)	BFD 42 (DAT 40)
Control	Mean 1)	9788	100097	11222	12938	15666	19463	17578	18928
	± SD	1256	2295	1406	2105	1297	3391	2722	2230
	% 3)	-	+3	+15	+32	+60	+99	+80	+93
BAS 762 02 F	Mean 1)	9928	9309	11841	14034	15694	18675	20475	20363
	± SD	1104	1908	3245	2620	2213	5812	4821	4342
	% 3)	-	-6	+19	+41	+58	+88	+106	+105
Reference item I	Mean 1)	9563	10153	11278	11109	11981	13050	13472	14513
	± SD	2101	2674	2936	3473	4254	5405	5377	3303
	% 3)	-	+6	+18	+16	+25	+36	+41	+52
Reference item II	Mean 2)	9619	6806	6806	9450	9956	13444	12263	14569
	± SD	1034	557	716	318	2466	5489	6364	3580
	% 3)	-	-29	-29	-2	+4	+40	+27	+51

DAT: days after treatment, BFD: Brood area fixing day
1) Mean of 4 replicates
2) Mean of two replicates
3) Relative change [%] in comparison with BFD 0 (DAT -2) calculated from the respective mean values.

General brood assessments – brood 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 groups developed within the range of natural variability in a comparable manner over the course of the study. During the first investigated brood cycle until BFD 22, the mean comb area covered with brood stages increased on a similar level in the control and test item treatment group and developed within the range of natural variability, amounting to 9798 (+10%) and 11319 cm²/colony (+26%) for the control and test item, respectively. In contrast, the mean brood area at BFD 22 amounted to 9360

and 9076 cm²/colony for the reference item I and reference item II, respectively, meaning a similar level compared to the pre-application level for reference item I and an increase of +2% for reference item II.
At the last assessment on BFD 42 the mean brood area amounted to 10262, 11629, 11242 and 12789 cm²/colony for the control, test item reference item I and reference item II, respectively. Compared to the pre-application level, the mean brood area increased by 16%, 30%, 20% and 44% in the control, test item, reference item I and reference item II, respectively.

Food stores
The comb area covered with food stores (nectar/honey and pollen) revealed similar levels for the control, test item and reference item groups. Overall, the nectar and pollen stores were on an acceptable level when compared to the size of the colonies and had no negative or limiting effect on brood or colony development throughout the study. The effects on brood and food development are summarized in
Table A **23**.

Table A 23: Brood and food development: Estimated total brood (eggs, larvae + pupae) or food (nectar + pollen) area per colony [cm²/colony] 1)

Treatment group		BFD 0 (DAT -2)	BFD 6 (DAT 4)	BFD 9 (DAT 7)	BFD 15 (DAT 13)	BFD 22 (DAT 20)	BFD 28 (DAT 26)	BFD 35 (DAT 33)	BFD 42 (DAT 40)
Eggs [cm²/colony]									
Control	Mean 1)	1599	1135	1186	2217	1805	1495	1882	1882
	± SD	321	694	509	342	179	215	522	778
	% 3)	-	-29	-26	+39	+13	-6	+18	+18
BAS 762 02 F	Mean 1)	1341	1521	1238	2037	1573	1521	1882	1289
	± SD	552	746	188	604	319	155	627	523
	% 3)	-	13	-8	+52	+17	13	+40	-4
Reference item I	Mean 1)	1392	1289	1418	1831	1418	1057	1418	1444
	± SD	480	450	528	284	464	555	627	658
	% 3)	-	-7	2	+31	+2	-24	+2	+4
Reference item II	Mean 2)	1341	361	619	1960	1289	1031	1341	1547
	± SD	146	219	292	146	73	292	438	0
	% 3)	-	-73	-54	+46	-4	-23	+0	+15
Larvae [cm²/colony]									
Control	Mean 1)	2759	2630	1908	2166	1960	2888	1779	2810
	± SD	1165	861	644	1114	700	1055	935	1115
	% 3)	-	-5	-31	-21	-29	5	-36	+2
BAS 762 02 F	Mean 1)	2862	1934	2037	2398	2914	2527	1882	2475
	± SD	586	643	860	991	479	342	893	1188
	% 3)	-	-32	-29	-16	+2	-12	-34	-14
Reference item I	Mean 1)	2682	1908	1805	1702	2037	2630	2295	2346
	± SD	938	924	562	801	736	702	1171	716
	% 3)	-	-29	-33	-37	-24	-2	-14	-13
Reference item II	Mean 2)	3352	1392	1135	1444	2321	2578	2785	3300
	± SD	511	73	583	146	365	0	292	146
	% 3)	-	-58	-66	-57	-31	-23	-17	-2
Pupae [cm²/colony]									
Control	Mean 1)	4512	5827	5337	5286	6034	6317	6523	5569
	± SD	1572	1793	1306	1655	1761	1743	2829	3623
	% 3)	-	29	18	+17	+34	40	+45	+23
BAS 762 02 F	Mean 1)	4770	6291	6188	5698	6833	8174	7787	7864
	± SD	615	326	720	1610	1556	330	765	567
	% 3)	-	32	30	+19	+43	71	+63	+65
Reference item I	Mean	5286	5930	4976	4306	5905	7400	7477	7452

Treatment group		BFD 0 (DAT -2)	BFD 6 (DAT 4)	BFD 9 (DAT 7)	BFD 15 (DAT 13)	BFD 22 (DAT 20)	BFD 28 (DAT 26)	BFD 35 (DAT 33)	BFD 42 (DAT 40)
	[1]								
	± SD	1099	2029	1601	1109	1415	1645	1759	1239
	% [3]	-	12	-6	-19	+12	40	+41	+41
Reference item II	Mean [2]	4177	5466	5054	3661	5466	7838	8303	7942
	± SD	656	729	583	219	1167	1313	1094	1167
	% [3]	-	31	21	-12	+31	88	+99	+90
Entire brood (eggs, larvae + pupae) [cm²/colony]									
Control	Mean [1]	8870	9592	8431	9669	9798	10701	10185	10262
	± SD	2271	3123	2149	2544	2470	2702	3881	3609
	% [3]	-	8	-5	+9	+10	21	+15	+16
BAS 762 02 F	Mean [1]	8973	9746	9463	10133	11319	12222	12170	11629
	± SD	367	1395	1469	2464	1275	509	720	1689
	% [3]	-	9	5	+13	+26	36	+36	+30
Reference item I	Mean [2]	9360	9128	8199	7838	9360	11087	11190	11242
	± SD	2424	2934	2544	1915	2002	2701	2610	2245
	% [3]	-	-2	-12	-16	+0	18	+20	+20
Reference item II	Mean [1]	8870	7220	6807	7065	9076	11448	12428	12789
	± SD	292	438	292	511	1604	1604	1823	1313
	% [3]	-	-19	-23	-20	+2	29	+40	+44
Nectar [cm²/colony]									
Control	Mean [1]	3636	2501	4229	3842	10365	14542	15290	17920
	± SD	1198	1382	803	1239	1180	2302	3164	3093
	% [3]	-	-31	16	+6	+185	300	+321	+393
BAS 762 02 F	Mean [1]	3765	2450	4306	6601	9643	14001	15161	16940
	± SD	1100	296	1512	1701	2232	4199	5023	4926
	% [3]	-	-35	14	+75	+156	272	+303	+350
Reference item I	Mean [2]	2475	1856	3842	3429	9437	12222	9824	13202
	± SD	1176	559	1168	1284	3723	4786	5318	5133
	% [3]	-	-25	55	+39	+281	394	+297	+433
Reference item II	Mean [1]	3455	3404	3094	2785	4538	6498	5105	8148
	± SD	802	292	146	583	729	3501	4449	3792
	% [3]	-	-1	-10	-19	+31	88	+48	+136
Pollen [cm²/colony]									
Control	Mean [1]	1805	1960	2089	1521	3171	2321	2372	3068
	± SD	924	438	296	213	398	309	512	441
	% [3]	-	9	16	-16	+76	29	+31	+70
BAS 762 02 F	Mean [1]	1470	1109	1676	1135	1728	1779	1676	2604
	± SD	805	432	424	326	308	893	991	1267
	% [3]	-	-25	14	-23	+18	21	+14	+77
Reference item I	Mean [2]	2114	1985	2166	1341	2037	1624	1753	1521
	± SD	727	508	223	438	1005	341	1120	472
	% [3]	-	-6	2	-37	-4	-23	-17	-28
Reference item II	Mean [1]	2372	1495	1238	4538	2424	1392	1135	1702
	± SD	875	73	0	5980	1386	1386	729	1240
	% [3]	-	-37	-48	+91	+2	-41	-52	-28
Entire food (nectar + pollen) [cm²/colony]									
Control	Mean [1]	5441	4461	6317	5363	13537	16863	17662	20988
	± SD	456	1180	721	1038	1469	2295	3490	2978

Treatment group		BFD 0 (DAT -2)	BFD 6 (DAT 4)	BFD 9 (DAT 7)	BFD 15 (DAT 13)	BFD 22 (DAT 20)	BFD 28 (DAT 26)	BFD 35 (DAT 33)	BFD 42 (DAT 40)
	% [3]	-	-18	16	-1	+149	210	+225	+286
BAS 762 02 F	Mean [1]	5234	3558	5982	7735	11371	15780	16837	19545
	± SD	573	574	1323	1843	2307	4934	5747	5733
	% [3]	-	-32	14	+48	+117	201	+222	+273
Reference item I	Mean [2]	4590	3842	6008	4770	11474	13846	11577	14723
	± SD	797	361	1292	1036	3869	4934	6157	5260
	% [3]	-	-16	31	+4	+150	202	+152	+221
Reference item II	Mean [1]	5827	4899	4332	7323	6962	7890	6240	9850
	± SD	73	219	146	5397	656	4886	5178	5032
	% [3]	-	-16	-26	+26	+19	35	+7	+69

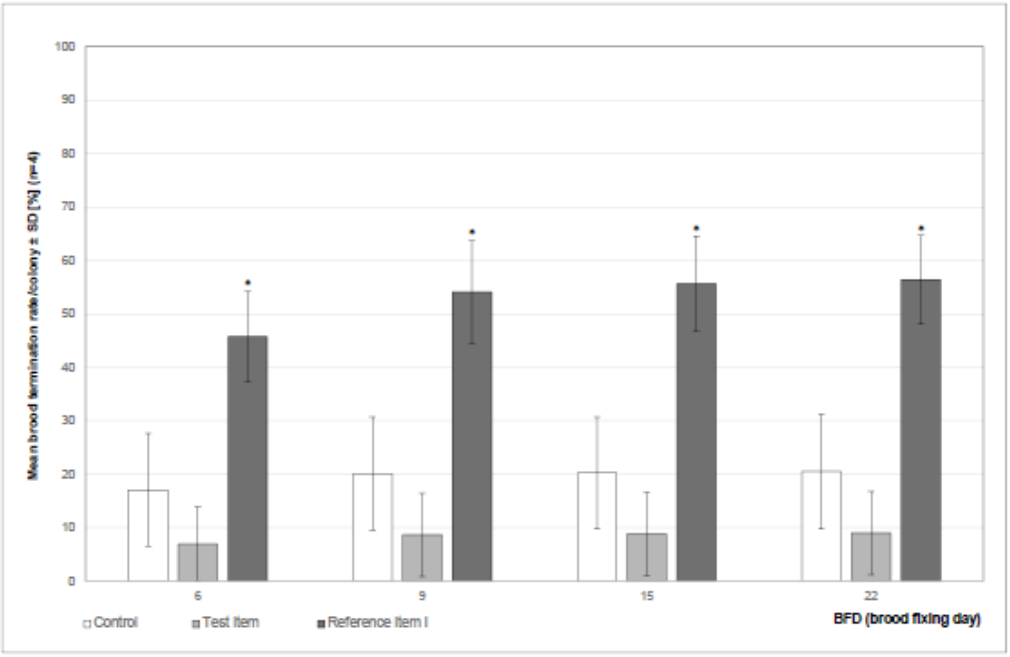
DAT: days after treatment, BFD: Brood area fixing day
[1] Mean of 4 replicates
[2] Mean of two replicates
[3] Relative change [%] in comparison with BFD 0 (DAT -2) calculated from the respective mean values.

Detailed brood development of individually labelled brood cells
The evaluation of the development of initially labelled brood cells (eggs) was expressed by the following brood indices: Brood termination rate [BTR], brood index [BI] and brood compensation index [BCI].

Brood termination rate [BTR]
The mean BTR of initially labelled eggs amounted to 20.5 and 9.0% for the control and test item groups, respectively, at final evaluation on BFD 22. Therefore, the termination of labelled eggs was on a similar level and within a natural range of variability, without any statistically significant differences between control and test item treatment (Student t-test, one-sided greater, $p < 0.05$).
In contrast, the reference item I treatment group revealed a high brood termination rate of 56.4%, which was statistically significantly different when compared to the control group (Student t-test, one-sided greater, $p < 0.05$). Summary of brood termination rate (BTR) is provided below.

Assessment day	Mean brood termination rate of initially labelled eggs [%]					
	Treatment group					
	Control		Test item		Reference item I	
	Mean[1]	± SD	Mean[1]	± SD	Mean[1]	± SD
BFD 6	17.1	10.5	6.9	7.0	45.8*	8.5
BFD 9	20.1	10.6	8.7	7.8	54.1*	9.6
BFD 15	20.3	10.5	8.8	7.8	55.7*	8.9
BFD 22	20.5	10.7	9.0	7.8	56.4*	8.3

BFD: Brood area fixing day; [1] mean of four replicates
* = statistically significantly different (STUDENT t-test) one-sided greater, p<0.05
Statistical analyses were performed with rounded values.
Detailed brood assessments were not conducted for reference item II



* = statistically significant in comparison to the control (STUDENT t-test, one sided greater, p<0.05)

Brood index [BI]

The BI displays a negative correlation with the BTR: the higher the BTR the lower the BI and vice versa. Therefore, the BI of initially labelled eggs at BFD 22 amounted to 4.0 and 4.6 for the control and test item groups, respectively, without any statistically significant differences during the study (Student t-test, one-sided smaller, $p < 0.05$).

In contrast, the reference item I treatment revealed a much lower brood index of 2.2, which is statistically significantly different compared to the control (Student t-test, one-sided smaller, $p < 0.05$). Summary of brood index (BI) is provided below.

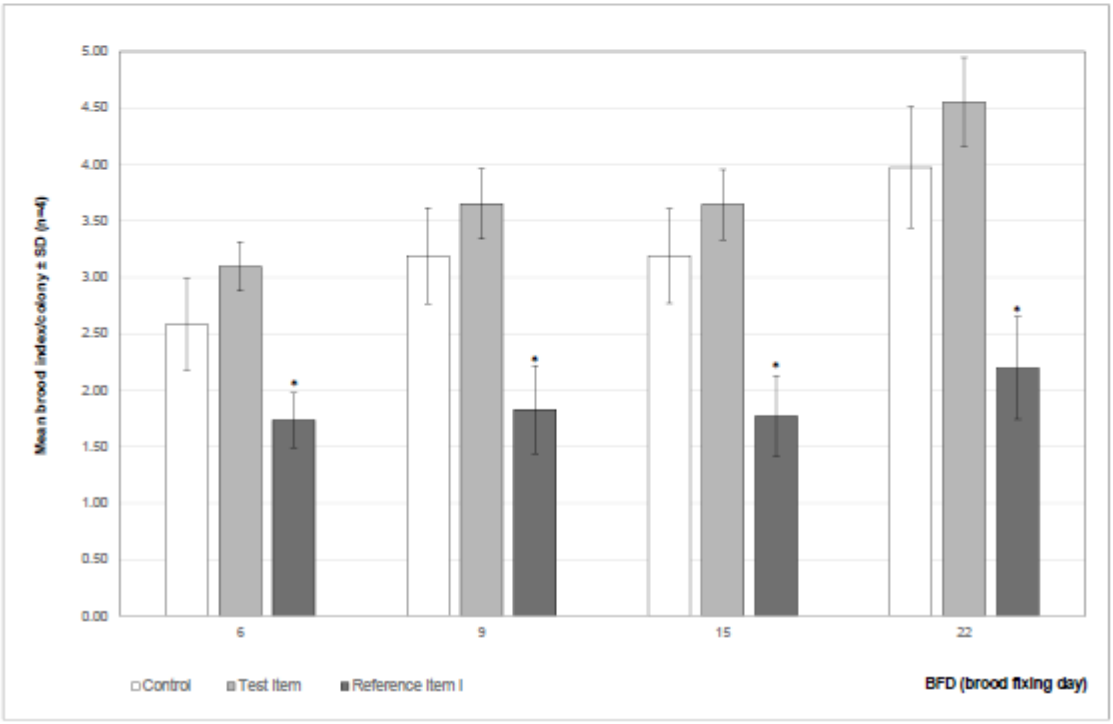
Assessment day	Mean brood index of initially labelled eggs [%]					
	Treatment group					
	Control		Test item		Reference item I	
	Mean[1]	± SD	Mean[1]	± SD	Mean[1]	± SD
BFD 6	2.6	0.4	3.1	0.2	1.7*	0.2
BFD 9	3.2	0.4	3.7	0.3	1.8*	0.4
BFD 15	3.2	0.4	3.7	0.3	1.8*	0.4
BFD 22	4.0	0.5	4.6	0.4	2.2*	0.5

BFD: Brood area fixing day; 1) mean of four replicates

* = statistically significantly different (STUDENT t-test) one-sided smaller, p<0.05)

Statistical analyses were performed with rounded values.

Detailed brood assessments were not conducted for reference item II



* = statistically significant in comparison to the control (STUDENT t-test, one sided smaller, p<0.05)

Brood compensation index [BCI]

The BCI was on a similar level for both control and test item treatment and amounted to 4.3 and 4.8 in the control and test item treatment, respectively and therefore without any statistically significant difference, indicating that most of the terminated brood-cells were refilled with new eggs (Student t-test, one-sided smaller, $p < 0.05$).

In contrast, the reference item I revealed a statistically significant lower brood compensation index of 3.6, which means that only few emptied cells were refilled with new eggs (Student t-test, one-sided smaller, $p < 0.05$). Summary of brood compensation index (BCI) is provided below.

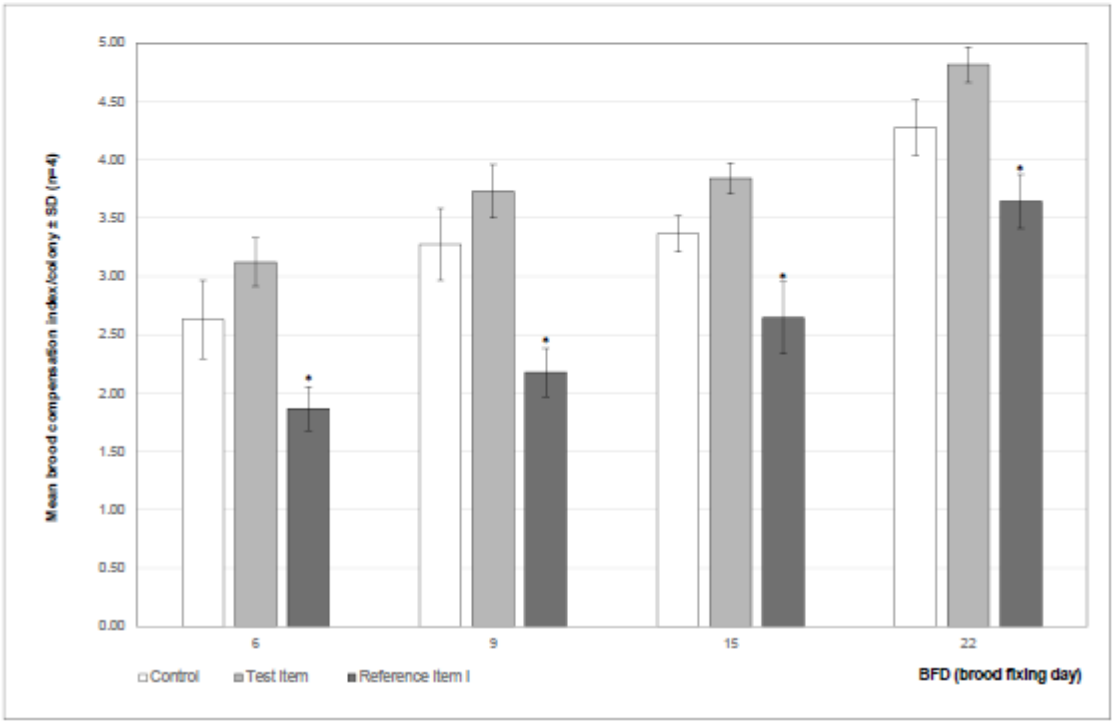
Assessment day	Mean brood compensation index of initially labelled eggs [%]					
	Treatment group					
	Control		Test item		Reference item I	
	Mean[1]	± SD	Mean[1]	± SD	Mean[1]	± SD
BFD 6	2.6	0.3	3.1	0.2	1.9*	0.2
BFD 9	3.3	0.3	3.7	0.2	2.2*	0.2
BFD 15	3.4	0.2	3.8	0.1	2.7*	0.3
BFD 22	4.3	0.2	4.8	0.2	3.6*	0.2

BFD: Brood area fixing day; [1)] mean of four replicates

* = statistically significantly different (STUDENT t-test) one-sided smaller, p<0.05)

Statistical analyses were performed with rounded values.

Detailed brood assessments were not conducted for reference item II



* = statistically significant in comparison to the control (STUDENT-t test, one sided smaller, p<0.05)

The overall summary of brood parameters is provided ~~results are summarized~~ in Table A 24 below.

Table A 24: Detailed brood developments (single cell assessments): BTR, BI and BCI on BFD 22 [1)]

Assessment	Control		BAS 762 02 F		Reference item I	
	Mean [2)]	± SD	Mean [2)]	± SD	Mean [2)]	± SD
Brood termination rate (BTR) [%]	20.5	10.7	9.0	7.8	56.4 *	8.3
Brood-index (BI)	4.0	0.5	4.6	0.4	2.2 *	0.5
Brood compensation index (BCI)	4.3	0.2	4.8	0.2	3.6 **	0.2

BFD: Brood area fixing day

1) At the last relevant assessment when development is expected to be completed, *i.e.* BFD 22 for marked eggs.

2) Mean of 4 replicates.

* Statically significant different (Students t-test; p<0.05, one-sided smaller).

** Statically significant different (Students t-test; p<0.05, one-greater smaller).

Quality criteria:

Quality criteria [1)]	**Obtained in this study**
Reference item treatment: brood termination > 50% and < 90% or distinct increase in pupal and adult mortality compared to the control	Ref. item I (fenoxycarb): 56.4% brood termination at BFD 22 21.8 dead pupae/colony/day (0 dead pupae/colony/day in the control) in the post-treatment phase Ref. item II (dimethoate): 43.7 dead adult bees/colony/day (11.9 dead adult bees/colony/day in the control) in the post-treatment
Flight density ≥ 5 bees/m² shortly before the application	6.3 to 7.3 bees/m² in all treatment groups

1) There are no validity criteria listed in OECD 75 (2007). Nevertheless, general criteria to assess the quality of honey bee (semi-)field studies can be considered.

III. CONCLUSION

Under semi-field conditions (tunnel test), BAS 762 02 F was applied in a single application at a rate of 1100 ml/ha (equivalent to 110 g BAS 750 F/ha and 220 g BAS 510 F/ha) to flowering *Brassica napus* L. during active foraging conditions. No unacceptable effects on mortality, foraging activity, colony development, colony strength or bee brood were observed after application. Overall, based on the results of this study, BAS 762 02 F does not adversely affect honey bee colonies.

A 2.3.1.6 KCP 10.3.1.6 Field tests with honeybees

As BAS 762 02 F poses no unacceptable risk to honey 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 performed in line with the respective guideline with no deviations. Reproduction assessment was not carried out in this study. All validity criteria were met and the study is considered acceptable with the following endpoint relevant for the risk assessment: LR_{50} > 3.0 L product/ha

Reference:	CP 10.3.2.1/1
Report	Effects of BAS 762 02 F on the predatory mite Typhlodromus pyri Scheuten in a laboratory test XXX, U., 2019 Report No 863052, 1948NTL0010 BASF DocID 2019/1061533 Authority registration No
Guideline(s):	IOBC (Bluemel et al. 2000) with recommendations given by GRIMM et al. (2001)
Deviations:	No
GLP:	Yes (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)
Acceptability:	Acceptable
Duplication (if vertebrate study)	No

Executive Summary

In a rate-response laboratory study, protonymphs of the mite *Typhlodromus pyri* (Acari: Phytoseiidae) were exposed to dried residues of BAS 762 02 F on glass plates. The test item was applied at application rates of 0.1875, 0.375, 0.75, 1.5 and 3.0 L BAS 762 02 F/ha. Additionally, the reference item BAS 152 11 I (dimethoate) was applied at a rate of 15 mL BAS 152 11 I/ha and a deionized water control was set up. All substances were applied in 200 L water/ha. Mite mortality was assessed 3 and 7 days after treatment (DAT).

After 7 days there was 1.0% mortality in the control, compared with 0.0%, 1.0%, 1.0%, 2.0% and 0.0% mortality in the 0.1875, 0.375, 0.75, 1.5 and 3.0 L BAS 762 02 F/ha treatment rates, respectively. When adjusted for the control treatment deaths, the corrected mortalities were -1.0%, 0.0%, 0.0%, 1.0% and -1.0% in the five respective test item treatments. No statistically significant differences between the test item treatments and the control were observed.

In a laboratory study with BAS 762 02 F, the LR_{50} for *Typhlodromus pyri* was > 3.0 L BAS 762 02 F/ha in 200 L water/ha.

I. MATERIALS AND METHODS

A. MATERIALS

Test item: BAS 762 02 F, batch no. FD-190207-0001, content of a.s.: mefentrifluconazole (BAS 750 F, Reg. No. 5 834 378): 96.2 g/L analyzed (nominal 100.0 g/L) and

boscalid (BAS 510 F, Reg. No. 300 355): 205.2 g/L analyzed (nominal 200.0 g/L), density: 1.130 g/cm^3.

B. STUDY DESIGN

Test species: *Typhlodromus pyri* Scheuten (predatory mite); protonymphs < 24 hours old; source (in the stage of eggs): "Katz Biotech AG", Baruth, Germany.

Test design: Exposure of mites to air-dried residues on treated glass plates. Seven treatment groups (5 test item rates, a deionized water control and a reference item) with 5 replicates per treatment group, each consisting of 20 mites. Assessments of mite mortality were made 3 and 7 days after treatment (DAT).

Endpoints: Mortality (LR_{50}).

Reference item: BAS 152 11 I (dimethoate, nominal 400 g/L, measured 429.0 g/L).

Test rates: Untreated control: deionized water
Test item:

Nominal application rates of BAS 762 02 F		
Based on the product BAS 762 02 F [L/ha]	**Based on the a.s. BAS 750 F [g/ha]**	**Based on the a.s. BAS 510 F [g/ha]**
0.1875	18.75	37.5
0.375	37.5	75
0.75	75	150
1.5	150	300
3.0	300	600

Reference item: BAS 152 11 I was applied at an application rate of 15 mL BAS 152 11 I/ha.
All substances were applied in 200 L water/ha via calibrated laboratory spraying equipment.

Test conditions: Temperature: 23 – 27°C; relative humidity: 67 - 72%; photoperiod: 16 h light : 8 h dark; light intensity: 2020 lux; food: pollen from pine (*Pinus nigra*) and birch (*Betula pendula*), 1:1.

Analytics: No analytical verification of the test item is required according to the current test guideline. Hence, no analytical verification was conducted.

Statistics: Descriptive statistics. The mortality data in each test-item treatment were compared to control data using Chi2 2x2 Test with Bonferroni Correction ($\alpha = 0.05$).

II. RESULTS AND DISCUSSION

After 7 days there was 1.0% mortality in the control, compared with 0.0%, 1.0%, 1.0%, 2.0% and 0.0% mortality in the 0.1875, 0.375, 0.75, 1.5 and 3.0 L BAS 762 02 F/ha treatment rates, respectively. When adjusted for the control treatment deaths, the corrected mortalities were -1.0%, 0.0%, 0.0%, 1.0% and -1.0% in the five respective test item treatments. No statistically significant differences between the test item treatments and the control were observed (Chi^2 2x2 Test with Bonferroni Correction, $\alpha = 0.05$). The results are summarized in Table A 25.

Table A 25 Effects on *Typhlodromus pyri* exposed to BAS 762 02 F under worst-case laboratory conditions

Treatment	Rate [1] [L/ha]	Mortality [2] [%]	Corrected mortality [3] [%]
Control	--	1.0	--
BAS 762 02 F	0.1875	0.0	-1.0
	0.375	1.0	0.0
	0.75	1.0	0.0
	1.5	2.0	1.0
	3.0	0.0	-1.0
		Endpoint [L BAS 762 02 F/ha]	
LR_{50}		> 3.0	

1) Application rate in 200 L water/ha.
2) Mortality after 7 days of exposure to BAS 762 02 F on glass plates.
3) Corrected mortality according to Abbott (1925).

In the reference item treatment, 77.0% mortality (76.8% corrected) was observed at 7 DAT.

Validity criteria:

Validity criteria according to Bluemel et al (2000)	Obtained in this study
Control mortality ≤ 20% on day 7	1.0%
Corrected mortality in the reference group 50-100% on day 7	76.8%

All validity criteria were met.

III. CONCLUSION

In a laboratory study with BAS 762 02 F, the LR_{50} for *Typhlodromus pyri* was > 3.0 L BAS 762 02 F/ha in 200 L water/ha.

A 2.3.3.2 Study 2

Comments of zRMS:	The study was performed in line with the respective guideline with a minor deviation. It was noted that the food source provided during the study was 25 % w/w aqueous fructose solution while the recommended food in the respective guideline is a 1:3 v/v solution of honey and water. However, this deviation is considered to have no impact on the outcome of the study since all validity criteria were met. Reproduction assessment was not carried out in this study. Overall, the study is considered acceptable with the following endpoint relevant for the risk assessment: LR_{50} > 3.0 L product/ha

Reference:	CP 10.3.2.1/2
Report	Effects of BAS 762 02 F on the parasitic wasp Aphidius rhopalosiphi (DESTEFANI-PEREZ) in a laboratory test XXX, U., 2019 Report No 863053, 1948NAL0010 BASF DocID 2019/1061532 Authority registration No
Guideline(s):	IOBC (MEAD-BRIGGS *et al.* 2000) with recommendations given by GRIMM et al. (2001) ~~ENV/MC/CHEM(98)17, GLP Principles of the German Chemikaliengesetz (Chemicals Act)~~
Deviations:	Minor deviation (see the commenting box above) ~~No~~

GLP:	Yes (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)
Acceptability:	Acceptable ~~Yes~~
Duplication (if vertebrate study)	No

Executive Summary

In a worst-case laboratory study, adults of the wasp *Aphidius rhopalosiphi* (Hymenoptera: Braconidae) were exposed to dried residues of BAS 762 02 F on glass plates. The test item was applied at application rates of 0.1875, 0.375, 0.75, 1.5 and 3.0 L BAS 762 02 F/ha. Additionally, the reference item BAS 152 65 I (dimethoate) was applied at a rate of 0.3 mL BAS 152 65 I/ha and a deionized water control was set up. All substances were applied in 200 L spray solution/ha. Wasp mortality was assessed after 2, 24 and 48 hours of exposure.

After 48 hours, there was 5.0% mortality in the control treatment, compared with 5.0, 2.5, 2.5, 5.0 and 5.0% mortality in the 0.1875,2 0.375, 0.75, 1.5 and 3.0 L BAS 762 02 F/ha treatment rates, respectively. When adjusted for the control treatment deaths, the corrected mortality in the respective test item treatments was 0%, -2.6%, -2.6%, 0% and 0%. No statistically significant differences between the test item treatments and the control were observed.

In a laboratory study with *Aphidius rhopalosiphi* the LR_{50} was > 3.0 L BAS 762 02 F/ha in 200 L water/ha.

I. MATERIALS AND METHODS

A. MATERIALS

Test item: BAS 762 02 F, batch no. FD-190207-0001, content of a.s.: mefentrifluconazole (BAS 750 F, Reg. No. 5 834 378): 96.2 g/L analyzed (nominal 100.0 g/L) and boscalid (BAS 510 F, Reg. No. 300 355): 205.2 g/L analyzed (nominal 200.0 g/L), density: 1.130 g/cm^3.

B. STUDY DESIGN

Test species: *Aphidius rhopalosiphi* (parasitoids); adults less than 48 h old; source (in the stage of mummies): "Katz Biotech AG", Baruth, Germany.

Test design: Exposure of the wasps to air-dried residues on treated glass plates. Seven treatment groups (5 test item rates, a deionized water control and a reference item) with 4 replicates per treatment, each consisting of 10 wasps (7 females, 3 males). Assessment of mortality was done 2, 24 and 48 hours after test initiation.

Endpoint: Mortality (LR_{50}).

Reference item: Dimethoate EC 400 (dimethoate, nominal 400 g/L, analyzed 429.0 g/L).

Test rates: Untreated control: deionized water

Test item:

Nominal application rates of BAS 762 02 F		
Based on the product BAS 762 02 F [L/ha]	**Based on the a.s. BAS 750 F [g/ha]**	**Based on the a.s. BAS 510 F [g/ha]**
0.1875	18.75	37.5
0.375	37.5	75
0.75	75	150
1.5	150	300
3.0	300	600

Reference item: BAS 152 65 I was applied at an application rate of 0.3 mL BAS 152 11 I/ha.
All substances were applied in 200 L spray solution/ha.

Test conditions: Temperature: 18- 22°C; relative humidity: 67 - 72%; photoperiod: 16 h light : 8 h dark; light intensity: 2030 lux; food: 25% aqueous fructose solution.

Analytics: No analytical verification of the test item is required according to the current test guideline. Hence, no analytical verification was conducted.

Statistics: Descriptive statistics. The mortality data in each test-item treatment were compared to the control data using Chi² 2x2 Table Test with Bonferroni Correction ($\alpha = 0.05$).

II. RESULTS AND DISCUSSION

After 48 hours, there was 5.0% mortality in the control treatment, compared with 5.0, 2.5, 2.5, 5.0 and 5.0% mortality in the 0.1875, 0.375, 0.75, 1.5 and 3.0 L BAS 762 02 F/ha treatment rates, respectively. When adjusted for the control treatment deaths, the corrected mortality in the respective test item treatments was 0%, -2.6%, -2.6%, 0% and 0%. No statistically significant differences between the test item treatments and the control were observed (Chi² 2x2 Table Test with Bonferroni Correction, $\alpha = 0.05$). The results are summarized in Table A 26.

Table A 26 Effects on *Aphidius rhopalosiphi* exposed to BAS 762 02 F under worst-case laboratory conditions after 48 hours of exposure

Treatment	**Rate [L/ha] [1)]**	**Mortality [%] [2)]**	**Corrected mortality [%] [3)]**
Control	--	5.0	--
BAS 762 02 F	0.1875	5.0	0
	0.375	2.5	-2.6
	0.75	2.5	-2.6
	1.5	5.0	0
	3.0	5.0	0
	Endpoint [L BAS 762 02 F/ha]		
LR_{50}	> 3.0		

1) Application in 200 L water/ha
2) Mortality after 48 h of exposure to BAS 762 02 F on treated glass plates.
3) Corrected mortality according to Abbott (1925).

In the reference item treatment, 100% mortality was observed at 48 h.

Validity criteria:

Validity criteria according to Mead-Briggs M. et al. (2009)	**Obtained in this study**
Control mortality <13 ~~10~~% (48 h)	5.0%
Corrected mortality in the reference item group 50 - 100% (48 h)	100%

All validity criteria were met.

III. CONCLUSION

In a laboratory study with *Aphidius rhopalosiphi* the LR_{50} was > 3.0 L BAS 762 02 F/ha in 200 L water/ha.

A 2.3.4 KCP 10.3.2.2 Extended laboratory testing, aged residue studies with non-target arthropods

As BAS 762 02 F poses no unacceptable risk to non-target arthropods, further studies are not necessary.

A 2.3.5 KCP 10.3.2.3 Semi-field studies with non-target arthropods

As BAS 762 02 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

As BAS 762 02 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

As BAS 762 02 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 submitted by the Applicant in support of this evaluation as for boscalid currently no EU agreed long-term toxicity endpoint for earthworms is available. The study was already evaluated at the EU level during the renewal process of boscalid and considered acceptable by the RMS (see first version of the DRAR of November 2018). Taking this into account the derived NOEC of 25 mg a.s./kg dws may be considered in the risk assessment performed in this report, bearing in mind that the renewal process of boscalid was not yet finalised and the endpoints reported in the first version of the DAR are subject of the discussion and may potentially change. The study summary is struck through and shaded as being not evaluated at the zonal level.

Reference:	CP 10.4.1.1/1
Report	Sublethal toxicity of BAS 510 F (Boscalid) to the earthworm Eisenia fetida in artificial soil XXX, S., 2014 Report No EU-141048055S, EU-429178,14 10 48 055 S BASF DocID 2014/1083454 Authority registration No
Guideline(s):	OECD 222 (2004)
Deviations:	No
GLP:	Yes (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)
Acceptability:	The study was already evaluated and accepted in the course of the EU renewal and was thus not re-evaluated at the zonal level.
Duplication (if vertebrate study)	No

~~**Executive Summary**~~

~~The effects of BAS 510 F (boscalid) on *Eisenia fetida* (Annelida: Oligochaeta) mortality, biomass development and reproduction were investigated in a chronic laboratory study over 56 days. Five test item concentrations (6.25, 12.5, 25, 50 and 100 mg a.s./kg dry soil) were incorporated into the soil (10% peat) with 4 replicates per treatment (each containing 10 worms). An untreated control with 8 replicates was included. The reference item was tested in a separate study. Assessment of worm mortality, biomass, and feeding activity was carried out after 28 days; assessment of reproduction (number of juveniles) was carried out after 56 days.~~

~~BAS 510 F did not show any statistically significant effects on mortality and biomass. The mortality of adult worms was between 0.0% and 2.5% in the test item treatments and 1.3% in the control group. The weight change of adult worms was between 26.7% and 29.2% in the test item treatments and 27.9% in the control group.~~
~~In the control, 135.6 juveniles were counted after 56 days. In the test item treatment groups, the number of juveniles ranged between 90.0 and 143.0. The reproduction rate was statistically significantly different compared to the control at 50 and 100 mg a.s./kg dry soil, the highest two treatment rates tested. No behavioral abnormalities were observed in any of the treatment groups. The feeding activity in all test item treated groups was comparable to the control.~~

In a 56-day reproduction study with boscalid (BAS 510 F ,Reg. No. 300355), no adverse effects on survival and biomass development could be determined at concentrations up to and including 100 mg a.s./kg dry soil. Statistically significant effects on the number of *Eisenia fetida* juveniles were determined at 50 and 100 mg a.s./kg dry soil. Therefore, the NOEC for mortality and biomass was ≥ 100 mg a.s./kg dry soil, and the NOEC for reproduction was determined to be 25 mg a.s./kg dry soil. The EC_{10} was determined to be 37 mg a.s./kg dry soil.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 510 F (boscalid, Reg. No. 300 355), batch no. COD 001035, analyzed purity: 99.4% (± 1.0%).

B. STUDY DESIGN

Test species: *Eisenia fetida*; adult worms with clitellum and weight of 300 – 499 mg, approximately 3 months old; source: W. Neudorff GmbH KG followed by in-house culture.

Test design: In a 56-day test, adults of *Eisenia fetida* were exposed to 5 concentrations of BAS 510 F in treated artificial soil according to OECD 222 (10% peat). In total, 6 treatment groups were set up (5 concentrations of the test item and 1 untreated control group) with 4 replicates for the test item treatments and 8 replicates for the control, 10 adult worms per replicate. The artificial soil was treated and filled into vessels, before the earthworms were introduced on the top of the soil.
Assessment of worm mortality, behavioral effects and weight change was done after 28 days of exposure, after an additional 28 days (56 days after application) reproduction (number of juveniles) was assessed.

Endpoints: Mortality, weight change, feeding activity, reproduction rate.

Reference item: Nutdazim 50 Flow (carbendazim, SC 500).

Test concentrations: Control, 6.25, 12.5, 25, 50 and 100 mg BAS 510 F/kg dry soil.

Test conditions: Artificial soil according to OECD 222 with 10% peat; pH 6.00 – 6.02 at test initiation, pH 5.77 – 5.81 at test termination; water content 54.9% – 55.2% of its maximum water holding capacity (WHC) at test initiation and 54.4% – 55.0% of WHC at test termination, temperature: 18.0°C – 21.7°C; photoperiod: 16 hours light : 8 hours dark, light intensity: 570 lux, feeding with horse manure.

Analytics: No analytical verification of the test item is required according to the current test guideline. Hence, no analytical verification was conducted.

Statistics: Descriptive statistics; Fisher's Exact Binomial test for mortality ($\alpha = 0.05$, one-sided greater). Williams t-test for weight change and reproduction data ($\alpha = 0.05$, one-sided smaller), Probit analysis (Finney 1971).

II. RESULTS AND DISCUSSION

Boscalid did not show any statistically significant effects on mortality and biomass (Fisher's Exact Binomial test for mortality, $\alpha = 0.05$, one-sided greater; Williams t-test for biomass, $\alpha = 0.05$, one-sided smaller). The mortality of adult worms was between 0.0% and 2.5% in the test item treatments and 1.3% in the control group. The weight change of adult worms was between 26.7% and 29.2% in the test item treatments and 27.9% in the control group.
In the control, 135.6 juveniles were counted after 56 days. In the test item treatment groups, the number of juveniles ranged between 90.0 and 143.0. The reproduction rate was statistically significantly different compared to the control at 50 and 100 mg a.s./kg dry soil, the highest two treatment rates tested (Williams t-test, $\alpha = 0.05$, one-sided smaller). No behavioral abnormalities were observed in any of the treatment groups. The feeding activity in all test item treated groups was comparable to the control.

The results are summarized in Table A 27.

Table A 27: Effects of BAS 510 F on *Eisenia fetida* in a 56-day reproduction study

BAS 510 F [mg a.s./kg dry soil]	**Control**	**6.25**	**12.5**	**25**	**50**	**100**
Mortality (day 28) [%]	1.3	0.0	2.5	0.0	2.5	0.0
Weight change (day 28) [%]	27.9	26.8	28.3	29.2	26.7	27.7
Number of juveniles (day 56)	135.6	143.0	139.3	129.8	113.5 *	90.0 *
Reproduction (day 56) [% of control]	–	105.4	102.7	95.7	83.7	6.4
	Endpoints [mg BAS 510 F/kg dry soil]					
NOEC (day 28)	≥ 100					
NOEC (day 56)	25					
EC_{50} (day 56)	> 100					
EC_{10} (56 d)	37					

* Statistically significantly different compared to the control (Williams t-test, $\alpha = 0.05$, one-sided smaller).

In a separate study with the reference item Nutdazim 50 Flow (carbendazim, SC 500), the number of juveniles was reduced by 39 and 100% at concentrations of 5 and 10 mg product/kg dry soil (mean number of juveniles = 77 and 0) after 8 weeks of test duration when compared to control (mean number of juveniles = 127).

Validity criteria:

Validity criteria according to OECD 222 (2016)	**Obtained in this study**
Adult mortality in the control ≤ 10%	1.3%
Number of juveniles per control replicate ≥ 30 (with 10 adults per replicate)	111 to 168
Coefficient of variation of reproduction in the control ≤ 30%	12.5%

All validity criteria were met.

III. CONCLUSION

In a 56-day reproduction study with BAS 510 F (Reg. No. 300 355), no adverse effects on survival and biomass development could be determined at concentrations up to and including 100 mg a.s./kg dry soil. Statistically significant effects on the number of *Eisenia fetida* juveniles were determined at 50 and 100 mg a.s./kg dry soil. Therefore, the NOEC for mortality and biomass was ≥ 100 mg a.s./kg dry soil, and the NOEC for reproduction was determined to be 25 mg a.s./kg dry soil. The EC_{10} was determined to be 37 mg a.s./kg dry soil.

A 2.4.1.1.2 Study 2

Comments of zRMS:	The study was performed in line with OECD 222 with no deviations. The test design was relevant to derive both NOEC and ECx values (8 concentrations, 8 replicates for control, 4 replicates per treatment group). Reliability of the EC_{10} value has been evaluated in line with recommendations of EFSA Supporting publication 2019:EN-1673: • NW (normalised width) of 0.42 was calculated, which results with rating "good" in line with Table E9 in EFSA Supporting publication 2019:EN-1673, • median EC_{10} is lower than $EC_{20,low}$, • the dose-response curve is shallow with steepness of 0.28 (i.e. <0.33). Based on above indications the calculated EC_{10} is considered to be sufficiently reliable. All validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment: 56d NOEC = 84.0 mg product/kg soil dw 56d EC_{10} = 86.0 mg product/kg soil dw

Reference:	CP 10.4.1.1/2
Report	Effects of BAS 762 02 F on the reproduction of the earthworm Eisenia andrei in artificial soil, XXX, S., 2020 Report No 863046, 1848TEC0048 BASF DocID 2020/1000741 Authority registration No
Guideline(s):	OECD 222 (2016)
Deviations:	No
GLP:	Yes (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)
Acceptability:	Acceptable
Duplication (if vertebrate study)	No

Executive Summary

The effects of BAS 762 02 F on mortality, biomass development and reproduction of *Eisenia andrei* (Annelida: Oligochaeta) were investigated in an extended laboratory study over 56 days. Eight test item concentrations (8, 14, 26, 47, 84, 151, 272 and 490 mg BAS 762 02 F/kg dry soil) were incorporated into the soil (10% peat) with 4 replicates per treatment (each containing 10 worms). An untreated control with 8 replicates was included. The reference item was tested in a separate study. Assessment of worm mortality, body weight, and feeding activity was carried out after 28 days; assessment of reproduction (number of juveniles) was carried out after 56 days.

After 28 days, no mortality was observed in the control group or any test item treatment group. The body weight in the test item treatment groups was not statistically significantly different compared to the control. The weight change of adult worms was 27.4 – 32.1% in the test item treated groups and 29.1% in the control group. The feeding activity in all test item treated groups was comparable to the control. The reproduction rate was statistically significantly different compared to the control at concentrations of 151, 272 and 490 mg test item/kg soil dry weight. No pathological symptoms and no further effects on behaviour of the worms were observed.

In a 56-day earthworm reproduction study with BAS 762 02 F, the NOEC for mortality and biomass was determined to be greater than or equal to 490 mg BAS 762 02 F/kg dry soil. The NOEC for reproduction was determined to be 84 mg BAS 762 02 F/kg dry soil. The EC_{10}, EC_{20} and EC_{50} values for reproduction were calculated to be 86, 135 and 321 mg BAS 762 02 F/kg dry soil, respectively.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 762 02 F, batch no. FD-190207-0001, content of a.s.: mefentrifluconazole (BAS 750 F, Reg. No. 5 834 378): 96.2 g/L analysed (nominal 100.0 g/L) and boscalid (BAS 510 F, Reg. No. 300 355): 205.2 g/L analysed (nominal 200.0 g/L), density: 1.130 g/cm^3.

B. STUDY DESIGN

Test species: *Eisenia andrei*; adult worms with clitellum and weight of 312 – 494 mg, approximately 4 months old; source: in-house culture.

Test design: In a 56-day test, adults of *Eisenia andrei* were exposed to the test item in treated artificial soil according to OECD 222 (10% peat). In total, 9 treatment groups were set up (8 concentrations of the test item and 1 untreated control group) with 4 replicates for the test item treatments and 8 replicates for the control, 10 adult worms per replicate. Assessment of worm mortality, behavioural effects and weight change was done after 28 days of exposure. After an additional 28 days (56 days after application), reproduction (number of juveniles) was assessed.

Endpoints: Mortality (LC_{50}, NOEC), weight change (NOEC), feeding activity and reproduction (number of juveniles, ($EC_{50/20/10}$, NOEC)).

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:

Nominal concentrations of BAS 762 02 F			
Based on the product BAS 762 02 F [mg/kg dry soil	**Based on total a.s. [mg/kg dry soil]**	**Based on the a.s. BAS 750 F [mg/kg dry soil]**	**Based on the a.s. BAS 510 F [mg/kg dry soil]**
8	2.1	0.7	1.4
14	3.8	1.3	2.5
26	6.9	2.3	4.6
47	12	4.1	8.3
84	22	7.4	15
151	40	13	27
272	72	24	48
490	130	43	87

The amounts of BAS 750 F and BAS 510 F were calculated with unrounded values and based on the nominal contents of the a.s. The density (1.130 g/cm³) was taken into account.

Reference item: Maypon Flow was applied at concentrations of 5 and 10 mg product/kg dry soil.

Test conditions: Artificial soil according to OECD 222 with 10% peat; pH: 5.93 - 5.98 at test initiation, pH 5.65 - 5.77 at test termination; water content: 56.0 - 56.2% of its maximum water holding capacity (WHC) at test initiation and 54.7% - 55.9% of WHC at test termination, temperature: 19.2 - 21.8°C; photoperiod: 16 hours light : 8 hours dark, light intensity: 650 lux, feeding with mixture of horse manure, straw and peat.

Analytics: No analytical verification of the test item is required according to the current test guideline. Hence, no analytical verification was conducted.

Statistics: Descriptive statistics; Dunnett-t-test for weight change and Williams-t-test for reproduction ($\alpha = 0.05$, one-sided smaller), 3-parametric normal cumulative distribution function (CDF) for calculation of EC_x values.

II. RESULTS AND DISCUSSION

After 28 days, no mortality was observed in the control group or any test item treatment group. The body weight in the test item treatment groups was not statistically significantly different compared to the control (Dunnett-t-test, $\alpha = 0.05$, one-sided smaller). The weight change of adult worms was 27.4 – 32.1% in the test item treated groups and 29.1% in the control group. The feeding activity in all test item treated groups was comparable to the control. The reproduction rate was statistically significantly different compared to the control at concentrations of 151, 272 and 490 mg test item/kg soil dry weight (Williams-t-test, $\alpha = 0.05$, one-sided smaller). No pathological symptoms and no further effects on behavior of the worms were observed. The results are summarized in Table A 28.

Table A 28 Effects of BAS 762 02 F on *Eisenia andrei* in a 56-day reproduction study

BAS 762 02 F [mg/kg dry soil]	**Control**	**8**	**14**	**26**	**47**	**84**	**151**	**272**	**490**
Mortality (day 28) [%]	0	0	0	0	0	0	0	0	0
Weight change (day 28) [%]	29.1	29.7	27.4	31.5	28.8	32.1	30.1	27.8	29.2
Number of juveniles (day 56)	313.0	334.5	311.0	339.8	298.8	281.0	258.5 *	174.3 *	131.3 *
Reproduction (day 56) [% of control]	100	106.9	99.4	108.5	95.4	89.8	82.6	55.7	41.9
Endpoints [mg BAS 762 02 F/kg dry soil]									
NOEC (day 28)	≥ 490								
NOEC (day 56)	84								
LC_{50} (day 28) [1)]	> 490								
EC_{10} (day 56) [2)]	86 (95% confidence limits: 70 - 106)								
EC_{20} (day 56) [2)]	135 (95% confidence limits: 116 - 159)								
EC_{50} (day 56) [2)]	321 (95% confidence limits: 299 - 349)								

* Statistically significantly different from control (Williams-t-test for reproduction, $\alpha = 0.05$, one-sided smaller)

1) Based on estimation of data.

2) Based on 3-parametric normal cumulative distribution function.

In a separate study the reference item Maypon Flow (carbendazim, SC 500), the number of juveniles was reduced by 58 and 99% at concentrations of 5 and 10 mg product/kg dry soil (mean number of juveniles = 71.3 and 1.5, respectively) after 8 weeks of test duration when compared to control (mean number of juveniles = 169).

Validity criteria:

Validity criteria according to OECD 222 (2016)	Obtained in this study
Adult mortality in the control ≤ 10%	0.0%
Number of juveniles per control replicate ≥ 30 (with 10 adults per replicate)	260 to 374
Coefficient of variation of reproduction in the control ≤ 30%	12.6%

All validity criteria were met.

III. CONCLUSION

In a 56-day earthworm reproduction study with BAS 762 02 F, the NOEC for mortality and biomass was determined to be greater than or equal to 490 mg BAS 762 02 F/kg dry soil. The NOEC for reproduction was determined to be 84 mg BAS 762 02 F/kg dry soil. The EC_{10}, EC_{20} and EC_{50} values for reproduction were calculated to be 86, 135 and 321 mg BAS 762 02 F/kg dry soil, respectively.

A 2.4.1.2 KCP 10.4.1.2 Earthworms - field studies

As BAS 762 02 F does not pose an 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 submitted by the Applicant in support of this evaluation as currently no EU agreed long-term toxicity endpoint for *Folsomia candida* is available for boscalid. The study was already evaluated at the EU level during the renewal process of boscalid and considered acceptable by the RMS (see first version of the DRAR of November 2018). Taking this into account the derived NOEC of 1000 mg a.s./kg dws may be considered in the risk assessment performed in this report, bearing in mind that the renewal process of boscalid was not yet finalised and the endpoints reported in the first version of the DAR are subject of the discussion and may potentially change. The study summary is struck through and shaded as being not evaluated at the zonal level.

Reference:	CP 10.4.2.1/1
Report	Effects of BAS 510 F (Boscalid) on the reproduction of the collembolan Folsomia candida XXX, S., 2014 Report No EU-141048066S, EU-429191,14 10 48 066 S BASF DocID 2014/1083456 Authority registration No
Guideline(s):	OECD 232 (2009)
Deviations:	No
GLP:	Yes (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)
Acceptability:	The study was already evaluated and accepted in the course of the EU renewal and was thus not re-evaluated at the zonal level.
Duplication (if vertebrate study)	No

~~**Executive Summary**~~

The effects of boscalid (BAS 510 F) on mortality and reproduction of the collembolan *Folsomia candida* were investigated in a chronic laboratory experiment over a time period of 28 days. The test item was mixed into artificial soil at rates of 62.5, 125, 250, 500 and 1000 mg a.s./kg dry soil. For the control, the soil was left untreated. 4 replicates were prepared for the treatment groups and 8 replicates were prepared for the control, each containing 10 collembolans. Assessment of mortality, reproduction and behavior was made 28 days after treatment.

No statistically significant effect on parental mortality was found for any concentration tested. Mortality rates of 2.5% to 5.0% were recorded in the test item treatment groups. In the control, the mortality rate was 5.0%.

No statistically significant effects on the number of juveniles compared to the control were recorded at any concentration tested. The mean reproduction in the control reached 734 juveniles. Reproduction rates in 62.5, 125, 250, 500 and 1000 mg BAS 510 F/kg dry soil were 713, 728, 737, 715 and 735 juveniles, respectively.

In a 28-day collembolan reproduction study with BAS 510 F (boscalid), the LC_{50} was determined to be > 1000 mg BAS 510 F kg dry soil. The NOEC based on mortality and reproduction was determined to be ≥ 1000 mg BAS 510 F/kg dry soil.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Boscalid (BAS 510 F, Reg. No. 300355), batch no.: COD 001035, analysed purity: 99.4% (± 1.0%).

B. STUDY DESIGN

Test species: Collembola (*Folsomia candida*), age: 9 - 12 days; source: in house culture.

Test design: 28 day test in treated artificial soil (with 5% peat); different concentrations of the test item were mixed homogeneously into the soil which was filled in glass vessels before collembolans were introduced on top of the soil. 6 treatment groups (5 test item concentrations, 1 control) were set up with 4 replicates for the test item treatments and 8 replicates for the control, each containing 10 juvenile collembolans. Feeding of collembola occurred with about 2 mg dry yeast at the beginning of the test for each test vessel and additional feeding on day 14. Assessment of adult collembolans mortality, reproduction rate (number of juveniles) and behavioural effects was carried out after 28 days.

Endpoints: Mortality and reproduction rate after 28 days.

Reference item: Boric acid (100% analysed). The effects of the reference item were investigated in a separate study.

Test concentrations: Control, 62.5, 125, 250, 500 and 1000 mg BAS 510 F/kg dry soil.

Test conditions: Artificial soil according to OECD 232 with a peat content of 5%; water content: 58.3% - 58.8% of the maximum water holding capacity (WHC) at test initiation and 57.1% - 58.3% of the maximum WHC at test termination; pH 6.02 - 6.08 at test initiation, pH 5.77 - 5.82 at test termination; temperature 18.1°C - 19.3°C; photoperiod: 16 h light : 8 h dark; light intensity 450 lux.

Analytics: No analytical verification of the test item is required according to the current test guideline. Hence, no analytical verification was conducted.

Statistics: Descriptive statistics. Fisher's Exact Binomial test with Bonferroni Correction for mortality (α = 0.05, one-sided greater), Williams t test for reproduction (α = 0.05, one-sided smaller).

II. RESULTS AND DISCUSSION

No statistically significant effect on parental mortality was found for any concentration tested (Fisher`s Exact Binomial Test with Bonferroni Correction, α = 0.05, one-sided greater).
Mortality rates of 2.5% to 5.0% were recorded in the test item treatment groups. In the control, the mortality rate was 5.0%.
No statistically significant effects on the number of juveniles compared to the control were recorded at any concentration tested (Williams t test, α = 0.05, one-sided smaller). The mean reproduction in the control reached 734 juveniles. Reproduction in 62.5, 125, 250, 500 and 1000 mg a.s./kg dry soil reached 713, 728, 737, 715 and 735 juveniles, respectively.

The results are summarized in Table A 29.

Table A 29: Effects of boscalid (BAS 510 F) on collembola (*Folsomia candida*) in a 28-day reproduction study

BAS 510 F [mg a.s./kg dry soil]	**Control**	**62.5**	**125**	**250**	**500**	**1000**
Mortality (day 28) [%]	5.0	5.0	2.5	5.0	2.5	2.5
No. of juveniles (day 28)	734	713	728	737	715	735
Reproduction (day 28) [% of control]	100	97	99	100	97	100
	Endpoints [mg a.s./kg dry soil]					
$NOEC_{mortality, reproduction}$	≥ 1000					
LC_{50}	> 1000					

Validity criteria:

Validity criteria according to OECD 232 (2016)	**Obtained in this study**
Mean adult mortality in the control ≤ 20%	5.0%
Mean number of juveniles per control replicate ≥ 100	734
Coefficient of variation of reproduction in the control ≤ 30%	10.9%

All validity criteria were met.

III. CONCLUSION

In a 28-day collembolan reproduction study with BAS 510 F (boscalid) the LC_{50} based was determined to be > 1000 mg BAS 510 F/kg dry soil. The NOEC based on mortality and reproduction was determined to be ≥ 1000 mg BAS 510 F/kg dry soil.

A 2.4.2.1.2 Study 2

Comments of zRMS:	The study was performed in line with OECD 232 with no deviations. The test design was relevant to derive both NOEC and ECx values (8 concentrations, 8 replicates for control, 4 replicates per treatment group). Reliability of the EC_{10} value has been evaluated in line with recommendations of EFSA Supporting publication 2019:EN-1673: • NW (normalised width) of 0.21 was calculated, which results with rating “good” in line with Table E9 in EFSA Supporting publication 2019:EN-1673,

	• median EC_{10} is lower than $EC_{20,low}$, • steepness of the dose-response curve could not be calculated since no effects >50% were observed. Although based on above indications the calculated EC_{10} might be considered to be sufficiently reliable, in opinion of the zRMS it should be treated with caution, since effect >10% were observed only at the highest concentration tested. All validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment: NOEC = 200 mg product/kg soil dw EC_{10} = 239.9 mg product/kg soil dw

Reference:	CP 10.4.2.1/2
Report	Effects of BAS 762 02 F on the reproduction of the collembolan Folsomia candida XXX, S., 2020 Report No 863047, 1948TCC0039 BASF DocID 2020/1000742 Authority registration No
Guideline(s):	~~2004/10/EC of 11 February 2004,~~ OECD 232 (2016)
Deviations:	No
GLP:	Yes (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)
Acceptability:	Acceptable
Duplication (if vertebrate study)	No

Executive Summary

The effects of BAS 762 02 F on mortality and reproduction of the Collembola *Folsomia candida* were investigated in a chronic laboratory study over 28 days. The test item was mixed into artificial soil at concentrations of 8.3, 14.1, 23.9, 40.7, 69.2, 117.6, 200.0 and 340.0 mg BAS 762 02 F/kg dry soil. For the control treatment, the soil was left untreated. 4 replicates were prepared for the treatment groups and 8 replicates for the control, each containing 10 collembolans. Assessments of mortality, reproduction (number of juveniles) and behavior were carried out 28 days after treatment.

Mortality rates of 0.0% - 15.0% were recorded in the test item treatment groups. In the control the mortality rate was 2.5%. The highest test item treatment group of 340.0 mg BAS 762 02 F/kg dry soil was statistically significantly different compared to the control with 15% mortality. The mean number of juveniles counted 28 days after introduction of the parental collembolans into the test vessels was 1529 in the control and 1551, 1562, 1490, 1559, 1567, 1463, 1460 and 1230 at concentrations of 8.3, 14.1, 23.9, 40.7, 69.2, 117.6, 200.0 and 340.0 mg BAS 762 02 F/kg dry soil, respectively. Statistically significant effects on the number of juveniles compared to the control were recorded at the highest test item concentration of 340.0 mg BAS 762 02 F/kg dry soil, with a reproduction rate of 80.4% of the control.

In a 28-day *Folsomia candida* reproduction study, the LC_{50} and the EC_{50} are estimated to be > 340.0 mg BAS 762 02 F/kg dry soil, the highest tested concentration. The NOEC for mortality and reproduction was determined to be 200 mg BAS 762 02 F/kg dry soil. The EC_{10} for reproduction was determined to be 239.9 mg BAS 762 02 F/kg dry soil.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 762 02 F, batch no. FD-190207-0001, content of a.s.: mefentrifluconazole (BAS 750 F, Reg. No. 5 834 378): 96.2 g/L analysed (nominal 100.0 g/L) and boscalid (BAS 510 F, Reg. No. 300 355): 205.2 g/L analysed (nominal 200.0 g/L), density: 1.130 g/cm^3.

B. STUDY DESIGN

Test species: Collembola *(Folsomia candida)*, age: 9 - 12 days; source: in-house culture.

Test design: 28-day test in treated artificial soil according to OECD 232; different concentrations of the test item were homogenously mixed into artificial soil (5% peat) and filled in glass vessels before collembolans were introduced on top of the soil. 9 treatment groups (8 test item concentrations, control) were set up with 4 replicates for the test item treatments and 8 replicates for the control, each containing 10 collembolans. Assessment of adult mortality, reproduction and behavioural effects was carried out after 28 days.

Endpoints: Mortality and reproduction rate after 28 days (NOEC, LC_{50}, EC_{10}, EC_{20}, EC_{50}).

Reference item: Boric acid (100.8% analysed) The effects of the reference item were investigated in a separate study.

Test concentrations: Untreated control
Test item:

Nominal concentrations of BAS 762 02 F			
Based on the product BAS 762 02 F [mg/kg dry soil]	**Based on total a.s. [mg/kg dry soil]**	**Based on the a.s. BAS 750 F [mg/kg dry soil]**	**Based on the a.s. BAS 510 F [mg/kg dry soil]**
8.3	2.20	0.73	1.47
14.1	3.74	1.25	2.49
23.9	6.36	2.12	4.24
40.7	10.8	3.60	7.21
69.2	18.4	6.12	12.25
117.6	31.2	10.4	20.8
200.0	53.1	17.7	35.4
340.0	90.3	30.1	60.2

The amounts of BAS 750 F and BAS 510 F were calculated with unrounded values and based on the nominal contents of the a.s. The density (1.130 g/cm³) was taken into account.

Reference item: Boric acid was applied at concentrations of 44, 67, 100, 150 and 225 mg/kg dry soil.

Test conditions: Artificial soil according to OECD 232 (with a peat content of 5%); pH 5.97 - 6.05 at test initiation, pH 5.74 - 5.79 at test termination; water content at test initiation 57.8 - 58.0% of maximum water holding capacity (WHC) and 56.1 - 57.1% of maximum WHC at test termination; temperature: 19.6 - 21.7°C; photoperiod: 16 h light : 8 h dark; light intensity: 620 lux; food: 2 mg dry yeast at the start of the test and on day 14.

Analytics: No analytical verification of the test item is required according to the current test guideline. Hence, no analytical verification was conducted.

Statistics: Descriptive statistics; Chi² 2x2 Table Test with Bonferroni Correction for mortality (α = 0.05, one-sided greater), Williams-t-test for reproduction (α = 0.05, one-sided smaller). 3-parametric normal cumulative distribution function (CDF) for reproduction.

II. RESULTS AND DISCUSSION

Mortality rates of 0.0% - 15.0% were recorded in the test item treatment groups. In the control the mortality rate was 2.5%. The highest test item treatment group of 340.0 mg BAS 762 02 F/kg dry soil was statistically significantly different compared to the control with 15% mortality (Chi² 2x2 Table Test with Bonferroni Correction, α = 0.05, one-sided greater). The mean number of juveniles counted 28 days after introduction of the parental collembolans into the test vessels was 1529 in the control and 1551, 1562, 1490, 1559, 1567, 1463, 1460 and 1230 at concentrations of 8.3, 14.1, 23.9, 40.7, 69.2, 117.6, 200.0 and 340.0 mg BAS 762 02 F/kg dry soil, respectively. Statistically significant effects on the number of juveniles compared to the control were recorded at the highest test item concentration of 340.0 mg BAS 762 02 F/kg dry soil, with a reproduction rate of 80.4% of the control (Williams-t-test, α = 0.05, one-sided smaller). The results are summarized in Table A 30.

Table A 30 Effect of BAS 762 02 F on Collembola (*Folsomia candid*) in a 28-day reproduction study

BAS 762 02 F [mg/kg dry soil]	**Control**	**8.3**	**14.1**	**23.9**	**40.7**	**69.2**	**117.6**	**200.0**	**340.0**
Mortality (day 28) [%]	2.5	2.5	2.5	2.5	0.0	0.0	0.0	2.5	15.0*
Mean no. of juveniles (day 28)	1529	1551	1562	1490	1559	1567	1463	1460	1230*
Reproduction in [%] of control (day 28)	100	101.5	102.1	97.4	102.0	102.5	95.7	95.5	80.4
	Endpoints [mg BAS 762 02 F/kg dry soil]								
$NOEC_{mortality/reproduction}$ (28 d)	200.0								
$LOEC_{mortality/reproduction}$ (28 d)	340.0								
LC_{50} (28 d) [1)]	> 340.0								
$EC_{10,reproduction}$ (28 d) [2)] (95 % confidence limits)	239.9 (215.5 – 264.8)								
$EC_{20,reproduction}$ (28 d) [2)] (95 % confidence limits)	343.2 (322.6 – 372.0)								
$EC_{50,reproduction}$ (28 d) [1)]	> 340.0								

Calculations were performed with unrounded values.

* Statistically significant differences compared to the control (Chi² 2x2 Table Test with Bonferroni Correction for mortality, α = 0.05, one-sided greater; Williams-t-test for reproduction, α = 0.05, one-sided smaller)

1) Based on estimation of the data.

2) Based on 3-parametric normal CDF.

In a separate study, the EC_{50} (reproduction) of the reference item boric acid was calculated to be 103 mg/kg dry soil. The LC_{50} was determined to be 161 mg/kg soil dry weight. The NOEC for mortality and for reproduction was determined to be 44 mg/kg soil dry weight. The EC_{50} value for the reproduction was close to the value of 100 mg/kg soil dry weight as stated in OECD 232 (2016). The EC_{50} therefore showed that the test system is sensitive.

Validity criteria:

Validity criteria according to OECD 232 (2016)	**Obtained in this study**
Mean adult mortality in the control ≤ 20%	2.5%
Mean number of juveniles per control replicate ≥ 100	1529
Coefficient of variation of reproduction in the control ≤ 30%	8.9%

All validity criteria were met.

III. CONCLUSION

In a 28-day *Folsomia candida* reproduction study, the LC_{50} and the EC_{50} are estimated to be > 340.0 mg BAS 762 02 F/kg dry soil, the highest tested concentration. The NOEC for mortality and reproduction was determined to be 200 mg BAS 762 02 F/kg dry soil. The EC_{10} for reproduction was determined to be 239.9 mg BAS 762 02 F/kg dry soil.

A 2.4.2.1.3 Study 3

Comments of zRMS:	The study was performed in line with OECD 226 with no major deviations. It was noted that at the test termination the pH value was 5.4 in the control and 6 out of 8 treatment groups while the minimum pH value required is 5.5. However, as all validity criteria of the study were met, this slight pH deviation is not considered to have any impact on the outcome of the study. The test design was relevant to derive both NOEC and ECx values (8 concentrations, 8 replicates for control, 4 replicates per treatment group). Overall, the study is considered acceptable with the following endpoint relevant for the risk assessment: $NOEC_{reproduction}$ = 352.9 mg product/kg soil dw EC_{10} could not be calculated since effects >10% were not observed at any of the concentrations tested.

Reference:	CP 10.4.2.1/3
Report	Effects of BAS 762 02 F on the reproduction of the predatory mite Hypoaspis aculeifer, XXX, L., 2020 Report No 863048, 1948THC0031 BASF DocID 2020/1000743 Authority registration No
Guideline(s):	OECD 226 (2016)
Deviations:	No
GLP:	Yes (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)
Acceptability:	Acceptable
Duplication (if vertebrate study)	No

Executive Summary

The effects of BAS 762 02 F on mortality and reproduction of the predatory mite *Hypoaspis aculeifer* were investigated in a chronic laboratory study over 14 days. The test item was mixed into artificial soil at concentrations of 14.6, 24.9, 42.3, 71.8, 122.1, 207.6, 352.9 and 600.0 mg BAS 762 02 F/kg dry soil. For the control treatment, the soil was left untreated. 8 replicates and 4 replicates were prepared for the control and test item treatment groups, respectively, each containing 10 adult soil mites (females). Assessments of adult mortality and reproduction were carried out after 14 days of exposure.

Mortality rates of 0.0% - 10.0% were recorded in the test item treatment groups. In the control the mortality rate was 3.8%. In the test item treatment groups, no statistically significant differences compared to the control were observed. The mean number of juveniles counted 28 days after introduction of the parental

collembolans into the test vessels was 292.1 in the control and 292.0, 285.0, 284.5, 271.0, 271.3, 250.3, 287.3 and 265.5 at concentrations of 14.6, 24.9, 42.3, 71.8, 122.1, 207.6, 352.9 and 600.0 mg BAS 762 02 F/kg dry soil., respectively. Only the highest test item treatment group of 600.0 mg BAS 762 02 F/kg dry soil was statistically significantly different compared to the control with a reproduction rate of 91% of the control.

In a 14-day *Hypoaspis aculeifer* reproduction study with BAS 762 02 F, the LC_{50}, EC_{10}, EC_{20} and EC_{50} were estimated to be > 600 mg BAS 762 02 F/kg dry soil. The NOEC for mortality was ≥ 600 mg BAS 762 02 F/kg dry soil. The NOEC for reproduction was determined to be 352.9 mg BAS 762 02 F/kg dry soil.

I. MATERIALS AND METHODS

A. MATERIALS

Test item: BAS 762 02 F, batch no. FD-190207-0001, content of a.s.: mefentrifluconazole (BAS 750 F, Reg. No. 5 834 378): 96.2 g/L analysed (nominal 100.0 g/L) and boscalid (BAS 510 F, Reg. No. 300 355): 205.2 g/L analysed (nominal 200.0 g/L), density: 1.130 g/cm^3.

B. STUDY DESIGN

Test species: Predatory mites (*Hypoaspis aculeifer)*, adults with an age difference of 3 days; source: in-house culture.

Test design: 14-day chronic laboratory test in treated artificial soil according to OECD 226. Different concentrations of the test item were mixed homogenously into artificial soil (5% peat) and used to fill vessels after which mites were introduced on top of the soil; 9 treatment groups (8 test item concentrations, control); 4 replicates for each test item treatment and 8 replicates for the control group, each containing 10 mites. Assessments of adult mortality and reproduction effects were carried out after 14 days of exposure.

Endpoints: Mortality (LC_{50}, NOEC), reproduction rate (number of juveniles, $EC_{50/20/10}$, NOEC).

Reference item: Dimethoate (98.8% ± 0.5% analysed). The effects of the reference item were investigated in a separate study.

Test concentrations: Untreated control

Test item:

Nominal concentrations of BAS 762 02 F			
Based on the product BAS 762 02 F [mg/kg dry soil]	**Based on total a.s. * [mg/kg dry soil]**	**Based on the a.s. BAS 750 F * [mg/kg dry soil]**	**Based on the a.s. BAS 510 F * [mg/kg dry soil]**
14.6	3.9	1.3	2.6
24.9	6.6	2.2	4.4
42.3	11.2	3.7	7.5
71.8	19.1	6.4	12.7
122.1	32.4	10.8	21.6
207.6	55.1	18.4	36.7
352.9	93.7	31.2	62.5
600.0	159.3	53.1	106.2

* Based on nominal contents of active substances and a test item density of 1.130 g/cm³, calculations were done with unrounded values.

Test conditions: Artificial soil according to OECD 226 (5% peat); pH 5.7 – 5.9 at test initiation,

pH 5.4 - 5.5 at test termination; water content at study initiation 46.36 - 49.16% of maximum water holding capacity and 46.32 - 48.44% of maximum WHC at test termination; temperature: 20.3 - 21.7°C; photoperiod: 16 h light : 8 h dark, light intensity: 478 lux; feeding: *Tyrophagus putrescentiae* (SCHRANK) at the beginning and *ad libitum* in the course of the test.

Analytics: No analytical verification of the test item is required according to the current test guideline. Hence, no analytical verification was conducted.

Statistics: Descriptive statistics; Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm ($\alpha = 0.05$, one-sided greater) for mortality, Williams-t-test for reproduction ($\alpha = 0.05$, one-sided smaller).

II. RESULTS AND DISCUSSION

Mortality rates of 0.0% - 10.0% were recorded in the test item treatment groups. In the control the mortality rate was 3.8%. In the test item treatment groups, no statistically significant differences compared to the control were observed (Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm, $\alpha = 0.05$, one-sided greater). The mean number of juveniles counted 28 days after introduction of the parental collembolans into the test vessels was 292.1 in the control and 292.0, 285.0, 284.5, 271.0, 271.3, 250.3, 287.3 and 265.5 at concentrations of 14.6, 24.9, 42.3, 71.8, 122.1, 207.6, 352.9 and 600.0 mg BAS 762 02 F/kg dry soil., respectively. The highest test item treatment group of 600.0 mg BAS 762 02 F/kg dry soil was statistically significantly different compared to the control with a reproduction rate of 91% of the control (Williams-t-test, $\alpha = 0.05$, one-sided smaller). The results are summarized in
Table A **31**.

Table A 31: Effects of BAS 762 02 F on predatory mite (*Hypoaspis aculeifer)* in a 14-day reproduction study

BAS 762 02 F [mg/kg dry soil]	**Control**	**14.6**	**24.9**	**42.3**	**71.8**	**122.1**	**207.6**	**352.9**	**600.0**
Mortality (day 14) [%]	3.8	0.0	0.0	5.0	2.5	2.5	10.0	2.5	7.5
No. of juveniles (day 14)	292.1	292.0	285.0	284.5	271.0	271.3	250.3	287.3	265.5 *
Reproduction in [%] of control (day 14)	100	100	98	97	93	93	86	98	91
	Endpoints [mg BAS 762 02 F/kg dry soil]								
$NOEC_{mortality}$ (14 d)	≥ 600								
$NOEC_{reproduction}$ (14 d)	352.9								
LC_{50} (14 d) [1)]	> 600								
$EC_{10,reproduction}$ (14 d) [1)]	> 600								
$EC_{20,reproduction}$ (14 d) [1)]	> 600								
$EC_{50,reproduction}$ (14 d) [1)]	> 600								

Calculations were performed with unrounded values.

* Statistically significant differences compared to the control (Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm for mortality, $\alpha = 0.05$, one-sided greater; Williams-t-test for reproduction, $\alpha = 0.05$, one-sided smaller).

1) Based on estimation of data.

In a separate GLP study, the EC_{50} (reproduction) of the reference item dimethoate was calculated to be 6.3 mg dimethoate/kg dry soil.

Validity criteria:

Validity criteria according to OECD 226 (2016)	**Obtained in this study**
Mean adult mortality in the control ≤ 20%	3.8%
Mean number of juveniles per control replicate ≥ 50	292.1
Coefficient of variation of reproduction in the control ≤ 30%	6.7%

All validity criteria were met.

III. CONCLUSION

In a 14-day *Hypoaspis aculeifer* reproduction study with BAS 762 02 F, the LC_{50}, EC_{10}, EC_{20} and EC_{50} were estimated to be > 600 mg BAS 762 02 F/kg dry soil. The NOEC for mortality was ≥ 600 mg BAS 762 02 F/kg dry soil. The NOEC for reproduction was determined to be 352.9 mg BAS 762 02 F/kg dry soil.

A 2.4.2.2 KCP 10.4.2.2 Higher tier testing

As BAS 762 02 F does not pose an 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 study was performed fully in line with OECD 216 with no deviations. All validity criteria were met and the study is considered acceptable. It may be concluded that the effects of the test item on soil nitrogen formation rates were < 25 % at the end of the study period (28 days) up to 30.0 mg product/kg soil dw

Reference:	CP 10.5/1
Report	Effects of BAS 762 02 F on the activity of soil microflora (Nitrogen transformation test) XXX, M., 2019 Report No 863056, Ju1948SMN0042 BASF DocID 2019/1061116 Authority registration No
Guideline(s):	OECD 216 (2000)
Deviations:	No
GLP:	Yes (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)
Acceptability:	Acceptable
Duplication (if vertebrate study)	No

Executive Summary

The effect of BAS 762 02 F on nitrogen transformation was tested in a lucerne-enriched silty-loamy sand soil. BAS 762 02 F was applied to samples of the soil in the laboratory at nominal rates of 6.0 and 30.0 mg BAS 762 02 F/kg dry soil. The treated soils and the untreated control soils were incubated at approx. 20°C in the dark for 28 days. Triplicate samples of each treatment were removed for analysis of NH_4-nitrogen to NO_3-nitrogen, 0, 7, 14 and 28 days after application.

No adverse effects of BAS 762 02 F on nitrogen transformation in soil could be observed at both test concentrations (6.0 and 30.0 mg BAS 762 02 F/kg dry soil) after 28 days (time interval 0-28). Only negligible deviations from the control of +5.9% (at 6.0 mg BAS 762 02 F/kg dry soil) and +7.1% (at 30.0 mg BAS 762 02 F/kg dry soil) were measured at the end of the 28-day incubation period (time interval 0-28).

Exposure of BAS 762 02 F in a field soil up to a test concentration of 30.0 mg BAS 762 02 F/kg dry soil, caused no adverse effects (deviation from control < 25 %, OECD 216) on the soil nitrogen transformation (measured as NO_3-N- production) at the end of the 28-day incubation period (time interval 0-28).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 762 02 F, batch no. FD-190207-0001, content of a.s.: mefentrifluconazole (BAS 750 F, Reg. No. 5 834 378): 96.2 g/L analysed (nominal 100.0 g/L) and boscalid (BAS 510 F, Reg. No. 300 355): 205.2 g/L analysed (nominal 200.0 g/L), density: 1.130 g/cm^3.

Test soil: Biologically active agricultural soil: silty-loamy sand (DIN 4220) soil: pH 6.0 (H_2O), 1.48% C_{org}, microbial mass: 4.13% of C_{org}, water holding capacity (WHC): 37.37 g/100 g dry soil.

Test design: Determination of the N-transformation (NO_3-nitrogen-production) in soil enriched with lucerne meal (concentration in the soil 0.5%; the C/N ratio was 13.2/1). Comparison of test item treated soil with a non-treated soil. NH_4-nitrogen formed from organically bound nitrogen and NO_3-nitrogen formed from the nitrification process was determined using an Autoanalyzer. Sampling scheme: 0, 7, 14 and 28 days after treatment. Sub-samples (3 replicates) were withdrawn from the bulk batches and subjected to the measurement.

Test concentrations: Control (untreated)
Test item:

Nominal concentrations of BAS 762 02 F			
Based on the product BAS 762 02 F [mg/kg dry soil]	**Based on total a.s. [mg/kg dry soil]**	**Based on the a.s. BAS 750 F [mg/kg dry soil]**	**Based on the a.s. BAS 510 F [mg/kg dry soil]**
6.0	1.59	0.53	1.06
30.0	7.96	2.65	5.31

The amounts of mefentrifluconazole (BAS 750 F) and boscalid (BAS 510 F) were calculated based on the nominal contents of the a.s. The density of 1.130 g/cm³ was taken into account.

Endpoints: Effects on the NO_3-nitrogen production after 7, 14 and 28 days of exposure.

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 27.2 mg dinoterb/kg dry soil.

Test conditions: Loamy sand soil: ~~soil moisture 45% of its maximum WHC,~~ measured water content: 16.34 - 16.71 g/100 g dry soil (equivalent to 43.73 - 44.71 % of WHC), pH 6.0 – 6.1. Soil samples were incubated at 19.4 – 21.0°C in the dark.

Analytics: No analytical verification of the test item is required according to the current test guideline. Hence, no analytical verification was conducted.

Statistics: Descriptive statistics.

II. RESULTS AND DISCUSSION

No adverse effects of BAS 762 02 F on nitrogen transformation in soil could be observed at both test concentrations (6.0 and 30.0 mg BAS 762 02 F/kg dry soil) after 28 days (time interval 0-28). Only negligible deviations from the control of +5.9% (at 6.0 mg BAS 762 02 F/kg dry soil) and +7.1% (at 30.0 mg BAS 762 02 F/kg dry soil) were measured at the end of the 28-day incubation period (time interval 0-28). The results are summarized in

Table A **32**.

Table A 32 Effects of BAS 762 02 F on soil micro-organisms (nitrogen transformation) for the intervals 0-7, 0-14 and 0-28 (rates for interval)

Soil (days)	**Control**	**6.0 mg BAS 762 02 F/kg dry soil**		**30.0 mg BAS 762 02 F/kg dry soil**	
	NO_3–N [mg/kg dry soil] 1)	**NO_3–N [mg/kg dry soil] 1)**	**% Deviation from control 2)**	**NO_3–N [mg/kg dry soil] 1)**	**% Deviation from control 2)**
Loamy sand (0 - 7 d)	30.40	31.83	+4.7	32.00	+5.3
Loamy sand (0 - 14 d)	42.80	43.53	+1.7	49.13	+14.8
Loamy sand (0 - 28 d)	56.77	60.10	+5.9	60.80	+7.1
Soil (days)	**NO_3–N [mg/kg dry soil/d] 3)**	**NO_3–N [mg/kg dry soil/d] 3)**	**% Deviation from control 2)**	**NO_3–N [mg/kg dry soil/d] 3)**	**% Deviation from control 2)**
Loamy sand (0 - 7 d)	4.34	4.55	+4.7	4.57	+5.3
Loamy sand (0 - 14 d)	3.06	3.11	+1.7	3.51	+14.8
Loamy sand (0 - 28 d)	2.03	2.15	+5.9	2.17	+7.1

The calculations were performed with unrounded values.

1) Measured values sampling day "x" - measured values sampling day 0, mean of 3 replicates.

2) Based on NO_3-nirogen production; - = inhibition; + = stimulation

3) Daily rates not given in the study report but recalculated based on the data for the interval.

In a separate study the reference item dinoterb produced a stimulation of nitrogen transformation of +34.2% at 13.60 mg/kg dry soil determined 28 days after application.

Validity criteria:

Validity criteria according to OECD 216 (2000)	**Obtained in this study**
Coefficient of variation in the control for NO_3-N ≤ 15%	max. 5.3% (silty-loamy sand)

All validity criteria were met.

III. CONCLUSION

Exposure of BAS 762 02 F in a field soil up to a test concentration of 30.0 mg BAS 762 02 F/kg dry soil, caused no adverse effects (deviation from control < 25 %, OECD 216) on the soil nitrogen transformation (measured as NO_3-N- production) at the end of the 28-day incubation period (time interval 0-28).

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

Tests on non-target plants have been conducted. The data point is covered by Appendix 2.6.2 (KCP 10.6.2).

A 2.6.2 KCP 10.6.2 Testing on non-target plants

A 2.6.2.1 Study 1

Comments of zRMS:	In general, the study was performed in line with OECD 227 with no major deviations regarding the environmental conditions, replication, observations, observation interval, parameters measured, application of the treatments, etc. It was noted that the number of plants per pot was 3 for lettuce, cabbage, oilseed rape, 2 for tomato, soybean, and corn and 6 for carrot, onion, ryegrass, and wheat. OECD 227 states that the number of plants per pot depends on the species, pot size and test duration, and should provide adequate and uniform growth conditions and avoid overcrowding and shading of plants by each other. For the 15 cm container (used in the study and indicated in OECD 227), 1-2 or 3 seeds should be sown for bigger plants and for smaller plants 5-10 seeds should be used. In principle, after the seeds have emerged, thinning should be completed so that there is only one plant per pot for larger-growing species, while for smaller growing species more than one plant per pot is allowed. Additionally, according to OECD 227, the replicate is defined as a pot but in certain cases a tray of multiple pots with one plant per pot can also be considered as a replicate. In this study 1-3 pots per replicate were used depending on the plant species to obtain 5 replicates per treatment (there were 30 plants for each species per treatment). Although for some species the number of plants per pot could be slightly too high, all plants in the control and the treatment group survived, no phytotoxic symptoms were observed for all tested plant species and the validity criteria of the test were met. Therefore, in the zRMS opinion these deviations had no significant impact on the outcome of the study. It was also noted in the analytical part of the study that for LC-MS/MS determination of BAS 750 F (mefentrifluconazole), accidentally the LC-MS/MS transitions 399 m/z → 70 m/z (quantitation) and 399 m/z → 182 m/z (monitored for confirmation) were applied. For this analyte, the original BASF method L0361/01 used the MS/MS transitions 398 m/z → 70 m/z (quantitation) and 398 m/z → 182 m/z (confirmation). As the applied parent ion 399 m/z is related to the compound specific hydrogenated ^{13}C satellite of the most intense molecular ion signal of BAS 750 F (relative intensity of ^{13}C satellite signal: approx. 20 %), selectivity (e.g. fragmentation pattern) and sensitivity of the LC-MS/MS determination of BAS 750 F is still given. Applicability of the modified method was also demonstrated by a successful method validation (linearity, accuracy and precision) for BAS 750 F within this study. In the case of preparation of final dilutions of application solutions for analysis significantly higher concentrations of the analyte were present in the application solutions compared to the analysis of water samples discussed in the original method L0361/01. Thus a modified procedure implementing serial dilution steps was used in this analytical phase. Also in the case of preparation of fortified solutions for concurrent recovery control (method validation) significantly higher concentrations of the analyte were present in the application solutions compared to the analysis of water samples discussed in the original method L0361/01. Thus a modified procedure implementing serial dilution steps was used in this analytical phase. According to the study report of the analytical part, these deviations had no significant impact on the outcome of the analysis since no analyte (above the calculated LOD for C_{Spray} of 0.010 g/L) or relevant interferences were detected in the LC-MS/MS analysis of a respective untreated application solution. Also, the recoveries in fortified samples were within the acceptable range of 70-110 % of the fortified concentration given in the SANCO Guideline 3029/99 rev. 4. No phytotoxic effects were observed on any of the species tested (with exception of 1%

	chlorosis on cabbage) and for this reason ER_{50} is estimated to be above the single rate tested (i.e. >1.0 L/ha). Overall, the study is considered acceptable with the following endpoint relevant for the risk assessment: ER_{50} > 1.0 L product/ha

Reference:	CP 10.6.2/1
Report	Effect of BAS 762 02 F on vegetative vigour of ten species of terrestrial plants under greenhouse conditions XXX, A., 2020a Report No 863050, AC/BASF/19/22 BASF DocID 2020/1000745 Authority registration No
Guideline(s):	OECD 227 July 2006, ~~OECD ENV/JM/MONO(2002)/9, EPA~~ OCSPP 850.4150 (2012)
Deviations:	Yes (see the commenting box above)
GLP:	Yes (certified by Land Brandenburg Ministerium der Justiz und fuer Europa und fuer Verbraucherschutz, Potsdam, Germany)
Acceptability:	Acceptable
Duplication (if vertebrate study)	No

Executive Summary

In a vegetative vigor test, six species of dicotyledonous plants (carrot, lettuce, oilseed rape, cabbage, soybean, tomato) and four species of monocotyledonous plants (onion, ryegrass, wheat, corn) were exposed to BAS 762 02 F to evaluate the phytotoxic potential. BAS 762 02 F was applied post-emergence at growth stage BBCH 12-14 in a limit test with a rate of 1.0 L BAS 762 02 F/ha. In addition, a control treatment with tap water was set up. After application, the plants were cultivated for 21 days under greenhouse conditions. Assessment of plant survival and phytotoxicity was done 7, 14 and 21 days after treatment (DAT) and assessment of plant length and shoot dry weight was done at study termination (21 DAT).

All control plants remained healthy throughout the entire trial period. No control mortality was observed. No negative impact of the application of 1.0 L BAS 762 02 F/ha at BBCH 12-14 on plant survival, plant length, dry biomass production and plant phytotoxicity was found for all tested species.

Based on the results of this study, conducted under greenhouse conditions, it can be concluded that BAS 762 02 F applied post emergence at BBCH 12-14 with a rate of 1.0 L/ha did not cause effects to plant phytotoxicity, plant survival, plant length and plant dry biomass for all tested plant species. The NOER for plant survival, plant length, dry biomass and plant phytotoxicity of all tested plant species is equal to or higher than the tested rate of 1.0 L BAS 762 02 F/ha. The ER_{50} for all plant species is > 1.0 L BAS 762 02 F/ha.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 762 02 F, batch no. FD-190207-0001, content of a.s.: mefentrifluconazole (BAS 750 F, Reg. No. 5 834 378): 96.2 g/L analyzed (nominal 100.0 g/L) and boscalid (BAS 510 F, Reg. No. 300 355): 205.2 g/L analyzed (nominal 200.0 g/L), density: 1.130 g/cm^3.

B. STUDY DESIGN

Test species: Carrot (*Daucus carota*), lettuce (*Lactuca sativa*), cabbage (*Brassica oleracea* var. *capitata f. alba*), oilseed rape (*Brassica napus*), tomato (*Solanum lycopersicum*), soybean (*Glycine max*), onion (*Allium cepa*), ryegrass (*Lolium multiflorum*), wheat (*Triticum aestivum*) and corn (*Zea mays*).

Test design: Greenhouse study; limit test; 2 treatment groups (1 test item rate, control); 5 replicates per treatment, 1 - 3 pots/replicate, each pot with 2 - 6 plants per pot; post-emergence application at growth stage BBCH 12-14 using a laboratory spray cabin at a mean output volume of 272 L/ha (CV of 1.78% for all tested plant species); assessment of plant survival and phytotoxicity was done 7, 14 and 21 days after treatment (DAT); assessment of plant length and shoot dry weight was done 21 DAT.

Endpoints: Survival, phytotoxicity, plant length and shoot dry weight (NOER, ER_{50}).

Test rates: Control (tap water) and 1.0 L BAS 762 02 F/ha.

Test conditions: Daily average temperature: 21.1 - 30.9°C; daily mean relative humidity: 49.2 - 69.7%; photoperiod: day length ≥ 16 hours; additional light supply automatically for 16 hours in maximum when indoor illumination was less than 300 µmol.

Analytics: Analytical verification of the a.s. BAS 750 H present in application solutions prepared from the test item BAS 762 02 F was conducted using a LC method with MS/MS detection (method no. L0361/01).

Statistics: Descriptive statistics. Depending on outcomes of pretesting sequences the limit concentration of BAS 762 02 F for survival, plant length and biomass was tested by pairwise comparison with the control. Metric data were tested by Two-sample t-test (Student t-test, one-sided smaller, p = 0.05). The NOER for phytotoxicity was estimated. Phytotoxicity values < 10% were considered as insignificant.

C. DESCRIPTION OF THE ANALYTICAL PROCEDURES

The concentration of BAS 750 F (contained in BAS 762 02 F) in the application solution was determined according to the analytical method L0361/01. The validation of the analytical method is described in the study report. The aqueous application solutions were diluted in two steps by a total factor of 100000 using acetonitrile/water (20/80 v/v) + 0.1% formic acid as solvent. Three replicate dilutions were analyzed for each selected application solution. The diluted application solutions were analyzed for the content of BAS 750 F by LC-MS/MS with external standardization. The limit of detection (LOD) was set to 0.01 g/L. Due to the high total dilution factor (100000), no relevant matrix effects were expected for the LC-MS/MS determination of BAS 750 F. Frozen storage stability for the analyte mefentrifluconazole (BAS 750 F) in aqueous solutions is demonstrated for 90 ± 1 days in a previous study communicated by the Sponsor. Details on measured fortification samples and obtained procedural recoveries for BAS 750 F are given in Table A 33.

Table A 33 Procedural recoveries for BAS 750 F

Matrix	Fortification level (g/L)	n	Mean (%)	RSD (%)
Mass Transition: 399 m/z → 70 m/z				
Application solution	0.275	5	94	4.64
Application solution	0.443	5	99	6.73

The average concentration of the active ingredient BAS 750 F (Mefentrifluconazole) obtained by LC-MS/MS analysis of the treated application solution derived from the test item BAS 762 02 F was 0.351 g/L equivalent to a recovery of 95 % of the expected concentration.

II. RESULTS AND DISCUSSION

All control plants remained healthy throughout the entire trial period. No control mortality was observed. No negative impact of the application of 1.0 L BAS 762 02 F/ha at BBCH 12-14 on plant survival, plant length, dry biomass production and plant phytotoxicity was found for all tested species. The results are summarized in Table A 34 and Table A 35.

Table A 34 Effects of BAS 762 02 F on survival, phytotoxicity, plant height and plant dry weight 21 DAT

BAS 762 02 F [L/ha]	Carrot	Lettuce	Cabbage	Oilseed rape	Tomato	Soybean	Onion	Ryegrass	Wheat	Corn
Plant survival [%]										
Control	100	100	100	100	100	100	100	100	100	100
1.0	100	100	100	100	100	100	100	100	100	100
Phytotoxicity [%]										
Control	0	0	0	0	0	0	0	0	0	0
1.0	0	0	1[C]	0	0	0	0	0	0	0
Plant length [% compared to control]										
Control	--	--	--	--	--	--	--	--	--	--
1.0	99.1	101.6	100.4	100.3	101.9	100.8	101.4	103.3	96.7	101.3
Plant dry weight [% compared to control]										
Control	--	--	--	--	--	--	--	--	--	--
1.0	94.4	101.5	101.4	105.3	102.7	103.5	103.0	104.0	98.5	106.7

C = Chlorosis

Table A 35 NOER and ER_{50} of BAS 762 02 F for non-target plants 21 DAT

BAS 762 02 F [L/ha]	Carrot	Lettuce	Cabbage	Oilseed rape	Soybean	Tomato	Onion	Ryegrass	Wheat	Corn
Plant survival										
NOER	≥ 1.0	≥ 1.0	≥ 1.0	≥ 1.0	≥ 1.0	≥ 1.0	≥ 1.0	≥ 1.0	≥ 1.0	≥ 1.0
ER_{50}	> 1.0	> 1.0	> 1.0	> 1.0	> 1.0	> 1.0	> 1.0	> 1.0	> 1.0	> 1.0
Phytotoxicity										
NOER	≥ 1.0	≥ 1.0	≥ 1.0	≥ 1.0	≥ 1.0	≥ 1.0	≥ 1.0	≥ 1.0	≥ 1.0	≥ 1.0
Plant length										
NOER	≥ 1.0	≥ 1.0	≥ 1.0	≥ 1.0	≥ 1.0	≥ 1.0	≥ 1.0	≥ 1.0	≥ 1.0	≥ 1.0
ER_{50}	> 1.0	> 1.0	> 1.0	> 1.0	> 1.0	> 1.0	> 1.0	> 1.0	> 1.0	> 1.0
Plant dry weight										
NOER	≥ 1.0	≥ 1.0	≥ 1.0	≥ 1.0	≥ 1.0	≥ 1.0	≥ 1.0	≥ 1.0	≥ 1.0	≥ 1.0
ER_{50}	> 1.0	> 1.0	> 1.0	> 1.0	> 1.0	> 1.0	> 1.0	> 1.0	> 1.0	> 1.0

Validity criteria:

Validity criteria according to OECD 227	Obtained in this study
Seedling emergence rate is at least 70%	yes (88% to 98%)
In the controls:	
The plants do not exhibit visible phytotoxic effects (e.g. chlorosis, necrosis, wilting, leaf and stem deformations). Plants exhibit only normal variation in growth and morphology for that particular species	yes (0%)
Mean plant survival at least 90% for the duration of the study	yes (100%)
Environmental conditions for a particular species are identical and growing media contain the same amount of soil matrix, support media, or substrate from the same source	yes

All validity criteria were met.

III. CONCLUSION

Based on the results of this study, conducted under greenhouse conditions, it can be concluded that BAS 762 02 F applied post emergence at BBCH 12-14 with a rate of 1.0 L/ha did not cause effects to plant phytotoxicity, plant survival, plant length and plant dry biomass for all tested plant species. The NOER for plant survival, plant length, dry biomass and plant phytotoxicity of all tested plant species is equal to or higher than the tested rate of 1.0 L BAS 762 02 F/ha. The ER_{50} for all plant species is > 1.0 L BAS 762 02 F/ha.

A 2.6.2.2 Study 2

Comments of zRMS:	In general, the study was performed in line with OECD 208 with no major deviations regarding the environmental conditions, replication, observations, observation interval, parameters measured, application of the treatments, etc. It was noted that the number of seeds per pot was 5 for lettuce, cabbage, oilseed rape, tomato, soybean, and corn and 10 for carrot, onion, ryegrass, and wheat. OECD 208 states that the number of plants per pot depends on the size of the seeds and the size of the container, and for the 15 cm container (used in the study and indicated in OECD 208), the number of seeds for bigger plants should be 1-2 or 3 and for smaller plants 5-10. Taking this into account, the plant density for bigger plants such as lettuce or cabbage could have been too high in this study. Additionally, according to OECD 208, the replicate is defined as a pot, and in this study, e.g. 2 pots per replicate were used for plants for which 5 seeds per pot were sown (but in the end there were 40 seeds for each plant species per treatment). Nevertheless, since all plants in the control and the treatment group survived and no phytotoxic symptoms were observed for all tested plant species, and the validity criteria of the test were met, in the zRMS opinion these deviations had no significant impact on the outcome of the study. It was also noted in the analytical part of the study that for LC-MS/MS determination of BAS 750 F (mefentrifluconazole), accidentally the LC-MS/MS transitions 399 m/z → 70 m/z (quantitation) and 399 m/z → 182 m/z (monitored for confirmation) were applied. For this analyte, the original BASF method L0361/01 used the MS/MS transitions 398 m/z → 70 m/z (quantitation) and 398 m/z → 182 m/z (confirmation). As the applied parent ion 399 m/z is related to the compound specific hydrogenated ^{13}C satellite of the most intense molecular ion signal of BAS 750 F (relative intensity of ^{13}C satellite signal: approx. 20 %), selectivity (e.g. fragmentation pattern) and sensitivity of the LC-MS/MS determination of BAS 750 F is still given. Applicability of the modified method was also demonstrated by a successful method validation (linearity, accuracy and precision) for BAS 750 F within this study. In the case of preparation of final dilutions for analysis of application solutions significantly higher concentrations of the analyte were present in the application solutions compared to the analysis of water samples discussed in the original method L0361/01. Thus a modified procedure implementing serial dilution steps was used in this analytical phase. Also in the case of preparation of fortified solutions for concurrent recovery control (method validation) significantly higher concentrations of the analyte were present in the application solutions compared to the analysis of water samples discussed in the original method L0361/01. Thus a modified procedure implementing serial dilution steps was used in this analytical phase. According to the study report of the analytical part, these deviations had no significant impact on the outcome of the analysis since no analyte (above the calculated LOD for C_{Spray} of 0.010 g/L) or relevant interferences were detected in the LC-MS/MS analysis of a respective untreated application solution. Also, the recoveries in fortified samples were within the acceptable range of 70-110 % of the fortified concentration given in the SANCO Guideline 3029/99 rev. 4. No phytotoxic effects were observed on any of the species tested (with exception of 1% chlorosis on cabbage) and for this reason ER_{50} is estimated to be above the single rate tested (i.e. >1.0 L/ha).

	Overall, the study is considered acceptable with the following endpoint relevant for the risk assessment: ER_{50} > 1.0 L product/ha

Reference:	CP 10.6.2/2
Report	Effect of BAS 762 02 F on seedling emergence and seedling growth of ten species of terrestrial plants under greenhouse conditions, XXX, A., 2020b Report No 863049, AC/BASF/19/21 BASF DocID 2020/1000744 Authority registration No
Guideline(s):	EPA 850.4100 - Seedling Emergence and Seedling Growth (2012), OECD 208 (2006), ~~OECD ENV/JM/MONO(2002)/9~~
Deviations:	Yes (see the commenting box above)
GLP:	Yes (certified by Land Brandenburg Ministerium der Justiz und fuer Europa und fuer Verbraucherschutz, Potsdam, Germany)
Acceptability:	Acceptable
Duplication (if vertebrate study)	No

Executive Summary

In a seedling emergence test, six species of dicotyledonous plants (carrot, lettuce, oilseed rape, cabbage, soybean and tomato) and four species of monocotyledonous plants (onion, ryegrass, wheat and corn) were exposed to BAS 762 02 F. The test item was applied pre-emergence at an application rate of 1.0 L BAS 762 02 F/ha. In addition, a control treatment with tap water was set up. Plants were cultivated under greenhouse conditions for 21 days (carrot and onion for 28 days). Assessments for seedling emergence, plant survival and phytotoxicity were done 7, 14 and 21 days after treatment (DAT) for all plants (14, 21 and 28 DAT for carrot and onion). Assessments for plant length and plant dry weight were done at study termination 21 DAT (for carrot and onion 28 DAT).

All control plants remained healthy throughout the entire trial period. No control mortality was observed. No negative impact of the pre-emergence application of 1.0 L BAS 762 02 F/ha on seedling emergence, plant survival, plant length, dry biomass production and plant phytotoxicity was found for all tested species.

Based on the results of this study, conducted under greenhouse conditions, it can be concluded that the fungicide BAS 762 02 F did not cause effects to plant survival and plant length of the tested plant species. The NOER for plant emergence, plant survival, plant length and biomass of all tested plant species is ≥ 1.0 L BAS 762 02 F/ha. The ER_{50} is > 1.0 L BAS 762 02 F/ha for all tested plant species.

I. MATERIALS AND METHODS

A. MATERIALS

Test item: BAS 762 02 F, batch no. FD-190207-0001, content of a.s.: mefentrifluconazole (BAS 750 F, Reg. No. 5 834 378): 96.2 g/L analyzed (nominal 100.0 g/L) and boscalid (BAS 510 F, Reg. No. 300 355): 205.2 g/L analyzed (nominal 200.0 g/L), density: 1.130 g/cm^3.

B. STUDY DESIGN

Test species:	Carrot (*Daucus carota*), lettuce (*Lactuca sativa*), cabbage (*Brassica oleracea* var. *capitata f. alba*), oilseed rape (*Brassica napus*), tomato (*Solanum lycopersicum*), soybean (*Glycine max*), onion (*Allium cepa*), ryegrass (*Lolium multiflorum*), wheat (*Triticum aestivum*) and corn (*Zea mays*).
Test design:	Greenhouse study; limit test; 2 treatment groups (1 test item rate, control); 4 replicates per treatment, 1 - 2 pots/replicate, each pot with 5 - 10 seeds per pot; pre-emergence application shortly after seeding using a laboratory spray chamber at a mean output volume of 265 L/ha (CV of 1.15% for all tested plant species); assessment of seedling emergence, plant survival and phytotoxicity was done 7, 14 and 21 days after treatment (DAT) (carrot and onion 14, 21 and 28 DAT); assessment of plant length and shoot dry weight was done 21 DAT (for carrot and onion 28 DAT).
Endpoints:	Seedling emergence, survival, phytotoxicity, plant length and plant dry weight (NOER, ER_{50}).
Test rates:	Control (tap water) and 1.0 L BAS 762 02 F/ha.
Test conditions:	Daily average temperature: 23.7 - 29.0°C; daily mean relative humidity: 52.0 - 68.6%; photoperiod: day length $\geq$ 16 hours; additional light supply automatically for 16 hours in maximum when indoor illumination was less than 300 µmol.
Analytics:	Analytical verification of the a.s. BAS 750 F present in application solutions prepared from the test item BAS 762 02 F was conducted using a LC method with MS/MS detection (method no. L0361/01).
Statistics:	Descriptive statistics; Depending on outcomes of pretesting sequences the limit concentration of BAS 762 02 F for emergence, survival, plant length, and biomass was tested by pairwise comparison with the control. Metric data were tested by Two sample t-test (Student t-test, one sided smaller, p = 0.05) and quantal data were tested by Two-sample Fisher's Exact test (one-sided greater, p = 0.05).

C. DESCRIPTION OF THE ANALYTICAL PROCEDURES

Concentrations of BAS 750 F (contained in BAS 762 02 F) in application solution were determined according to the analytical method L0361/01. The validation of the analytical method is described in the study report. The aqueous application solutions were diluted in two steps by a total factor of 100 000 using acetonitrile/water (20/80 v/v) + 0.1% formic acid as solvent. Three replicate dilutions were analyzed for each selected application solution. The diluted application solutions were analysed for the content of BAS 750 F by LC-MS/MS with external standardization. The limit of detection (LOD) was set to 0.01 g/L. Due to the high total dilution factor (100000), no relevant matrix effects were expected for the LC-MS/MS determination of BAS 750 F. Frozen storage stability for the analyte mefentrifluconazole (BAS 750 F) in aqueous solutions is demonstrated for 90 ± 1 days in a previous study communicated by the Sponsor. Details on measured fortification samples and obtained procedural recoveries for BAS 750 F are given in

Table A **36**.

Table A 36 Procedural recoveries for BAS 750 F

Matrix	Fortification level (g/L)	n	Mean (%)	RSD (%)
Mass Transition: 399 m/z → 70 m/z				
Application solution	0.273	5	99	3.24
Application solution	0.447	5	100	3.10

The average concentration of the active ingredient BAS 750 F (Mefentrifluconazole) obtained by LC-MS/MS analysis of the treated application solution derived from the test item BAS 762 02 F was 0.403 g/L equivalent to a recovery of 107 % of the expected concentration.

II. RESULTS AND DISCUSSION

All control plants remained healthy throughout the entire trial period. No control mortality was observed. No negative impact of the pre-emergence application of 1.0 L BAS 762 02 F/ha on seedling emergence, plant survival, plant length, dry biomass production and plant phytotoxicity was found for all tested species (Two-sample Fisher's Exact test, one-sided greater, p=0.05). The results are summarized in Table A 37 and

Table A **38**.

Table A 37 Effect of BAS 762 02 F on seedling emergence, survival, phytotoxicity, plant length and plant dry weight 21 DAT (for carrot and onion 28 DAT)

BAS 762 02 F [L/ha]	Carrot 1)	Lettuce	Cabbage	Oilseed rape	Tomato	Soybean	Onion 1)	Ryegrass	Wheat	Corn
Seedling emergence [% for control and % compared to control in test item groups]										
Control	90	98	95	93	80	98	95	88	95	95
1.0 2)	103	100	103	108	103	97	92	94	97	100
Survival [%]										
Control	100	100	100	100	100	100	100	100	100	100
1.0	100	100	100	100	100	100	100	100	100	100
Phytotoxicity [%]										
Control	0	0	0	0	0	0	0	0	0	0
1.0	0	0	0	0	0	0	0	0	0	0
Plant length [% compared to control]										
Control	--	--	--	--	--	--	--	--	--	--
1.0	97.9	99.0	101.0	99.7	95.3	97.5	102.2	97.4	101.6	101.0
Plant dry weight [% compared to control]										
Control	--	--	--	--	--	--	--	--	--	--
1.0	99.1	93.1	105.4	103.7	99.0	95.5	94.2	92.2	98.1	99.5

1) Carrot and onion 28 DAT
2) Compared to control

Table A 38 NOER and ER_{50} of BAS 762 02 F for non-target plants 21 DAT (for carrot and onion 28 DAT)

BAS 762 02 F [L/ha]	Carrot [1)]	Lettuce	Cabbage	Oilseed rape	Soybean	Tomato	Onion [1)]	Ryegrass	Wheat	Corn
Seedling emergence										
NOER	≥ 1.0	≥ 1.0	≥ 1.0	≥ 1.0	≥ 1.0	≥ 1.0	≥ 1.0	≥ 1.0	≥ 1.0	≥ 1.0
ER_{50}	> 1.0	> 1.0	> 1.0	> 1.0	> 1.0	> 1.0	> 1.0	> 1.0	> 1.0	> 1.0
Survival										
NOER	≥ 1.0	≥ 1.0	≥ 1.0	≥ 1.0	≥ 1.0	≥ 1.0	≥ 1.0	≥ 1.0	≥ 1.0	≥ 1.0
ER_{50}	> 1.0	> 1.0	> 1.0	> 1.0	> 1.0	> 1.0	> 1.0	> 1.0	> 1.0	> 1.0
Phytotoxicity										
NOER	≥ 1.0	≥ 1.0	≥ 1.0	≥ 1.0	≥ 1.0	≥ 1.0	≥ 1.0	≥ 1.0	≥ 1.0	≥ 1.0
Plant length										
NOER	≥ 1.0	≥ 1.0	≥ 1.0	≥ 1.0	≥ 1.0	≥ 1.0	≥ 1.0	≥ 1.0	≥ 1.0	≥ 1.0
ER_{50}	> 1.0	> 1.0	> 1.0	> 1.0	> 1.0	> 1.0	> 1.0	> 1.0	> 1.0	> 1.0
Plant dry weight										
NOER	≥ 1.0	≥ 1.0	≥ 1.0	≥ 1.0	≥ 1.0	≥ 1.0	≥ 1.0	≥ 1.0	≥ 1.0	≥ 1.0
ER_{50}	> 1.0	> 1.0	> 1.0	> 1.0	> 1.0	> 1.0	> 1.0	> 1.0	> 1.0	> 1.0

1) Carrot and onion 28 DAT

Validity criteria:

Validity criteria according to OECD 208	Obtained in this study
Seedling emergence is at least 70% in the control	yes (80% to 98%)
Seedlings do not exhibit visible phytotoxic effects (e.g. chlorosis, necrosis, wilting, leaf and stem deformations) in the control and control plants exhibit only normal variation in growth and morphology for that particular species	yes (0%)
Mean survival of emerged control seedlings at least 90% for the duration of the study	yes (100%)
Environmental conditions for a particular species are identical and growing media contain the same amount of soil matrix, support media, or substrate from the same source	yes

All validity criteria were met.

III. CONCLUSION

Based on the results of this study, conducted under greenhouse conditions, it can be concluded that the fungicide BAS 762 02 F did not cause effects to plant survival and plant length of the tested plant species. The NOER for plant emergence, plant survival, plant length and biomass of all tested plant species is ≥ 1.0 L BAS 762 02 F/ha. The ER_{50} is > 1.0 L BAS 762 02 F/ha for all tested plant species.

A 2.6.3 KCP 10.6.3 Extended laboratory studies on non-target plants

As BAS 762 02 F poses no unacceptable risk to non-target plants, further tests are not necessary.

A 2.6.4 KCP 10.6.4 Semi-field and field tests on non-target plants

As BAS 762 02 F poses no unacceptable risk to non-target plants, further tests are not necessary.

A 2.7 KCP 10.7 Effects on other terrestrial organisms (flora and fauna)

No new studies available.

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 762 02 F or of the active substances.